Measurement of physical activity, sedentary time and continuous glucose concentrations: novel techniques for behavioural profiling

by

Andrew Paul Kingsnorth

Doctoral Thesis

Submitted in partial fulfilment of the requirements
for the award of Doctor of Philosophy of Loughborough University

December 2016

Acknowledgements

Without the many, the last three years would not have been possible. I would like to take this opportunity to thank friends and colleagues that have dedicated their time, experience and friendship throughout my PhD.

Firstly, I would like to thank Dr Dale Esliger for his encouragement to undertake a PhD and for his mentorship throughout the whole process. Your enthusiasm and support to achieve the best has made the last three years challenging but has put me in a great position for a career in academia. I would also like to acknowledge Dr Lauren Sherar, who despite being a late addition to the supervisory team has been a great mentor from the very beginning. Without their guidance, I would not have had the wide variety of experiences and developed the necessary skills to complete this thesis.

Many thanks to my other postgraduate colleagues, who have helped me over the last three years to stay focussed (or not) and to have something to model my own successes on. There are too many to mention but a special thanks to James and Adam who have always provided welcome relief from work.

Another special mention must go to the staff at the Respiratory Biomedical Research Unit in Leicester and to Mark, as he was someone I could always count on for sound advice and scientific rigour. I would also like to thank Maxine, as my last study would not have been possible without her support.

I would like to thank my friends outside of the PhD who have kept me going with questions like ‘have you finished yet?’ and ‘when are you going to get a job?’. Also to my Mum and Dad, whom I cannot thank enough for their unwavering confidence in my ability and the much-needed support over my wandering journey through academia. Be assured... this is the end of the road of being a student.

Finally, my deepest thanks go to Lucy for being there through the highs and lows and providing me with much needed ‘breaks’ from my thesis (being her third TA). She has also sacrificed many things whilst I have been completing this PhD and I hope we can now look forward to the next chapter of our lives together.

Table of Contents

Thesis Contributions

All work within this thesis was conducted by the author under the supervision of Dr Dale Esliger and Dr Lauren Sherar. The author was directly involved in all aspects of protocol design, ethical submissions, recruitment, measurement and data analysis for all studies reported within this document.

This thesis comprises of three studies from two data sources. Studies One (Chapter Three) and Two (Chapter Four) utilised data from the Physical Activity and Respiratory Health (PhARaoH), which was a cross-sectional study funded by research facilitation funds from National Health Service (NHS) England. The study was devised, managed and conducted in collaboration between Loughborough University, University Hospitals of Leicester NHS Trust and the Respiratory Biomedical Research Unit at Glenfield Hospital. The data was collected in conjunction with Mark Orme, a student from the Physical Activity and Public Health Research Group (PAPHRG) at Loughborough University. It is the aim of the Co-investigators that the dataset be utilised for other research questions and is currently available through an application process.

Study Three was conducted with another student from the PAPHRG (Maxine Whelan) to increase efficiency by utilising the same set of participants. Each study had a separate set of aims but used the same data from the physiological and behavioural sensors.

Finally, data manipulation and statistical analysis was completed solely by the author, except for Chapter Four (Study Two) where help was sought to write a computer program to calculate entropy. Sam Adema, a Research student in the Advanced Virtual Reality Research Centre provided the computer script which calculated the entropy scores.

Thesis Outputs

Journal articles

Orme, M. W., Esliger, D. W., Kingsnorth, A. P., Steiner, M. C., Singh, S. J., Malcolm, D., Morgan, M. D. and Sherar, L. B. (2016) Physical Activity and Respiratory Health (PhARaoH): Data from a Cross-Sectional Study. Open Health Data, 4(1), p. 4.

Oral and poster presentations (relating to this thesis)

Kingsnorth, A. P., Sherar, L. B., Whelan, M. E. and Esliger, D. W. Are blood sugar levels influenced by your physical activity? (Poster). Influence & Impact Conference, Loughborough University, October 2016

Kingsnorth, A. P., Whelan, M. E. and Esliger, D. W. Bio-behavioural Feedback: Integrating continuous measures to achieve better health (Oral). Café Academique, Loughborough University, June 2015

Kingsnorth, A. P. and Esliger, D. W. Health technology: meaningful feedback to change behaviour (Oral). Health & Wellbeing Conference, Loughborough University, February 2014

Kingsnorth, A. P. and Esliger, D. W. Health technology: meaningful feedback to change behaviour (Oral). Designed to Move, Leeds Metropolitan, February 2014

Journal articles in preparation (relating to this thesis)

Kingsnorth, A. P., Whelan, M. E., Sherar, L. B. and Esliger, D. W. Physical activity, sedentary behaviour and glycaemic variability: are daily estimates of behaviour and glucose associated?

Kingsnorth, A. P., Adema, S., Orme, M. W., Sherar, L. B. and Esliger, D. W. Associations between cardiometabolic risk factors and behaviour complexity using sample entropy.

Kingsnorth, A. P., Orme, M. W., Sherar, L. B. and Esliger, D. W. Physical activity, sedentary behaviour and their relation to diabetes risk score in adults.

Whelan, M. E., Morgan, P. S., Sherar, L. B., Kingsnorth, A. P., Magistro, D. and Esliger, D. W. Brain responses to personalised behavioural and physiological feedback from wearable self-monitoring technology: An fMRI study.

Orme, M. W., Singh, S. J., Esliger, D. W., Kingsnorth, A. P., Morgan, M. D. L., Steiner, M. C. and Sherar, L. B. Being physically inactive and being sedentary are distinct behaviours in COPD.

Orme, M. W., Singh, S. J., Esliger, D. W., Kingsnorth, A. P., Morgan, M. D. L., Steiner, M. C. and Sherar, L. B Cross-sectional characteristics of individuals with mild-moderate COPD reporting contrasting self-reported symptom burdens.

List of Tables

List of Figures

List of Equations

Abbreviations

BMI	Body mass index
CGM	Continuous glucose monitor
COPD	Chronic obstructive pulmonary disease
CPM	Counts per minute
HbA1c	Glycated haemoglobin
HDL	High-density lipoprotein
ISWT	Incremental Shuttle Walk Test
LDL	Low-density lipoprotein
LVPA	Light to vigorous physical activity
MAGE	Mean amplitude of glycaemic excursions
MARD	Mean absolute relative difference
mCAFT	Modified Canadian Aerobic Fitness Test
METs	Metabolic equivalents
MGluc	Mean daily glucose
MVPA	Moderate to vigorous physical activity
OGTT	Oral glucose tolerance test
PhARaoH	Physical Activity and Respiratory Health Study
StDevG	Standard deviation of glucose
VIF	Variance inflation factor

Chapter One - Introduction

1.1 Introduction

It is well known that physical activity is important in the primary and secondary prevention of chronic diseases such as hypertension, diabetes and cardiovascular disease (Warburton et al., 2006). Despite the documented benefits, large representative samples confirm that only a small percentage of individuals are sufficiently active (Troiano et al., 2008; Craig et al., 2009; Colley et al., 2011). When measured objectively, the amount of individuals meeting the physical activity guidelines was as little as 5% in an English cohort (Craig et al., 2009). Reductions in occupation-related energy expenditure, increased motorised transport and a built environment not conducive to being physically active are some of the factors that go towards explaining a reduction in physical activity levels over the last few decades (Katzmarzyk and Mason, 2009; Church et al., 2011). The direct economic cost to health-care systems worldwide has been conservatively estimated at $53.8 billion in 2013 which contributes to losses of productivity of $13.7 billion (Ding et al., 2016). As the fourth leading risk factor for global mortality (World Health Organization, 2009), insufficient physical activity levels are a challenge that needs to be addressed in order to prevent an increase in the incidence of largely preventable chronic diseases and the economic burden upon health services.

Diabetes is a highly prevalent chronic disease that can be attributed to a lack of physical activity (Helmrich et al., 1991) and according the International Diabetes Federation an estimated 7.3 billion people are suffering from diabetes worldwide (International Diabetes Federation, 2015). Vital for targeted prevention, objective measurements of physical activity aid researchers in establishing patterns of behaviour that have cross-sectional associations with diabetes risk. Additionally, as the field has progressed, the measurement of physical activity is now typically assessed using technologies such as accelerometry that can provide volume, frequency and duration estimates of behaviour. A large amount of research has previously been focussed on the quantification of volume or ‘dose’, however newer methods allow for the modelling of behavioural replacement, the interrogation of behavioural profiles and the use of devices at varying locations on the body (e.g. wrist vs waist). Additionally, whilst individuals can accumulate the same volume of physical activity, distinct behavioural ‘types’ such as ‘Weekend Warriors’ and ‘Busy Bees’ have been identified from large data samples (Metzger et al., 2008; Marschollek, 2014), which could have distinct relationships with health.

Current UK physical activity guidelines state that adults should complete 150 minutes of moderate to vigorous physical activity (MVPA), or 75 minutes of vigorous intensity, or a combination of both in a week to reduce the risk of developing chronic disease later in life (Department of Health, 2011a). As few are meeting the recommended level of physical activity, shown by nationally representative samples (Troiano et al., 2008; Craig et al., 2009; Colley et al., 2011), perhaps the current activity promotion paradigm of promoting a delayed reward may not be enough of a motivating factor to induce behaviour change. Temporal discounting describes how the value of a reward decreases as the delay to attainment increases (Green et al., 1996). Consequently, as the 'reward' (e.g. decreased morbidity and mortality risk) occurs so far into the future, the immediate costs outweigh the future benefits and as such, individuals are unlikely to use preventative measures to avoid an unhealthy lifestyle (Chapman and Elstein, 1995). Acute physiological responses, such as reduced glucose concentrations can be induced after walking (Dunstan et al., 2012), activity breaks (Peddie et al., 2013) and standing for an afternoon (Buckley et al., 2014). Providing objective feedback on glucose concentrations in response to physical activity may be more influential on behaviour change than promising a potential risk reduction that will occur sometime in the future.

Technological advances have allowed a proliferation of devices that allow individuals to self-monitor not only their behaviour continuously, but also their glucose concentrations up to a 2 week period at a time. Preliminary work equating behaviour with glucose responses has solely focused on people with type 2 diabetes. Yet, providing personalised glucose feedback and counselling on expected activity-related glucose reductions from role models resulted in a significant increase in moderate activity minutes and a decrease in sedentary and light minutes (Allen et al., 2008). Similarly, sedentary time has been shown to be associated with time spent in hyperglycaemia, although only over a very short period of time (3-5 days) (Fritschi et al., 2016). Opening up new opportunities for self-monitoring of behaviours, this technique could also be used as a preventative measure in a non-diabetic population. However, for this information to be truly effective, the coupling of behaviour and glucose data in a free-living environment needs to be achieved. Therefore, it is worthwhile investigating the association between physical activity, sedentary time and glucose to see whether any activity related decline in glucose concentrations can be identified within a sample of non-diabetic adults.

1.2 Thesis aims and outline

The aim of this thesis was to profile sedentary time and physical activity behaviours in relation to diabetic risk, cardiometabolic health and glucose control using novel measurement and analytical methods.

The main objectives were as follows:

- To objectively profile sedentary time and physical activity using wearable devices (Chapters Three, Four and Five)
- To determine the relationship between sedentary time and physical activity with cardiometabolic risk factors and glucose concentrations (Chapters Three, Four and Five)
- To understand how the behavioural profile of each individual can influence cardiometabolic health (Chapters Three and Four)
- To examine if the novel processing of behaviour and glucose data can highlight any unexplored relationships with health (Chapters Four and Five)

Chapter Two - Literature Review

2.1 Terminology

Before proceeding into the main body of this literature review, it is important to operationally define important terminology that will be used throughout the thesis.

2.1.1 Physical activity, exercise, sedentary and inactivity

Physical activity and exercise are key terms used within the literature to describe the basic construct of physical movement. Physical activity is described as any bodily movement produced by the skeletal muscles that causes an increase in energy expenditure and whilst a sub component of physical activity, exercise on the other hand is planned, structured, repetitive and has the primary goal of improving or maintaining fitness (Caspersen et al., 1985).

Sedentary behaviour is described as any behaviour whilst in a sitting or reclining posture with an energy expenditure of ≤ 1.5 metabolic equivalents (METs) (Sedentary Behaviour Research Network, 2012). Often used interchangeably, inactivity is not the same as sedentary behaviour, but should be used to describe those who are not meeting the current physical activity guidelines by performing insufficient levels of moderate to vigorous physical activity (MVPA) (Sedentary Behaviour Research Network, 2012). In this thesis, sedentary time is used to describe time spent in activity of low counts, which is indicative of sedentary behaviour as you cannot accurately confirm if a person is sitting/reclining or in a standing posture from count based accelerometer data, the latter classifying true sedentary behaviour.

2.1.2 UK physical activity guidelines

Guidelines have been proposed by the Department of Health that give the general population a minimum target of physical activity, per week, that individuals should obtain to get health benefits. Age specific guidelines are available, however this thesis will focus on the adult (19-64) guidelines (Department of Health, 2011a):

- 150 minutes of MVPA in bouts of 10 minutes or more, 75 minutes of vigorous physical activity or a combination of both
- Strength improving exercises at least 2 days a week
- Minimise the time being sedentary

2.1.3 Physical fitness

Whilst movement relates to the behaviour being performed, physical fitness is a set of attributes that you attain, through continued practice of exercise which can be further sub divided into two components: skill attainment and health related fitness (Caspersen et al., 1985). The latter component can also be further divided into sub-domains: cardiorespiratory endurance, body composition, muscular strength, muscular endurance and flexibility (Caspersen et al., 1985). In this thesis, fitness is used to refer to cardiorespiratory endurance or the ability to supply fuel by the circulatory and respiratory systems during prolonged physical activity (Caspersen et al., 1985).

2.1.4 Validity and reliability

To assess validity, a device should firstly be evaluated first for its reliability, as a test cannot be valid if it is not reliable (Thomas and Nelson, 1996). In this thesis, validity is referred to as accuracy or the degree of closeness between a true value of the 'measurand' (quantity to be measured) and the measurement (Squara et al., 2015). In contrast, reliability is used to describe measurement trueness or the quantification of the agreement between replicate results under specified conditions for the same method (Squara et al., 2015).

2.1.5 Diabetes

Diabetes as a chronic disease can be subdivided into two main distinct conditions: type 1 and type 2 diabetes. Whereas type 1 is classified by a loss of β-cell functionality and absolute insulin deficiency, type 2 is characterised by insulin resistance that may lead to a loss of insulin secretion (American Diabetes Association, 2016). Lifestyle related factors are thought to be influential in the development of type 2 diabetes (International Diabetes Federation, 2015), and will be referred to by the use of the word diabetes within this thesis.

2.2 Physical activity and health

Undertaking regular physical activity is widely accepted to reduce the risk of developing chronic diseases such as cardiovascular disease, diabetes, cancer, hypertension, obesity, depression and osteoporosis (Warburton et al., 2006). Several chronic and acute biological adaptions have been cited as being responsible for the reductions in chronic disease risk by undertaking regular physical activity. These are summarised within Figure 2.1 (Warburton et al., 2006).

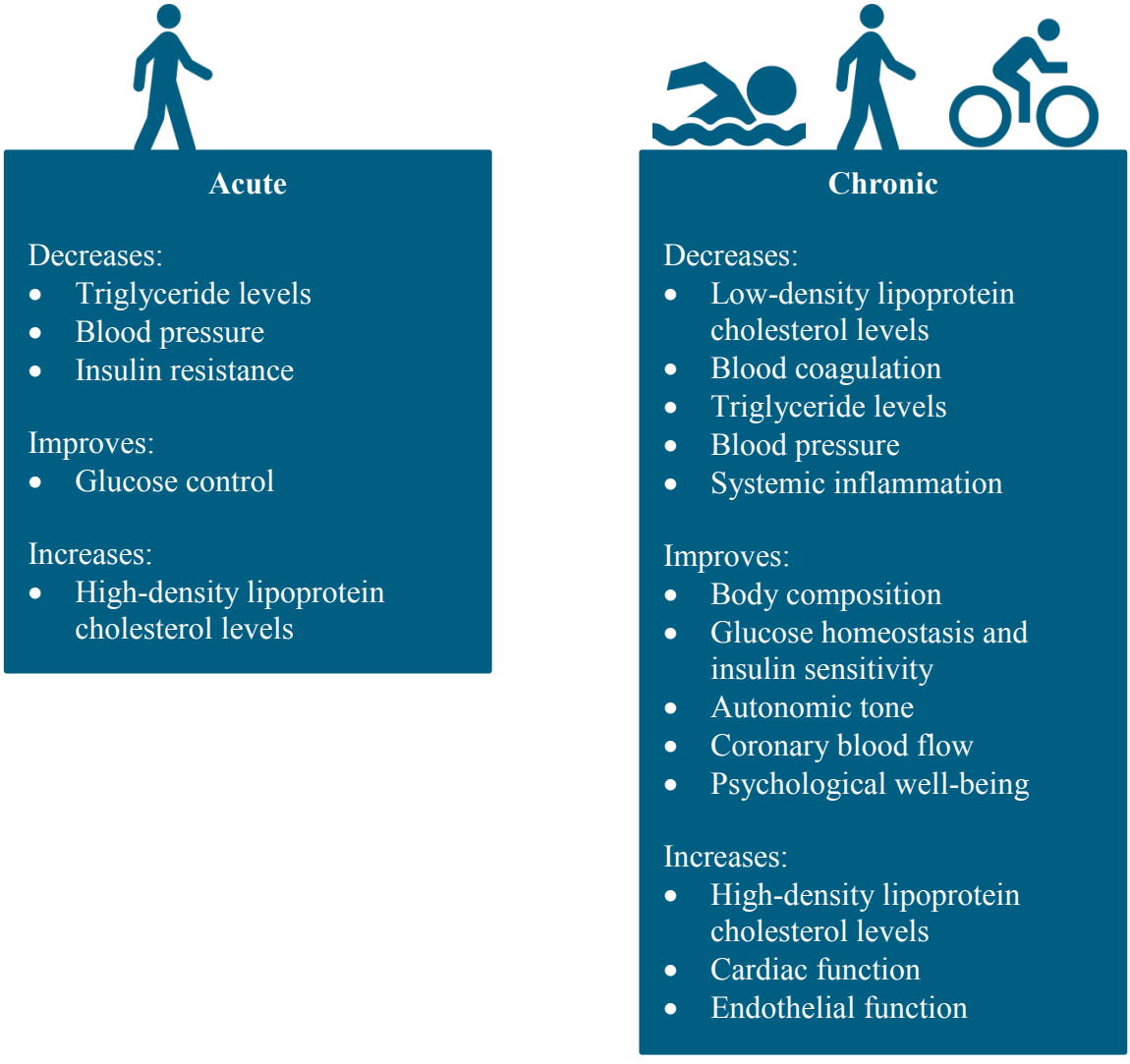


Figure 2.1. The acute and chronic benefits of being more physically active. Summarised from Warburton et al, (2006): 'Health benefits of physical activity: the evidence'.
Icons taken from www.flaticon.com. Designers: Scott de Jonge (walking man), Freepik (swimmer) and Google (cyclist).

Many of the chronic adaptations to being physically active occur over an extended period of time and cannot be immediately quantified. The work within Chapter Six (Study Three) aims to explore the acute physiological changes that are induced by being physically active within a free-living environment. This is to ascertain if there is indeed a relationship that is measureable, as this information could be used to motivate people to become more physically active in future research studies. The acute response to being more physically active is based upon the literature that suggests a single session of physical activity has been shown to improve cardiometabolic risk factors such as glucose concentrations. This literature will now be discussed in the following section.

2.2.1 Acute response of glucose concentrations to physical activity

Physical activity can induce acute physiological health benefits on health parameters such as blood glucose, even after a single bout of activity (Thompson et al., 2001). A study that assessed postprandial glucose and insulin levels following either uninterrupted sitting, uninterrupted sitting with either 2 minute bouts of light walking or moderate intensity walking every 20 minutes in overweight/obese adults, found that compared to sitting, the area under the glucose and insulin curve was reduced ($p < 0.01$) (Dunstan et al., 2012). In a randomised cross over fashion, each participant was asked to refrain from caffeine, alcohol and exercise 48 hours before each activity condition. The postprandial spike was caused by a standardised test drink taken 2 hours into each trial.

Postmeal hyperglycaemia is an independent risk factor for microvascular disease and it is recommended that it is reduced to benefit cardiovascular health (Ceriello et al., 2008), i.e. a lower area under the curve is beneficial for health. Subsequent studies have replicated these methods and have found similar findings. In adult males with type 2 diabetes, breaking up sedentary time with either three 15 minute bouts of activities of daily living (post meal strolling at 3 METs) or one bout of 45 minutes reduced postprandial areas under the curve for both conditions ($p < 0.001$ one bout; $p < 0.05$ activities of daily living) (van Dijk et al., 2013). In this trial, moderate activity of a continuous fashion created higher acute health benefits. However, matching for total duration in separated bouts could be an effective way to induce changes of glucose by embedding lifestyle physical activity for those that cannot perform continuous bouts.

Attenuations in blood glucose concentrations are also evident for lower intensity behaviours such as standing (Buckley et al., 2014; Thorp et al., 2014). In a sample of office workers, 10 employees were asked to sit and then stand for an afternoon on separate workdays whilst standardising their food intake. A 43% attenuation in blood glucose values was established and at the end of the day, blood glucose was 1.7 mmol/L and 0.5 mmol/L higher than baseline for both the seated and standing condition respectively (Buckley et al., 2014). Associations with accelerometry within a standardised condition has also revealed a 0.06 mmol/L · 9h decrease in glucose area per every 100 counts (Peddie et al., 2013) showing that the effect of physical activity on glucose metabolism has a wealth of evidence to support it. Nevertheless, studies have utilised controlled methods and mainly have been conducted within a laboratory setting where sedentary conditions were rigorously enforced i.e. only rising from a seated position to go to the toilet. If benefits are constrained by a necessity for standardisation by controlling food intake or isolating behaviours i.e. comparing uninterrupted sitting with a period of activity, then the feasibility of applying these techniques out of controlled settings is reduced. Studies should now progress to ascertain whether activity related glucose reductions can be extended into a free living environment. If increased variability does not eliminate the relationship between acute health and physical activity, the relationship between sedentary time, physical activity and glucose is likely to be robust enough for remote monitoring purposes.

Behind the acute responses to physical activity, there are underlying mechanisms which help explain why being physically active is beneficial. In healthy adults, glucose concentrations are kept within a stable range through a process called homeostasis, which requires a combination of metabolic events: sufficient insulin secretion, the stimulation of glucose uptake and the suppression of hepatic glucose production (Bouchard et al., 2012). The body uses two hormones to regulate this process: glucagon when there is not enough glucose and insulin when there is an abundance of glucose (Figure 2.2) (Kirk et al., 2008). Increased glucose uptake is initiated by an increased translocation of the GLUT4 glucose transporter, which is signalled through the independent pathways of insulin and muscle contractions (Bouchard et al., 2012). Once across the muscle membrane, glucose is either used as energy in glycolysis or synthesised into glycogen using glycogen synthase (Bouchard et al., 2012).

The increased uptake of glucose is therefore an important by-product of activity and one strategy to utilise the glucose uptake mechanism could be by the way of introducing ‘physical

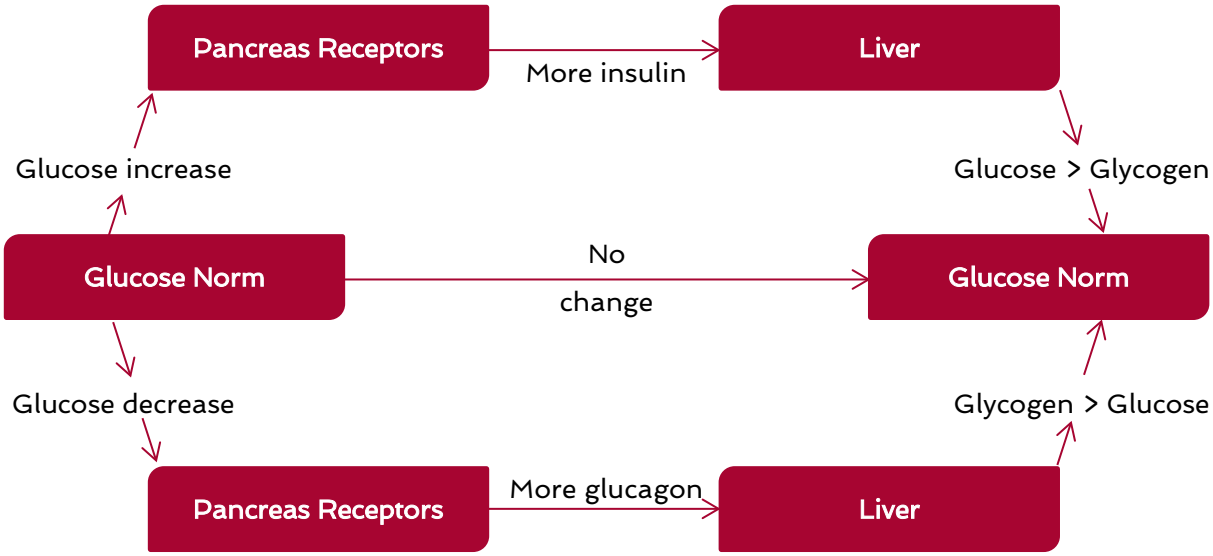


Figure 2.2. The process of blood glucose homeostasis outlining the use of insulin and glucagon within the body (Kirk et al., 2008).

activity snacks', which aim to continuously top up glucose intake throughout the day. Completing brief (1 minute) but intense (90% maximum heart rate) physical activity 30 minutes before meals attenuated 3 hour postprandial glucose concentrations for breakfast and dinner compared to energy matched sessions of 30 minutes of moderate walking (60% maximum heart rate) before breakfast and dinner (−1.4 mmol/L $p = 0.02$; −0.7 mmol/L $p = 0.04$) (Francois et al., 2014). Whilst it has also been suggested that physical activity should be completed after meals (Chacko, 2014), it is clear that introducing physical activity 'snacks' throughout the day could be an effective strategy for reducing postprandial glucose concentrations.

2.3 Diabetes and the association with physical activity

According to the World Health Organisation (WHO), 314 million more people were living with diabetes in 2014 than in 1980 (108 million) (World Health Organization, 2016). This number is expected to rise to 439 million by 2030 (Shaw et al., 2010), and other estimates put this number at 642 million by 2040 at a prevalence of 10.4% (8.5-13.5%) (International Diabetes Federation, 2015). Diabetes spend is estimated to account for 5-20% of total health care expenditure for the majority of countries with the human cost being 5 million deaths in 2014 (International Diabetes Federation, 2015), indicating that diabetes is a major global health burden.

The WHO recommends diabetes be diagnosed if one or more of the following criteria is met: a fasting glucose concentration ≥ 7.0 mmol/L or glucose concentrations ≥ 11.1 mmol/L following a 75g oral glucose tolerance test (OGTT) (World Health Organization, 2016). Additionally, individuals that are at higher risk but not currently meeting the threshold for diabetes can be categorised either into Impaired Glucose Tolerance or Impaired Fasting Glucose (World Health Organization, 2016):

- Impaired Glucose Tolerance
  - Fasting levels of glucose < 7.0 mmol/L **AND**
  - Glucose concentrations ≥ 7.8 and < 11.1 mmol/L 2 hours after ingestion of 75g oral glucose load
- Impaired Fasting Glucose
  - Fasting levels of glucose 6.1 to 6.9 mmol/L **AND**
  - Glucose concentrations < 7.8 mmol/L 2 hours after ingestion of 75g oral glucose load

The term pre-diabetes has been used to describe those individuals that fall within the impaired tolerance or impaired fasting criteria (American Diabetes Association, 2016), yet this phrase has been discouraged in the past as this attaches stigma to the condition by association with the word diabetes (World Health Organization, 2006). Nevertheless, the American Diabetes Association still continues to use the term pre-diabetes and has also lowered their criteria to denote classification at 5.6-6.9 mmol/L fasted state or 7.8-11.0 mmol/L following a OGTT (American Diabetes Association, 2016).

2.3.1 Risk factors for diabetes

Although non modifiable risk factors for diabetes exist such as age, genetics and ethnicity, many are considered lifestyle modifiable and include: excess body weight, physical inactivity and poor nutrition (International Diabetes Federation, 2015). Physical activity has been associated with a decreased risk of developing type 2 diabetes (Helmrich et al., 1991; Bassuk and Manson, 2005), with data from 20 longitudinal studies indicating that a substantial reduction in risk (20-30%) can occur with regular physical activity (Gill and Cooper, 2008). Indeed, a well cited study that compared the risk reduction between lifestyle embedded physical activity and a pharmacological intervention found that lifestyle changes were more effective than Metformin (Diabetes Prevention Program Research Group, 2002). Physical activity should therefore be used in the primary and secondary prevention of diabetes (Warburton et al., 2006).

Ethnicity as a non-modifiable risk factor is also important as it has been observed that in certain ethnic groups, such as the South Asian population, the prevalence of diabetes can be 15% higher than Europeans (McKeigue et al., 1991) and diabetes is more prevalent at an earlier age (Mather and Keen, 1985). A recent study has identified that South Asian individuals may also need to participate in 10-15 minutes more of MVPA on top of the current adult guidelines to obtain the same level of reduction in risk as Europeans (Iliodromiti et al., 2016). This would suggest that population level screening is important to identify those currently at high risk of developing diabetes in order to intervene early and reduce their risk of developing the condition.

2.3.2 Diabetes risk scores

In order to prevent further incidence and identify current prevalence of diabetes in the wider population, effective screening of risk factors should be undertaken. Risk scores are a more feasible method of detecting at risk individuals than the considered gold standard OGTT. The Leicester Diabetes Risk Assessment Score was developed in a multi-ethnic UK population to identify those at high risk of impaired glucose regulation and type 2 diabetes (Gray et al., 2010). The following criteria: age, sex, ethnicity, family history of type 2 diabetes, waist circumference, body mass index (BMI) and antihypertensive medication or high blood pressure are used to estimate risk (Gray et al., 2010). A modified version of the risk score (excluding waist circumference as this is not routinely measured) has been implemented into primary care and has been shown to identify a higher number of people with abnormal

glucose tolerance (Gray et al., 2012). The risk score is a useful and feasible way to identify those at risk of developing diabetes.

From this overview, it is clear that whilst diabetes risk screening is important, the effective measurement of risk factors such as physical activity is also of significance in order to identify modifiable behaviours that can reduce the risk of chronic disease.

2.4 Measurement of physical activity

The measurement of physical activity behaviours using valid and reliable techniques is required in order to confirm the determinants and health associations of being physically (in)active (Dishman et al., 2001). Measurement is the quantification of an unknown measurand using experimental methods (Squara et al., 2015), and in terms of physical activity can be expressed as either measuring the behaviour or the physiological response (energy cost) of the behaviour (Lamonte and Ainsworth, 2001). Figure 2.3 represents a conceptual model between energy expenditure, the behaviour and assessment tools that can measure physical activity (movement). Health associations have been shown to be better predicted by energy expenditure than physical activity behaviours (Lee and Paffenbarger, 1998; Manson et al., 1999), therefore before quantifying the effects upon health outcomes, activity is often extrapolated to units of energy expenditure (Lamonte and Ainsworth, 2001). However, before extrapolation can take place the valid and reliable measurement of physical activity is required, which can be achieved by an ever increasing list of methods or devices.

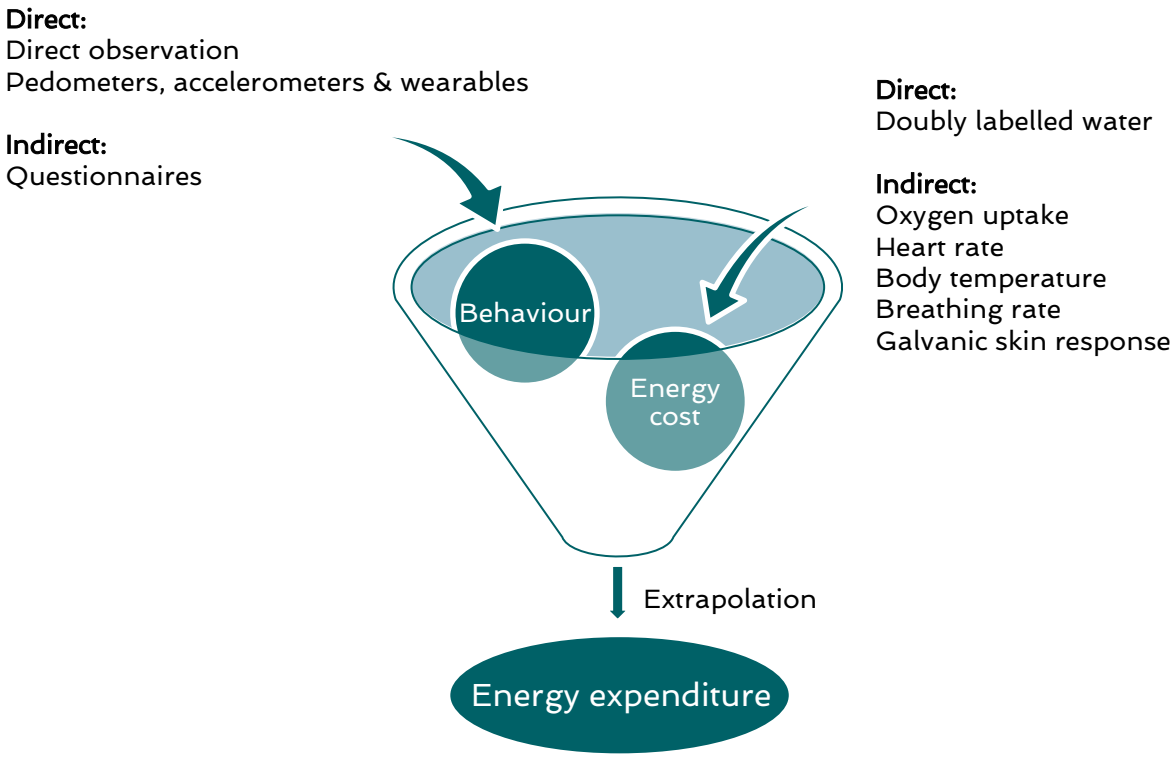


Figure 2.3. A diagram representing the assessment tools associated with the quantification of the behaviour and energy cost related to the extrapolation to energy expenditure. Adapted from (Lamonte and Ainsworth, 2001).

2.4.1 How behaviour is measured and analytical parameters to consider

Physical activity measurement can be broadly summarised into the following categories: subjective methods (questionnaires, diaries) and objective methods or portable monitors (pedometers, accelerometers, posture sensors) (Troiano, 2005). Nonetheless, there is often a feasibility and validity trade off where a device may be highly feasible by requiring low resources, but considered less valid. For instance direct observation can provide a wealth of information pertaining to the type, frequency and duration of physical activity but lacks feasibility in large scale population studies due to involved deployment costs and potential reactivity (Sylvia et al., 2014). Similarly, self-report measures such as questionnaires are highly feasible and easy to administer (Strath et al., 2013), but estimations can be influenced by social desirability (Shephard, 2003; Adams et al., 2005), which have the potential to result in an overestimation of physical activity (Adams et al., 2005). Therefore, the choice of measurement technique should be chosen specific to each research question and resources (time, budget and staff) available (Dishman et al., 2001).

Small devices such as accelerometers are now widely used to objectively measure physical activity and are thought to remove certain limitations of self-reported measures (Prince et al., 2008). Accelerometers are devices worn typically for seven days on the waist, wrist, upper arm, thigh or ankle that can estimate energy expenditure and intensity by measuring bodily acceleration (Chen and Bassett, 2005; Troiano, 2005). The monitors collect physical acceleration data at set time periods (e.g. sampling frequency of 100 Hz = 100 times a second) and after filtering has taken place to remove potential artefacts (biologically implausible levels of acceleration) in the signal (band pass filtering), data are converted into 'counts' which are then averaged over a specific time window (epoch) depending on the research requirements (Chen and Bassett, 2005). An epoch of 1 minute (60 seconds) is frequently used in adults, however depending on the study population, shorter epochs can be used to prevent sporadic activity being lost or different intensities being averaged over the same time window (Chen and Bassett, 2005).

Before accelerometry data can be associated with health outcomes, a number of analytical parameters need to be applied to the data. One of the most influential decisions is which cut-points to use that classify physical activity into an energy expenditure category of intensity. Accelerometer counts are converted into METs through prediction equations that assess the relationship between count values and energy expenditure. These relationships have been

identified within a laboratory setting using gold standard measures such as indirect calorimeters (Chen and Bassett, 2005; Strath et al., 2013). Many cut-points exist that have been developed for use within specific populations such as children, adults and older adults. However, as cut-point choice can vastly influence the volume of physical activity quantified (Orme et al., 2014), care must be taken when comparing studies that use different cut-points.

Once cut-points have been chosen, researchers must also decide on a set of criteria for wear that constitutes a valid day and how many valid days denotes a valid file. Frequently used criteria are: valid day ≥ 10 hours and a valid file ≥ 4 valid days (Troiano et al., 2008). Whilst appearing not to be originally statistically derived, the valid day criterion has been consistently applied, especially within the National Health and Nutritional Examination Survey 2003-2006 cycles, with the number of valid days to denote a valid file varying somewhat more (Tudor-Locke et al., 2012). Depending on the amount of data available a pragmatic decision may have to be made to include the majority of the sample without compromising on quality. However recent research has suggested that as little as 1 day randomly selected from a sampled week can provide stable group level means of physical activity (Wolff-Hughes et al., 2016).

Accelerometer devices can measure physical activity within one to three orthogonal planes (anteroposterior, mediolateral, and vertical) (Chen and Bassett, 2005); however, older accelerometers were only equipped to measure activity in one plane (vertical). As activity can occur in more than one plane, vector magnitude has been posited as a way of measuring cross-axial physical activity. Providing a composite measure of acceleration of the three axis, vector magnitude is calculated by taking the square root of the sum of squares of each of the axes (Aguilar-Farías et al., 2014). However, estimations of behaviour from vector magnitude cannot be compared to singular axis measurements as by technical specification; vector magnitude is measuring additional acceleration in the antero-posterior and medio-lateral and not just the vertical planes (Aguilar-Farías et al., 2014). In addition to cut-points, care must be taken to identify the axis that behaviour has been derived from, as studies will not be comparable.

Another parameter that has to be defined when using accelerometers is the non-wear criteria. This criteria is an algorithm that defines when a biologically implausible string of zero counts is present, which is likely attributable to a person not wearing the sensor. A common non-

wear criteria is for 60 seconds of consecutive zeros to be present in the data with allowance of up to 2 minutes (2 x 60 second epochs) for non-zero interruptions (Troiano et al., 2008). This decision can be traced back to research that showed 60 minutes of consecutive zeros optimised the sample size compared with 20 minutes (Mâsse et al., 2005; Tudor-Locke et al., 2012). A higher criterion of 90 minutes has also been proposed to classify non-wear time (Choi et al., 2011). Deciding which criteria to use can influence the amount of sedentary behaviour measured, as too strict (lower amount of time) may decrease sedentary behaviour detection by removing time as non-wear, and too liberal (higher amount of time) may over estimate sedentary behaviour as there is less chance for the algorithm to find the duration of non-zeros. Again, researchers must be aware of this effect when choosing a criteria; however, clear reporting can increase transparency and the chance for true comparisons between studies.

Although accelerometers provide researchers with an objective measurement of physical activity, the knowledge required to analyse the data is often a steep learning curve, which can reduce the feasibility for those without sufficient knowledge or skills. There are also additional limitations of the devices, including:

- Deployment challenges: Monitors can be expensive, loss or damage is not uncommon and wearing monitors can be considered burdensome.
- Analytical decisions: As described above, the decisions researchers make with the data can influence the conclusions.
- Sources of error: Errors can be caused by technical, human or measurement sources e.g. spurious data, incorrect deployment, reactivity or lacking the ability to measure context.
- Applicability: As sensors are calibrated slightly differently, inter-monitor comparisons cannot be compared directly.

The preceding section highlights the analytical challenges facing researchers by using accelerometers to quantify behaviour. Analytical decisions must be made to process accelerometry data; however, these may often be influenced by the need or want to be able to compare results between studies. The analytical decisions are briefly summarised in Table 2.1. While the term accelerometer is commonly used to describe a device that measures levels of physical activity, it should be stressed that despite most monitors utilising the same

Table 2.1. Accelerometry data collection and analytical parameters.

Deployment parameters	
Axis analysed	The equations that extrapolate from acceleration to energy expenditure are accelerometry axis dependent i.e. if developed using from uni-axial data, only data from that axis can be used. Researchers should be mindful of the source of analytical parameters.
Cut-points	Cut-point choice can impact behavioural estimates. Choice should be made depending on the population and if comparisons are required against large national samples.
Device axis	Most new devices are tri-axial; however, older models can be uniaxial or dual-axial. To obtain vector magnitude estimates of physical activity, tri-axial devices must be used.
Device model	Device outputs can vary by brand, model and generation of accelerometer. Comparisons to large nationally representative samples may dictate the device you use.
Epoch analysed	As data are commonly measured in a higher resolution than normally required, data needs to be collapsed into more appropriate units in order to be analysed. The most common epoch for accelerometry research is 60 seconds, although smaller time periods can be used if more sporadic behaviours are sought.
Monitor start time	At certain times, accelerometers can be initialised without being worn straight away. If analysed without adjustment, an over estimation of behaviour can occur. Start times can be adjusted to the appropriate temporal location by either a manual adjustment/trimming of the file or using software that can use an additional 'read in file' to change the start date for analysis.
Non-wear criteria	Non-wear criteria is an algorithm that removes accelerometry data that is deemed biologically implausible to achieve. Most commonly applied as any period of accelerometry data that is characterised by 60 minutes of consecutive zeros, with allowance of up to 2 minutes of non-zero interruptions.
Number of deployments	If a device is found to be faulty, the number of times a device is deployed could be problematic. Devices should be deployed equally.

Table 2.1. Accelerometry data collection and analytical parameters.

Deployment parameters	
Number of devices	The number of devices can limit the speed of deployment. Although costly, preparations should be made for lost or 'faulty' devices.
Sample rate	Sample rate is how many times a second acceleration is measured and it can be limited by both battery capacity and device memory. Newer devices can measure up to 100 times a second (100Hz) over seven days and it is better analytically to be able to measure at a higher frequency than needed.
Valid day criterion	If participants do not wear the devices for the required deployment period, the inclusion of their data may skew associations. Additionally, those that do not comply with the wear instructions, may be unique in their physical activity levels. To be included within analyses, the amount threshold is commonly set at > 600 minutes or 10 hours of wear. If wear is very problematic, this threshold can be lowered using a sensitivity analysis, although this should be done pragmatically if trying to establish habitual activity behaviours.
Valid file criterion	To be sure of the associations between habitual physical activity and health, measurements by accelerometry should be a reliable and valid assessment of each individual. Four or more valid days are commonly required to denote a valid file; however, this can be altered if compliance is low, although studies have shown that more often 5 or more days are required.
Wear instructions	A common protocol is to ask the participants to wear the device for all waking hours apart from when engaging in water-based activities such as swimming or bathing. Although removed before sleep, the non-wear time cannot be considered sleep without the use of diaries or a sleep detection algorithm as non-wear is not necessarily sleep time.
Wear location	Most estimations of energy expenditure have been derived from waist worn estimations; however, wrist worn accelerometers are now being used more often. Wear location can dictate what samples the collected data can be compared to.

hardware (accelerometers), it is the firmware, software or location of a device that dictates if the data are analysed as measuring energy expenditure or body position (Kang and Rowe, 2015). Information relating to the firmware of each accelerometer device is usually proprietary to the specific manufacturer (Byrom and Rowe, 2016), and this makes it hard for researchers to have full control over the processing of their data. Additionally, although count based estimations of physical activity have been popular amongst researchers, it has also been shown that estimations of energy expenditure from this method can result in substantial errors at the individual level (Staudenmayer et al., 2009). This has led other researchers to develop data analysis techniques that remove some of the uncertainty regarding measurement parameters. Methods such as pattern recognition or machine learning can improve the calculation of energy expenditure (Staudenmayer et al., 2009); however, these often require further specialist computing and analysis knowledge and therefore are not currently widespread in their use. As machine learning techniques are not used within this thesis, the methods are acknowledged as an alternative methodology, but will not be described in detail.

2.4.2 Use in epidemiological research

In the past, it has been suggested that accelerometers were not feasible in large scale studies due to the relatively high cost of each unit and the difficulties in interpreting the data (Wood, 2000). Technological advancement has reduced these concerns and accelerometers are now feasible within large scale epidemiological surveys (Esliger and Tremblay, 2007; Lee and Shiroma, 2014) and cycles of national surveys including the Health Survey for England (Craig et al., 2009), National Health and Nutritional Examination Survey (Troiano et al., 2008), Canadian Health Measures Survey (Colley et al., 2011), amongst others. Within the English sample of 2008, adults and children were assessed using accelerometers over 7 days and also by questionnaire. The results showed a large discrepancy of physical activity guidelines compliance between questionnaire and accelerometer assessed physical activity. Self-reported estimates reported that 42% and 31% of men and women met the physical activity guidelines, however when measured objectively, only 6% and 4% of men and women met the guidelines, respectively (Craig et al., 2009). Whilst self-reported guideline compliance is already low, the number of adults meeting the current guidelines when measured objectively is cause for concern. Moreover, the capacity of accelerometers to provide in depth analysis of behaviour i.e. a minute-by-minute account, indicates that they should always be considered when selecting an assessment tool to measure physical activity levels within a sample.

2.4.3 Behaviour replacement models

In order to ascertain the benefits in health status conferred by changing physical activity behaviours, studies are required to track health behaviours longitudinally. More recently, researchers have been able to model these relationships in cross-sectional data using a technique borrowed from the nutritional epidemiological field called isotemporal substitution. The technique uses accelerometry data and by holding the total wear time of each individual constant, intensities of physical activity (sedentary, light, MVPA) are entered into a regression model and systematically dropped or removed from the analysis, leaving certain intensities within the model. For every one unit change in the dropped variable, the regression coefficients represent the change in the dependent variable if behaviour was reallocated to another intensity e.g. sedentary to either light or MVPA (Mekary et al., 2009). For many analyses, the dependent variable represents a cardiometabolic risk factor such as BMI, waist circumference or glucose. For ease of interpretation, the volume of physical activity can be subdivided into meaningful chunks of time e.g. 10 minutes, which can then be used to suggest the health benefits of reallocating 10 minutes of one behaviour to another. Since the introduction to the field of physical activity research, the technique has been applied by a number of studies that have investigated the effect of behaviour substitution upon health (Buman et al., 2010, 2014; Mekary et al., 2013; Falconer et al., 2015; Healy et al., 2015; Stamatakis et al., 2015; Yates et al., 2015; Ekblom-Bak et al., 2016a). The rise of the popularity of this statistical technique is due to the ability to model behavioural adjustment using cross-sectional observational data, which can help to unearth the amounts of physical activity increase or sedentary time reduction that can have beneficial consequences on health. Whilst cross-sectional associations cannot be deemed causal, the assessment of behavioural modification is important for behaviour change research as context is given about how much is required to positively influence health.

2.4.4 Wrist worn accelerometry

Accelerometers can be placed around the waist, wrist, upper arm, thigh or ankle, yet most are commonly deployed round the waist (Kamada et al., 2016), as this is the location where many of the energy expenditure extrapolation equations have been developed. Aimed at increasing compliance (Troiano et al., 2014), wrist worn monitors may be able to capture more non-ambulatory activities than a traditional waist deployment. However, as most of the seminal work connected to objective measurement of physical activity has been conducted at the waist, there is still a lack of widely accepted analysis parameters such as cut-points and it is

still unknown what effect the wrist location will have upon physical activity measurements (Shiroma et al., 2016). Advances in understanding have provided various methods of estimating wrist worn behaviour using raw signal analysis that does not have limitations of using pre-defined epochs or intensity categories. Techniques such as the sedentary sphere estimate sedentary time by posture classification (Rowlands et al., 2014) and Euclidean Norm Minus One adjusts data by removing gravitation components before generating estimates of behaviour (Bakrania et al., 2016b). Nevertheless, these methods currently require a higher level of understanding of skill and knowledge to process, therefore current count based metrics will still need to be calculated on wrist data, in order to increase feasibility and also comparability with existing literature.

When sedentary time measured via a wrist worn accelerometer (ActiGraph GT3X+) was compared against a criterion thigh worn accelerometer (activPal), wrist-estimated sedentary time was deemed optimal at < 1853 counts per minute for the non-dominant wrist (Kappa = 0.57) and < 2303 for the dominant wrist (Kappa = 0.58) (Koster et al., 2016), indicating moderate agreement (Landis and Koch, 1977). Comparing wrist and waist worn accelerometry directly, another study investigated the mean differences between sedentary time and MVPA in an older adult women sample. Demonstrating a higher cut-point for sedentary time (< 2000), the authors also revealed that mean differences were the lowest for MVPA between ≥ 7500 and ≥ 8250 vector magnitude counts per minute (Kamada et al., 2016). Comparing allocation into count based activity quintiles, agreement was considered moderate (Kappa = 0.56) yet only classified individuals into the same quintile 46.8% of the time (Kamada et al., 2016). Cut-points were evaluated using waist vector magnitude counts per minute (CPM) calculations (Sedentary < 200 CPM, MVPA ≥ 2690 CPM); therefore, caution should be taken when comparing wrist-based results against waist worn uniaxial estimations. The possible trade-off between validity and feasibility whilst using these methods may be problematic; nevertheless, as this field is in its infancy, these ranges will aid researchers in classification of physical activity and sedentary time in samples using wrist worn accelerometry. The described cut-points are summarised in Table 2.2.

2.4.5 More than just volume

Physical activity is often expressed as minutes spent within an intensity and whilst MVPA can be accrued sporadically, only bouts ≥ 10 minutes of MVPA can be considered guideline compliant activity (Department of Health, 2011a). As a guideline, this suggests that bouts

Table 2.2. Cut-points for wrist worn accelerometry.

Study	Intensity	Counts
Koster (2016)	Sedentary	0-1853
Kamada (2016)	Sedentary	0-1999
	Light 1	2000-7499
	Light 2	2000-8249
	MVPA 1	≥ 7500
	MVPA 2	≥ 8250

Abbreviation: MVPA, moderate to vigorous physical activity.

lasting less than the recommended number are not as influential for health. However, evidence now suggests that similar cardiovascular benefits can be conveyed by accruing sporadic MVPA of activity lasting < 10 minutes including positive associations with high-density lipoprotein (HDL) ($\beta = 0.87$, $p = 0.001$) and negative associations with waist circumference ($\beta = -0.86$, $p < 0.0001$), BMI ($\beta = -0.30$, $p < 0.0007$) and triglycerides ($\beta = -4.42$, $p < 0.0001$) (Glazer et al., 2013). Similarly, a longitudinal study showed that individuals were 31% less likely to develop hypertension after 5 years if they were within the highest tertile of MVPA short bouts (White et al., 2015). Emphasising the notion that one size does not fit all, physical activity can be accrued in many different ways, which can now be quantified using objective tools and innovative data reduction methods.

There is now a growing amount of evidence that recognises physical activity as a multidimensional behaviour that can also be broken down into an additional parameter of regularity (how often), as well as intensity (sedentary, light, MVPA) and duration (minutes) of physical activity (Marschollek, 2013). Similarly, distinct behavioural profiles have been identified that show types of behavioural patterns. Metzger and colleagues (2008) investigated different activity profiles using latent class analysis and found five distinct classes, including those that predominantly undertook their physical activity at the weekend ('Weekend Warriors'). Indeed, a recent evaluation of mutually exclusive categories including 'Busy Bees' (low sedentary, highly active), 'Light Movers' (low sedentary, low activity),

'Sedentary Exercisers' (highly sedentary, highly active) and 'Couch Potatoes' (high sedentary, low activity) in a Health Survey for England dataset found that compared to the couch potatoes, the most beneficial category for reductions in BMI and waist circumference was in the 'Busy Bees' group (Bakrania et al., 2016a). Whilst examining the interactions between sedentary time and physical activity, groupings were based upon a calculation of sedentary behaviour-to-light-intensity physical activity ratio (sedentary status) and MVPA (active status), and did not use any reflection on the regularity of physical activity accrual.

Another study investigated three parameters of intensity, duration and regularity together against cardiometabolic risk factors (Marschollek, 2016). After identifying clusters using an algorithm (*x*-Means), significant differences in BMI were observed both between the most and the least active, but also those that are irregular and less extensively active (Marschollek, 2016). As an example of the clusters identified, cluster one was interpreted as having a very high intensity overall, with regular low-intensity activity but more irregular high-intensity physical activities (Marschollek, 2016). Compared to cluster four which showed an opposing relationship, it is unclear from these analyses whether a particular parameter is mediating the relationship with health as all clusters vary somewhat and have not been matched in an iterative process.

Whilst many behavioural profiling techniques exist, most rely upon applying a number of analytical parameters to the data in order to quantify behaviour. Other raw signal methods have been mentioned briefly within this thesis, although most determine the duration within certain intensity or identifying the context of the behaviour. As this section has reported that regularity of behaviour may influence health parameters, it is logical that these relationships are investigated further. Techniques are becoming more available to applied researchers that were previously constrained to engineering applications. Entropy is a measure of signal complexity (Pincus, 1991), and assesses the probability that the sequences are the same for a given number of points (Richman and Moorman, 2000). High entropy values denote less system order (Pincus and Goldberger, 1994) whilst lower values indicates higher regularity (Richman and Moorman, 2000). This technique was utilised within investigations into the complexity of step counts in older adults, where the highest active participants had the highest entropy scores, leading the authors to conclude that highly complex patterns offers additional information of walking behaviours, supplementary to the total volume of activity (Cavanaugh et al., 2010). Entropy has also been used for research into count values for

walking impairment in patients with multiple sclerosis (Sosnoff et al., 2010), assessments of postural sway (Ramdani et al., 2009) and for heart rate variability analysis (Lake et al., 2002). Relating the method to physical activity behaviours could characterise those that are more complex in their behaviour i.e. more irregular or individuals that are less complex i.e. more sedentary.

Calculating entropy on physical activity data can circumnavigate certain limitations encountered from wrist positioning of the accelerometer and offers a relatively unprocessed view of behaviour. However, there is a paucity of research in this domain, yet knowing if complexity or regularity of behaviour is an important factor in physical activity accrual would aid researchers and clinicians to prescribe the right type of physical activity to treat ill health.

2.5 Measurement of physical activity and glucose

The measurement of physical activity and sedentary time has now evolved into a well-established field, although much is still to be understood between movement and health. Physiological sensor technologies are also advancing at an accelerated rate and now provide researchers with the ability to quantify the biological implications of behaviour e.g. physical activity. Opening up new possibilities for behavioural research, the quantification of both behaviour and physiology on health will help researchers advance the understanding of the interaction between sedentary time, physical activity and health.

2.5.1 Behaviour discounting

Current health promotion messaging campaigns such as 'Change4Life' list the benefits of being physically active as 'keeps your heart healthy, reduces your risk of serious illness and strengthens muscles and bones' (National Health Service, 2016). Health enhancing behaviours such as being physically active often do not convey rewards straight away, and somewhat require short term minor inconveniences to achieve (Hall and Fong, 2003). Behavioural economics research has highlighted how future events can be devalued or discounted by an individual when compared with an immediate reinforcer and how distant rewards decrease in value with increasing time periods (Bickel and Marsch, 2001; Tate et al., 2015). Discounting has also been shown to persist even if the future reward is significantly larger, as individuals that are high 'discounters' will opt for immediate rewards instead of future benefits (Tate et al., 2015).

There is a paucity of research upon physical activity behaviour discounting rates, however high discounting rates have been shown to induce lower rates of vigorous physical activity participation (Bradford, 2010). Similarly, another study aimed to compare discounting rates and self-reported vigorous exercise participation in older adults living in the community (n = 137). Participants that reported exercising > 50 minutes a week of vigorous physical activity had significantly lower discounting rates than those who participated in less activity (Tate et al., 2015). Although the number of participants considered to be 'exercisers' was similar to 'non-exercisers' (n = 68 vs. n = 69), when it has previously been reported that national samples show many people do not engage in the recommended level of activity, the number completing over 50 minutes is likely overestimated. However, as one of the first studies of its kind, the research has shown that there may be a temporal discounting issue with physical activity participation that should be explored further. Strategies such as providing real time

feedback to highlight the immediate benefits of moving more could motivate those deemed as high discounters to be more physically active.

Given the interdisciplinary nature of this thesis, which provides knowledge and depth surrounding cardiometabolic risk factors, behavioural measurement, signal processing and glucose indices, behaviour change theory is mentioned but will not be a main focus of the work presented.

2.5.2 Measurement of glucose

People with type 1 diabetes often measure their blood glucose concentrations using finger capillary samples to calculate the necessary dose of insulin to prevent hyper (high) or hypo (low) glycaemia. Additionally, it is essential for people with type 2 diabetes to maintain a good control of blood glucose to avoid or delay further complications such as heart disease, vision loss or renal failure (Clar et al., 2010). A venous sample is considered the gold standard of glucose measurement, but due to its invasive nature, the management of glucose concentrations is typically done either using a capillary blood measurement device or a continuous glucose monitor (CGM). First commercially available in 2000, CGMs have advantages over capillary blood measurements as they can give the user a wealth of information on their glucose concentrations including, the current glucose concentration, rate of change and warnings of hypo/hyperglycaemia (Rodbard, 2016). Most devices are semi-invasive and use a micro fibre needle that is inserted into the interstitial space under the skin. A common way to measure glucose is by using enzyme electrodes that quantify the current produced by a glucose oxidase reaction (Oliver et al., 2009). The result of the reaction is hydrogen peroxide and this is directly proportional to the concentration of glucose (Oliver et al., 2009). Readings can be taken up to a frequency of every 5 minutes which increases their utility as a device that can measure the effects of sleep, medication, diet and exercise (Vigersky and Shrivastav, 2016). The development of CGMs aids in the medical management of diabetes; however, they have their limitations. Considered an adjunct to regular self-monitoring (Blevins et al., 2010), CGMs are similarly invasive and require frequent calibrations using traditional capillary blood devices (two to four times per day) and sensors have to be changed every few of days (Vashist, 2013). This comes at significant cost to the user if not covered by either a public health service or a medical insurance policy.

Although there are no widely accepted standards to assess the accuracy of CGMs (Damiano et al., 2013; Kropff et al., 2015), the mean absolute relative difference (MARD) is often reported in the literature. MARD is calculated as the absolute error between measured and reference values as a percentage of the reference value (Kovatchev et al., 2008). The more accurate devices will have a lower percentage (Burge et al., 2008), with most CGMs typically performing between 10 to 20% MARD (Vashist, 2013). Recent technology has reduced MARD errors to ±10%, allowing for accurate adjustments in medication (Rodbard, 2016). Another limitation of CGM devices that utilise interstitial methods to measure glucose is that there is a lag time of 5 to 10 minutes which can be heightened during rapid changes of glucose concentrations (Garg et al., 2010), although further advancements in algorithms are reducing this issue (Rodbard, 2016). Challenges associated with sensor life, calibration, cost and invasiveness, mean that CGMs in their current form might not be feasible for research purposes; however, successive developments are likely to rectify these issues.

One such development is the creation of 'Flash Glucose technology' that has opened up the monitoring of glucose to patients with type 2 diabetes by providing a device that can measure glucose up to 14 days at a time, requires no capillary blood calibrations (factory calibrated) and is at a heavily reduce price compared to existing CGMs (Heinemann and Freckmann, 2015). Proven to have similar accuracy against blood samples measured using a Yellow Springs Instrument analyser (MARD 11.0%), the device however had a lower accuracy for the first day of monitoring attributed to an inflammatory response after sensor insertion (Bailey et al., 2015). The study was conceptualised to gain CE marking (Heinemann and Freckmann, 2015), a process to ensure a product complies with European safety, health and environmental requirements (GOV.UK, 2012), and therefore there is a lack of independent supporting evidence regarding the accuracy and reliability of the devices. Nevertheless, studies carried out as part of the prototyping phase have shown promise with factory calibration (MARD = 10.4% and 12.2%) (Hoss et al., 2013, 2014). To use the flash glucose monitoring Freestyle Libre device (Abbott Laboratories, Illinois, USA), users are required to wear a sensor that is applied to the upper arm and to carry a reader device with them. A measurement is taken when the user holds the reader near the sensor and the information is transferred using near field communication (wireless transfer). Unlike CGMs, information is not automatically synced to the reader and therefore data loss can occur as the sensor can only hold 8 hours of glucose measurements. Additionally, Abbott is taking steps to reduce the burden of carrying another device by developing a smart phone app (LibreLink) and there are

also early reports of another app (LibreLinkUp) that will allow parents and care providers to self-monitor glucose concentrations remotely. Clearly, the technology is developing rapidly and will be well suited to be used successfully within clinical and research trials to assess the influence of self-monitoring glucose on clinical outcomes.

Looking into the future of glucose measurement a recent report (May 2016) by the Horizon and Scanning Research & Intelligence Centre highlighted 16 non-invasive glucose monitors that have utilised optical, transdermal and electrochemical techniques (Horizon Scanning Research & Intelligence Centre, 2016). One such device consists of a disposable patch (sugarBEAT, Loughborough, UK) that can measure glucose up to four times an hour and transmit information to a watch device such as an Apple watch. Though many of the identified technologies are still in their development stage, reducing the invasiveness will open up the technology to a wider audience and to those who are interested in monitoring their health.

2.5.3 Glucose analytics

Once measured, data must be analysed and then interpreted. CGMs can capture data of both great quantity and complexity (Clarke and Kovatchev, 2009); however, there are many ways to characterise glucose concentrations from the data. As seen in physical activity profiles, individuals with the same average glucose values can have very different glucose profiles e.g. number, duration and magnitude of glucose excursions (Siegelaar et al., 2010). The amount that glucose concentrations vary from day to day can be influenced by many factors such as food and physical activity. Higher variability in glucose concentrations following meals has been associated with diabetic microvascular complication and disease (Ceriello et al., 2008); therefore, quantifying the inter-day variation is of interest to ascertain the level of glucose control. Many statistical techniques and indices have been proposed that measure glycaemic variability and include average daily risk ratio, continuous overlapping net glycaemic action, High Blood Glucose Index, Lability Index, Low Blood Glucose Index, mean amplitude of glycaemic excursions (MAGE), mean of daily differences, mean daily glucose (MGluc) and standard deviation of glucose (StDevG), amongst others (Hill et al., 2011). Many of the aforementioned techniques were developed with infrequent measurements in mind (capillary samples) and the introduction of the CGMs has opened up new possibilities for the measurement and monitoring of glucose over longer durations. The availability of so much choice has hindered rather than helped clarify which measures to use, with some new

techniques actually describing an established metric under a different name (Rodbard, 2012). To simplify the understanding of glycaemic variability for both clinicians and patients, it is beneficial to use measures that are not only easily understood but also have established clinical importance.

The StDevG has been suggested as one of the easiest measures of glucose variability to understand (Siegelaar et al., 2010), with 69% of an expert panel of diabetes specialists also agreeing it was the most commonly used index (Bergenstal et al., 2013). Measuring the spread of data points around the mean, StDevG indicates how variable the data are and whether glucose is well controlled (i.e. low SdDevG = closer to the mean). Rodbard (2009) has also furthered the use of SdDevG on glucose data, outlining the use of the calculation to determine within day, between time points, within series or between daily means variability.

MAGE is another measure of glucose variability that has been suggested as the gold standard of glucose fluctuations (Monnier et al., 2007). It measures the average height of glucose excursions above 1 standard deviation (Hill et al., 2011) and was designed to measure glucose excursions related to meals (DeVries, 2013). Developed by Service et al (1970), it is recommended to be calculated by computerised methods (Baghurst, 2011), and there are now many software packages that can calculate MAGE (Glyculator, Fritzsche, Baghurst and Easy GV) (Sechterberger et al., 2014). Agreement between software derived MAGE calculations have been shown to result in varying correlation coefficients (0.79 to 0.99), although not compared to a manual calculation of the technique which was posited by the author as the true gold standard (Sechterberger et al., 2014). MAGE has been criticised on a number of points including whether it is comparable to other methods to determine postprandial excursions (e.g. area under the curve), the calculation is operator and criteria dependent, there is a high correlation with SdDevG and it has been questioned whether only including excursions above one standard deviation has clinical significance (DeVries, 2013). However, MAGE is not dependent on the glucose average and assesses peaks that could be seen as higher importance (i.e. greater excursions) than minor fluctuations in the data (Monnier et al., 2008). If transparently calculated, MAGE as a measure of glycaemic control should be calculated on available CGM datasets.

Finally, a less commonly written about measure of glucose variability is MGluc. As a simple measure of the sum of glucose values divided by the number of measurements, the resultant

value represents inter-day variability and a day that is characterised by higher peaks or more time spent at higher glucose values would result in a higher mean value.

In summary, whilst there are numerous ways of calculating glycaemic variability, there should be a greater focus on those that are easily understood, as they are more likely to be resonant to both clinicians and users of blood glucose monitoring devices.

2.5.4 Self-monitoring of glucose

The use of CGMs is not a new strategy for the management of diabetes. The self-monitoring of blood glucose can help patients connect behaviours such as eating to the subsequent effect on glucose concentrations (McAndrew et al., 2007). Individuals with type 1 diabetes regularly measure their blood glucose concentrations, yet it is not commonly carried out as part of type 2 diabetes medical management. Glycated haemoglobin (HbA1c) is a measure of a glycaemic control over the previous 2-3 months (World Health Organization, 2006), and can be used as a diagnostic criterion for diabetes (World Health Organization, 2011a). A Cochrane review of blood glucose self-monitoring in individuals with type 2 diabetes stated that in established cases (over 1 year) a significant decrease in glycated haemoglobin (HbA1c), of 0.3% was evident at 6 months; however, the effect was attenuated after 12 months (0.13%) and became non-significant (Malanda et al., 2012). Protocols included within the review only sampled glucose at low frequencies and at varied time points and therefore do not entirely prohibit the use of newer technologies such as CGM.

Interventions using CGM devices have shown significant declines in HbA1c (1.0%) at 12 weeks (Ehrhardt et al., 2011; Vigersky et al., 2012), which was also found to be sustained at 52 weeks (0.8%) compared to self-monitoring of blood glucose using finger stick measurements (Vigersky et al., 2012). It has been discussed that the cost associated with the supply of CGM equipment outweighs the benefits of self-monitoring in individuals with type 2 diabetes; however, assessments advocate real time glucose monitoring as a cost effective treatment ($9319 per life year gained) (Fonda et al., 2016). Though based upon a small sample size (n = 100), the evidence showing CGMs as being cost effective will undoubtedly increase their utility as a treatment tool for individuals with diabetes.

2.5.5 Existing literature on the coupling of behaviour and glucose

In the previous sections, it has been demonstrated that the measurement of glucose in response to a behavioural stimulus could perhaps be an effective motivational aid. Only a few studies have investigated the coupling of both behaviour and glucose within an intervention. A randomised control trial by Allen and colleagues (2008) measured physical activity and glucose concentration by accelerometer and CGMs in 52 people with type 2 diabetes (non-insulin dependent). After wearing the glucose monitor for 3 days, participants in the intervention group received individual education that included feedback on the participant's own expected areas of activity-related glucose reduction, the showcasing of role model examples and an individually prescribed physical activity programme. The intervention group significantly increased average moderate activity by 5 minutes ($p < 0.05$) and decreased sedentary/light activity by 5 minutes ($p < 0.05$) (Allen et al., 2008). A subsequent study in 29 women with type 2 diabetes resulted in no significant changes in physical activity after 12 weeks (Allen et al., 2011). Importantly, both studies only relied on the use of CGMs for a very short duration (3 days) and expected participants to anticipate the decline in blood sugar levels rather than being provided with real time feedback. Not providing an immediate reinforcer may be a detriment to these studies, although they have laid the foundations for future research to provide such measures. This conclusion is supported by a recent study that utilised real time CGMs to show pre-diabetic individuals how prescribed exercise sessions affected their glucose concentrations (Bailey et al., 2016). Participants using CGMs had greater program attendance to structured exercise sessions compared to a standard care condition (Bailey et al., 2016).

Before interventions can be successful at using CGMs as a motivation aid, the objectively and continuous relationship between physical activity and glucose needs to be established. To the author's knowledge, only one study has objectively measured behaviour and glucose in a free-living environment. Measuring sedentary time by wrist worn accelerometry (Actiwatch-Score, Phillips, USA) and glucose using CGMs (iPro, Medtronic, USA), time spent in hyperglycaemia (high glucose, > 8.9 mmol/L) was significantly predicted by sedentary time ($b = 0.12$) (Fritschi et al., 2016). It was posited by the authors that an additional 60 minutes of sedentary time increases time in hyperglycaemia by 7.4 minutes. Nonetheless, sedentary time within this sample was estimated using wrist worn accelerometry but had waist worn cut-points applied to the data and therefore should be treated with caution. The next logical step is to ascertain whether physical activity shows an opposing relationship with glucose and if

the magnitude of the change is linear i.e. will an increase in physical activity be the same magnitude of change to sedentary time but in the opposing direction, or will the difference be heightened. Newer technologies that can measure physical activity over a longer time period should also be utilised to reduce the impact of short term variability of behaviour on acute health changes such as blood glucose.

2.5.6 Fitness and glucose

In addition to modifying the effect of physical activity upon glucose, large prospective cohorts have reported associations between cardiorespiratory fitness and the risk and incidence of diabetes (Wei et al., 1999; Sawada et al., 2003). Sawada and colleagues (2003) used a submaximal exercise ergometer test to determine fitness levels in a sample of 4,747 non-diabetic Japanese men and after a 14 year follow up, individuals in the highest fitness quartile had a 75% decreased risk of developing type 2 diabetes (age adjusted) compared to the lowest fitness quartile. Likewise, a recent meta-analysis revealed an inverse relationship between fitness and risk of diabetes (Zaccardi et al., 2015). Finally, changes in cardiorespiratory fitness have been reported to predict reductions in HbA1c ($\beta = -0.422$, $p = 0.002$) and also decrease HbA1c by −0.4% after aerobic training in individuals with type 2 diabetes (Bacchi et al., 2012). Therefore, the above evidence advocates fitness as an important factor to consider when assessing any associations between diabetes and health.

2.5.7 mHealth

Wearable technologies such as fitness trackers and home medical devices now allow individuals to monitor their own physical activity and health, capturing a wealth of information that has not been previously available. This has increased the opportunities for the integration of patient data into the current health service infrastructure which could improve patient care. Wearable devices now contain multiple sensors and most commonly are able to track heart rate either for a specific workout period or over a whole day. Indeed recently, monitoring using a generic fitness tracker helped to diagnose a heart condition in an individual within an emergency department whilst they were being treated for another diagnosed condition (Rudner et al., 2016). As new multi-sensor technologies are developed, further opportunities will open up for the management or prevention of chronic diseases through the integration of wearable data; therefore, strategies to integrate the wealth of data available should be encouraged.

The cost effectiveness of remote health monitoring was evaluated by the Whole Systems Demonstrator study which assessed the impact of tele-monitoring against usual care within primary care patients (Henderson et al., 2013). Overall, this study found that the added information provided by tele-health was not found to be cost effective compared to usual care (Henderson et al., 2013); however, it is unclear which technologies were used (varied per care plan) or how frequent the readings were. Both these factors could influence participant engagement, cost and utility especially if technologies were not user friendly or outdated. Moreover, the current clinical infrastructure may have been another potential limiting factor of the study as primary care trusts may not be adequately equipped or have the resources to handle large amounts of information in an effective and actionable manner. In its current form, remote monitoring places a large burden upon the user. Yet, with technology rapidly advancing, the old adage 'necessity is the mother of invention' could lead to more efficient platforms and integrations which may aid in the detection and monitoring of a wide range of health conditions. More work is needed to develop newer technologies with fully integrated platforms before tele-health can be discounted as a means on improving health care.

Collecting an increasing amount of personal data presents several issues and challenges that have not been previously encountered. Access rights to data, data security and storage and data governing policies are all issues that need to be addressed and thought through before tele-monitoring can be used in practice (Meingast et al., 2006). Privacy is a major concern for most people and will be a fundamental challenge to overcome if remote monitoring is to be acceptable to large numbers of people. This privacy concern is not unjustified as the data collected by an individual could also be used to harm the intended individual, if the technology utilises tracking location for example (Al Ameen et al., 2012). Steps can be taken to anonymise data; however, this would not work in cases where identification is necessary, for care provision for example. There are additional ways to protect data from unauthorised access such as data encryption, authentication, firewalls and by limiting the number of users that have access to the data (Al Ameen et al., 2012), but there is always the chance of a security breach of data for those who have the means. So far, the mHealth dialogue has primarily focused on the provision of a singular measurement of health at one time point and how this can be integrated into the current infrastructure. Monitoring cardiometabolic risk factors without any additional context perhaps does not fulfil the potential for early intervention in the prevention of chronic diseases. Quantifying the effect that behaviour has upon physiology over multiple time periods could offer information for counselling and could

close the gap between action and consequence when it comes to physical activity and health. Yet, this debate is perhaps only academic at the current time as there is a lack of literature that outlines the relationships between behaviour and acute physiology such as glucose concentrations. Consequently, the coupling of data needs to be achieved to establish a relationship, before it could be used for remote monitoring purposes to reduce the burden of chronic disease.

2.6 Thesis aims

The aim of this thesis was to profile sedentary time and physical activity behaviours in relation to diabetic risk, cardiometabolic health and glucose control using novel measurement and analytical methods. The gaps in the literature and the aim(s) of each study will now be presented below.

2.6.1 Aims of Chapter Three (Study One)

Gap in the literature: Whilst increasing physical activity has been reported to reduce the risk of diabetes, there is a lack of studies assessing whether physical activity is linked to diabetes risk generated from screening measurements. Physical activity behaviours and sedentary time are mutually exclusive, as participation within a specified intensity prevents a behaviour of a different intensity from being undertaken at that point in time. Isotemporal substitution models can assess the effect of behavioural replacement i.e. substituting sedentary for light activity, upon singular cardiometabolic health risk factors, whilst holding total wear time constant. Therefore, this technique could be applied to accelerometry data to identify if, and what levels of behaviour replacement modifies diabetes risk in adults.

Aims: To examine the relationships among diabetes risk scores, sedentary time and physical activity measured using wrist worn accelerometry, and to model the changes in risk scores by reallocating movement behaviours from lower to a higher intensity.

2.6.2 Aims of Chapter Four (Study Two)

Gap in the literature: Many research studies have already established an intensity and duration association between physical activities spend in sedentary, light or moderate to vigorous physical activity (MVPA). A gathering body of evidence suggests individuals may accrue their activity in varying ways.

Aims: To calculate a novel method of behavioural regularity called sample entropy on wrist worn accelerometry and to ascertain whether there are associations with cardiometabolic risk factors in adults.

2.6.3 Aim of Chapter Five (Study Three)

Gaps in the literature: Changes in postprandial glucose have been shown to be initiated by physical activity in a well-controlled environment. It is still unknown whether any inducements of glucose change are evident in a free-living situation and over an extended period of time. If associations with behaviour can be established, the information could be used as an effective motivational aid to increase physical activity.

Aim: To ascertain if there is a relationship between objectively measured sedentary time, physical activity and glucose variability using glucose monitoring in an adult population.

Physical activity, sedentary time and the association with diabetes risk in adults

Chapter overview

This chapter reports the results of a cross-sectional analysis of 251 participants from Leicestershire (UK), which aimed to investigate the association between physical activity, sedentary time and a calculated diabetes risk score. Data were collected between March and September 2014, which comprised of cardiometabolic risk factors and objectively monitored sedentary time and physical activity. Isotemporal substitution analysis quantified the effect behavioural replacement models had upon diabetes risk scores. This was calculated for the whole sample and individuals deemed to have the 'most to gain' i.e. least active. The chapter concludes that the greatest reductions in diabetes risk were observed for those individuals who were measured as being the least active of the sample.

3.1 Abstract

Introduction: Insufficient physical activity is a major risk factor for developing type 2 diabetes. Using isotemporal substitution models, the influence of replacing modest durations of sedentary time with physical activity on diabetes risk scores can be studied. The aims of this study were to examine the relationship between diabetes risk scores, sedentary time and physical activity measured using wrist worn accelerometry, and to model the changes in risk scores by reallocating movement behaviours from lower to a higher intensity.

Methods: Data from 251 (93 males; aged 56.7 ± 8.8) participants from a mixed ethnicity cohort from Leicestershire, UK were selected for analysis. The relationship between diabetes risk (using the Leicester Diabetes Risk Assessment Score), physical activity and sedentary time was identified using multiple linear regressions and isotemporal substitution analysis. Models were calculated for main effects and also adjusted for peak oxygen uptake (VO_2) and accelerometer wear time.

Results: Both unadjusted and adjusted models revealed that diabetes risk was inversely related to sedentary time, and positively related to light and moderate to vigorous physical activity (MVPA) ($p < 0.0005$). Unadjusted, the replacement of sedentary time with 10 minutes of either light or MVPA resulted in a reduction in diabetes risk score of −0.22 and −0.54, respectively. There was an eight to nine times greater reduction in risk for the same MVPA replacement models when the least active participants were compared to the pooled analysis (3.601 unadjusted).

Conclusion: Diabetes risk is associated with sedentary time and physical activity estimated from wrist worn accelerometry. The replacement of sedentary time with MVPA is most beneficial for the least active individuals.

3.2 Introduction

Diabetes is a major chronic disease estimated to be affecting 422 million adults in 2014 (World Health Organization, 2016) and due to its slow onset, 50% of diabetic cases may be undiagnosed (Forouhi and Wareham, 2014). Insufficient physical activity is a major risk factor for developing diabetes (Warburton et al., 2006) and those with type 2 diabetes are less likely to engage in the recommended physical activity (Nelson et al., 2002; Plotnikoff et al., 2006; Zhao et al., 2011). A recent systematic review and meta-analysis revealed non-linear relationships between walking, leisure time, vigorous physical activity and resistance exercise with the risk of developing type 2 diabetes (Aune et al., 2015). However, as estimates were calculated as hours per week, it is still unclear what level of risk reduction could be exhibited from modest increments in physical activity that may be undertaken by insufficiently active individuals.

Physical activity behaviours are commonly classified into mutually exclusive intensity categories of sedentary time, light, moderate or vigorous physical activity. These categories by their very nature are compositional, that is to say that spending time in one intensity results in less time remaining in the day to spend in other intensity categories (Chastin et al., 2015). A technique called isotemporal substitution allows for the replacement or reallocation of behaviour from one intensity to another, to be modelled upon health parameters. The 10 minute replacement of sedentary time with moderate to vigorous physical activity (MVPA) has been shown to be associated with cardiometabolic risk factors such as a reduction in body mass Index (BMI) ($b = -0.39$) and an increase in high-density lipoprotein (HDL) ($b = 0.037$) (Hamer et al., 2014). Additionally, even the reallocation of a single minute of sedentary time to either light activity or MVPA has been shown to significantly change metabolic syndrome risk in a cross-sectional study of older adults (50-64 years) (Ekblom-Bak et al., 2016a). However, there is limited evidence on whether the reallocation of sedentary time to either light or MVPA can reduce diabetes risk, predicted using common risk factors. Knowing this information will help optimise public health messages that promote more active and/or less sedentary lifestyles.

Estimates of physical activity and sedentary time can be measured using objective methods such as accelerometry. Most quantifications of behaviour are obtained using a waist worn deployment, as the majority of energy expenditure algorithms have been derived from this location. However, many researchers have started to collect data at the wrist including the US

National Health and Nutrition Examination Survey (Troiano et al., 2014), and the UK Biobank (Sudlow et al., 2015). Posited as a way to increase compliance (Troiano et al., 2014), wrist worn locations also aid in the sampling of non-ambulatory activities. Still in its infancy, there are a lack of widely accepted cut-points that can classify behaviour into intensity variables (Shiroma et al., 2016). More research is needed into the relationship between wrist worn derived activity and health outcomes. Therefore, the aim of this study is to examine the relationship between sedentary time, physical activity and diabetes risk using wrist worn accelerometry. An additional aim is to model the change in risk scores generated by the reallocation of movement behaviours from lower to higher intensity.

3.3 Methods

3.3.1 Sample

Data were used from the Physical Activity and Respiratory Health Study (PhARaoH), a cross-sectional, observational study that aimed to quantify the associations between health and physical activity behaviours in adults (ages 40-75 years) with and without a diagnosis of chronic obstructive pulmonary disease (COPD) living in Leicestershire and Rutland, UK. More details can be found at Orme et al. (2016). The study sample for this paper came from the control sample of UK adults without COPD. Participants were recruited through local media distribution (posters and leaflets), newspaper and radio advertisements (February to March 2014). All participants gave their written informed consent and the study was approved by the National Research Ethics Service Committee East Midlands Nottingham-2. The participant information sheet is contained within Appendix A. Those without a confirmed diagnosis of COPD or type 2 diabetes, had valid accelerometry (described in the next section) and no missing covariates were used for the current study.

3.3.2 Study design

This cross-sectional, observational study required participants to attend one 2 hour appointment at a timeslot of their choosing. The study flow is detailed in Figure 4.1. Participants were asked to refrain from eating or drinking anything except water for at least 4 hours and also to not take part in exercise on the day of the visit. Participants were reimbursed for their travel to the study location.

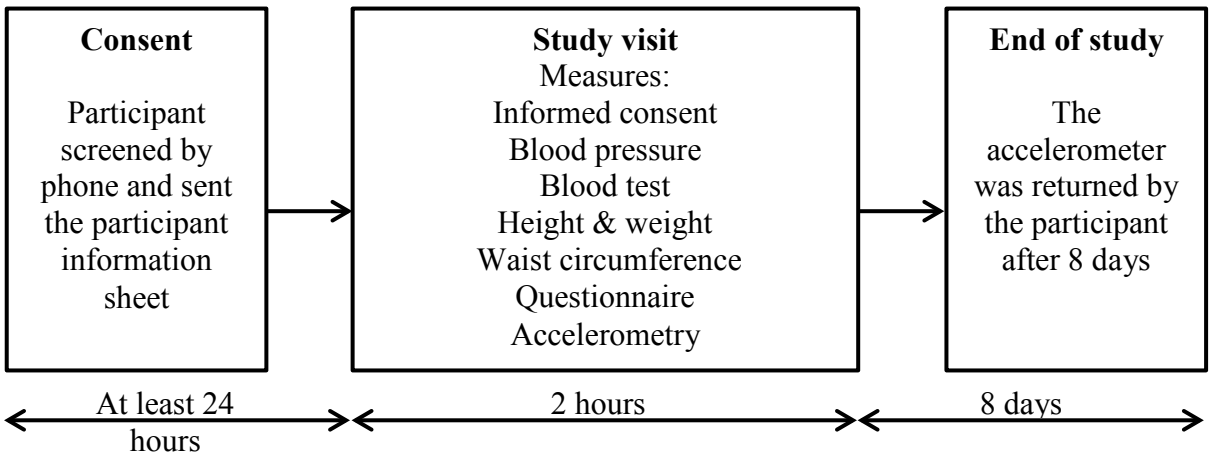


Figure 4.1. Study flow of the Physical Activity and Respiratory Health (PhARaoH) study. Each appointment lasted 2 hours and the study ended after the accelerometer was returned.

3.3.3 Study measurements

All study measurements were taken by trained research personnel at the Respiratory Biomedical Research Unit located at Glenfield Hospital, Leicester from March to September 2014. After informed consent, a number of measurements were taken on all participants including a seated blood pressure assessment (Omron 705IT, Omron, UK), a fasted venous blood sample, height (SECA 213, SECA, Germany), weight (Tanita MC780MA, Tanita, The Netherlands), waist circumference (HaB International Ltd, UK) and an Incremental Shuttle Walk Test (ISWT). Fasting time was chosen due to the consecutive appointment schedule where a visit could either be early morning or late afternoon. Eating status was confirmed at the start of each visit, with any that had not adhered to the pre-testing guidelines being excluded from the analyses.

Demographic, general health, family disease history, medication and chronic disease information, physical activity and sitting time were collected using sections from the Health Survey for England 2008 and UK Biobank questionnaires (NatCen, 2009; UK Biobank, 2009). For more detail on the study methods, please see Appendix B.

3.3.4 Accelerometry

Physical activity and sedentary time were measured by accelerometer (ActiGraph wGT3X-BT, ActiGraph, Pensacola, USA) that was worn for 7 days on non-dominant wrist. Participants were asked to wear the devices at all times and only to remove it when engaging in water based activities such as showering or swimming. The accelerometers were set to collect data at 100Hz and were deployed in a delayed state, with recording starting at midnight on the first day of wear. Devices were initialised and downloaded into 60-second .agd files using version 6.10.1 of ActiLife Software (ActiGraph, Pensacola, USA) and subsequently analysed using KineSoft (KineSoft, Loughborough, UK) version 3.3.80. For detailed data collection and analytical parameters, the reader is directed to Table 4.1. Participants were provided with a pre-paid envelope to return their accelerometer to the Respiratory Biomedical Research Unit.

The continuous wear protocol allows for greater adherence as there is reduced chance of device removal; however, without sleep diaries, relying on the runtime algorithm alone could under/overestimate sleep duration and therefore under/overestimate the duration of sedentary time. To overcome this, a semi-automated approach was adopted based upon sleep research

Table 4.1 Accelerometry data collection and analytical parameters

Data collection parameters	
Device manufacturer	ActiGraph
Model	wGT3X-BT
Number of axes	Triaxial
Number of devices	109
Average unit deployment	2 (ranging from 1-6)
Measurement frequency	100Hz
Wear location	Non-dominant wrist (non-writing hand)
Wear period	8 days (not activated on day 1)
Monitor start time	00:00 Day 2
Monitor stop time	23:59 Day 8
Wear instructions	All waking hours (except water based activities: swimming or bathing)
Initialisation software	ActiLife version 6.10.1
Analysis parameters	
Axes used for analyses	Vector magnitude
Epoch analysed	60 seconds
Imputation	None
Valid day criterion	≥ 600 minutes or 10 hours
Valid file criterion	≥ 4 valid day
Non-wear criteria	≥ 90 minutes of consecutive zeros (2 minutes non-zero interruption allowance)
Cut-points	
Sedentary Koster	0-1853 counts
Sedentary Kamada	0-1999 counts
Light Kamada1	2000-7499 counts
Light Kamada2	2000-8249 counts
MVPA Kamada1	≥ 7500 counts
MVPA Kamada2	≥ 8250 counts
Sleep removal method	Sleep algorithm and visual inspection
Analysis software	KineSoft version 3.3.80

Abbreviations: MVPA, Moderate to vigorous physical activity; Koster cut-points were derived calculated against an inclinometer comparison (Koster et al., 2016); Kamada cut-points were derived from a comparison between wrist and waist placements (vector magnitude) (Kamada et al., 2016).

literature, which identified the start of sleep (INBED) and ceasing of sleep (OUTBED) using a vector magnitude count based threshold algorithm (Carney et al., 2004).

Assessing each epoch, a sustained 90% reduction for the proceeding 15 epochs (minutes) in vector magnitude counts between the hours of 21:00 and 23:59 signified an INBED time. Similarly, a sustained increase in vector magnitude counts of 75% during the hours of 06:00 and 09:00 for five consecutive epochs (minutes) denoted an OUTBED time. The epochs containing the original 90% reduction or 75% increase in vector magnitude counts were used as the time stamps for sleep. For those days where an INBED time could not be determined, or if there were activity spikes within 1 hour of the INBED derived timestamp, the files were flagged for visual inspection to ascertain the actual time of sleep. Sleep was removed from the files and resaved as .dat files to allow further analyses to take place.

Currently there are no widely accepted cut-points for wrist accelerometry as there is a lack of validation studies assessing the metabolic cost of physical activities measured at the wrist. Comparisons between wrist and waist based models have been estimated in a free living environment and vector magnitude counts per minute (CPM) thresholds have been proposed that minimise the mean difference between device locations. Compared with waist accelerometry, wrist based estimates of sedentary time and MVPA had the lowest mean differences at < 2000 CPM for sedentary and between ≥ 7500 and ≥ 8250 CPM for MVPA (Kamada et al., 2016).

This study therefore characterised sedentary time as any behaviour at a threshold < 2000 CPM, Light activity between 2001-7499 CPM and MVPA ≥ 7500 CPM. The higher cut-points for MVPA were calculated (Kamada 2) for all analyses for comparison purposes and these are presented within Appendix C. Within this study, the lower cut-points will be referred to solely as Kamada. Additionally, in the interest of exploring cut-points for sedentary time, sedentary time was calculated using the threshold of 1853 CPM which has been derived against an activPAL comparison on the non-dominant wrist (Sedentary Koster) (Koster et al., 2016). Waist worn analysis typically uses 4 or more days of physical activity to classify a ‘valid file’ (Troiano et al., 2008) and therefore this guideline was applied for this study.

3.3.5 Leicester Diabetes Risk Assessment Score

The Leicester Diabetes Risk Assessment Score (from hereon, diabetes risk score) was developed in a multi-ethnic UK sample to identify individuals at risk of developing type 2 diabetes (Gray et al., 2010). A seven-question assessment uses age, gender, ethnicity, familial history of diabetes, waist circumference, BMI and treatment for hypertension to derive a risk score. A composite score is then calculated to place individuals at low risk, increased risk, moderate risk and high risk (Table 4.2). These points then correspond to the risk of having type 2 diabetes now and in 10 years. Scores were calculated for all participants with a complete set of variables.

Table 4.2. Interpretation of risk levels for Leicester Diabetes Risk Assessment Scores.

Risk level	Risk Score	Chances of having type 2 diabetes now	Chances of high blood glucose now, meaning risk of type 2 in 10 years
Low	0-6	1 in 200	1 in 20
Increased	7-15	1 in 50	1 in 10
Moderate	16-24	1 in 33	1 in 7
High risk	25-47	1 in 14	1 in 3

The Leicester Diabetes Risk Assessment Score (Gray et al. 2010).

3.3.6 Main statistical tests

The focus of this paper was to examine the relationships between sedentary time, physical activity and diabetes risk and also to model the influence of behaviour replacement upon diabetes risk scores. Therefore to complete these aims, appropriate statistical tests were sought; namely multiple linear regression and isotemporal substitution analyses. These will now be described in the following section.

This section assumes a basic knowledge of statistics and will only describe general statistical information that will explain the use of the test within this document as it is beyond the scope of this thesis to describe fundamental statistical details in full.

Multiple linear regression analysis

In research, often the relationship between two variables is of interest. As an extension of a basic correlation, regression techniques can be used to calculate a prediction of one variable from another (Field, 2013).

Most statistics can be underpinned mathematically by Equation 4.1, where the particular statistical model (made up of variables and parameters) and an amount of error can be used to predict the data we have collected (Field, 2013). These statistical models are then used to predict an outcome in wider a population using models fitted to the collected sample (Field, 2013).

$$Outcome_i = (model) + error_i$$

Equation 4.1. An equation representing a basic model of data prediction. Taken from (Field, 2013).

The above equation can be considered a linear model as it is the equation for a straight line, which gives us information relating to the gradient and intercept of the relationship (Field, 2013). Both of these parameters then allow researchers to predict what the resultant outcome would be by substituting variables within the model and solving the equation (Field, 2013). Multiple linear regression analyses is an extension of the simple linear model, that uses several predictor variables within the model to predict an outcome (Field, 2013).

Linear models are calculated so that the outcome can be generalised to a wider population than sampled; however, this relies on a number of statistical assumptions that need to be met in order to be confident in the generated equation (Field, 2013). A brief description of the limitations that have been described by Field (2013) are:

- Any predictors should be linearly related to the outcome and be additive; adding the predictors together explains their combined effect.
- The residuals (difference between observed value and predicted value) should be independent.
- All residuals should have the same variance (homoscedasticity).
- Residuals should be normally distributed (mean of 0).
- Predictors are uncorrelated with any other external values.

- Variables should be quantitative or categorical.
- Predictor variables (two or more) should not correlate too highly with each other (multicollinearity).
- The predictors should vary in value.

If any of these assumptions are not met, then the model, confidence intervals and significance tests may be invalid (Field, 2013).

As described above, predictors should be linearly related but more important they should be theoretically related to the outcome variable (Field, 2013). This ensures the generated model has sound theoretical underpinnings. Additionally, if other variables are known to heavily influence a particular outcome, omitting them from the regression analyses may not provide an accurate representation of the population. Therefore, these should be entered into the model to adjust the regression coefficients of all predictors and will be referred to as 'controlling for' within this thesis. To measure the influence, different models can be used that show the effect of a covariate within the analyses. Entering predictors into the 1st model (model 1) and then covariates into the 2nd model (model 2), which includes all predictors from the 1st and 2nd models, will show how the inclusion of the covariates influences the significance and amount of variance explained by the predictor variables of interest.

If a particular model violates an assumption, robust methods can be used to mostly overcome the identified issues (Field, 2013). One such method is called bootstrapping. If researchers are unsure of the normality of their data, bootstrapping takes multiple smaller samples from entire sampled dataset to calculate parameter estimates such as the mean (Field, 2013). This can be repeated up to 2000 times and the resulting calculations estimate the limits where 95% of the parameter estimates fall for all samples (Field, 2013).

Isotemporal substitution analysis

Behavioural activities can be considered mutually exclusive, as whilst an individual partakes in a particular activity, they cannot take part in another at the same time (Mekary et al., 2009). Additionally, as time per day is finite, researchers have become interested in whether substitution of certain behaviours such as sedentary time with activities of a light or MVPA intensity is beneficial for weight and cardiovascular risk factors (Mekary et al., 2009; Buman et al., 2014).

The technique is based upon the work of Willett and colleagues who used energy partition models in nutritional epidemiology to account for total energy intake in their analyses (Willett and Stampfer, 1986; Willett et al., 1997). Having a similar challenge in physical activity and health research, researchers often wish to see whether changing behaviour has an influence on chronic disease risk or positively affects cardiometabolic risk factors. Mekary and colleagues (2009) extended this technique to physical activity research by describing a method that models the substitution of a behaviour with another of the same duration. This is achieved using a standard multiple linear regression model, entering all measured activities, total activity time and any covariates as predictors (Mekary et al., 2009). By removing one of the activity predictors, the coefficient values represent the consequence of substituting the removed predictor for any of the predictors left within the model, keeping total activity constant (Mekary et al., 2009). Coefficients will represent a one-unit change and if activity is divided into 10 minute periods, any behaviour replacement will be a 10 minute substitution. To aid in interpretation, an example of this technique is detailed in Equation 4.2.

Outcome = predictor 1 + predictor 2 + predictor 3 + total activity + covariates
= sedentary time + light activity + MVPA + total activity + age + sex + BMI
= light activity + MVPA + total activity + age + sex + BMI

Equation 4.2. An example of the isotemporal substitution model. In this example, sedentary time is dropped and the coefficients for predictors left within the model represent a one-unit replacement of sedentary time with light activity or MVPA. Adapted from (Mekary et al., 2009).

3.3.7 Statistical analysis

Analysis of variance tests with Bonferroni adjusted comparisons were used to analyse diabetes risk score group differences for demographic, cardiometabolic and physical activity continuous variables (trends reported) and Chi-squared analyses were used for categorical variables. Multiple linear regression models were used to examine the relationship between physical activity, sedentary time and diabetes risk score. Behaviour variables were assessed independently as predictors in model one then adjusted for wear time in model two. As evidence suggests an inverse relationship between diabetes mortality and cardiorespiratory fitness (Lavie et al., 2013), ISWT derived peak VO_2 (ml/kg/min) was also included within model two (Dourado et al., 2013). For more information on the ISWT, please see the Appendix B. Age, sex and BMI were not included as covariates as these were already part of

the composite score. Additionally, wear CPM was not adjusted for accelerometer wear time as the duration of wear is already factored into the calculation of the variable. Assumptions of distributed residuals were checked visually using plots and multicollinearity was assessed using variance inflation factors (VIFs). All analyses were conducted within SPSS version 22 for Windows (IBM, Armonk, NY, USA) and significance was set at $p < 0.05$.

Isotemporal substitution was calculated by entering all intensity classifications (sedentary, light and MVPA) in addition to accelerometer wear time within a regression model (Mekary et al., 2013). In this study, intensity variables were divided into 10 minute chunks to both align with the current physical activity guidelines (Department of Health, 2011b), and to represent achievable goals for individuals to aspire to. Analyses were conducted in an iterative process, with univariate Isotemporal substitutions being assessed in Model one and then adjusted in model two for ISWT derived peak VO_2 (ml/kg/min). Due to a lack of consensus on the accelerometry cut-points, isotemporal substitution modelling was also completed on derived light to moderate physical activity (LVPA) variables in order to assess the effect of substituting physical activity of any intensity on diabetic risk scores. Finally, the effect of substituting sedentary time for varying durations (10, 20 or 30 minutes) was also modelled using linear regressions.

Isotemporal substitution analysis assumes a linear relationship between predictors and outcomes variables, therefore the changes in diabetes risk score will be the same for all participants regardless of their initial levels of physical activity or sedentary time. To investigate whether replacement of sedentary time into either light or MVPA causes greater reductions in risk for those currently engaging in large amounts of sedentary time and low MVPA, a sensitivity analysis was performed. Based upon another study that made mutually exclusive categories (Bakrania et al., 2016a), participants were categorised by their sedentary time and light activity ratio (sedentary time ÷ light activity), and whether they had completed 150 minutes of MVPA in a week. Participants were labelled as having low physical activity if they resided within quartiles two to four of the sedentary/light ratio and if they did not complete 150 minutes of MVPA per week.

3.4 Results

3.4.1 Sample characteristics

A sub-set of the 436 individuals from the PhARaoH study, without a diagnosis of COPD, ≥ 4 days of valid accelerometry, without a diagnosis of diabetes, with a valid diabetes risk score and peak VO_2 (n = 251) were selected to be used in the analyses (Figure 4.2).

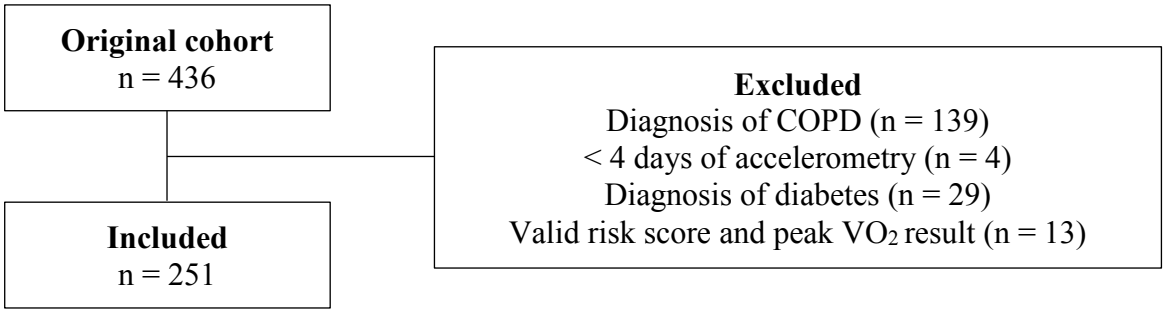


Figure 4.2. The number of participants excluded from analyses.

Descriptive statistics are presented in Table 4.3. The sample comprised mostly of women (63%) and those of a white origin (61%); however, South Asians were the next predominant ethnicity within the sample (34%). Compared to the 2011 census, the sample had a higher representation of South Asian and fewer white participants than those reported to be living within Leicestershire and the City of Leicester (14% and 78% respectively) (Office for National Statistics, 2012). Accelerometry compliance was high, with an average of 7 valid days for the cohort and 91% of individuals had 7 days of valid wear with 16 having 6 valid days and three having 4 and 5 valid days of accelerometer wear.

The two highest prevalent risk categories were increased (37%) and moderate (36%) risk. Diabetes risk groups differed by all demographic, cardiometabolic and behavioural variables with the exception of maternal diabetes, wear minutes and the number of valid days. Pairwise comparisons revealed differences between low, moderate and high risk groups but not increased risk for cardiometabolic risk factors such as age (low < mod/high, $p < 0.0005$), percent body fat (low < mod/high, $p < 0.004$), BMI (low < inc/mod/high, $p < 0.0005$) and glycated haemoglobin (HbA1c) (low < mod/high, $p < 0.003$). Group means for light and MVPA followed a pattern where the increased group had more favourable estimates which then declined linearly to the moderate and high risk categories i.e. Light minute means: low = 425, increased = 430, moderate = 397, high = 374, which could be a factor of a small sample size for the low category.

Table 3.3. Descriptive statistics (Mean ± SD) for the whole sample and broken down into diabetes risk groups.

	Whole group n = 251	Low risk n = 28	Increased risk n = 92	Moderate risk n = 90	High risk n = 41	
Demographics						
Age (Years)	56.7 ± 8.8	50.9 ± 4.9	54.5 ± 8.3	58.5 ± 9.0	61.7 ± 8.0	***
Gender Male (n)	93	5	24	41	23	***
Ethnicity White (n)	152	25	60	48	19	*
Ethnicity South Asian (n)	85	3	26	36	20	
Ethnicity Other (n)	14	0	6	6	2	
Anthropometrics						
Body Fat (%) n = 248	30.0 ± 8.1	25.2 ± 6.7	29.2 ± 7.4	31.0 ± 8.4	33.3 ± 8.2	***
BMI (kg/m^2)	26.9 ± 5.2	22.5 ± 1.7	25.0 ± 3.6	28.1 ± 4.9	31.6 ± 5.8	***
Waist circumference (cm)	89.9 ± 13.7	77.5 ± 6.3	82.9 ± 10.0	94.1 ± 11.2	104.8 ± 12.5	***
Cardiometabolic						
HbA1c (%) n = 248	5.5 ± 0.4	5.3 ± 0.2	5.4 ± 0.3	5.5 ± 0.4	5.7 ± 0.4	***
Glucose (mmol/L) n = 228	5.0 ± 0.5	5.0 ± 0.4	4.9 ± 0.3	5.2 ± 0.6	5.1 ± 0.6	*
Systolic blood pressure (mmHg)	132.9 ± 17.7	121.0 ± 16.6	129.0 ± 16.4	138.0 ± 16.2	138.7 ± 18.9	***
Diastolic blood pressure (mmHg)	79.6 ± 9.5	73.3 ± 8.5	78.3 ± 8.9	82.9 ± 9.2	80.0 ± 9.1	***
Peak VO_2 (ml/kg/min)	24.1 ± 6.9	28.8 ± 7.0	26.0 ± 6.5	22.2 ± 6.0	20.8 ± 7.0	***
Father had diabetes (yes)	39	1	12	13	13	*
Mother had diabetes (yes)	39	0	14	18	7	
Sibling had diabetes (yes)	27	0	6	13	8	*
Currently taking blood pressure medication (yes)	52	0	4	26	22	***
Have a hypertension diagnosis (yes)	57	0	5	28	24	***
Diabetes Risk Score	16.4 ± 7.7	3.9 ± 2.2	11.7 ± 2.3	19.6 ± 2.4	28.5 ± 3.1	***

Table 4.3. Descriptive statistics (Mean ± SD) for the whole sample and broken down into diabetes risk groups.

	Whole group n = 251	Low risk n = 28	Increased risk n = 92	Moderate risk n = 90	High risk n = 41	
Behaviour						
Wear minutes PD	973.0 ± 51.8	964.2 ± 49.2	969.7 ± 46.7	972.4 ± 51.7	987.9 ± 62.9	
Wear counts total PD (x100,000)	23.7 ± 5.7	25.2 ± 3.5	25.5 ± 6.3	22.9 ± 5.5	20.8 ± 4.0	***
Wear counts total PM (x1000)	2.4 ± 0.6	2.6 ± 0.4	2.6 ± 0.6	2.4 ± 0.5	2.1 ± 0.4	***
Sed Kos minutes PD	509.1 ± 98.0	478.5 ± 70.4	476.5 ± 98.4	524.3 ± 98.6	569.9 ± 74.7	***
Sed Kam minutes PD	527.1 ± 98.4	497.1 ± 71.2	494.2 ± 99.1	542.1 ± 98.6	588.3 ± 75.0	***
Li Kam minutes PD	408.4 ± 84.9	424.6 ± 55.1	430.2 ± 89.0	396.7 ± 87.5	374.2 ± 72.0	**
MV Kam minutes PD	37.4 ± 30.7	42.5 ± 22.3	45.2 ± 38.1	33.5 ± 26.4	25.3 ± 19.0	**

Reported as means and standard deviations unless otherwise stated; diabetes risk groups calculated according to Gray et al. (2010); abbreviations: BMI, body mass index / HbA1c, glycated haemoglobin / VO2, oxygen uptake / PD, per day / PM, per minute / Sed, sedentary / Kos, Koster / Kam, Kamada / Li, light / MV, moderate to vigorous physical activity; significance only reported for trend; Ethnicity reported once; Chi-square significance is reported as exact.

*p < 0.05, **p < 0.005, ***p < 0.0005

3.4.2 Associations between movement behaviours and diabetes risk score

Univariate linear regressions revealed significant associations between diabetes risk score and all movement behaviour variables ($p < 0.0005$) and are represented in Table 4.4. These associations persisted within covariate adjusted models (wear time, ISWT derived peak VO_2 (ml/kg/min)). Whilst sedentary time exhibited positive associations with diabetes risk score, physical activity variables were negatively associated. For instance, per one unit change (1 minute per day) in sedentary time (Kamada), diabetes risk score increased by 0.028 (unadjusted) or 0.024 (adjusted). Univariate analysis explained between 6 to 13% of the explained variance in the risk score with an additional 10-17% explained by the inclusion of the covariates. Sedentary time and wear CPM explained the most variance (unadjusted), however 87% for univariate and 76-77% was still left unexplained from adjusted models.

Table 3.4. Associations between diabetes risk score and physical activity intensity variables.

	Unstandardised Coefficients		Standardised Coefficient		95% Confidence Intervals		
	b	**SE**	**β**	**Sig.**	**Lower**	**Upper**	**R^2**
MODEL ONE							
Wear counts PD	−0.000004	0.000001	−0.329	< **0.0005**	−0.000006	−0.000003	0.108
Wear counts PM	−0.005	0.001	−0.363	< **0.0005**	−0.006	−0.003	0.132
SedKos PD	0.028	0.005	0.362	< **0.0005**	0.019	0.038	0.128
SedKam PD	0.028	0.005	0.361	< **0.0005**	0.019	0.037	0.130
LiKam PD	−0.022	0.006	−0.248	< **0.0005**	−0.033	−0.011	0.061
MVKam PD	−0.069	0.015	−0.277	< **0.0005**	−0.099	−0.039	0.076
MODEL TWO							
Wear counts PD	−0.000004	0.000001	−0.298	< **0.0005**	−0.000006	−0.000002	0.242
Wear counts PM	−0.004	0.001	−0.295	< **0.0005**	−0.006	−0.002	0.231
SedKos PD	0.024	0.005	0.302	< **0.0005**	0.015	0.033	0.234
SedKam PD	0.024	0.005	0.303	< **0.0005**	0.015	0.033	0.243
LiKam PD	−0.024	0.005	−0.268	< **0.0005**	−0.035	−0.014	0.226
MVKam PD	−0.052	0.015	−0.207	< **0.0005**	−0.081	−0.023	0.201

Model one represents unadjusted associations and model two is adjusted for peak VO_2 derived from the Incremental Shuttle Walk Test (ISWT); regression coefficients represent the change in diabetes risk score per one unit change (1 minute) in behavioural variables; abbreviations: sig, significance / PD, per day / PM, per minute / Sed, sedentary / Kos, Koster / Kam, Kamada / Li, light / MV, moderate to vigorous physical activity; significant results are in bold; R^2 = coefficient of determination; wear counts PM is not adjusted for wear minutes as it is already a factor of time.

3.4.3 Isotemporal substitution analysis

Removing the light and MVPA intensity cut-points allows for the evaluation of total movement and not just within a certain intensity category. This was achieved by reanalysing the accelerometry data. Instead of demarcating the intensity cut-points, behaviour was categorised into either sedentary, or LVPA. Using isotemporal substitution modelling, LVPA was found to be associated with diabetes risk scores for both Kamada and Koster estimates of LVPA. Results are presented in Table 4.5. Substituting 10 minutes per day of sedentary time for LVPA results in a 0.237-0.279 reduction of diabetes risk score (Kamada adjusted and unadjusted respectively). Splitting LVPA into light and MVPA showed that substitution into higher intensity activity results in greater reductions in diabetes risk scores (Table 4.6). Substituting light physical activity for MVPA did not reach statistical significance, despite coefficients being of a similar magnitude to the replacement from sedentary to light activity. Substituting sedentary time with MVPA produced the greatest decreases in diabetes risk, as for every 10 minutes replacement of sedentary time with MVPA, diabetes risk scores were decreased by 0.540-0.377 (unadjusted–adjusted). Furthermore, modelling the reallocation of 10, 20 or 30 minutes of either light activity or MVPA resulted in greater decreases in diabetes risk scores. These are presented within Figure 4.3.

Adjusting for peak VO_2, derived from ISWT, reduced the regression coefficients as it accounted for a large proportion of the variance. Calculating the associations between diabetes risk scores and Kamada 2 cut-points resulted in larger decreases of diabetes risk. These are presented within Appendix C.

VIF values were less than two in all but two models, suggesting low multicollinearity, however large VIF values were revealed for isotemporal substitution models where MVPA was the reference. Caution should be taken when interpreting the results of that model within Table 4.6.

Table 3.5. Isotemporal substitution associations between diabetes risk score and light to moderate physical activity (LVPA).

	Sedentary			**LVPA Kamada**			**LVPA Koster**		
		95% Confidence Intervals			**95% Confidence Intervals**			**95% Confidence Intervals**	
	b	**Lower**	**Upper**	**b**	**Lower**	**Upper**	**b**	**Lower**	**Upper**
MODEL ONE									
SedKam PD		**Reference**		**−0.279*** **	- 0.374	−0.184	**−0.281*** **	−0.376	−0.186
LVPAKam PD	**0.279*** **	0.184	0.374		**Reference**			**N/A**	
LVPAKoster PD	**0.281*** **	0.186	0.376		**N/A**			**Reference**	
MODEL TWO									
SedKam PD		**Reference**		**−0.237*** **	- 0.327	−0.148	**−0.238*** **	−0.328	−0.148
LVPAKam PD	**0.237*** **	0.148	0.327		**Reference**			**N/A**	
LVPAKoster PD	**0.238*** **	0.148	0.328		**N/A**			**Reference**	

Model one represents unadjusted associations and model two is adjusted for peak VO_2 derived from the Incremental Shuttle Walk Test (ISWT); regression coefficients represent the substitution of 10 minutes of one behaviour into another of a different intensity; abbreviations: B, unstandardised regression coefficient / Sed, sedentary / Kam, Kamada / PD, per day / LVPA, light to vigorous physical activity / Kos, Koster; reference represents the behaviour being ‘dropped’ from isotemporal analysis; significant results are in bold.
*** < 0.0005

Table 3.6. Associations between diabetes risk score and isotemporal substitution replacement into intensity variables of sedentary, light and moderate to vigorous physical activity (MVPA).

	Sedentary			**Light**			**MVPA**		
	b	**95% Confidence Intervals**		**b**	**95% Confidence Intervals**		**b**	**95% Confidence Intervals**	
		Lower	**Upper**		**Lower**	**Upper**		**Lower**	**Upper**
MODEL ONE									
SedKam PD		**Reference**		**−0.221*** **	−0.335	−0.107	**−0.540** **	−0.843	−0.238
LiKam PD	**0.221*** **	0.107	0.335		**Reference**		−0.319[a]	−0.670	0.031
MVKam PD	**0.540** **	0.238	0.843	0.319[a]	−0.031	0.670		**Reference**	
MODEL TWO									
SedKam PD		**Reference**		**−0.208*** **	−0.315	−0.101	**−0.377*[b]**	−0.666	−0.087
LiKam PD	**0.208*** **	0.101	0.315		**Reference**		−0.168	−0.501	0.165
MVKam PD	**0.377*[b]**	0.087	0.666	0.169	−0.164	0.501		**Reference**	

Model one represents unadjusted associations and model two is adjusted for peak VO_2 derived from the Incremental Shuttle Walk Test (ISWT); regression coefficients represent the substitution of 10 minutes of one behaviour into another of a different intensity; abbreviations: B, unstandardised regression coefficient / PD, Per Day / Sed, Sedentary / Kam, Kamada / Li, Light / MV, moderate to vigorous physical activity; reference represents the behaviour being 'dropped' from isotemporal analysis; significant results are in bold. * < 0.05, ** < 0.005, *** < 0.0005, a p = 0.074, b p = 0.011

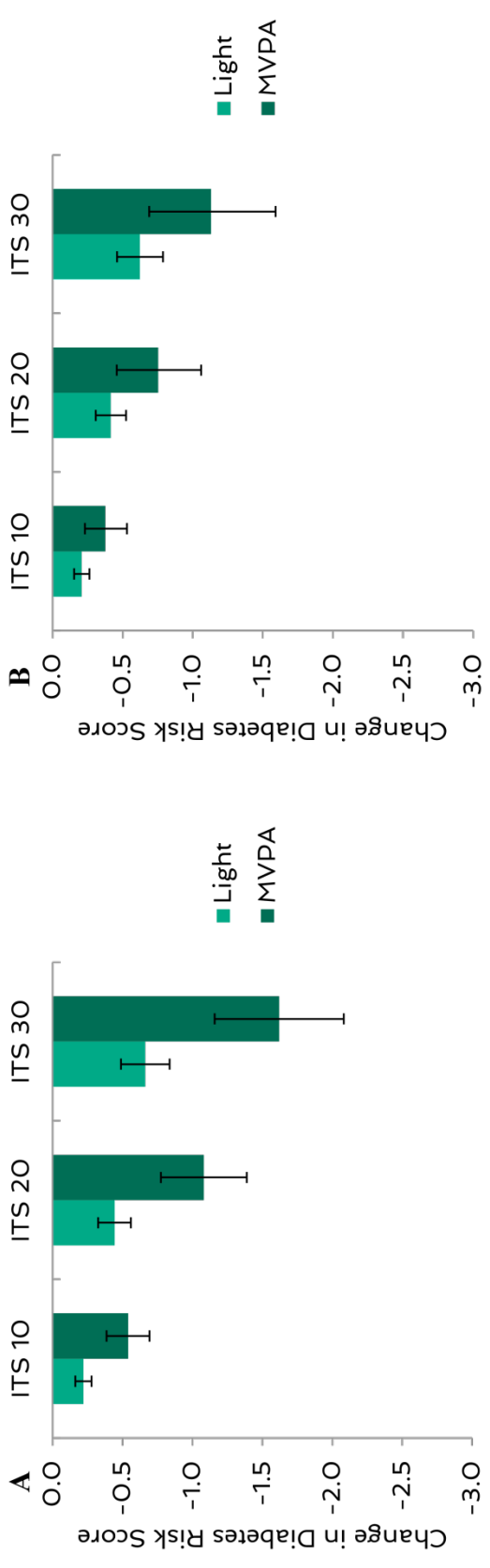


Figure 4.3. Bar graphs representing the change in diabetes risk if sedentary time was substituted with light or moderate to vigorous physical activity (MVPA) in blocks of 10 (ITS 10), 20 (ITS 20) or 30 (ITS 30) minutes per day.
Error bars = standard error of coefficients. All relationships are < 0.05.
A = unadjusted Kamada threshold variables (sedentary:0-1999, light: 2000-7499, MVPA: ≥ 7500)
B = adjusted Kamada threshold variables

3.4.4 Isotemporal substitution analysis – stratified

Separate isotemporal substitution modelling for the low physical activity group is presented in Table 4.7. Splitting the group by behavioural status reveals larger changes in diabetes risk scores for low physical activity individuals (n = 88) when replacing 10 minutes of sedentary time to MVPA (−3.145 adjusted, −3.145 adjusted); however, replacement into light activity was not significant. For the 'Else' category (n = 163), reductions are slightly attenuated from estimates provided within Table 4.6 and are of a lower magnitude compared to low activity coefficients. From these results, it suggests that the replacement of sedentary time into MVPA for low activity individuals can be eight to nine times (−3.601/−0.470 unadjusted, −3.145/−0.365 adjusted) more potent than more active and less sedentary participants.

Table 4.7. Associations between diabetes risk score and isotemporal substitution replacement of sedentary time into light and moderate to vigorous physical activity (MVPA), split by low physical activity high sedentary group and else.

		Model One		**Model Two**	
		Low PA	**Else**	**Low PA**	**Else**
Light	**b**	−0.188	**−0.204**	−0.171	**−0.209**
	Sig.	0.189	**0.004**	0.213	**0.001**
	95% Lower CI	−0.470	−0.342	−0.442	−0.337
	95% Upper CI	0.094	−0.067	0.100	−0.082
MVPA	**b**	**−3.601**	**−0.470**	**−3.145**	**−0.365**
	Sig.	**0.026**	**0.011**	**0.043**	**0.036**
	95% Lower CI	−6.755	−0.832	−6.192	−0.706
	95% Upper CI	−0.447	−0.108	−0.099	−0.025

Model one represents unadjusted associations and model two is adjusted for peak VO_2 derived from the Incremental Shuttle Walk Test (ISWT); regression coefficients represent the substitution of 10 minutes of one behaviour into another of a different intensity; abbreviations: B, unstandardised regression coefficient / PA, physical activity / Sig, significance / CI, confidence intervals; significant results are in bold.

3.5 Discussion

3.5.1 Main discussion

This study aimed to examine the relationship between diabetes risk scores, physical activity and sedentary time measured using wrist worn accelerometry, and to model the changes in risk scores by reallocating movement behaviours from lower to a higher intensity using isotemporal substitution modelling. Results revealed that objectively measured physical activity and sedentary time were associated with diabetes risk and whereas sedentary time and light activity have similar magnitude of coefficients, MVPA was shown to have the strongest relationship to diabetes risk. Additionally, substituting 10 minutes of sedentary time with either light or MVPA significantly reduced diabetes risk score, again with the strongest reduction occurring for an exchange to MVPA. Interestingly, substituting light activity with MVPA was not significant, indicating most benefits are obtained by initiating changes from sedentary time to any physical activity rather than increasing the activity intensity of those already engaging in some light activity.

Previous studies using substitution models have found that the replacement of sedentary time conveys a beneficial association with cardiovascular biomarkers, with MVPA being a more potent reduction (Buman et al., 2014). In the present study, a substitution of 10 minutes of sedentary time into light activity or MVPA reduced diabetes risk score by −0.221 or −0.540, respectively (unadjusted). These adjustments are modest as to put it into perspective, individuals would have to replace sedentary time with either 204 minutes of light or 84 minutes of MVPA per day (unadjusted and rounded) to modify risk enough to cross a risk category from the middle of another e.g. 20 to 15 (moderate to increased risk) whilst holding everything else constant. When controlling for peak VO_2, an additional daily 13 minutes of light and 36 of MVPA per day is required to initiate those changes. Based upon previous isotemporal substitution literature, these estimates are not inconsistent. Hamer and colleagues (2014) reported a reduction of Hba1c of 0.024% for a 10 minute reallocation from sedentary time to MVPA. A large amount of sedentary time would have to be reallocated (216 minutes) to reach a suggested clinically significant change in HbA1c of 0.5% (Little et al., 2011). Though achievable, considering that many individuals do not meet physical activity guidelines (Craig et al., 2009), a substantial daily change may be difficult to implement and may have to be combined with other lifestyle intervention strategies. The replacement of sedentary time with light activity in this study was associated with a reduction in diabetes risk score and compared to MVPA, the levels of risk reduction was between 45 to 59%

(unadjusted / adjusted) less when substituting sedentary time into light activity. More activity would have to be undertaken to confer the same reduction in risk score, nonetheless, light activity may be more achievable for certain individuals.

Splitting the group by behavioural status decreases the amount of reallocation required to have an impact upon diabetes risk scores for those currently undertaking low physical activity but high sedentary time. The replacement of sedentary time to MVPA was associated with up to eight to nine times greater risk reduction compared to the pooled analyses. Therefore, only 13-15 minutes (adjusted-unadjusted) of replacement of sedentary to MVPA would be required to reduce diabetes risk by five points, a substantial reduction in the requirement to attain the same changes in diabetes risk scores. Similar to the findings of Yates and colleagues (2015), where impaired glucose regulation status modified substitution from sedentary time to light activity (light x impaired glucose regulation interaction $p = 0.0001$) compared to normal glucose metabolism participants. A curvilinear dose response relationship between total activity, MVPA and HbA1c has been demonstrated with a stronger relationship with HbA1c at lower levels of physical activity and when MVPA makes up a higher percent of total activity (Gay et al., 2016). Supporting the findings from this study, the relatively low amount of MVPA required for low physical activity participants is more attainable than the large estimates previously reported, and could be incorporated by making small changes in behaviour.

Within the cohort a larger amount of participants are either classified as ‘increased risk’ or ‘moderate risk’. Furthermore, as risk increases a higher proportion of South Asians are present within each group. It has been reported that the South Asian population may develop diabetes up to 10 years earlier due to a two to four times higher risk of developing diabetes (Sattar and Gill, 2015). A recent study has suggested ethnicity specific physical activity guidelines whereby South Asian individuals are required to undertake 10-15 minutes more MVPA per day to have the same levels of cardiometabolic risk as White European Individuals (Iliodromiti et al., 2016). The substitution of behaviour and diabetes risk scores within this sample may therefore need to be broken down into ethnic groups, to explore any regression coefficients reductions, which would represent a higher requirement of physical activity to confer a similar risk reduction.

The choice of cut-points used within the study have not been validated, yet provide a starting point for researchers to explore wrist relationships with health. The sensitivity analysis approach taken by including varying thresholds, allows for choice to be evaluated. It is clear that the 146 count difference between sedentary classifications does not impact on the relationship between diabetes risk and behaviour; however for both the light and MVPA categories, coefficients increase in size for both the diabetes risk and isotemporal substitution models. Removing potential cut-point choice bias, associations for LVPA and wear counts total and per minute variables are significant, indicating the issue is of identifying where along the wrist worn intensity continuum the magnitude of the relationship changes and not if the relationship exists. The thresholds were derived from an older population with a mean age of 71.9 compared to 56.7 within this sample (Kamada et al., 2016), therefore caution must be taken when interpreting the coefficients. More research is needed to study the classification of behaviour at the wrist in a younger population as if the thresholds are too conservative or liberal, modelled relationships between health related outcomes would be affected.

Replacement models represent a 10 minute reallocation of one behaviour to another. Yet, as behaviour is finite (Chastin et al., 2015), it is more likely that an isolated change in one behavioural intensity would be unachievable, considering some light activity would have to occur on the transition to MVPA. Additionally, differing clusters of behaviours have already been investigated within accelerometry and have identified a number of behaviour types or patterns in the data (Metzger et al., 2008; Marschollek, 2014). Much research has focused on the length of bouts, with many concluding that shorter bouts can convey similar benefits to those lasting for longer than 10 minutes (Glazer et al., 2013). Few have addressed objectively measured frequency of physical activity, although literature from the sedentary breaks area reveals that breaking up sedentary time is associated with metabolic risk factors (Healy et al., 2008a). Allowing individuals to have more choice about how they accrue their physical activity, increases the likelihood that they can create a lifestyle embedded physical activity change, though any advice has to be based on sufficient credible evidence.

3.5.2 Limitations & Strengths

This study is cross-sectional in nature and therefore causality cannot be assumed. Additionally, much is still unknown about wrist worn estimates of physical activity and sedentary time. Compared to an activPAL device, an ActiGraph placed on the non-dominant wrist underestimated sedentary time on average by 22.6 minutes a day (Koster et al., 2016),

which represents 4.4% of average sedentary time for the sample. Confidence intervals indicate a consistent underestimation (0.5 to 44.6 minutes per day), however inspection of Bland Altman plots suggests both under and overestimation. The measurement error could influence the strength of the relationships between sedentary time replacement and diabetes risk within this sample.

Similarly, wrist based estimates of sedentary time and physical activity may be inflated by non-ambulatory wrist movement. Despite similar behavioural patterns, a comparison between wrist and waist deployments found consistently higher median counts for wrist deployments (Shiroma et al., 2016). This should be taken into account when looking at the present analyses; however, pattern recognition algorithm developments should reduce the influence of this limitation going forward. Additionally, the sleep detection method has not been extensively validated and therefore estimates of physical activity and sedentary time may be underestimated/overestimated in certain individuals who do not follow typical diurnal patterns of behaviour. This study adds to the limited evidence of the association between diabetes risk and wrist worn objectively measured physical activity. The sample included a large proportion of south Asian individuals (33%) who have been shown to have an increased risk of diabetes (Sattar and Gill, 2015). Finally, this study has used a novel way of removing sleep and is one of the first studies to use wrist worn accelerometry to associate sedentary time and physical activity to diabetes risk scores and peak VO_2.

3.5.3 Conclusion

This study shows that there are associations between wrist worn estimates of physical activity and diabetes risk in a UK population. Substituting sedentary time with physical activity resulted in a greater reduction in diabetes risk; however, moving from light to MVPA did not always result in significant risk reductions. Risk reductions were eight to nine times greater for those individuals characterised as conducting lower physical activity and higher sedentary time. Further research should also investigate whether reductions in risk are similar in different ethnicities. Finally, work should also be carried out using different behavioural profiles to assess if similar reductions in diabetes risk are obtained.

Association between cardiometabolic risk factors and behaviour complexity using sample entropy

Chapter overview

The previous chapter re-established the links between sedentary time, physical activity and diabetes risk scores in an adult cohort from Leicestershire. Within this study, behaviour was categorised into intensity categories of sedentary time, light activity and moderate to vigorous physical activity; however, recent literature suggests individuals can accrue their physical activity levels quite differently, despite the volume remaining the same. This chapter aimed to analyse behaviour using a novel technique of complexity called sample entropy, to ascertain if the way individuals accrue their activity is associated with cardiometabolic risk factors. This chapter reports the results of a cross-sectional analysis using the same primary dataset collected from Study One (Chapter Three), but included 290 individuals due to differences in the sample inclusion criteria. The chapter concludes that whilst high-density lipoprotein (HDL) cholesterol, triglycerides and glycated haemoglobin (HbA1c) were associated with entropy scores, more research is needed to understand what level of entropy is considered average and how entropy scores can be used in combination with duration and intensity to form a detailed behaviour profile of an individual.

4.1 Abstract

Introduction: Most associations between physical behaviours and health are assessed using intensity and duration based estimations; however, individuals accrue physical activity in differing ways and behavioural profiles have been linked with varying cardiometabolic risk factors. The frequency or regularity of behaviour may hold additional relationships with health, but have not been extensively explored. Accelerometers provide researchers with a large stream of raw data to analyse. The aim of this paper was to calculate a novel method of behavioural regularity called sample entropy from wrist worn accelerometry and to ascertain whether there are associations with cardiometabolic risk factors in adults.

Methods: Data from 290 (107 males; aged 57.0 ± 8.8) participants from a mixed ethnicity cohort from Leicestershire, UK were selected for analysis. Entropy scores were calculated using 60-second count data within MATLAB. The relationship between entropy scores, physical activity, sedentary time and cardiometabolic risk factors was identified using multiple linear regressions. Models were calculated for main effects and also adjusted for age, sex, accelerometer wear time and body mass index (BMI).

Results: Sample entropy scores were significantly related to high-density lipoprotein (HDL) cholesterol ($b = 0.148$, $p = 0.042$), triglycerides ($b = -0.293$, $p = 0.042$) and glycated haemoglobin (HbA1c) ($b = -0.225$, $p = 0.006$), even after adjustment for confounding variables. Traditional intensity estimates of physical activity were not associated; however, the frequency of breaks in sedentary time were significantly related to entropy scores ($b = 0.004$, $p = 0.002$).

Conclusion: Using a novel measure of signal complexity, associations have been revealed with cardiometabolic risk factors; however further analysis in a larger, more diverse dataset is required to ascertain the utility of this technique within behavioural research and if so, what constitutes typical/average levels of entropy within a population.

4.2 Introduction

Physical activity behaviours can be objectively measured using accelerometry in large scale epidemiological studies as advancements in technology have made them less expensive and able to provide more information such as raw acceleration data (Troiano et al., 2014). As a result, a large body of evidence now exists on the relationship between objectively measured physical activity and health (Buman et al., 2010; Camhi et al., 2011; Healy et al., 2008b; Henson et al., 2013). However, the majority of the research has focused on quantifying the intensity and duration of physical activity with little attention being paid to the frequency of bouts or their regularity (Marschollek, 2013). Metzger and colleagues (2008) were one of the first to use accelerometry to investigate different patterns of accumulation using latent class analysis and found five distinct classes, including those that predominantly undertook their physical activity at the weekend or 'Weekend Warriors'. Associating different patterns of physical activity accumulation with cardiometabolic risk factors, significant differences have been found in body mass index (BMI), waist circumference, glycated haemoglobin (HbA1c) and high-density lipoprotein (HDL) cholesterol (Bakrania et al., 2016a; Marschollek, 2016). Although much can be learned by determining the time someone spends in each intensity category (e.g., sedentary, light, moderate, and vigorous), there are no universally agreed upon cut-points that demarcate intensity categories (Lee and Shiroma, 2014). That is to say, cut-points can vary widely from liberal to conservative depending on the device and the population under study, among other things. Therefore, other techniques should be explored that are not constrained by having to use predefined intensity cut-points.

Entropy is a measure of signal complexity (Pincus, 1991) and for a given number of points, entropy assesses the probability that adjacent sequences of data are the same with a lower value of entropy indicating higher similarity/regularity and vice versa (Richman and Moorman, 2000). By definition, a system with a high degree of randomness (i.e., high entropy) represents less system order (Pincus and Goldberger, 1994). In terms of physical activity, higher entropy data may characterise an individual's behaviour that is more like a 'Busy Bee', partaking in frequent short bouts of physical activity. On the opposite end of the movement continuum, being sedentary is characterised by any waking behaviour in a sitting or reclining posture of a low energy expenditure (≤ 1.5 METs) (Sedentary Behaviour Research Network, 2012), which may lead to more system order. Ascertaining if regularity is associated with cardiometabolic risk factors would add to the knowledge surrounding

physical activity prescription, by quantifying if individuals should be less regular in order to beneficially impact their health.

Entropy can be calculated using either approximate entropy or sample entropy and although similar, approximate entropy has been reported to be more reliant on the number of data points within a file and lacks relative consistency (Richman and Moorman, 2000). Entropy has been applied to biological signals such as heart rate variability (Lake et al., 2002), posture analysis (Ramdani et al., 2009), and physical activity data (Cavanaugh et al., 2010; Sosnoff et al., 2010), albeit not in a health association context. In a sample of older community-dwelling adults, those who were more active (≥ 10,000 steps) were reported to have more complex patterns of behaviour when measured by approximate entropy (0.5010) compared to inactive individuals (< 5000 steps, entropy = 0.2813) (Cavanaugh et al., 2010). An advantage of using entropy with accelerometry data is that processing decisions pertaining to the intensity of the activity are nullified as the raw signal is analysed and not post processed data. However, in order to gain a greater understanding of the measurement, entropy scores should still be related to more commonly used units such as minutes spent in certain intensities.

It is currently unknown whether this under researched metric will reveal any new information about behavioural profiles or whether the information can already be explained by current methods. Nevertheless, regularity of physical behaviours is important to investigate as this information will explore the varying ways individuals can accrue physical activity, in addition to existing intensity and duration estimates. Therefore, the aim of this paper is to calculate sample entropy from a sample of wrist worn accelerometry and associate the outcomes with cardiometabolic risk factors. Additionally, sample entropy will be evaluated against traditional physical activity estimates to see if the analysis is highlighting a different association with health.

4.3 Methods

4.3.1 Sample

Data were used from the Physical Activity and Respiratory Health Study (PhARaoH), a cross-sectional, observational study that aimed to quantify the associations between health and physical activity behaviours in adults (ages 40-75 years) with and without a diagnosis of chronic obstructive pulmonary disease (COPD) living in Leicestershire and Rutland, UK. More details can be found at Orme et al. (2016). All participants gave their written informed consent and the study was approved by the National Research Ethics Service Committee East Midlands Nottingham-2. The study sample for this paper came from the control sample of UK adults without COPD and had valid accelerometry and entropy score (described in the next section) and no missing covariates.

4.3.2 Study measurements

All measurements were taken at Glenfield Hospital from March to September 2014 at the Respiratory Biomedical Research Unit by members of the study team. Blood pressure was measured three times, in 1 minute intervals, in the upper right arm using a digital blood pressure monitor (Omron 705IT, Omron, UK). Height, weight, waist circumference and percent body fat were measured using a portable stadiometer (SECA 213, SECA, Germany), tape measure (HaB International Ltd, UK) and bio electrical impedance machine (Tanita MC780MA, Tanita, The Netherlands). A fasting blood test was obtained by a trained phlebotomist after participants had fasted for over 4 hours. This fasting time was chosen pragmatically as appointments could be scheduled either early morning or late afternoon. Though it is recommended to fast for > 8 hours for a glucose test and > 9 hours for a lipid profile (National Health Service, 2015), non-fasted lipid profiles (< 8 hours) have been shown to be associated with disease risk (Bansal et al., 2007; Langsted et al., 2008) and HbA1c was also measured which does not require the individual to be in a fasted state. Fasting time was confirmed and any participants that had eaten < 4 hours were excluded from the blood analysis (apart from HbA1c). If fasting data were missing, the participant was coded as not fasting > 4 hours. For a more detailed description of these methods, the reader is directed to Appendix B.

4.3.3 Accelerometry

Physical activity and sedentary time were measured by an accelerometer (ActiGraph wGT3X-BT, ActiGraph, Pensacola, USA) worn on the non-dominant wrist for seven full

days. Participants were instructed to wear the device at all times and only to remove the device when bathing or swimming. Devices were deployed with a delayed start time of 00:00 of day 2, which would then collect data for the next seven full days. Acceleration data were captured at 100Hz, initialised and downloaded into 60 second .agd files using version 6.10.1 of ActiLife Software (ActiGraph, Pensacola, USA). Processed files were then analysed using KineSoft (KineSoft, Loughborough, UK) version 3.3.80.

As the accelerometer was worn continuously, even during sleep, this behaviour needed to be removed from the data. As the protocol did not include sleep diaries, a vector magnitude count based threshold algorithm was used. Assessing each epoch, a sustained 90% reduction for the proceeding 15 epochs (minutes) in vector magnitude counts between the hours of 21:00 and 23:59 signified an INBED time. Similarly, a sustained increase in vector magnitude counts of 75% during the hours of 06:00 and 09:00 for five consecutive epochs (minutes) denoted an OUTBED time. The epochs containing the original 90% reduction or 75% increase in vector magnitude counts were used as the time stamps for sleep. For those days where an INBED time could not be determined, or if there were activity spikes within 1 hour of the INBED derived timestamp, the files were flagged for visual inspection to ascertain the actual time of sleep. For a detailed description of accelerometry data collection and analytical parameters, the reader is directed to section 4.3.4.

Sedentary time was characterised as any behaviour at a threshold below 2000 counts per minute (CPM), Light activity between 2001-7499 CPM and moderate to vigorous physical activity (MVPA) ≥ 7500 CPM (Kamada et al., 2016). Also, an additional sedentary time cut-point was calculated using the threshold of 1853 CPM which has been derived against an activPAL comparison on the non-dominant wrist (Sedentary Koster) (Koster et al., 2016). A higher cut-point (≥ 8500 CPM) was also calculated for MVPA (Kamada 2); however, the threshold of ≥ 7500 CPM will be solely referred to as Kamada throughout this study.

Finally, removing the light and MVPA intensity cut-points allows for the evaluation of total movement above being sedentary and not just within a certain intensity category. This was achieved by reanalysing the accelerometry data and behaviour was categorised into either sedentary (< 2000 Kamada), or LVPA (≥ 2000 Kamada). From these new variables, the daily number of breaks (LVPA bouts), duration of breaks (LVPA minutes) and the sum of counts for all breaks (LVPA counts), from being sedentary, were calculated.

4.3.4 Entropy processing

Through a semi-automated process of sleep identification and removal, files were prepared for analysis using Microsoft Excel (Microsoft Corporation, Washington, USA). For more detail, please see Appendix D. Once the files were in the correct format to be processed, they were run through an entropy script and output files were generated containing entropy scores. Entropy analysis requires a set of user defined parameters for *'m'* or number of time points that are evaluated as being similar and *'r'* which is the degree of tolerance allowed for matching to occur (Richman and Moorman, 2000). Entropy has been confined largely to heart rate variability or gait analysis and whilst no widely accepted values exist for entry parameters, widely used values are a $m = 2$ and $r = 0.1\text{-}0.25$ (Lake et al., 2002; Yentes et al., 2013). Given this, for the current study m was set at 2 and r at 0.2.

The entropy outputs were transferred to SPSS (IBM, NY, USA) where syntax was written to match physical activity data with the outputs. For some files, entropy scores could not be calculated due to the fact that self-matching windows could not be found within the tolerance. Valid days of data where this occurred were coded as missing. Valid entropy scores and physical activity data were defined as the following:

- Entropy: no missing information
- Physical activity: ≥ 600 minutes of wear

Entropy scores were then matched with temporally ordered physical activity data and only if a day had valid entropy and physical activity data, this information was then averaged to create 'per valid day' variables. Only individuals with four or more matched days were carried forward for analysis. Additionally, to ensure fair comparisons, physical activity was recoded into different variables using SPSS if there was a corresponding valid entropy score. If a day did not have a valid entropy score, the value was set as missing. The new variables were then used for regression analyses.

Entropy is a measure of complexity, though it is unclear whether scores are influenced by the magnitude of the data or the number of changes within the data. To explore this notion further, a file manipulation approach was taken to assess if entropy was associated with the peaks of activity, by removing magnitude (count values) from the data. A sample of 10 randomly selected files (five men & five women) were chosen and then minute by minute

count data were coded as either one for sedentary (< 2000), two for light (2000-7500 CPM) or three for MVPA (≥ 7500 CPM). A representation is shown within Figure 5.1. The information generated through this process was then plotted onto a scatter plot.

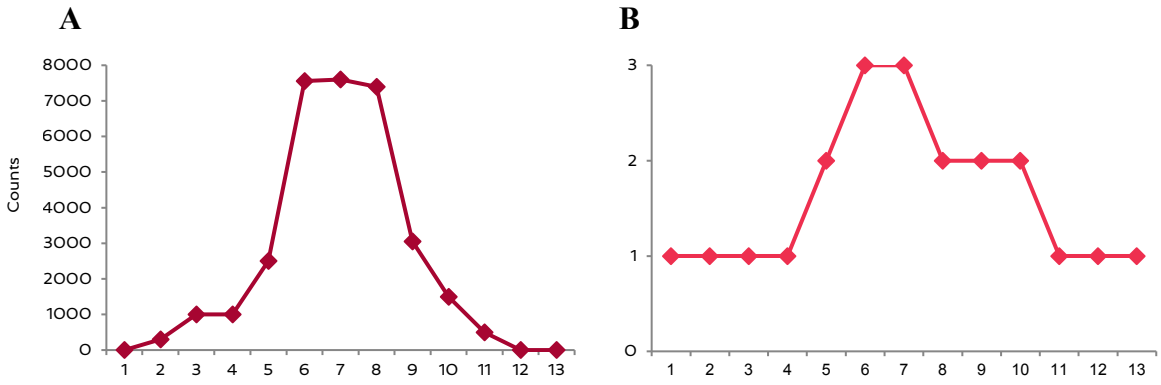


Figure 5.1. Graphs representing the classification of physical behaviours into intensity coding over 13 epochs (minutes).
Graph A is the vector magnitude trace and Graph B is the same data classified as either 1 for sedentary (<2000 counts per minute (CPM)), 2 for light (2000-7500 CPM) or 3 for moderate to vigorous physical activity (MVPA) (≥7500 CPM) (Kamada et al., 2016). The line changes in Graph B represents a change of intensity category. In this example, there are 4 changes in intensity at epoch 4, 5, 7 and 10.

Undertaking further analysis into the interaction between physical activity and entropy scores on cardiometabolic health, groupings were calculated to represent the interaction between activity levels and entropy scores. Activity and entropy categories were formed by splitting recalculated LVPA and entropy scores into quantiles and by combining the categories to form four distinct groups. The groups are outlined in Figure 5.2. LVPA was used to reduce any possible threshold bias in the analysis which may be present when classifying activity into either light or MVPA. These grouping were then used within the statistical analysis.

4.3.5 Main statistical tests

The focus of this paper was to examine the relationships between cardiometabolic risk factors and sample entropy. Therefore to complete these aims, appropriate statistical tests were sought, namely multiple linear regression and binary logistic regression. These will now be described in the following section.

		Entropy Grouping	
		1	2
Activity Grouping	1	Low Entropy Low Activity (1)	High Entropy Low Activity (2)
	2	Low Entropy High Activity (3)	High Entropy High Activity (4)

Figure 5.2. Physical activity and entropy groupings.
1 = lowest quantile and 2 = highest quantile.

This section assumes a basic knowledge of statistics and will only describe general statistical information that will explain the use of the test within this document as it is beyond the scope of this thesis to describe fundamental statistical details in full.

Regression analyses

This paper utilises the same multiple linear regression techniques as Chapter Three (Study One) and the reader is directed there for a more detailed explanation of the technique. In this study, logistic regression is utilised, which is similar to multiple regression but has an outcome variable that is categorical (Field, 2013). This statistical test aims to predict the membership of a certain individual to a categorical outcome and when there are only two outcomes (e.g. Obese vs. Not Obese), it can be referred to as binary logistic regression (Field, 2013). Binary logistic regression is calculated in a similar way to multiple linear regression but instead of predicting a value from predictor variables, the statistic calculates the probability of the membership to a group from known values of variables (Field, 2013). Binary logistic regression is interpreted using odds ratios which explain the change in the odds of something occurring from a unit change in the predictor (Field, 2013). An odds ratio greater than one denotes that as the predictor increases, the odds of the outcome also increases whilst an odds ratio less than one indicates the opposite (decreases) (Field, 2013).

Similar to multiple linear regression analyses, there are certain assumptions that need to be met. A brief description of the limitations that have been described by Field (2013) are:

- Linearity between the log of the outcome variable and any continuous predictors.
- Errors should be independent i.e. cases of data should not be related.
- Predictors should not be highly correlated.

4.3.6 Statistical analysis

Multiple linear regression models were used to assess the relationship between per valid day sample entropy scores (dependent variable) and common cardiometabolic outcomes of mean systolic/diastolic blood pressure, mean waist circumference, total cholesterol, low-density lipoprotein cholesterol (LDL), HDL, Triglycerides, fasting glucose (> 4 hours) and HbA1c (predictors). Additionally, to ascertain if there is a relationship between signal variability and traditional estimates of physical behaviour, physical activity, sedentary time and breaks in sedentary time (bouts, duration and counts) were entered as predictors into multiple linear regressions models. For all analyses, regressions were calculated as separate models and model one represents univariate associations with the outcomes and model two was adjusted for wear minutes, age, sex and BMI. The inclusion of wear minutes accounts for the differences of monitor wear time as an individual has more opportunity to gain higher/lower complexity values if worn for longer period of time. Age, sex and BMI have been shown to influence physical activity levels in a UK national sample (Craig et al., 2009) and were also included as covariates. Binary logistic regressions also assessed the associations between cardiometabolic risk factors and the four distinct activity groupings shown in Figure 5.2. Cardiometabolic risk factors were divided by the median value into two groups and then entered into binary logistic regressions. Odds ratios were calculated as the odds of being within the highest quantile or most favourable quantile of the cardiometabolic risk factor. Regressions were adjusted by wear time apart from HbA1c, which was found to violate the linearity assumption.

All analyses was conducted within SPSS version 22 for Windows (IBM Armonk, NY, USA) and significance was set at $p < 0.05$. Analysis was conducted using pairwise deletion and specific sample sizes are stipulated in the results section. Assumptions of distributed residuals were checked visually using plots and multicollinearity was assessed using variance inflation factors (VIFs). VIF values were less than two in all but two models, suggesting low

multicollinearity. The visual inspection of the residual plots, histograms and P-P plots identified potential problematic cardio-metabolic variables (Triglycerides, HDL, Glucose and HbA1c). Exhibiting either a minor positive skew or deviation from the central line of the P-P plots, these variables were bootstrapped to provide adjusted regression coefficients. Additional assumptions for logistic regressions (i.e. linearity of the logit and multicollinearity) were also undertaken. One continuous predictor violated the linear assumption and so was omitted from the analysis; however, multicollinearity tests produced VIF values less than two for all binary regressions.

4.4 Results

4.4.1 Sample characteristics

A sub-set of the 436 individuals measured from the PhARaoH study, without a diagnosis of COPD, with 4 or more valid physical activity and entropy days and no missing covariates was included within the analyses (Figure 5.3). Descriptive statistics for the sample are outlined in Table 5.1.

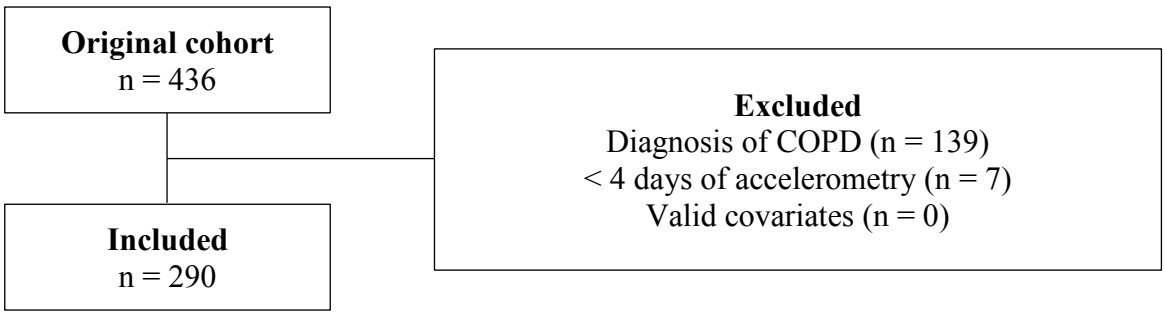


Figure 5.3. The number of participants excluded from analyses.

The 290 participants had a mean age of 57 ± 8.8 years and 63% of the sample were female. Participants on average were overweight with a BMI of 27.1, and had a high but not hypertensive (133.2 mmHg) blood pressure. Mean HbA1c (5.6) is 0.1 mmol/L away from being placed within the pre-diabetic range according to the American Diabetes Association guidelines (American Diabetes Association, 2016). Accelerometry compliance was good as 265 (91%) individuals had 7 days of valid wear (Mean = 6.9). The average number of valid activity and entropy days were also high; however, the percentage of people having 7 valid days dropped to 248 (86%). This suggests that more days were lost due to the calculation of sample entropy than through the application of the accelerometry valid day criterion.

4.4.2 Association between entropy and cardiometabolic risk factors

Multiple linear regressions revealed significant relationships between per valid day entropy scores and HDL cholesterol ($b = 0.188$, $p = 0.018$), Triglycerides ($b = -0.363$, $p = 0.018$) and HbA1c ($b = -0.227$, $p = 0.013$). The full analyses are presented in Table 5.2. The inclusion of the covariates explained on average 20% more variance (waist circumference 82% – LDL cholesterol 3.6%). For those cardiometabolic risk factors in model one that were significant, BMI explained the most additional variance for HDL (6.1%), Triglycerides (6.1%) and HbA1c (3.3%).

Table 5.1. Descriptive statistics (Mean ± SD) for the whole sample.

	Sample n = 290			
Demographics				
Age (Years)	57.0	±	8.8	
Gender Females (n / %)	183		63	%
Anthropometrics				
Body Fat (%) n = 287	30.4	±	8.0	
BMI (kg/m^2)	27.1	±	5.2	
Waist circumference (cm)	90.4	±	14.0	
Cardiometabolic risk factors				
Mean systolic blood pressure (mmHg)	133.2	±	18.0	
Mean diastolic blood pressure (mmHg)	79.5	±	9.7	
Total cholesterol (mmol/L) n = 268	5.2	±	1.0	
LDL cholesterol (mmol/L) n = 260	2.9	±	0.8	
HDL cholesterol (mmol/L) n = 266	1.6	±	0.5	
Triglycerides (mmol/L) n = 268	1.4	±	1.0	
Glucose (mmol/L) n = 261	5.1	±	0.6	
HbA1c (%) n = 284	5.6	±	0.6	
Fasting time (minutes) n = 271	477.4	±	252.3	
Behaviour				
Wear minutes per day	976.4	±	51.1	
Wear counts total per day (x100,000)	23.7	±	6.0	
Wear counts total per minute	2426.1	±	598.8	
Sedentary Koster minutes per day	513.1	±	104.2	
Sedentary Kam minutes per day	531.1	±	104.6	
Light Kam minutes per day	408.4	±	89.4	
MVPA Kam minutes per day	36.7	±	31.1	
Entropy per day	1.1	±	0.3	
Valid accelerometry days (n)	6.9	±	0.4	
4 (n)	2			
5 (n)	4			
6 (n)	19			
7 (n)	265			
Valid entropy days (n)	6.8	±	0.5	
4 (n)	2			
5 (n)	7			
6 (n)	25			
7 (n)	256			
Valid activity & entropy days (n)	6.8	±	0.5	
4 (n)	5			
5 (n)	5			
6 (n)	32			
7 (n)	248			

Means and standard deviations unless otherwise stated; valid accelerometry ≥ 600 minutes; valid entropy = no missing data; abbreviations: BMI, body mass index / LDL, low-density lipoprotein / HDL, high-density lipoprotein / HbA1c, glycated haemoglobin / Kam, Kamada / MVPA, moderate to vigorous physical activity; average fasting time calculated on all that had fasted > 4 hours; missing fasting data were coded as missing.

Table 4.2. Entropy multiple linear regressions associations with cardiometabolic risk factors.

		Unstandardised Coefficients		Standardised Coefficient		95% Confidence Intervals		
	n	**b**	**SE**	**β**	**Sig.**	**Lower**	**Upper**	**R^2**
MODEL ONE								
Mean waist circumference (cm)	290	−2.416	2.498	−0.057	0.334	−7.331	2.500	0.003
Mean systolic blood pressure (mmHg)	290	3.334	3.224	0.061	0.302	−3.011	9.679	0.004
Mean diastolic blood pressure (mmHg)	290	−0.300	1.736	−0.010	0.863	−3.718	3.117	< 0.000
Total cholesterol (mmol/L)	268	−0.011	0.190	−0.003	0.956	−0.386	0.364	< 0.000
LDL cholesterol (mmol/L)	260	−0.060	0.159	−0.024	0.706	−0.372	0.252	0.001
HDL cholesterol (mmol/L)	**266**	**0.188**	**0.076**	**0.136**	**0.018**	**0.034**	**0.344**	**0.018**
Triglycerides (mmol/L)	**268**	**−0.363**	**0.156**	**−0.123**	**0.018**	**−0.703**	**−0.098**	**0.015**
Glucose (mmol/L)	261	−0.158	0.104	−0.082	0.136	−0.362	0.064	0.007
HbA1c (%)	**284**	**−0.227**	**0.091**	**−0.131**	**0.013**	**−0.410**	**−0.053**	**0.017**
MODEL TWO								
Mean waist circumference (cm)	290	0.582	1.062	0.014	0.584	−1.508	2.673	0.824
Mean systolic blood pressure (mmHg)	290	3.995	2.935	0.073	0.175	−1.783	9.773	0.007
Mean diastolic blood pressure (mmHg)	290	0.518	1.674	0.018	0.757	−2.777	3.814	0.094
Total cholesterol (mmol/L)	268	−0.029	0.187	−0.009	0.875	−0.397	0.338	0.064
LDL cholesterol (mmol/L)	260	−0.047	0.158	−0.018	0.765	−0.357	0.263	0.037
HDL cholesterol (mmol/L)	**266**	**0.148**	**0.071**	**0.107**	**0.042**	**0.015**	**0.302**	**0.192**
Triglycerides (mmol/L)	**268**	**−0.293**	**0.147**	**−0.099**	**0.042**	**−0.594**	**- 0.045**	**0.102**
Glucose (mmol/L)	261	−0.146	0.103	−0.076	0.150	−0.367	0.101	0.043
HbA1c (%)	**284**	**−0.225**	**0.083**	**−0.131**	**0.006**	**−0.389**	**- 0.066**	**0.141**

Model one represents a univariate association and model two is adjusted for wear minutes, age, sex and body mass index (BMI); abbreviations: n, sample size for analysis (pairwise deletion) / SE, standard error / Sig, significance; BMI, body mass index / LDL, low-density lipoprotein / HDL, high-density lipoprotein / HbA1c, glycated haemoglobin; significant results are in bold; R^2 = coefficient of determination of single predictor model (model one) and total explained variance for adjusted model (model two).

Given that entropy scores for physical activity are limited in the literature, the regression equation can be solved using exemplar data to aid the readers' understanding.

$$HbA1c = Constant + (B_0*Entropy) + (B_1*WearMin) + (B_2*Age) + (B_3*Sex) + (B_4*BMI)$$
$$Hba1c = 2.803 + (-0.225*Entropy) + (0.002*WearMin) + (0.016*Age) + (-0.046*Sex) + (0.020*BMI)$$

Equation 5.1. The prediction equation for HbA1c including entropy as a predictor variable.

For a male that has average wear minutes, age and BMI, manipulating the entropy score between the lowest value in the sample (0.44) and the highest (2.31) produces a HbA1c score of 6.1% and 5.6%, a difference of 0.5% which has been suggested as clinically significant (Little et al., 2011). However, without knowing what a high entropy score relates to, it is unknown whether the difference between the high and low entropy values for the sample is indeed a large difference.

Binary logistic regressions were utilised to assess the associations between cardiometabolic risk factors which were significant in model one of Table 5.2 (HDL, Triglycerides and HbA1c) and the four distinct activity groupings shown in Figure 5.2. Figure 5.4 represents the binary logistic regressions odds ratios for HDL, HbA1c and Triglycerides. Compared to group one, the odds of being within the most favourable HDL quantile were greater for groups two (OR = 2.192, p = 0.023), three (OR = 2.451, p = 0.10) and four (OR = 3.192, p = 0.001). The high entropy and high LVPA group were 3.2 times more likely to be within the higher HDL quantile. Whilst a similar trend in odds ratios were observed for HbA1c and Triglycerides, no significant results were obtained.

4.4.3 Association between entropy and traditional physical activity estimates

Multiple linear regressions between entropy scores and time spent in varying intensities of physical activity revealed no significant results. These are presented in Table 5.3. Regression coefficients do not follow our initial assumptions of entropy, that sedentary time is positively associated with entropy scores and vice versa for light or MVPA. Based upon these results, it seems to suggest that intensity and volume estimates of behaviour is not related/associated. Kamada 2 cut-points are also available for comparison purposes within Appendix E.

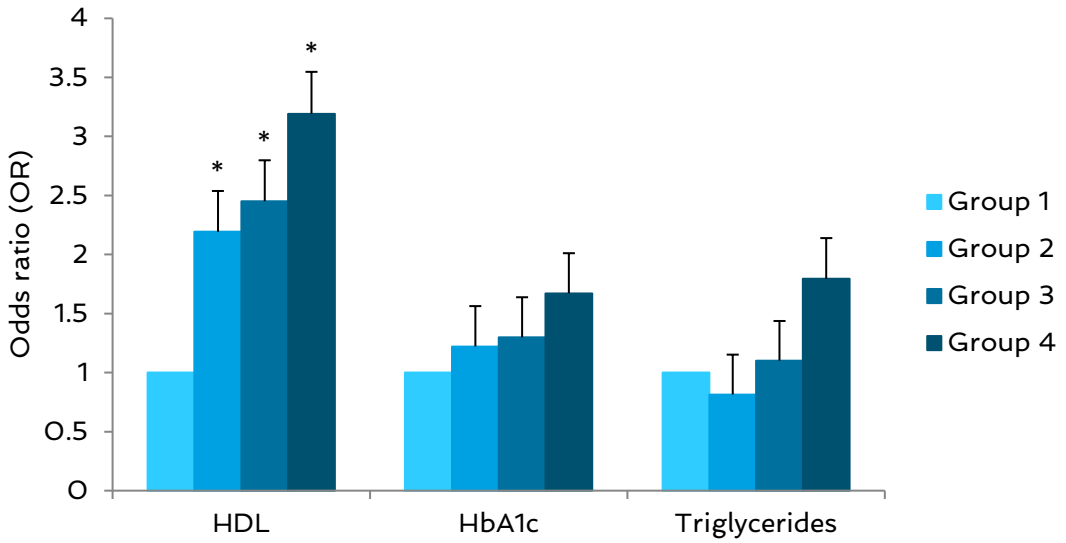


Figure 5.4. A bar plot representing the relationships between cardiometabolic risk factors and the 4 combined physical activity and entropy groupings controlled for wear time (not glycated haemoglobin (HbA1c)).
Each bar represents the odds of being within the more favourable cardiometabolic quantile (high-density lipoprotein (HDL) cholesterol: high; HbA1c: low; triglycerides: low). Error bars = standard error. Significant odds ratios were revealed for HDL but not HbA1c or triglycerides. Reference group = Group 1.

Group 1 = low entropy and low light to vigorous physical activity (LVPA)
Group 2 = high entropy and low LVPA
Group 3 = low entropy and high LVPA
Group 4 = high entropy and high LVPA

To ascertain if the relationship between behaviour and entropy was being hidden amongst intensity classifications, physical activity was collapsed into breaks in sedentary time variables i.e. if activity was not deemed to be below the sedentary cut-point, it would be indicate a break in sedentary time. This was achieved by using behaviour categorised into sedentary time or LVPA. The daily number of sedentary bouts (number of times going over the sedentary cut-point), counts and minutes were then associated against entropy scores using multiple linear regressions and are presented within Table 5.4.

The number of breaks in sedentary time (bouts) were positively associated with entropy scores (B x 10,000 = 40.0, p = 0.002) but only explained 3.2% of explained variance. Neither the counts or minutes of sedentary breaks were significantly related to entropy, suggesting only the amount of times individuals cross the sedentary threshold (cut-point) to complete physical activity of a light to vigorous intensity is related to entropy but not the duration or

Table 4.3. Entropy multiple linear regressions associations with intensity variables.

	Unstandardised Coefficient (x10,000)		Standardised Coefficient		95% Confidence Intervals (x10,000)		
	b	**SE**	**β**	**Sig.**	**Lower**	**Upper**	**R^2**
MODEL ONE							
Wear counts total PD	−0.0001	0.0003	−0.026	0.653	−0.0008	0.0005	0.001
Wear counts total PM	−0.18	0.32	−0.033	0.577	−0.82	0.46	0.001
Sedentary Koster PD	1.2	1.86	0.038	0.518	−2.46	4.87	0.001
Sedentary Kam PD	1.41	1.85	0.045	0.447	−2.24	5.06	0.002
Light Kam1 PD	0.15	2.17	0.004	0.945	−4.13	4.43	< 0.000
Light Kam2 PD	−0.34	2.05	−0.010	0.867	−4.39	3.7	< 0.000
MVPA Kam1 PD	−8.33	6.2	−0.079	0.180	−20.53	3.87	0.006
MVPA Kam2 PD	−8.63	9.19	−0.055	0.348	−26.72	9.46	0.003
MODEL TWO							
Wear counts total PD	−0.0003	0.0003	−0.049	0.437	−0.001	0.0004	0.011
Wear counts total PM	−0.33	0.35	−0.060	0.351	−1.03	0.37	0.012
Sedentary Koster PD	2.1	2.12	0.067	0.322	−2.07	6.28	0.015
Sedentary Kam PD	2.34	2.11	0.074	0.269	−1.82	6.51	0.016
Light Kam1 PD	−1.65	2.41	−0.045	0.495	−6.39	3.1	0.014
Light Kam2 PD	−2.13	2.28	−0.061	0.352	−6.62	2.37	0.015
MVPA Kam1 PD	−10.66	6.64	−0.101	0.110	−23.73	2.42	0.021
MVPA Kam2 per day	−11.28	9.86	−0.072	0.253	−30.69	8.13	0.017

Model one represents a univariate association and model two is adjusted for wear minutes, age, sex and body mass index (BMI); abbreviations: SE, standard error / Sig, significance / PD, per day / PM, per minute / MVPA, moderate to vigorous physical activity; regression coefficients represent the change in diabetes risk score per 1 unit change (1 minute) in behavioural variables; R^2 = coefficient of determination; unstandardised coefficients and standard errors have been divided by 10,000; wear counts total PM is not adjusted for wear minutes as it is already a factor of time.

Table 4.4. Entropy multiple linear regressions associations with breaks in sedentary time.

	Unstandardised Coefficient (x10,000)		Standardised Coefficient		95% Confidence Intervals (x10,000)		
	b	**SE**	**β**	**Sig.**	**Lower**	**Upper**	**R^2**
MODEL ONE							
Sedentary breaks bouts	**40.0**	**10.0**	**0.179**	**0.002**	**10.0**	**60.0**	**0.032**
Sedentary breaks counts	−0.0003	0.0003	−0.057	0.332	−0.0009	0.0003	0.003
Sedentary breaks minutes	−0.65	1.89	−0.020	0.729	−4.37	3.06	<0.000
MODEL TWO							
Sedentary breaks bouts	**40.0**	**10.0**	**0.184**	**0.004**	**10.0**	**60.0**	**0.041**
Sedentary breaks counts	−0.001	0.0003	−0.104	0.108	−0.001	0.0001	0.021
Sedentary breaks minutes	−2.0	2.0	−0.073	0.268	−6.51	1.82	0.016

Model one represents a univariate association and model two is adjusted for wear minutes, age, sex and body mass index (BMI); abbreviations: SE, standard error / Sig, significance; sedentary breaks calculated as any activity > 2000 counts per minute; regression coefficients represent the change in diabetes risk score per 1 unit change (1 minute) in behavioural variables; unstandardised coefficients and standard errors have been divided by 10,000; significant results are in bold; R^2 = coefficient of determination.

intensity of sedentary breaks. When assessed against the significant cardiometabolic risk factors from Table 5.2, only Triglycerides revealed a significant relationship with the number of breaks (bouts) in sedentary time (b = −0.007, p = 0.030), but not HbA1c or HDL.

4.4.4 Entropy file manipulation

Average daily entropy scores were calculated using the manipulated data files in addition to average daily transitions between intensities for the 10 participants. Plotted onto a scatter graph (Figure 5.5), the data points depict a positive linear relationship between the variables. As the number of transitions increases i.e. from sedentary to light or light to MVPA, average daily entropy scores also increase, indicating the number of intensity transitions has an influence on the magnitude of daily entropy scores. Nevertheless, collapsing the data into intensity categories has reduced the magnitude of the entropy scores as the variability in-between epochs has been eliminated i.e. count values will vary much more than the transitions from one, two or three.

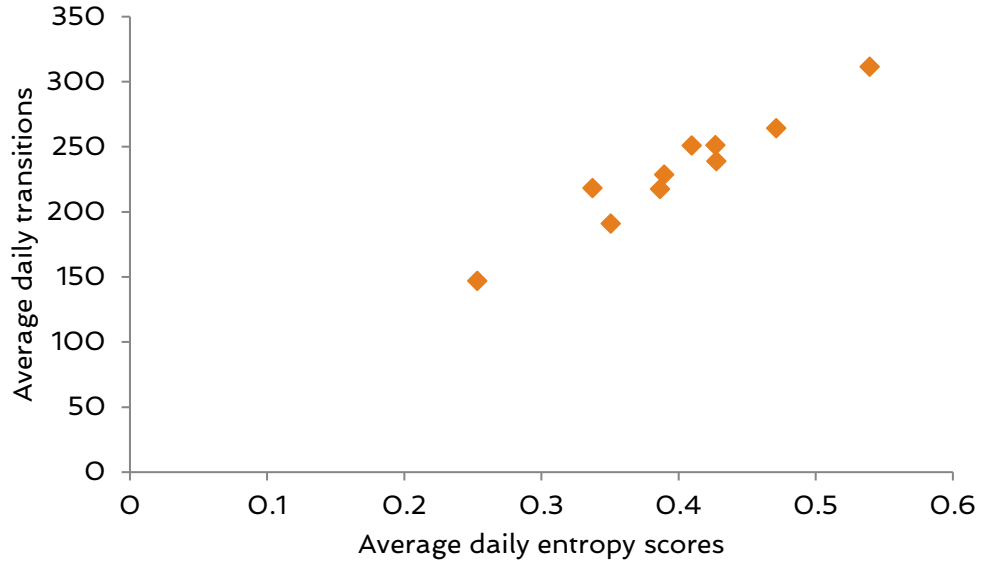


Figure 5.5. A scatter plot of average daily entropy scores against the average daily coding transitions.
Graph represents the data from a sub sample of participants (n=10).

4.5 Discussion

4.5.1 Main discussion

Entropy was calculated to ascertain whether behaviour regularity has any influence on health (cardiometabolic risk factors) and whether the new measurement has any relation to traditional physical activity estimates. Sample entropy, as a measure of regularity, was not associated with physical activity intensity or duration; therefore, it may not be measuring the amount of behaviour but rather the number of times behaviour changes. This assumption is supported by the significant positive associations between the number of sedentary breaks. Breaks in sedentary time have been positively associated with waist circumference, BMI, triglycerides, 2-h plasma glucose, HDL cholesterol and insulin (Healy et al., 2008a, 2011). The number of intensity classification transitions were shown to be related to entropy scores as shown by the file manipulation, albeit not statistically. Frequent changes to the signal (counts), by constantly changing to a differing intensity may be driving the relationship between entropy and cardiometabolic risk factors and therefore seems to indicate that being a 'Busy Bee' may confer health benefits. Because sample entropy is assessing self-similarity, if count values stay at the same magnitude (although not realistically possible), complexity will fall as the signal is indeed similar. This has important implications, as two behavioural patterns could have similar entropy scores but have been completed at different intensities. Therefore, entropy should be combined with other measures of behaviour to gain an overall picture of the behavioural profile.

HbA1c, triglycerides and HDL cholesterol were the only cardiometabolic risk factors that were significantly related to sample entropy, even after adjustment for BMI. BMI has been linked to lower physical activity levels (Hansen et al., 2013), yet as a cross-sectional sample it is unknown whether a decrease in physical activity has been caused by or a result of a high BMI. Caution should be taken when interpreting the adjusted models, if BMI is not a particular focus. Investigating the combined influence of entropy and physical activity upon cardiometabolic risk factors revealed that those with the highest entropy and LVPA were the most likely to be included within the more favourable quantile of HDL. Whilst the odds ratios were greater as the groups moved away from the reference group (low entropy/low activity), groups two (high entropy/low activity) and three (low entropy/high activity) only differed slightly in the likelihood of being included within the highest quantile of HDL. This suggests that the same benefits can either be obtained by being more complex in behavioural accrual but conduct less physical activity as those that are highly active but less complex. The higher

odds of being within the most favourable quantile for group three, compared to group two, indicates that the duration of activity may be more important in the relationship with health than the regularity of behaviour.

Both sample entropy and approximate entropy have been shown to be sensitive to manipulation of the length of the data being compared (*m)* and the tolerance for matching (*r)*, especially in short data sets (Yentes et al., 2013). To the best knowledge of the author, there is no guidance on how to optimise the choice of the correct *m* and *r* values (Lake et al., 2002), particularly within accelerometry processing studies. For comparison purposes the choice of values was based upon common choices within the literature and what previous authors have used within physical activity analysis (Cavanaugh et al., 2010; Sosnoff et al., 2010). To be sure of robust associations between health parameters and sample entropy, further exploratory analysis should model the effect of parameter choice on the direction and strength of the relationships.

Currently there is no gauge of what constitutes high, medium or low entropy scores. However, from the analyses presented within this paper, it is clear that the measurement of entropy captures an additional aspect of behaviour that cannot be solely explained by volume or breaks in sedentary time. Compared to the Health Survey for England 2008 dataset, participants accrued greater light activity (408 vs. 222 minutes), greater MVPA (37 vs. 28 minutes) but lower sedentary time (531 vs. 590 minutes) per day (Craig et al., 2009). The greater wear time within this sample (976 vs. 839 minutes) allowed for additional behaviour to be measured; however, activity accounted for 46% of total behaviour compared to 30% within the HSE dataset. Due to the greater volume of behaviour within both light and MVPA intensity classifications, it is unclear how entropy scores would be influenced, if less active individuals were included. Additional work is required using larger datasets to ascertain how entropy sits within the toolbox of the behavioural scientist and whether the additional effort to process the data is warranted.

4.5.2 Limitations & Strengths

As the sample were only required to fast for 4 hours or more, caution should be taken when interpreting those cardiometabolic risk factors that are sensitive to fasting status (glucose, total cholesterol, LDL, HDL and triglycerides). Whilst wrist worn accelerometry offers great potential for capturing a wider range of ambulatory behaviours, the cut-points and sleep data

removal process used for this study has not been validated. Every effort was made to reduce the effect of this by a manual check of sleep data removal and by using cut-points of varying levels. This is the first study to the author's' knowledge using sample entropy within a cardiometabolic risk factor context from wrist worn accelerometry data. The novel processing techniques to remove sleep data and for calculating sample entropy can serve as a guide of how to carry out this technique.

4.5.3 Conclusion

Using a novel measurement of signal complexity, associations have been revealed with cardiometabolic risk factors. Entropy scores are associated with the number of breaks in sedentary time, yet may be measuring a different feature of behaviour such as the number of changes of intensity. Regression analyses suggest that whilst the volume of activity may have a greater influence on the activity-entropy relationship with cardiometabolic risk factors, having high entropy levels but low activity is more favourable than low entropy and low activity. As a preliminary investigation into this technique, further analysis in a larger, more diverse dataset would be beneficial to ascertain whether the technique will be of use within behavioural research and what is the average level of entropy within the population.

Physical activity, sedentary time and glycaemic variability: are daily estimates of behaviour and glucose associated?

Chapter overview

Chapters Four and Five (Study One and Two) used cross-sectional data to establish the associations between sedentary time, physical activity and health. However, it is clear from large surveys that people are failing to be sufficiently active enough to benefit their health. Previous literature has suggested how using continuous glucose monitors can increase adherence to a physical activity programme by showing individuals how their glucose concentrations are influenced by activity, perhaps showing the benefits of being more active sooner rather than later. Nonetheless, before self-monitoring technologies can be used to influence behaviour, the coupling of sedentary time, physical activity and glucose concentrations must be investigated. This chapter takes a slightly different approach from the previous chapters by investigating the coupling of the aforementioned variables and develops data processing techniques to understand the data. Data were collected on 29 individuals recruited from Loughborough, UK during May to September 2016 using accelerometers and flash glucose technology.

5.1 Abstract

Introduction: Acute physiological changes such as reductions in postprandial glucose excursions have been demonstrated within experimental studies that have compared being physically active to sedentary conditions. However, for this information to be truly useful, the coupling of behaviour and glucose data in a free-living environment needs to be achieved. The aim of the study was to ascertain if there is a relationship between objectively measured physical activity, sedentary time and glucose variability using glucose monitoring in an adult population.

Methods: Data from 29 participants recruited from a mixed gender sample from Leicestershire, UK were selected for analysis. Physical activity, sedentary time and interstitial glucose was measured continuously over 14 days using an accelerometer and the Freestyle Libre flash glucose monitor. Daily time (minutes) spent sedentary, and in light activity and moderate to vigorous physical activity (MVPA) were regressed against glycaemic variability indices including daily mean (average) glucose, standard deviation and mean amplitude of glycaemic excursions (MAGE). Generalised Estimating Equations were calculated between behaviour and glycaemic variability variables. Models were calculated for main effects and also adjusted for age, gender and accelerometer wear time.

Results: Physical activity and sedentary time were associated with measures of glucose variability, however low fitness individuals showed a stronger relationship between MVPA and MAGE (MAGE: whole sample $b = -0.002$, low fitness $b = -0.012$. Additionally, after adjustment for covariates, sedentary time was positively associated with a higher daily mean glucose ($b = 0.001$, $p = 0.001$) and MAGE ($b = 0.002$, $p < 0.0005$) for the low fitness group. MVPA was negatively associated with mean glucose ($b = -0.004$, $p < 0.0005$) and MAGE ($b = -0.012$, $p < 0.0005$); however, standard deviation of glucose was not associated with behaviour of any intensity. The magnitudes of the relationships were small, although participants were non-diabetics and exhibited relatively good glucose control i.e. minimal fluctuations in daily glucose variability.

Conclusion: This study shows that sedentary time, physical activity and glucose variability are related. Despite supporting the previous laboratory research, it is uncertain whether any changes in glucose will reliably occur in all individuals. MVPA confers the largest reductions

in glucose variability indices, yet as one of the few studies to couple behaviour and glucose data, more research is needed on larger and more diverse samples.

5.2 Introduction

It is well known that physical activity is important in the primary and secondary prevention of chronic diseases such as hypertension, diabetes and cardiovascular disease (Warburton et al., 2006). Despite the documented benefits, large representative samples confirm that only a small percentage of individuals are sufficiently active (Craig et al., 2009; Colley et al., 2011; Troiano et al., 2008). When measured objectively, as little as 6% of men and 4% of women met the recommended physical activity guidelines of 150 moderate to vigorous physical activity (MVPA) in the 2008 Health Survey for England dataset (Craig et al., 2009). The UK physical activity guidelines suggest that regular physical activity can reduce the risk of developing chronic diseases such as type 2 diabetes and coronary heart disease (Department of Health, 2011a). However as many people are not completing the recommended amount of physical activity, perhaps the deferred reward of reduced risk is not enough to motivate people to be more active.

A concept from psychology called temporal discounting describes how the value of a reward decreases as the delay to attainment increases (Green et al., 1996). Consequently as the reward (e.g. decreased morbidity and mortality risk) occurs so far into the future, the immediate costs outweigh the future benefits and as such, individuals are unlikely to use preventative measures to avoid an unhealthy lifestyle (Chapman and Elstein, 1995). In addition to reducing risk of disease, physical activity has been shown to induce acute physiological changes to the body over a relatively short time period. A study investigated the differences in postprandial glucose between uninterrupted sitting, 2 minutes of light (walking at 3.2km/h) and moderate intensity (5.8-6.4 km/h) activity breaks every 20 minutes for 5 hours. Compared to uninterrupted sitting, both activity conditions lowered the net glucose response to a test drink (light: −1.7 mmol/L; moderate: −2.0 mmol/L) (Dunstan et al., 2012). Similarly, significant attenuations of postprandial glucose were also shown in a large trial of normal weight adults when regular activity breaks were undertaken (−866.7 IU/L · 9 h) (Peddie et al., 2013), and in office workers when standing for an afternoon (43% lower excursion to a standardised lunch) (Buckley et al., 2014). Despite the encouraging results, there is a lack of sufficient evidence to suggest a relationship between physical activity and glucose in the real world or out of a laboratory setting.

Recent technological advances have allowed a proliferation of devices that allow individuals to self-monitor physical activity behaviours in addition to acute physiology such as glucose

concentrations. Continuous glucose monitors (CGMs) have been developed that can display readings in real time and can provide both patients and clinicians with detailed information such as glucose concentration, direction and rate of change (Blevins et al., 2010). Using CGMs to provide feedback on acute physiology in response to physical activity is becoming more feasible and studies are now investigating the potential of this technology for behaviour change. In one study, after 3 days of monitoring using an accelerometer and CGMs in individuals with type 2 diabetes, participants were given counselling on their expected glucose reductions due to being physically active. Participants significantly increased their MVPA (5 mins) and decreased their sedentary/light minutes (5 mins), glycated haemoglobin (HbA1c) (1.2%) and body mass index (BMI) (0.53 kg/m^2) (Allen et al., 2008). The use of CGMs in type 2 diabetes is not routinely offered within the health care systems, nevertheless one study has deemed self-monitoring to be cost effective for those not on insulin (Fonda et al., 2016). CGMs in non-diabetic individuals on the other hand are relatively unexplored, yet offer great potential as a feedback mechanism to prevent further advancement of diabetes. As 50% of current diabetics could be undiagnosed (Forouhi and Wareham, 2014), more applied research is needed in order to transfer the knowledge gained from clinical populations into preventative action.

To ensure this new level of information is not discarded, associations between both behaviour (physical activity) and physiology (glucose) must be established empirically and more importantly, in the environment of intended use. Physical activity data using accelerometers is now routinely assessed in many studies yet the deployment of CGMs can be expensive, with costs up to $1000 not being uncommon (Vashist, 2013). Nevertheless, costs are reducing due to the development of newer technologies that should make the collection of glucose information more feasible. In addition to costs, there is also the decision of what physiological information should be assessed. In one of the few studies that has looked into behaviour and glucose responses, time spent in hyperglycaemia was significantly and positively associated with sedentary time in type 2 diabetics (Fritschi et al., 2016). However, glucose information can provide a depth of information about the physiological state and metrics should be explored that are population specific i.e. time in range may not be as important for individuals that do not deviate out of range very often.

There is a considerable gap in our understanding of the acute physiological changes that physical activity can have upon glucose using objective monitoring in a free-living

environment as most studies have been completed within laboratory conditions. The coupling of data in real time to provide actionable feedback to the user is not currently feasible with the technology and knowledge available; however, an investigation into whether there is a relationship between behaviour and glucose data in a post deployment fashion will enable researchers to take the next step towards providing real time feedback. Therefore, the aim of this study was to ascertain if there is a relationship between objectively measured physical activity and measures of glucose variability using glucose monitoring in non-diabetic individuals. The results of this study will increase the knowledge of the acute benefits that physical activity can have on health, using current and novel technologies.

5.3 Methods

5.3.1 Sample

Data used for this study was collected as part of the Sensing Interstitial Glucose to Nudge Active Lifestyles study that aimed to collect physical activity, sedentary time and glucose information using current sensing technologies, in order to explore the interactions over time; to find out what types of behaviour are most effective at controlling glucose concentrations. As a two part project, the data collected was then fed back to individuals within a Functional MRI (fMRI) machine to understand whether health feedback caused activation in the brain region related to behaviour change. This study will only present findings relating to the objective collection of physical activity, sedentary time and glucose data.

The study took place at the National Centre for Sports and Exercise Medicine at Loughborough University during May to September 2016. Recruitment consisted of flyers, internal and external adverts on the University website and email lists around departments. The study inclusion and exclusion criteria are presented in
Table 6.1 and within the participant information sheet (Appendix F). The criteria aimed to recruit those individuals that are not regularly active (not students) and without a confirmed

Table 6.1. Inclusion and exclusion criteria of the study.

Inclusion Criteria	Exclusion Criteria
• Between the ages of 30 and 60 • Right handed (for fMRI standardisation) • Non diabetic (confirmed diagnosis of type 1 or 2)	• Were pregnant • Taking diabetes medication • Have any permanent metal items in their body (fMRI protocol) • Has mobility related musculoskeletal problems • Not willing to give signed consent • Has any psychological disorders e.g. claustrophobic (fMRI protocol) • Could not adhere to the study protocol • Regularly active or currently involved in any structured exercise training • Students

diagnosis of diabetes in order to be representative of the general population. Due to the standardisation for the fMRI machine, potential participants were excluded if they did not meet standard fMRI safety criteria. All participants gave their written informed consent and the study was approved by the Loughborough University Human Participants Ethical Sub-Committee (R15-P142). At the end of the study, participants were given a comprehensive health report for their participation (Appendix G).

5.3.2 Study design

Participants attended a 2-hour morning appointment and were asked to adhere to the following pretesting guidelines:

- Not to eat or drink (except water) 8 hours prior to the appointment time
- Drink a glass of water at least 1 hour before the appointment
- Refrain from any strenuous activity 24 hours before the appointment

These were confirmed at the start of the appointment. A number of measurements were then conducted including anthropometrics, blood pressure, a fasting capillary blood test, strength and fitness tests. After the initial appointment, participants were fitted with two monitors, a glucose monitor and an accelerometer. The study flow is detailed in Figure 6.1.

5.3.3 Study measurements

After arrival, participants were reminded of the procedures and informed consent was taken. Prior to the commencement of any study measures, a Physical Activity Readiness Questionnaire (PAR-Q) was completed to ensure participant safety (Warburton et al., 2013). Any positive answers were dealt with by a clinically trained member of the study team.

Once cleared for participation, a seated blood pressure reading (Omron 705IT, Omron, UK) and then a fasting capillary blood test were undertaken. Two samples were collected and analysed using point of care devices for total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol (LDL), triglycerides, glucose (Lipid Profile•Glucose Cartridge, Cholestech LDX® Analyzer) and HbA1c (mmol/mol and %) (Afinion HbA1c, Afinion Analyzer, Alere, Massachusetts, USA).

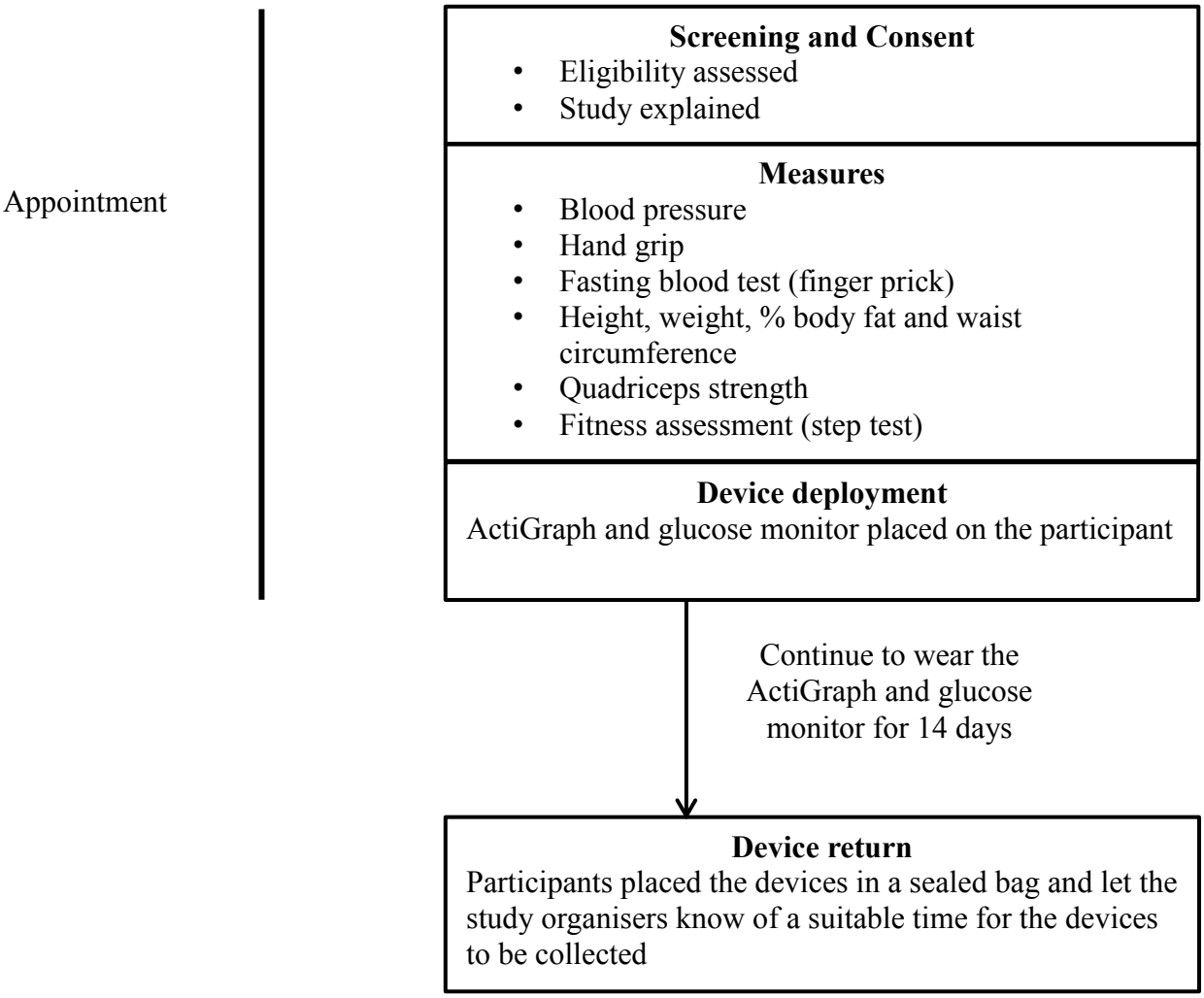


Figure 6.1. Study flow of the research study.
Each appointment lasted 2 hours and the monitoring portion of the study ended after both devices were returned after 14 days of wear.

Height, waist circumference, weight and body composition analysis was measured by a stadiometer (SECA 213, SECA, Germany), a tape measure (HaB International Ltd, UK) and a bio-electrical impedance machine (Tanita MC780MA, Tanita, The Netherlands). Fitness assessments in the form of hand grip (Takeii analogue dynamometer, Takei Scientific Instruments Co., LTD, Japan), quadriceps maximal voluntary contraction (DAVID Health Solution Ltd., Finland) and a sub maximal fitness test (Modified Canadian Aerobic Fitness Test (mCAFT)) were conducted on all participants. After completion of all study profiling measurements, participants were then given two devices to wear for 14 days, an

accelerometer and a glucose monitor device. For more detailed description of these methods, the reader is directed to Appendix H.

5.3.4 Accelerometry

ActiGraph accelerometers (wGT3X-BT Monitor, ActiGraph, Pensacola, USA) were deployed around the waist (right side) during waking hours for 14 whole days (relative to appointment time). Participants were asked to only remove devices during the day if engaging in water-based activities such as swimming or bathing. All devices were initialised 1 hour into the appointment time and were given a stop time of 23:59 on the last day of wear to account for any potential issues in deployment at the appointment. Measurement frequency was 100Hz and devices were downloaded into 60 second epoch files using ActiLife version 6.13.2 (ActiGraph, Pensacola, USA). Files were then processed using KineSoft (KineSoft, Loughborough, UK) version 3.3.80.

Non-wear was defined as 60 seconds of consecutive zeros with allowance of 2 minutes of non-zero interruptions and a valid day was defined as ≥ 600 minutes of valid monitor wear (Troiano et al., 2008). Counts per minute (CPM) cut-points were used to define sedentary time (0-99 CPM), light (100-2019 CPM) and MVPA (≥ 2020 CPM) (Troiano et al., 2008). Finally, temporality in the accelerometer data were restored using a semi-automated process to enable day comparisons with glucose data.

5.3.5 Glucose monitoring

A glucose monitor (Freestyle Libre, Abbott Laboratories, Illinois, USA) was deployed on the back of the upper left arm for 14 whole days. The device consists of a sensor that is attached to the arm via an adhesive patch and a handheld device that downloads the data from the sensor via near field communication. The sensor is able to capture interstitial glucose concntrations at each point in time or by default, every 15 minutes. Other devices require frequent calibration using a capillary blood sample every 4-12 hours. Utilising new technology the Freestyle Libre is factory calibrated and does not require any finger pricks over the wear period, without significant loss of accuracy (Hoss et al., 2013). It has also been shown to be accurate in individuals with type 1 and type 2 diabetes against capillary blood measurements and not affected by BMI or age (Bailey et al., 2015).

Before deployment, an alcohol wipe was used to prepare the insertion site. After deployment, a Tegaderm patch (3M, Minnesota, USA) was then applied over the sensor to ensure firm attachment, as initial pilot identified that some certain sensors failed to adhere for the full 14 days. Additional Tegaderm patches were provided to participants in the event that the patches became dirty or started to peel off. As the device is marketed as a 'Flash Glucose Monitor' and not a traditional CGM, data points are not continuously saved to the reader. Instead, the sensor has a limited memory and requires information to be 'pulled' within an 8 hour window otherwise it will be overwritten on the device. To reduce this, participants were asked to scan at least every 7 hours, more often if they liked, however no more than six to seven times a day. It was anticipated that there may be some missing data due to sleep occurring for more than 8 hours. Participants were encouraged to scan before going to sleep and once they had woken up to minimise data loss.

Participants were given a charging cable and instructed to charge the reader every 3-4 days or if the battery had run down. At any time, if the sensor was removed prematurely due to an adhesion issue or a sensor error, a decision was made whether a re-deployment took place. If a sensor failed during the first few days, a re-deployment took place; however, if > 10 days were captured, no re-deployment took place but the participant was asked to continue to wear the accelerometer. The number of instances is described in the results section. At the end of the 14 day period, the sensor stopped working and participants were provided with a biohazard bag to put the sensor into, or alternatively an appointment was made for one of the team to remove it.

To associate the glucose information captured by the glucose monitor, three measures of glucose variability were used within this study: mean daily glucose (MGluc), standard deviation of glucose (StDevG) and mean amplitude of glucose excursions (MAGE). MGluc and StDevG were indicated as the most common and easily interpreted metrics by an expert panel of diabetes experts (Bergenstal et al., 2013), and MAGE is considered the 'gold standard' for glucose variability measurement (Monnier et al., 2007). Choosing the most understandable and relevant indices will aid in the dissemination of knowledge to both clinicians and end users that could use this information to monitor their health.

To be able to calculate the glucose indices, glucose data had to be downloaded, prepared and then processed using a semi-automated approach. The glucose device records information

under two sub types: an automatic scan and a user scan type. Only the automatic scans were used within the analysis to ensure readings were taken every 15 minutes. If any data were missing, a manual adjustment using a user scan data point within ±3 minutes was made or data were replaced using linear interpolation if < three adjacent data points were missing. Consecutive values ≥ 90% for the day denoted a valid file and was carried forward for analysis. This decision was made in order to be sure that a true representation of glucose parameters was evaluated against physical activity. The largest block of data points was then analysed using EasyGV software to calculate measures of glycaemic variability which included MGluc, StDevG and MAGE (Hill 2010). For a more detailed description of these methods, the reader is directed to Appendix H.

5.3.6 Main statistical test

The focus of this paper was to examine the relationship between sedentary time, physical activity and interstitial glucose concentrations. As the analysis was based upon multiple days' worth of participants data, a statistical test was chosen that could account for repeated measures data called Generalised Estimating Equations (GEEs). These will now be described in the following section.

This section assumes a basic knowledge of statistics and will only describe general statistical information that will explain the use of the test within this document as it is beyond the scope of this thesis to describe fundamental statistical details in full.

Linear regression modelling assumes that the errors for each participant or observation are independent of each other (Field, 2013, p. 311); however, this type of model can be considered inappropriate for repeated measure designs as observations are likely to be somewhat correlated (Burton et al., 1998). Data could be aggregated into a summary statistic, such as the mean (Burton et al., 1998), yet, this approach does not allow for the assessment of how variables change over time.

GEEs can account for within subject correlations and missing data for repeated measure designs and were first applied to repeated observation datasets by Liang and Zeger (1986) (Zorn, 2001). The statistical technique estimates the degree of correlation within individuals, and then adjusts the regression coefficients and standard errors accordingly (Burton et al., 1998). Additionally, GEEs can process models with missing data, provided the missing

information is missing at random (Burton et al., 1998). In the context of glucose monitoring, GEEs can therefore process data, which may be missed due to a lack of scanning by the participant.

Researchers can even specify a correlation structure (i.e. how the repeated measurements are related to each other, and the various types that are available are outlined below (Burton et al., 1998):

- Autoregressive - observations that are close in time are more closely correlated.
- Exchangeable - observations are equally correlated within an individual.
- Independent - observations are uncorrelated within an individual.
- Unstructured - correlations have no assumptions and are estimated from the data.
- User specified - correlation coefficients are fixed by the researcher.

There is no certain way to determine which correlation structure to choose (Burton et al., 1998), nonetheless, GEEs have been deemed to be robust, even when correlation matrix is miss specified (Liang and Zeger, 1986). GEEs have previously been utilised within repeated measures sedentary time and glucose research (Fritschi et al., 2016) and are therefore appropriately applied to undertake an analysis of the relationship between sedentary time, physical activity and interstitial glucose concentrations within this research study.

5.3.7 Statistical analysis

Daily estimates of sedentary time, physical activity and glucose variability were transposed from a wide to a long format (variables to cases) which allowed daily behaviour to be compared to daily glucose values. For each participant, up to 13 days (cases) were available for each participant as days 1 and 15 were not valid due to being half days. GEEs were used to estimate associations between sedentary time, light physical activity and MVPA with MGluc, StDevG and MAGE. Models were calculated univariately and also adjusted for age, gender and accelerometer wear time. This analysis extended further to investigate whether fitness related differences existed for the associations between behaviour and glycaemic variability. Additional, GEE analysis was conducted for those individuals who were deemed at having low fitness levels or had fitness levels within the 'Needs improvement', 'Fair' and 'Good' health benefit zones of the mCAFT. Finally, to investigate whether there was significant fitness related differences in physical activity behaviours; Analysis of Covariance tests were conducted, adjusted for wear time (where appropriate).

5.4 Results

5.4.1 Sample characteristics

A total of 35 individuals took part in the study but 29 individuals were carried forward for analyses. All study characteristics are presented in Table 6.2. On average participants were 44.9 years of age, were slightly overweight (BMI = 25.3) and of a white ethnicity. All participants had fasted blood glucose concentrations < 7.0 mmol/L; however, four had fasted glucose concentrations ≥ 5.6, which could be considered within the pre-diabetic range. Accelerometry wear was high (895.1 minutes per day) and sedentary time accounted for on average 9.6 hours a day or 64% of daily behaviour. The sample on average had a 'Good' level of fitness, which is calculated from the health benefit zone from the mCAFT using the average sample age. For more information please see Appendix H.

5.4.2 Deployment

Participants were asked to at minimum, scan every 7 hours to prevent data loss; however, many participants scanned more often. The average scan rate is presented in Figure 6.2. The number of scans steadily increased to a peak of 13 for days 5 and 6 but then declined before another short-term rise before the sensor stopped working.

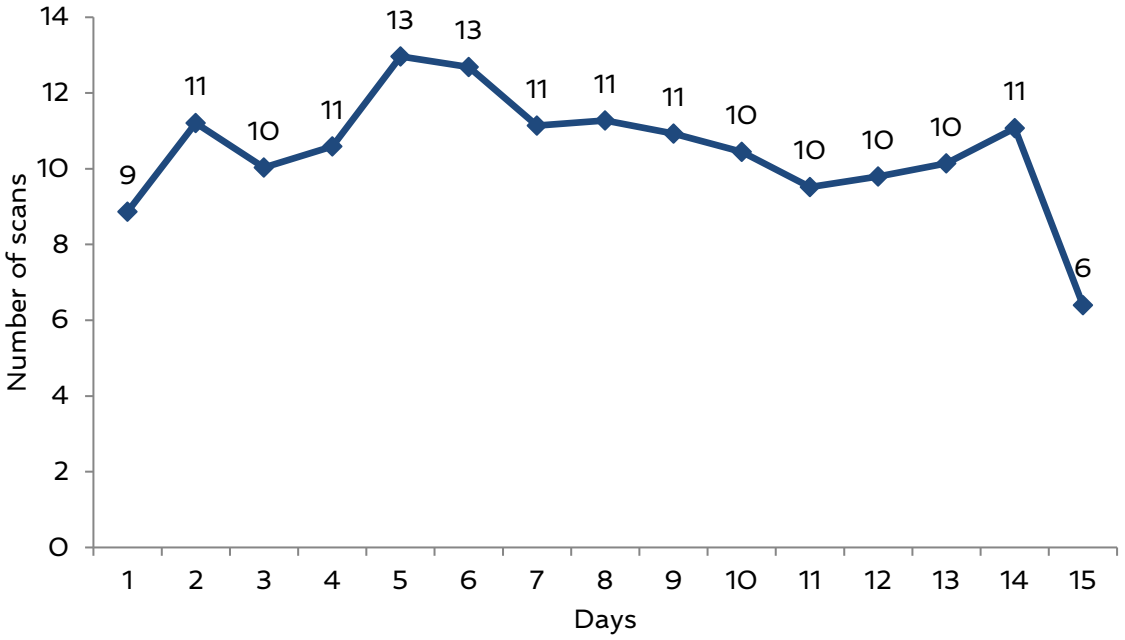


Figure 6.2. A graph representing the average number of scans per day for the whole sample (n=29).
Fifteen days are represented due to the deployment schedule as the first and last days could be half days.

Table 5.2. Characteristics of the study sample.

	n = 29					n = 29		
Demographics					**Number of valid days**			
Age (Years)	44.9	±	9.1		Valid accelerometry days (n)	13.8	±	0.6
Gender Male (n / %)	12		41	%	< 12 (n)	0		
Ethnicity - white (n / %)	26		89	%	12 (n)	2		
Anthropometrics					13 (n)	3		
Body Fat (%)	27.0	±	9.7		14 (n)	24		
BMI (kg/m^2)	25.3	±	4.1		Valid glucose days (n)	11.7	±	1.5
Waist circumference (cm)	85.0	±	11.2		< 7 (n)	0		
Cardiometabolic risk factors					7 (n)	1		
Mean SBP (mmHg)	122.4	±	11.7		8 (n)	1		
Mean DBP (mmHg)	75.6	±	7.0		9 (n)	0		
Total cholesterol (mmol/L)	4.7	±	0.8		10 (n)	2		
LDL cholesterol (mmol/L) n = 28	2.9	±	0.5		11 (n)	7		
HDL cholesterol (mmol/L)	1.5	±	0.4		12 (n)	8		
Triglycerides (mmol/L) n = 28	0.9	±	0.2		13 (n)	10		
Glucose (mmol/L)	4.9	±	0.6		Valid analysis days (n)	11.6	±	1.5
HbA1c (%)	5.3	±	0.4		< 7 (n)	0		
Fitness score	375.1	±	84.3		7 (n)	1		
Grip strength (Combined kg)	71.9	±	22.4		8 (n)	1		
QMVC (Nm)	139.7	±	51.8		9 (n)	0		
Behaviour					10 (n)	2		
Monitor wear (mins)	895.1	±	58.8		11 (n)	8		
Sedentary time (mins)	576.4	±	67.8		12 (n)	8		
Light physical activity (mins)	269.1	±	59.8		13 (n)	9		
MVPA (mins)	49.6	±	29.9					

Reported as means and standard deviations unless otherwise stated; valid accelerometry ≥ 600 minutes; valid glucose ≥ 90% of daily data points; abbreviations: n, sample size / BMI, body mass index / SBP, systolic blood pressure / DBP, diastolic blood pressure / LDL, low-density lipoprotein / HDL, high-density lipoprotein / HbA1c, glycated haemoglobin / QMVC, quadriceps maximal voluntary contraction / Nm, Newton Metre / MVPA, moderate to vigorous physical activity.

A number of participants had sensor redeployments (n = 4) due to a sensor error or sensors were removed prematurely by being caught on something or if the adhesive failed (n = 2). However, if a participant did not scan within the 8 hours, data were lost from the device and the next available data point was the first available automatic scan, 8 hours prior to the latest scan. This often resulted in a temporal drift of the data and for this reason, the amount of data points could be 95 or 97, instead of 96 for a complete file (4 scans per hour). Additionally, as the deployment strategy meant that the first and last days of monitoring were half days, data processing figures have been calculated for full days only (up to 13). For 29 participants over 13 days of complete wear, 36192 data points should be present within each file ((96 per day x 13) x 29); however, the number of data points available for this sample was 36035 and missing data represented 3% of the total data points. An outline of all data processing information is contained within Table 6.3.

Table 6.3. Glucose data processing details.

Average available data points per day (n)	93	/96
Average available data points per day (%)	97	%
Average largest continuous block of data (n)	91	/96
Average largest continuous block of data (%)	95	%
Average number of valid days	12	/13
Total data points replaced (n)	28	/96
Total data points Interpolated (n)	58	/96
Total missing values not replaced or interpolated (n)	973	/36035
Sensors removed due to a lack of adhesion (n)	2	
Sensors removed due to an error message (n)	3	
Sensors removed due to discomfort (n)	1	
Redeployments (n)	4	
Sensors not redeployed (n)	2	

Due to the way the sensor was deployed, data processing information was calculated using full days only (days 2-13) and sensor information refers to the whole monitoring period; abbreviations: n, number of data points; replaced values were taken from user scans if ± 3 minutes; data points were interpolated if two or less adjacent values were missing; missing values represent data points not replaced or interpolated; sensors were removed if they 'fell off' or were caught on something, had an error message or if the participant requested a redeployment due to discomfort; sensors were not always re-deployed and decisions were made on an individual basis on the risk vs whether enough data had been collected; if a sensor was not redeployed, data were treated as missing.

On average, each participant had 34 missing data points over the 13 days, equivalent to 8.5 hours. Two participants accounted for 43% of the missing data (420 data points) and removing their data reduces the average to 6.75 hours. Only four participants had no missing data out of the sample and an additional five had below 2 hours of missing data. Nonetheless, as these figures have been calculated on those individuals who met the valid accelerometry and glucose valid day criteria, the number of missing data from the total recruited sample would naturally be higher and therefore the above figures are not a true reflection of full deployment feasibility.

Accelerometry compliance was high with 83% of the sample achieving 14 valid days (> 600 minutes) of wear. Nevertheless, missing glucose data resulted in a lower number of analysis days as only 34% of participants achieved 13 full days of valid glucose wear.

5.4.3 Associations between glycaemic variables and behaviour

Table 6.4 presents unadjusted and adjusted models from the GEE analysis between sedentary time, light activity, MVPA and glycaemic variables. Univariate analysis showed no significant associations between daily MGluc and either sedentary time, light activity or MVPA, however once adjusted for wear minutes, age, and sex, sedentary time was positively associated with mean glucose. MAGE was significantly and negatively associated with sedentary time ($p < 0.0005$) and remained significant after adjustment. Light was negatively and MVPA positively associated with MVPA, with the direction changing after adjustment. Only adjusted sedentary time was significantly associated with MGluc and there were no significant associations between StDevG and behaviour.

An age and gender adjusted ‘health benefit zone’ was calculated for all participants after the mCAFT submaximal fitness test. It was noticed that within the sample there was a gender by fitness interaction as a higher proportion of males (73%) were classified as highly fit (‘Very Good’ and ‘Excellent’), compared with females (25%). A sensitivity analysis was calculated to ascertain the effect of only using participants categorised as low fitness (‘Needs improvement’, ‘Fair’ and ‘Good’). GEE results for this lower fitness group are presented in Table 6.5. Unadjusted mean glucose and MAGE was negatively associated with MVPA ($p = 0.0001$) and positively associated with sedentary time ($p = 0.038$). Interestingly, after adjustment for covariates, all relationships for MGluc and MAGE were significant, apart

Table 5.4. Univariate associations between physical activity and glycaemic variables for the whole sample.

	MGluc			StDevG			MAGE		
	b	**SE**	**Sig.**	**b**	**SE**	**Sig.**	**b**	**SE**	**Sig.**
MODEL ONE									
Sedentary	−0.0001	0.0002	0.688	−0.0001	0.0002	0.409	**−0.002**	**0.0004**	**< 0.0005**
Light	0.0003	0.0003	0.303	0.0001	0.0003	0.601	−0.001	0.0010	0.380
MVPA	−0.0003	0.0004	0.483	−0.0004	0.0004	0.321	0.0002	0.0011	0.849
MODEL TWO									
Sedentary	**0.001**	**0.0002**	**0.001**	0.0002	0.0003	0.546	**−0.002**	**0.0005**	**< 0.0005**
Light	−0.001	0.0003	0.053	0.0001	0.0002	0.783	**0.003**	**0.0009**	**0.001**
MVPA	−0.001	0.0009	0.148	−0.0006	0.0005	0.224	**−0.002**	**0.0009**	**0.017**

Model one represents a univariate association and model two is adjusted for wear minutes, age, and sex; abbreviations: MGluc, mean glucose / StDevG, standard deviation of glucose / MAGE, mean amplitude of glycaemic excursions / B, unstandardised regression coefficient / SE, standard Error / Sig, significance; MVPA, moderate to vigorous physical activity; significant results in bold.

Table 5.5. Univariate associations between physical activity and glycaemic variables for the low fitness group.

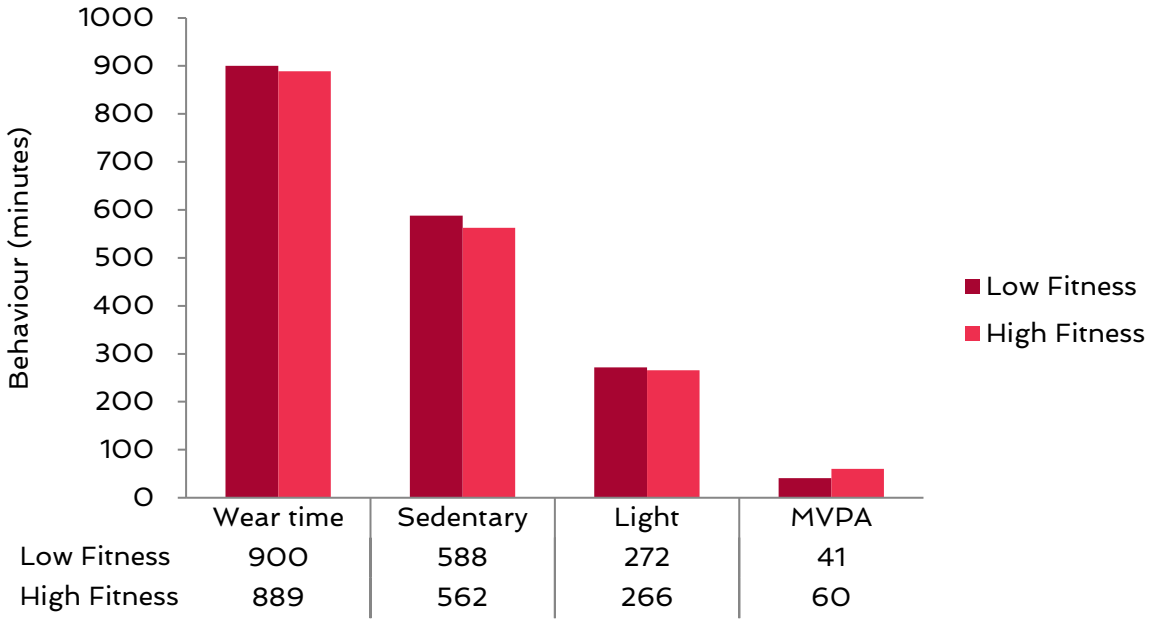
	MGluc			StDevG			MAGE		
	b	**SE**	**Sig.**	**b**	**SE**	**Sig.**	**b**	**SE**	**Sig.**
MODEL ONE									
Sedentary	0.0002	0.0002	0.328	−0.0001	0.0001	0.508	**0.001**	**0.0006**	**0.038**
Light	−0.0002	0.0005	0.612	−0.0001	0.0003	0.703	−0.001	0.0017	0.761
MVPA	**−0.005**	**0.0015**	**0.001**	−0.0001	0.0006	0.832	0.005	0.0040	0.170
MODEL TWO									
Sedentary	**0.001**	**0.0004**	**0.001**	0.0002	0.0005	0.653	**0.002**	**0.0006**	**< 0.0005**
Light	−0.001	0.0005	0.053	−0.0005	0.0005	0.333	**−0.002**	**0.0006**	**0.002**
MVPA	**−0.004**	**0.0009**	**< 0.0005**	0.0011	0.0008	0.201	**−0.012**	**0.0034**	**< 0.0005**

Model one represents a univariate association and model two is adjusted for wear minutes, age, and sex; abbreviations: MGluc, mean glucose / StDevG, standard deviation of glucose / MAGE, mean amplitude of glycaemic excursions / B, regression coefficient / SE, standard Error / Sig, significance; MVPA, moderate to vigorous physical activity; significant results in bold.

from mean glucose and light activity ($p = 0.053$). The strongest relationship was between MVPA and both mean glucose ($b = -0.004$, $p < 0.0005$) and MAGE ($b = -0.012$, $p < 0.0005$). Even though highly fit participants were excluded, StDevG did not reach significance in either the univariate or adjusted models.

To put the associations into context, the average daily change in mean glucose and MAGE was calculated only using day values that had at least one adjoining data point (prior). Daily differences in mean glucose fluctuations for the whole sample were low (0.023 mmol/L) and were also low for MAGE (0.013 mmol/L, representing on average a 5% daily change compared to individual MAGE values). Compared to normative values, most ($n = 28$) were above average MAGE values for their respective ethnicities, though the comparisons are against a relatively short period of monitoring (≤ 72 hours) (Hill et al., 2011). To model the effect of changing daily physical activity profiles, if sedentary time was increased by 60 minutes representing the same time as an average TV show, mean daily glucose could rise by 0.06 mmol/L in the low fitness group. Alternatively, increasing MVPA by 60 minutes per day could decrease mean glucose by 0.24 mmol/L which is greater than the daily mean glucose fluctuations. Additionally, the 60 minutes of MVPA could also decrease MAGE by 0.72 which over time could result in favourable changes in the glucose profile and in the case of a healthy Caucasian individual represents 51% of mean MAGE values (1.4 mmol/L) (Hill et al., 2011).

No significant differences in behaviour were observed between groups, though MVPA was trending towards significance ($p = 0.059$). These are presented within Figure 6.3.



	Wear time	Sedentary	Light	MVPA
Low Fitness	900	588	272	41
High Fitness	889	562	266	60

Figure 6.3. A bar plot outlining the behavioural profiles of low and high fit participants. The bars represent the amount of accelerometer wear time and the amount of sedentary, light and moderate to vigorous physical activity (MVPA). Analysis of variance tests adjusted for wear time (not wear time) revealed no significant differences between all behaviour variables, though MVPA was trending towards significance (p=0.059).

5.5 Discussion

5.5.1 Main discussion

Using objective methods to measure physical activity, sedentary time and glucose, our analysis revealed that there is a relationship between behaviour and glycaemic variability. This relationship is complex and it is clear that fitness may play a role within the glycaemic responses to physical activity such as increasing the magnitude of the relationship between MVPA and MAGE. Indeed, a recent paper using data from the UK Biobank assessed whether the relationship between physical activity and mortality was modified by grip strength or cardiorespiratory fitness and concluded that those with the lowest strength or fitness could benefit the most from interventions (Celis-Morales et al., 2016). Additionally, when adjusting sedentary time for either light activity or MVPA, those with lower fitness (Women < 32 & men < 35 ml/kg/min) and higher glucose (≥ 6.1 mmol/L) benefited more than high fitness participants, even after adjustment for sex, age, educational level, smoking and psychosocial stress (Ekblom-Bak et al., 2016b). It appears to support the hypothesis that whilst those with lower fitness may see daily differences in their glucose initiated by physical activity, those of higher fitness may keep their glucose concentrations within healthy ranges as a result of physiological adaptions, such as changes in insulin sensitivity (O'Gorman et al., 2006; Kirwan et al., 2009). It is unclear whether glucose monitoring will be beneficial in a highly fit population; however, the use of glucose devices may still be of interest to those that are interested in self-monitoring their health for interest alone. Nonetheless, more work is needed to confirm these findings.

Not all associations between behaviour and glycaemic variability reached significance, even for the lower fitness group. Previously, researchers have focused on the total area of glucose related reductions to physical activity (Dunstan et al., 2012; Peddie et al., 2013). Though useful to researchers, without a standardised normative value, values can often be confusing. Glycaemic measures were chosen for this study to be more resonant with the general user and also because of the clinical relevance. Being physically active, especially after meals is associated with a blunted glucose response as a result of the body using up the supply of glucose already in the blood stream (Chacko, 2014) which could explain the observed reduction in MAGE. Over time, a consistent reduction in MAGE could also lower MGluc by blunting the peak glucose concentrations or by initiating a faster return to 'normal' or euglycaemia.

StDevG was not related to behaviour of any intensity within this study though this index has not been extensively studied within a normoglycemic population. Compared to normative values for normoglycaemic individuals the study population have below average standard deviation values, suggesting a small amount of standard deviation in the glucose concentrations per day. Describing the spread around the mean, the amount of deviation may not be enough in this population to detect behaviour related changes over time. The average daily deviations were −0.0102 and −0.0001 mmol/L for both the low and high fit groups respectively. Demonstrating a very low level of deviation in this study, StDevG could not be the most representative example of glycaemic control within a population absent of diabetes and large fluctuations in blood glucose.

It is acknowledged that the relationship between behaviour and glucose variability is complex and may differ depending on the amount of data captured, which is a product of sensor wear. Figure 6.4 represents daily summaries for behaviour (sedentary, light and MVPA) and MAGE for a random participant with 13 days of wear from the study. Whilst the threshold for valid glucose data was set at ≥ 90% of daily values, accelerometer wear time was set at ≥ 600 minutes in line with many studies that have assessed habitual physical activity. It is clear to see that although most days are well above the threshold of valid day criterion, there is indeed some variation within the amount of time spent wearing the monitor. It is unknown whether any reduction in wear is due to non-wear intentionally or an extended period of sleep time and if the reason is the former, it would have important implications for the associations with acute health outcomes such as glucose. For instance, between day 6 and 7 there is a difference in wear of 3 minutes but an increase in MAGE (day 6 = 2.39 mmol/L, day 7 = 3.78 mmol/L). It is hard to determine why the increase has been brought about in this instance, and whether it is because of activity not captured by the accelerometer despite high wear across both days. Additionally, between days 9 and 10 there is a difference in wear of 155 minutes but a decrease in MAGE (day 9 = 3.36 mmol/L, day 10 = 1.7 mmol/L). Although wear time was adjusted for within the analyses, if it is imperative to wear the devices for all waking hours to show activity related decline in glucose then engagement may be more difficult with users as traditional wearable monitors can accrue steps intermittently, but still sum up to a goal at the end of the day. Reinforcing the need for wear time to be included within statistical models, 24 hour monitoring could be a better way of reducing the influence of missing data due to non-wear. Figure 6.5 and Figure 6.6 depict similar visual representations for MGluc and StDevG, respectively.

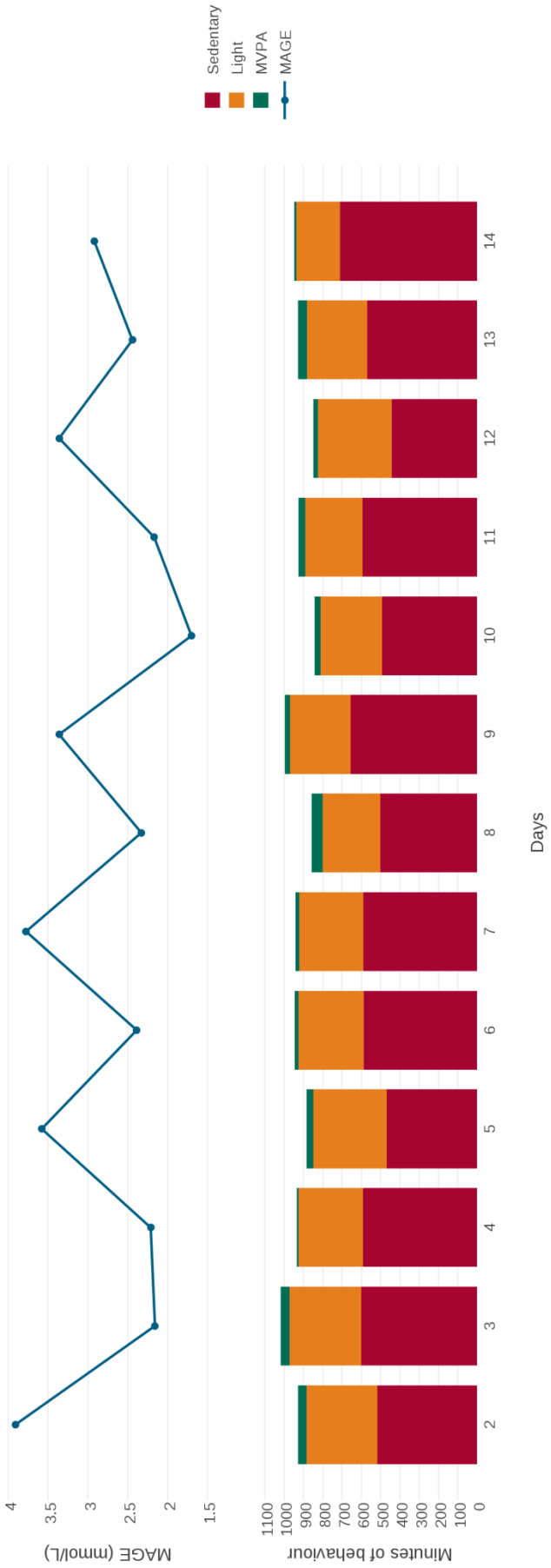


Figure 6.4. A combined graph representing the number of minutes of sedentary, light and moderate to vigorous physical activity, plotted against mean amplitude of glycaemic excursions (MAGE) over 13 days.
Days 1 and 15 were half days and were omitted as there lacked sufficient data to be deemed valid. The participant represented was chosen at random and is of low fitness and female.

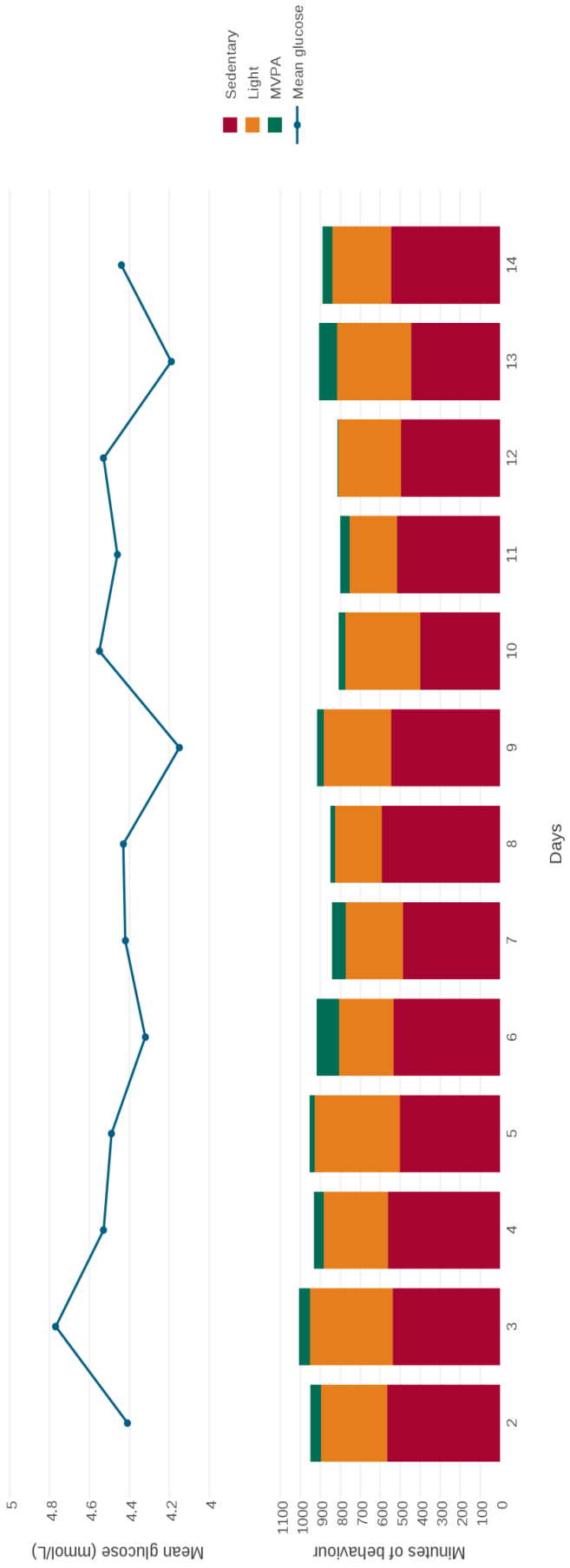


Figure 6.5. A combined graph representing the number of minutes of sedentary, light and moderate to vigorous physical activity, plotted against mean glucose over 13 days.
Days 1 and 15 were half days and were omitted as there lacked sufficient data to be deemed valid. The participant represented was chosen at random and is of low fitness and female.

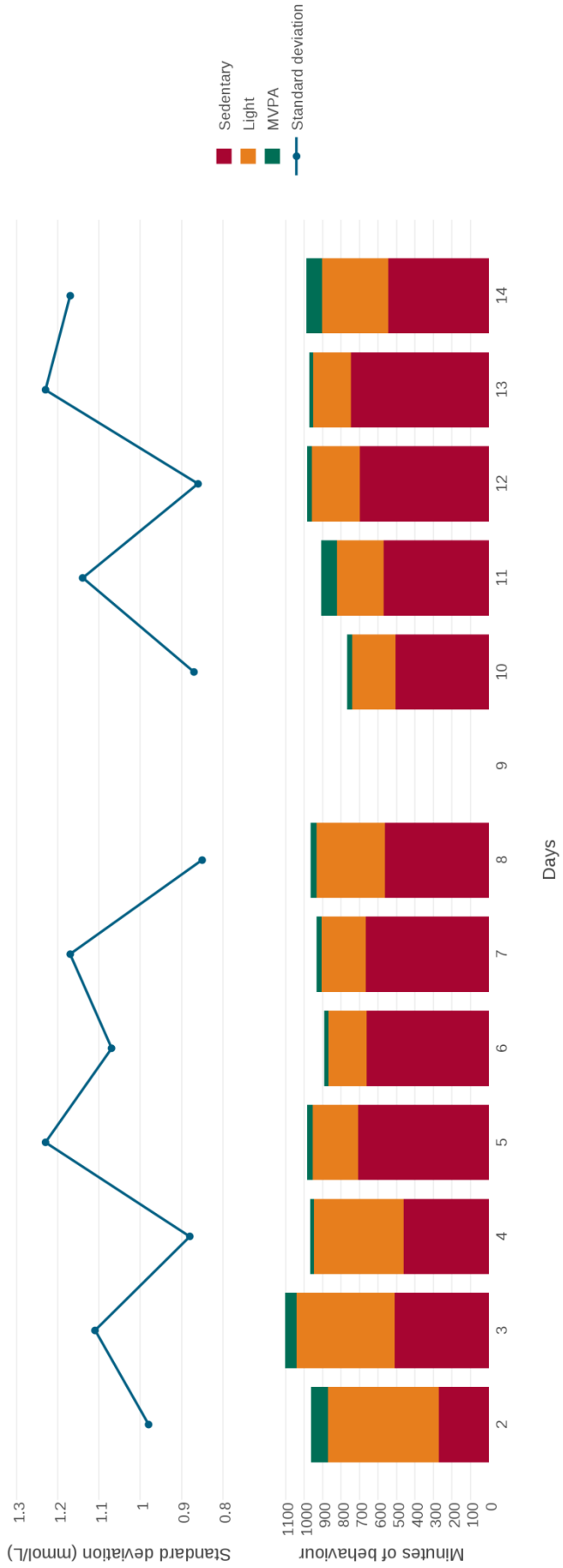


Figure 6.6. A combined graph representing the number of minutes of sedentary, light and moderate to vigorous physical activity, plotted against standard deviation of glucose over 13 days.
Days 1 and 15 were half days and were omitted as there lacked sufficient data to be deemed valid. Day 9 did not have sufficient CGM data. The participant represented was chosen at random and is of low fitness and male.

The study faced a number of CGM deployment issues that required a number of glucose sensors to be redeployed due to sensor errors and adhesion issues. Coupled with the amount of missing data, the invasiveness and recurring costs of the glucose monitor, there are a number of hurdles that researchers need to be aware of when using this particular device within a research study. Additionally, as one of the first studies utilising physiological feedback, we are unable to determine whether the information given back to the participants had any influence on the direction of both the glucose and the behaviour. Alternative glucose monitoring technologies and future iterations of devices could perhaps reduce these hurdles; nevertheless, the glucose device is a useful tool that should still be utilised to investigate the relationship between acute health and behaviour.

5.5.2 Limitations & Strengths

The small number of participants within this study was chosen to balance feasibility and cost, although the participant pool could be considered homogenous (University location and ethnicity). More women took part and comparatively, the men achieved a higher fitness score than the women. Food diary information (paper format) was collected during the study for 4 days at the start of monitor wear. The information was deemed unreliable due to the amount of foods missing from the food-coding database. Alternative methods of food collection should be utilised in the future, although self-reported dietary diaries have been called into question (Archer and Blair, 2015). Sedentary time was measured using count based accelerometry, which has the limitation of not being able to detect specific postural changes (Atkin et al., 2012), and is actually measuring inactivity not whether someone is sitting or lying for instance. Similarly the choice of cut-points can influence the data (Orme et al., 2014) and should be taken into account when drawing conclusions. For a gold standard measure of sedentary time, devices that can analyse posture should be used. The valid day criterion for glucose data was chosen to be conservative but has not been validated. More work is required to ascertain what level of missing data is acceptable and which does not introduce too much variability. However, this study is one of the first to investigate glycaemic variability and physical activity behaviours using objective monitoring technologies over an extended period of wear (13 days).

5.5.3 Conclusion

The present study shows that there is a relationship between behaviour and glycaemic variability within a small sample of white, mostly female, middle aged adults. Dichotomising the participants by fitness levels revealed stronger associations between MAGE and MVPA for the lower fitness group. Further evidence should focus on the collection of glucose data using methods to minimise data loss in order to confirm associations and the true magnitude of the relationship. Currently, it is unknown whether any activity related decline in glucose may occur for everyone and not just for the lowest fitness individuals therefore a more behaviourally and fitness diverse participant population should be utilised.

Chapter Six - Discussion

Chapter overview

This thesis has presented three studies, which add to the literature on the measurement of sedentary time and physical activity and the associations with diabetes risk and acute health parameters. The aim of this thesis was to profile physical activity behaviours in relation to diabetic risk, cardiometabolic health and glucose concentrations using novel measurement and analytical methods. By utilising novel methodologies, additional relationships with physical behaviours have been revealed that both strengthens the case for exercise as medicine but also that acute monitoring technologies could be used to promote increased physical activity. In the following sections, a summary of each of the chapters is presented alongside the key findings with specific reference to wider issues of measurement. A summary is provided alongside the strengths and limitations in Table 7.1. Finally, a general discussion is then provided, with future directions and closing comments concluding the chapter.

Table 6.1. A summary of the purpose, method, key findings, strengths and limitations of Chapters Three, Four and Five.

	Chapter Three	Chapter Four	Chapter Five
Purpose	1) Examine associations between behaviour and diabetes risk. 2) Model the effect of substituting behaviour to a higher intensity using isotemporal substitution analysis.	1) Calculate novel methods of behavioural complexity. 2) Associate complexity with cardiometabolic risk factors. 3) Ascertain if complexity relates to volumetric doses of behaviour.	1) Identify the relationship between objectively measured sedentary time, physical activity and gluose concentrations over 14 days in an adult normoglycemic population.
Method	Primary data collection from the Physical Activity and Respiratory Health (PhARaoH) study (sub sample n = 252). Wrist worn accelerometry (non-dominant wrist). Multiple linear regression and isotemporal substitution. Cross-sectional.	Primary data collection from the Physical Activity and Respiratory Health (PhARaoH) study (sub sample n = 290). Wrist worn accelerometry (non-dominant wrist). Sample entropy and multiple linear regression analyses. Cross-sectional.	Primary data collection from the Sensing Interstitial Glucose to Nudge Active Lifestyles study (n = 29). Waist worn accelerometry and continuous glucose monitoring. Generalised estimating equations. Cross-sectional.
Findings	Increasing physical activity and reallocating sedentary time to either light or MPVA reduces the risk of diabetes, with larger reductions observed in individuals with low levels of physical activity and high levels of sedentary time.	Higher sample entropy scores were associated with higher high-density lipoprotein (HDL) cholesterol levels but lower triglycerides and HbA1c levels; however, entropy is not related to volumetric doses of physical activity.	Behaviour was associated with measures of glycaemic variability and relationships of a more logical directional were observed for lower fitness individuals.

Table 7.1. A summary of the purpose, method, key findings, strengths and limitations of Chapters Three, Four and Five.

	Chapter Three	Chapter Four	Chapter Five
Strengths	Adds to the limited evidence of wrist worn accelerometry and diabetes risk. Use of isotemporal substitution modelling. Ethnically diverse sample.	Novel measurement of behaviour complexity.	Novel coupling of physical activity and glucose concentrations using acute health monitoring.
Limitations	Wrist worn accelerometry is still in its infancy. Sleep was removed through a semi-automated approach that has not been extensively validated.	Sample entropy has not been widely calculated. Wrist worn estimates may be affected by non-ambulatory wrist movements e.g. reading or working at a computer. Fasting time was only > 4 hours.	Findings cannot be generalised because of the small sample. Valid day and missing data criteria have not been validated.

6.1 Findings from Chapters Three, Four and Five

6.1.1 Chapter Three - Study One

In Chapter Three, objectively monitored physical activity and sedentary time collected from a primary sample of adults from Leicestershire and Rutland was associated with diabetes risk. Additionally, the effect of replacing sedentary time into physical activity was modelled using isotemporal substitution modelling. A linear relationship between sedentary time, light and moderate to vigorous physical activity (MVPA) was demonstrated for diabetes risk scores, and statistically substituting behaviour to a higher intensity resulted in a reduction of risk. Interestingly, reallocation from light intensity to MVPA did not always result in a significant change in diabetes risk scores, indicating that most benefits are perhaps gained from moving behaviour from sedentary time to either light or MVPA, but not moving light intensity to MVPA. An eight to nine times greater reduction in diabetes risk was revealed for those individuals currently undertaking low physical activity and high sedentary time, hinting at a curvilinear dose response effect of physical activity.

6.1.2 Chapter Four - Study Two

Despite physical activity guidelines being based upon volumetric doses of physical activity, studies are now identifying different physical activity patterns (Metzger et al., 2008; Marschollek, 2013), that have distinct relationships with cardiometabolic risk factors (Marschollek, 2016). Sample entropy is a measure of signal complexity and Chapter Four aimed to examine if complexity of accelerometry data was associated with cardiometabolic risk factors and traditional volume estimates of physical activity. Measured by wrist worn accelerometry, an increase in sample entropy scores (higher complexity) was associated with a lower glycated haemoglobin (HbA1c) score within a sample of UK adults. Whilst associations were evident, it is unclear what entropy scores are comparable to, which makes it difficult to ascertain what an average entropy score should be. Further research should be conducted in a larger and more diverse sample, perhaps at a national level.

6.1.3 Chapter Five - Study Three

After reaffirming the benefits that physical activity has on health, whether through volume (Chapter Three) or through complexity (Chapter Four), Chapter Five aimed to measure the acute influence of sedentary time and physical activity on glucose concentrations. Acute health monitoring through the measurement of glucose concentrations in real time may offer an alternative to activity tracking as it allows individuals to assess the impact of behavioural

choices in real time. However, to be confident of the relationship between behaviour and glucose concentrations, both data streams need to be associated using objective monitoring. Study Three measured sedentary time, physical activity and glucose concentrations over a 2 week period in 29 adults. Significant associations were evident between some measures of glycaemic variability and physical behaviours over multiple days of monitoring. Stronger associations were observed between MVPA and mean amplitude of glycaemic excursions (MAGE) for low fitness individuals. Showing proof of principle, more research should be conducted in larger samples to confirm the findings.

6.2 General discussion

Despite the large amount of evidence that physical activity has a positive effect upon health (Warburton et al., 2006), it is well known that the number of individuals completing the current recommended levels of physical activity is low in many well developed countries (Craig et al., 2009; Colley et al., 2011; Troiano et al., 2008). The measurement of behaviour reaffirms the associations between sedentary time, physical activity and chronic disease, though more focus is required upon novel ways to increase activity levels in an ever increasingly busy society that responds to immediacy. As advances are made in measurement techniques, it is clear that in addition to monitoring population levels of physical activity, research should also focus on how chronic disease risk factors can be mitigated within sustainable interventions.

6.2.1 The dose response

Chapter Three aimed to establish the reduction in diabetes risk of reallocating sedentary time to activities of a light or moderate to vigorous intensity. A considerable amount of reallocation was required to modify the risk of diabetes across risk categories (204 minutes Light and 84 minutes of MVPA). Despite supporting the literature that has found positive benefits of replacing sedentary time with standing, walking (Stamatakis et al., 2015) and MVPA (Buman et al., 2014; Hamer et al., 2014; Stamatakis et al., 2015) on all-cause mortality and cardiometabolic risk factors, the amount could be considered a hard sell to those that are not currently undertaking enough physical activity. Splitting the sample by low activity status revealed larger reductions in diabetes risk from substituting sedentary time to MVPA, with 13-15 minutes extra a day reducing the risk score of diabetes by five points, enough to cross a risk score category.

Although there is a general consensus within the field that even a little is good; more is better (Lee, 2007), there is evidence for a dose response curve where the larger risk reductions are obtained at lower ends of physical activity. A meta-analysis of nine prospective cohort studies found that whilst the risk of developing coronary heart disease was lower with a higher volume of leisure time physical activity, the largest risk reductions were observed in those completing the guidelines of 150 minutes of MVPA per week (14%) compared to individuals completing no leisure time physical activity (Sattelmair et al., 2011). As levels of leisure time physical activity increased, the additional amount of risk reduction was modestly lower (300 minutes = 20%, 750 = 25%) (Sattelmair et al., 2011). Similar results were

obtained for those with higher risk of diabetes (based on age and body mass index (BMI)) as the association between HbA1c and physical activity was stronger at lower physical activity levels (Gay et al., 2016). Depending on the public health message that is being conveyed, these results may either be encouraging or discouraging i.e. it may be perceived that most benefits are gained by becoming more active and it is less worth the effort to increase activity levels further. However, due to the low level of physical activity currently undertaken by the general population, facilitating and motivating individuals to increase their activity levels only slightly would have huge implications for public health.

Isotemporal substitution modelling facilitates the assessment of behavioural replacement upon a specified outcome such as cardiometabolic risk factors. Utilised within Chapter Three to show the reduction in diabetes risk, the technique is based heavily upon linear regression techniques and the calculations assume linearity and symmetry within the coefficients i.e. replacement of sedentary to MVPA has the same magnitude of change as the reciprocal relationship (Chastin et al., 2015). Despite this limitation, these techniques are valuable as they acknowledge that behaviours do not happen in isolation, but are inter-connected. Daily time is finite, behaviours including sleep, sedentary time and physical activity should be seen as compositional, with time spent in one decreasing the ability to spend it in another (Chastin et al., 2015). As a result, minimising time spent sedentary will increase time spent in another behaviour e.g. physical activity. Many studies still examine physical activity behaviour in isolation, whereas each behaviour should be examined in context with its neighbouring intensity as for instance, any association of light activity will be affected by both sedentary time and time spent in MVPA.

Sedentary time, or sitting, has been dubbed the new smoking yet a recent meta-analysis showed that conducting enough MVPA (60-75 minutes per day) was shown to offset the risk of mortality from high sitting time, with the risk of mortality still being lower for people sitting over 8 hours a day, the more MVPA they accrued (Ekelund et al., 2016). Conducting this level of physical activity is more than double the UK guidelines, however the guidelines should be seen as the minimum, with the WHO recommending 300 minutes a week of MVPA for additional benefits (World Health Organization, 2010). The basis of evidence that is used to encourage people to be more active needs to be balanced between what is true and what is achievable. The volume of activity to offset the mortality of sitting may be too high

and may be discouraging, yet as researchers we should not over sell the benefits of small amounts of physical activity, if a large volume is required.

The analysis presented within Chapter Three does not take into account the level of diabetes risk when calculating any reduction in risk due to physical activity behaviours. It is logical to think that whilst there is a linear relationship between diabetes risk scores, sedentary time, light and MVPA, the risk reduction will likely also reduce as they move from category to category. Yet, the findings within this thesis advocate that those who have the most to gain have the greatest reductions in risk. However, these results have come from a small, relatively healthy sample and need to be confirmed in a larger dataset, perhaps with a larger variation in health and levels of physical activity and sedentary time.

6.2.2 More than just volume

Much of the current literature on physical activity has focused on volumetric estimations of physical activity, yet behaviour often occurs sporadically, at multiple time points and in numbers of bouts. With the development of technology, objective measurements of physical activity by use of accelerometer have shown distinct patterns of behaviours between individuals. Metzger and colleagues (2008) wrote one of the first papers to quantify physical activity patterns using accelerometry data, defined by latent class analysis. Though five classes of MVPA were defined, the majority displayed parallel patterns, separated by incremental volume of activity per day; apart from a weekend warrior class that had moderate levels of MVPA per day but higher bouted MVPA at the weekend. Only focusing on intensity of behaviour ignores the additional information that the accelerometer can provide in addition to the possibility of being influenced by the cut-point choice.

A further attempt at behavioural profiling was developed that computed duration, intensity and also regularity of physical activity (Marschollek, 2013). Termed the ATLAS Index, distinct groups based upon the duration, intensity and regularity parameters have been associated with statistically different group differences in BMI and C Reactive Protein (Marschollek, 2016). Nonetheless, it is unclear how the calculations of each parameter influence group allocation and how they relate to individuals outside of the sample.

To further investigations into behavioural profiling, Chapter Four explored the use of Sample Entropy as a novel measure of physical activity behaviour. Not influenced by cut-points,

calculations are based upon count data per epoch (60 seconds) and look for self-similar patterns in the data. Though associations between entropy and cardiometabolic risk factors were observed for unadjusted and adjusted (age, sex and BMI) models, like previous attempts, it is unclear what entropy behaviourally represents. Significantly associated to the number of LVPA bouts, LVPA bouts were not related to HbA1c which seems to suggest in terms of HbA1c, entropy characterises a slightly different facet of behaviour.

High entropy represents less system order (Pincus and Goldberger, 1994) and behaviourally could indicate individuals that not only change their volume, but also their intensity at the same time. For a sequence to be self-similar, change in activity counts has to either rise and fall in the same pattern, or keep at a certain intensity for an extended duration of time. The combination together of rise and change in intensity will bring about changes in the signal that will not be self-similar in nature. Figure 7.1 represents this notion in a visual way. If a correct interpretation, two distinct patterns of behaviour could have the same or similar entropy scores. Though difficult in practice to match behaviourally, an individual with the

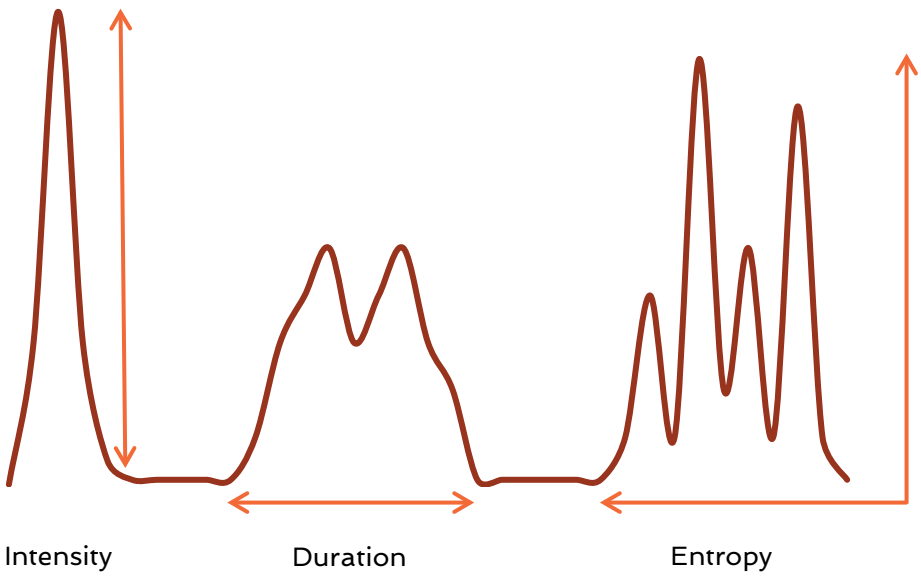


Figure 7.1. A schematic diagram representing an interpretation of what entropy represents. Whilst intensity denotes how many counts are achieved for a certain epoch, duration the length of an activity for a behavioural intensity, entropy may represent both the change in intensity and duration.

same pattern but at a higher intensity could have the same entropy score as someone at a lower intensity. As entropy ignores units attributed to the data, this could be problematic when categorising individuals on entropy alone. Data from Study Two suggests that additional information such as duration should be used, perhaps in conjunction with intensity to gain an understanding of overall behaviour.

As a strategy for physical activity promotion, changing both the intensity and duration of activity by introducing micro bouts could be an effective way to introduce less system order and increase entropy scores. Indeed evidence now exists on the breaking up of sedentary time and the benefits on cardiometabolic risk factors (Healy et al., 2008b, 2011). Though Chapter Four showed that high entropy-low activity was better than low entropy-low activity, the volume of physical activity seemed to be the persisting factor. There is insufficient evidence from a relatively small sample that entropy scores should be routinely measured; however, measuring physical activity profiles using novel measurements can provide valuable insights in the types of behaviours that can positively influence health.

6.2.3 Bio-behavioural feedback

It was confirmed within Study One and Two that there is a relationship between physical activity and health, though low levels of physical activity are still an ever increasing problem for national health services. Previous research has suggested levels of discounting (devaluing distant rewards) as a reason why people do not undertake sufficient physical activity, as a high level of discounting has been linked with low physical activity behaviours (Bradford, 2010). Laboratory work has revealed positive changes in acute glucose concentrations from standing and walking compared with enforced sedentary time or sitting. Displaying such positive effects of being physically active using a term called bio-behavioural feedback in an immediate context was the rationale behind the work within Chapter Six.

Controlled experimental work is valuable to establish the theoretical foundations of theory; however, without the introduction of sufficient variation it is hard to tell if associations between glucose and physical activity will be evident within free-living situations. Introducing noise (variability) into Study Three presented many problems with wear time, data loss and an uncertainty of knowing if glucose related declines were solely linked to behaviour. The approach taken within this thesis was to use daily summaries as a way to measure both glucose and behaviour, but a more informative and immediate method could be

to let the user know how glucose concentrations are influenced by specific bouts of physical activity. The quantification of bout-related glucose-related increases/decreases has advantages over daily summaries; however, the relationships between biological systems and behaviour does not always happen systematically. For instance, if a bout of physical activity is initiated, interrupted and then recommenced after a period of time, any change in glucose cannot be solely attributed to the first or second bout. Further investigations using bout analysis would be useful to establish the effect of any interruptions.

An assumption throughout Chapter Six is that glucose will rise due to sedentary time and decrease for any physical activity of a light or moderate to vigorous intensity. However, for prolonged bouts of activity, blood glucose concentrations are maintained through two processes that either break down glycogen (glycogenolysis) that is stored in the liver or form glucose from non-carbohydrate precursors (glyconeogensis) (Jeukendrup and Gleeson, 2004). At higher intensities (80% VO_2 max or more), blood concentrations can be elevated above resting levels as the liver starts to produce more glucose than is being utilised by the muscle (Jeukendrup and Gleeson, 2004). Conversely, the increased effect of insulin sensitivity within the muscle after physical activity causes an uptake of glucose from the bloodstream to synthesise glycogen (Jeukendrup and Gleeson, 2004). Indices used within Study Three such as daily mean glucose and MAGE may not be able to distinguish between those days where blood glucose remained constant throughout activity and those that had a slight rise in glucose due to completing activity at a higher intensity. Causing a drop in glucose concentrations after exercise may be the enhanced learning point that should be used i.e. tracking the effect that one bout of activity has on glucose concentrations for the next few hours, day or overnight period. Although bout analysis has limitations, it may provide greater context than daily summaries and be more of an immediate response to the behavioural stimuli.

Whilst significant associations were found between behaviour and MAGE in the pooled analysis, for lower fitness individuals, a stronger relationship was observed. Though fitness is related to diabetes risk (Wei et al., 1999; Sawada et al., 2003), data came from a non-diabetic and relatively healthy sample, who should not have abnormal glucose regulation. The results from Study Three perhaps suggest that fitness levels should be measured when assessing the effect of behaviour on glucose indices such as mean, standard deviation of glucose and MAGE. Still, for widespread application, results should be applicable to everyone and not in

specific populations or under certain conditions. The use of advanced computational algorithms such as machine learning could provide user specific solutions; although it is also acknowledged that the time and effort to set up such a system may not be feasible. Additionally, to establish sufficient behaviour change, feedback must be accurate, impermeable to large amounts of variation and not hindered by the amount of wear. The consequences of not meeting those specifications is that any device, whilst informative on health parameters, may end up taking the same path as other fitness trackers that stop being used by a third of users after 6 months (Ledger and McCaffrey, 2014).

Another issue of providing acute physiological feedback is the current invasiveness of continuous glucose monitoring. Though advancements of technology have made work in this area more practicable, such as the elimination of frequent calibrations and a sensor that can last up to 2 weeks, the process of capturing the physiological information still puts burden onto the user. A recent horizon scanning review of new and emerging non-invasive glucose technologies identified 40 devices, 16 of which were described as continuous glucose monitors (CGMs) (Horizon Scanning Research & Intelligence Centre, 2016). These technologies will allow for more feasible deployment and assessment of acute health changes, but to really be effective, they must be coupled with efficient data processing platforms. The work within Study Three only provided feedback at the end of the protocol, but to be truly effective, both behavioural and physiological data should be able to be accessed in real time. Whilst the fitness consumer market has led the way in providing physical activity feedback, capturing glucose information is not an easy process. Online platforms are now available that can take glucose information, but this still requires the user to use another piece of equipment to do so. An open source, crowd initiated project called ‘Nightscout’ is facilitating the remote access of glucose information via the internet and can transmit this data to wearbles such as the Apple Watch and the Pebble Smartwatch. These initiatives are filling the void not currently taken up by the glucose monitoring manufacturers, but developments are likely to advance rapidly. There is still much to learn about the coupling of behaviour and physiology and research should focus on the use of this novel information within an intervention, to see if bio-behavioural feedback can increase physical activity behaviours, to benefit the health of those that receive the feedback.

6.3 Future directions

This section will outline some recommendations for future work based on some limitations and natural progressions of the work presented within this thesis.

6.3.1 Chapter Three - Study One

The objective measurement of physical activity is routinely quantified using accelerometry; however, it is now generally accepted that the measurement of sedentary behaviour should be conducted using devices that can analyse postural data. Allowing for greater precision of the coefficients, posture data would provide an opportunity to investigate the change of diabetes risk scores if sitting was reallocated to standing. Additionally, due to sleep diaries not being part of the deployment protocol, sleep was removed through a semi-automated process. As a known risk factor for type 2 diabetes (Yaggi et al., 2006), a proper interrogation of sleep using sleep diaries and/or sleep devices would allow for true compositional analysis to be undertaken. Finally, the sample was recruited from primary care, local and internal advertisements. Every effort was made to recruit a physiologically and behaviourally diverse population, yet as only 12% of participants presented with three or more and 21% with two risk factors for the metabolic syndrome, the number of people with multiple comorbidities was low. Future studies should seek to replicate Study Three within a more demographically and physiologically diverse sample.

6.3.2 Chapter Four - Study Two

Aiming to ascertain whether cardiometabolic risk factors were influenced by the complexity of physical activity, activity levels were estimated using wrist worn physical activity. The basis of understanding physical activity has traditionally relied upon waist worn estimates. It is unknown whether the physical activity estimates at the wrist are heavily influenced by non-ambulatory movement e.g. seated wrist movement. A comparison at differing sites, including waist worn accelerometry would highlight any discrepancies in entropy scores. Similarly, entropy scores can be influenced by the parameter choice of *'m'* and *'r'* (Yentes et al., 2013). Identifying how parameter choice influences the association with cardiometabolic risk will allow for any modification to be factored into any interpretation of the data. Furthermore, as Chapter Four suggests entropy is capturing a different aspect of behaviour, calculating a composite score including entropy could unearth whether the additional effort to measure the new index is scientifically beneficial.

6.3.3 Chapter Five - Study Three

Glucose was shown to be associated with measures of glycaemic variability within Study Three. Further work should be conducted relating to the data processing and proof of principle within an intervention. Daily summaries were utilised, yet it is unknown how glucose indices are influenced by individual bouts. Another important factor to consider is whether acute physiological changes can be induced by a summation of short bouts of activity to reflect activities of daily living (ADLs) or whether it is solely purposeful activity. The modelling of bouts may reduce the issues surrounding wear time as instead of having to capture enough behavioural or physiological data, reductions or increases in glucose could be anchored to a specific event or point in time. This type of analysis would require more complex computation algorithms as any processing would need to take into account the following issues, amongst others:

- Previous physical activity or sedentary time - hourly, daily or weekly.
- Previous glucose concentrations - influenced by physical activity, food or hormones.
- Bout behaviour duration - what time duration is considered a bout.
- Bout effect duration - how long does the increase/decrease in glucose last for and is it modified by the intensity of behaviour.
- User characteristics - levels of type 2 diabetes risk, fitness, gender and age.

Perhaps a focus of future research could investigate a number of the mentioned issues, in order to increase the robustness of the associations.

The utility of bio-behavioural feedback as a motivational aid still has many hurdles that need to be addressed before the technology and infrastructure are ready for deployment. Alongside data-driven approaches to the physiological feedback, a feasibility intervention should be carried out to investigate whether providing physical activity, sedentary time and glucose information in real time changes physical activity levels. After establishing baseline measurements, enterprise partnerships between technology companies and researchers could be utilised to couple both streams of data within one application. A further follow up period could then establish if any short term increase or decrease in physical activity occurred. The protocol could make use of commercial wearables such as fitness trackers and CGMs to increase feasibility and connectivity of devices. Whilst the ActiGraph models are capable of remote monitoring, the advancements by companies such as Fitbit have made significant leaps in their data platforms and application programming interfaces (APIs) which make it

possible to use their data in a variety of ways. Whilst ideas for further research have been presented here, there are still many more possibilities, which may yield important information concerning the relationship between behaviour and acute physiological information such as glucose concentrations.

6.4 Final comments

Most individuals are still not sufficiently active to benefit their health, even though the negative consequences are widely known and researched. This thesis presents evidence confirming 1) the association between behaviour and risk of diabetes 2) that volume is not the only parameter related to health and 3) that behaviour can have acute influences on daily glucose variability parameters. It is clear that the work within Study Three, Four and Five are by no means the final product and future work should aim to refine the conclusions using larger and more diverse samples. The health of the nation is likely to continue to need improvement for some time, which is going to take every effort from scientists, policy makers and the commercial sector. The novel use of acute health monitoring technologies within this thesis sits amongst a very small amount of research that aims to explore the coupling of behavioural and acute physiological data. This is something that is likely to change in near future due to advances in technology. Lessons need to be learnt in the promotion of physical activity, but the measurement of behaviour is still important for the field, to further understand the complex interconnections with health and to move forward to design efficacious interventions that motivate people to become more physically active.

References

Adams, S. A., Matthews, C. E., Ebbeling, C. B., Moore, C. G., Cunningham, J. E., Fulton, J. and Hebert, J. R. (2005) 'The effect of social desirability and social approval on self-reports of physical activity.', *American journal of epidemiology*, 161(4), pp. 389–98.

Aguilar-Farías, N., Brown, W. J. and Peeters, G. M. E. E. G. (2014) 'ActiGraph GT3X+ cut-points for identifying sedentary behaviour in older adults in free-living environments.', *Journal of science and medicine in sport*, 17(3), pp. 293–9.

Alere (2016a) *Afinion HbA1c - Alere Afinion HbA1c Technical Brief*. Available at: http://www.alere.com/en/home/product-details/afinion-hba1c.html (Accessed: 10 October 2016).

Alere (2016b) *Alere Cholestech LDX® - TC GLU Cassettes Package Insert*. Available at: http://www.alere.com/en/home/product-details/cholestech-ldx-system.html (Accessed: 10 October 2016).

Allen, N. A., Fain, J. A., Braun, B. and Chipkin, S. R. (2008) 'Continuous glucose monitoring counseling improves physical activity behaviors of individuals with type 2 diabetes: A randomized clinical trial.', *Diabetes research and clinical practice*. NIH Public Access, 80(3), pp. 371–9.

Allen, N., Whittemore, R. and Melkus, G. (2011) 'A continuous glucose monitoring and problem-solving intervention to change physical activity behavior in women with type 2 diabetes: a pilot study.', *Diabetes technology & therapeutics*, 13(11), pp. 1091–9.

American Diabetes Association (2016) '2. Classification and Diagnosis of Diabetes.', *Diabetes care*, 39 Suppl 1(Supplement 1), pp. S13-22.

Archer, E. and Blair, S. N. (2015) 'Implausible data, false memories, and the status quo in dietary assessment.', *Advances in nutrition*. American Society for Nutrition, 6(2), pp. 229–30.

Atkin, A. J., Gorely, T., Clemes, S. A., Yates, T., Edwardson, C., Brage, S., Salmon, J., Marshall, S. J. and Biddle, S. J. H. (2012) 'Methods of Measurement in epidemiology: sedentary Behaviour.', *International journal of epidemiology*. Oxford University Press, 41(5), pp. 1460–71.

Aune, D., Norat, T., Leitzmann, M., Tonstad, S. and Vatten, L. J. (2015) 'Physical activity and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis.', *European journal of epidemiology*, 30(7), pp. 529–42.

Bacchi, E., Negri, C., Zanolin, M. E., Milanese, C., Faccioli, N., Trombetta, M., Zoppini, G., Cevese, A., Bonadonna, R. C., Schena, F., Bonora, E., Lanza, M. and Moghetti, P. (2012) 'Metabolic effects of aerobic training and resistance training in type 2 diabetic subjects: a randomized controlled trial (the RAED2 study).', *Diabetes care*, 35(4), pp. 676–82.

Baghurst, P. A. (2011) 'Calculating the mean amplitude of glycemic excursion from continuous glucose monitoring data: an automated algorithm.', *Diabetes technology & therapeutics*. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA, 13(3), pp. 296–302.

Bailey, K. J., Little, J. P. and Jung, M. E. (2016) 'Self-Monitoring Using Continuous Glucose Monitors with Real-Time Feedback Improves Exercise Adherence in Individuals with Impaired Blood Glucose: A Pilot Study.', *Diabetes technology & therapeutics*. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA, 18(3), pp. 185–93.

Bailey, T., Bode, B. W., Christiansen, M. P., Klaff, L. J. and Alva, S. (2015) 'The Performance and Usability of a Factory-Calibrated Flash Glucose Monitoring System.', *Diabetes technology & therapeutics*. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA, 17(11), pp. 787–94.

Bakrania, K., Edwardson, C. L., Bodicoat, D. H., Esliger, D. W., Gill, J. M. R., Kazi, A., Velayudhan, L., Sinclair, A. J., Sattar, N., Biddle, S. J. H., Khunti, K., Davies, M. and Yates, T. (2016a) 'Associations of mutually exclusive categories of physical activity and sedentary time with markers of cardiometabolic health in English adults: a cross-sectional analysis of the Health Survey for England.', *BMC public health*, 16(1), p. 25.

Bakrania, K., Yates, T., Rowlands, A. V., Esliger, D. W., Bunnewell, S., Sanders, J., Davies, M., Khunti, K. and Edwardson, C. L. (2016b) 'Intensity Thresholds on Raw Acceleration Data: Euclidean Norm Minus One (ENMO) and Mean Amplitude Deviation (MAD) Approaches.', *PloS one*. Edited by M. Buchowski, 11(10), p. e0164045.

Bansal, S., Buring, J. E., Rifai, N., Mora, S., Sacks, F. M. and Ridker, P. M. (2007) 'Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women.', *JAMA*, 298(3), pp. 309–16.

Bassuk, S. S. and Manson, J. E. (2005) 'Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease.', *Journal of applied physiology*, 99(3), pp. 1193–204.

Bergenstal, R. M., Ahmann, A. J., Bailey, T., Beck, R. W., Bissen, J., Buckingham, B., Deeb, L., Dolin, R. H., Garg, S. K., Goland, R., Hirsch, I. B., Klonoff, D. C., Kruger, D. F., Matfin, G., Mazze, R. S., Olson, B. A., Parkin, C., Peters, A., Powers, M. A., Rodriguez, H., Southerland, P., Strock, E. S., Tamborlane, W. and Wesley, D. M. (2013) 'Recommendations for standardizing glucose reporting and analysis to optimize clinical decision making in diabetes: the Ambulatory Glucose Profile (AGP).', *Diabetes technology & therapeutics*, 15(3), pp. 198–211.

Bickel, W. and Marsch, L. (2001) 'Toward a Behavioral Economic Understanding of Drug Dependence: Delay Counting Process', *Addiction*, 96(February 2000), pp. 73–86.

Blevins, T. C., Bode, B. W., Garg, S. K., Grunberger, G., Hirsch, I. B., Jovanovič, L., Nardacci, E., Orzeck, E. A., Roberts, V. L., Tamborlane, W. V, AACE Continuous Glucose Monitoring Task Force and Rothermel, C. (2010) 'Statement by the American Association of Clinical Endocrinologists Consensus Panel on continuous glucose monitoring.', *Endocrine practice*, 16(5), pp. 730–45.

Bouchard, C., Blair, S. and Haskell, W. (2012) *Physical activity and health*. 2nd Ed. Chapmaign, IL: Human Kinetics.

Bradford, W. D. (2010) 'The association between individual time preferences and health maintenance habits.', *Medical decision making*, 30(1), pp. 99–112.

Buckley, J. P., Mellor, D. D., Morris, M. and Joseph, F. (2014) 'Standing-based office work shows encouraging signs of attenuating post-prandial glycaemic excursion.', *Occupational and environmental medicine*, 71(2), pp. 109–11.

Buman, M. P., Hekler, E. B., Haskell, W. L., Pruitt, L., Conway, T. L., Cain, K. L., Sallis, J. F., Saelens, B. E., Frank, L. D. and King, A. C. (2010) 'Objective light-intensity physical activity associations with rated health in older adults.', *American journal of epidemiology*. Oxford University Press, 172(10), pp. 1155–65.

Buman, M. P., Winkler, E. A. H., Kurka, J. M., Hekler, E. B., Baldwin, C. M., Owen, N., Ainsworth, B. E., Healy, G. N. and Gardiner, P. A. (2014) 'Reallocating time to sleep, sedentary behaviors, or active behaviors: associations with cardiovascular disease risk biomarkers, NHANES 2005-2006.', *American journal of epidemiology*. Oxford University Press, 179(3), pp. 323–34.

Burge, M. R., Mitchell, S., Sawyer, A. and Schade, D. S. (2008) 'Continuous glucose monitoring: The future of diabetes management', *Diabetes Spectrum*, pp. 112–119.

Burton, P., Gurrin, L. and Sly, P. (1998) 'Extending the simple linear regression model to account for correlated responses: an introduction to generalized estimating equations and multi-level mixed modelling.', *Statistics in medicine*, 17(11), pp. 1261–91.

Byrom, B. and Rowe, D. A. (2016) 'Measuring free-living physical activity in COPD patients: Deriving methodology standards for clinical trials through a review of research studies.', *Contemporary clinical trials*, 47, pp. 172–84.

Camhi, S. M., Sisson, S. B., Johnson, W. D., Katzmarzyk, P. T. and Tudor-Locke, C. (2011) 'Accelerometer-determined moderate intensity lifestyle activity and cardiometabolic health', *Preventive Medicine*, 52(5), pp. 358–360.

Carney, C. E., Lajos, L. E. and Waters, W. F. (2004) 'Wrist actigraph versus self-report in normal sleepers: sleep schedule adherence and self-report validity.', *Behavioral sleep medicine*, 2(3), pp. 134-43–7.

Caspersen, C. J., Powell, K. E. and Christenson, G. M. (1985) 'Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research.', *Public health reports (Washington, D.C. : 1974)*. Association of Schools of Public Health, 100(2), pp. 126–31.

Cavanaugh, J. T., Kochi, N. and Stergiou, N. (2010) 'Nonlinear analysis of ambulatory activity patterns in community-dwelling older adults.', *The journals of gerontology. Series A, Biological sciences and medical sciences*, 65(2), pp. 197–203.

Celis-Morales, C. A., Lyall, D. M., Anderson, J., Iliodromiti, S., Fan, Y., Ntuk, U. E., Mackay, D. F., Pell, J. P., Sattar, N. and Gill, J. M. R. (2016) 'The association between physical activity and risk of mortality is modulated by grip strength and cardiorespiratory fitness: evidence from 498 135 UK-Biobank participants.', *European heart journal*, 33(6 Suppl), pp. S379–S399.

Ceriello, A., Colagiuri, S., Gerich, J., Tuomilehto, J. and Guideline Development Group (2008) 'Guideline for management of postmeal glucose.', *Nutrition, metabolism, and cardiovascular diseases*, 18(4), pp. S17-33.

Chacko, E. (2014) 'Timing and intensity of exercise for glucose control.', *Diabetologia*, 57(11), pp. 2425–6.

Chapman, G. B. and Elstein, A. S. (1995) 'Valuing the future: temporal discounting of health and money.', *Medical decision making*, 15(4), pp. 373–86.

Chastin, S. F. M., Palarea-Albaladejo, J., Dontje, M. L. and Skelton, D. A. (2015) 'Combined Effects of Time Spent in Physical Activity, Sedentary Behaviors and Sleep on Obesity and Cardio-Metabolic Health Markers: A Novel Compositional Data Analysis Approach.', *PloS one*, 10(10), p. e0139984.

Chen, K. Y. and Bassett, D. R. (2005) 'The technology of accelerometry-based activity monitors: current and future.', *Medicine and science in sports and exercise*, 37(11 Suppl), pp. S490-500.

Choi, L., Liu, Z., Matthews, C. E. and Buchowski, M. S. (2011) 'Validation of accelerometer wear and nonwear time classification algorithm.', *Medicine and science in sports and exercise*. NIH Public Access, 43(2), pp. 357–64.

Church, T. S., Thomas, D. M., Tudor-Locke, C., Katzmarzyk, P. T., Earnest, C. P., Rodarte, R. Q., Martin, C. K., Blair, S. N. and Bouchard, C. (2011) 'Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity.', *PloS one*, 6(5), p. e19657.

Clar, C., Barnard, K., Cummins, E., Royle, P., Waugh, N. and Aberdeen Health Technology Assessment Group (2010) 'Self-monitoring of blood glucose in type 2 diabetes: systematic review.', *Health technology assessment*, 14(12), pp. 1–140.

Clarke, W. and Kovatchev, B. (2009) 'Statistical tools to analyze continuous glucose monitor data.', *Diabetes technology & therapeutics*, 11 Suppl 1, pp. S45-54.

Colley, R. C., Garriguet, D., Janssen, I., Craig, C. L., Clarke, J. and Tremblay, M. S. (2011) 'Physical activity of Canadian adults: accelerometer results from the 2007 to 2009 Canadian Health Measures Survey.', *Health reports*, 22(1), pp. 7–14.

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F. and Oja, P. (2003) 'International physical activity questionnaire: 12-country reliability and validity.', *Medicine and science in sports and exercise*, 35(8), pp. 1381–95.

Craig, R., Mindell, J. and Hirani, V. (2009) 'Physical activity and fitness Health Survey for England 2008'.

CSEP (2003) 'The Canadian Physical Activity, Fitness and Lifestyle Approach (CPAFLA) 3rd edition', p. (7)24-31.

Damiano, E. R., El-Khatib, F. H., Zheng, H., Nathan, D. M. and Russell, S. J. (2013) 'A comparative effectiveness analysis of three continuous glucose monitors.', *Diabetes care*, 36(2), pp. 251–9.

Department of Health (2011a) 'Start Active, Stay Active A report on physical activity for health from the four home countries' Chief Medical Officers'.

Department of Health (2011b) *UK physical activity guidelines - Publications - GOV.UK*. Available at: https://www.gov.uk/government/publications/uk-physical-activity-guidelines (Accessed: 28 September 2016).

DeVries, J. H. (2013) 'Glucose Variability: Where It Is Important and How to Measure It', *Diabetes*, 62(5), pp. 1405–1408.

Diabetes Prevention Program Research Group (2002) ‘Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin’, *New England Journal of Medicine*. Massachusetts Medical Society, 346(6), pp. 393–403.

van Dijk, J.-W., Venema, M., van Mechelen, W., Stehouwer, C. D. A., Hartgens, F. and van Loon, L. J. C. (2013) ‘Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes.’, *Diabetes care*, 36(11), pp. 3448–53.

Ding, D., Lawson, K. D., Kolbe-Alexander, T. L., Finkelstein, E. A., Katzmarzyk, P. T., van Mechelen, W., Pratt, M. and Lancet Physical Activity Series 2 Executive Committee (2016) ‘The economic burden of physical inactivity: a global analysis of major non-communicable diseases.’, *Lancet*, 388(10051), pp. 1311–24.

Dishman, R. K., Washburn, R. A. and Schoeller, D. A. (2001) ‘Measurement of Physical Activity’, *Quest*. Routledge, 53(3), pp. 295–309.

Dourado, V. Z., Guerra, R. L. F., Tanni, S. E., Antunes, L. C. de O. and Godoy, I. (2013) ‘Reference values for the incremental shuttle walk test in healthy subjects: from the walk distance to physiological responses.’, *Jornal brasileiro de pneumologia*. Sociedade Brasileira de Pneumologia e Tisiologia (Brazilian Thoracic Society), 39(2), pp. 190–7.

Dunstan, D. W., Kingwell, B. A., Larsen, R., Healy, G. N., Cerin, E., Hamilton, M. T., Shaw, J. E., Bertovic, D. A., Zimmet, P. Z., Salmon, J. and Owen, N. (2012) ‘Breaking up prolonged sitting reduces postprandial glucose and insulin responses.’, *Diabetes care*. American Diabetes Association, 35(5), pp. 976–83.

Ehrhardt, N. M., Chellappa, M., Walker, S. M., Fonda, S. J. and Vigersky, R. A. (2011) ‘The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus.’, *Journal of diabetes science and technology*, 5(3), pp. 668–75.

Ekblom-Bak, E., Ekblom, Ö., Bergström, G. and Börjesson, M. (2016a) ‘Isotemporal substitution of sedentary time by physical activity of different intensities and bout lengths, and its associations with metabolic risk.’, *European journal of preventive cardiology*, 23(9), pp. 967–74.

Ekblom-Bak, E., Ekblom, Ö., Bolam, K. A., Ekblom, B., Bergström, G. and Börjesson, M. (2016b) 'SCAPIS Pilot Study: Sitness, Fitness and Fatness - Is Sedentary Time Substitution by Physical Activity Equally Important for Everyone's Markers of Glucose Regulation?', *Journal of physical activity & health*, 13(7), pp. 697–703.

Ekelund, U., Steene-Johannessen, J., Brown, W. J., Fagerland, M. W., Owen, N., Powell, K. E., Bauman, A., Lee, I.-M., Lancet Physical Activity Series 2 Executive Committe and Lancet Sedentary Behaviour Working Group (2016) 'Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women.', *Lancet*, 388(10051), pp. 1302–10.

Esliger, D. W. and Tremblay, M. S. (2007) 'Physical activity and inactivity profiling: the next generation.', *Canadian journal of public health*, 98 Suppl 2(S2E), pp. S195-207.

Falconer, C. L., Page, A. S., Andrews, R. C. and Cooper, A. R. (2015) 'The Potential Impact of Displacing Sedentary Time in Adults with Type 2 Diabetes.', *Medicine and science in sports and exercise*, 47(10), pp. 2070–5.

Field, A. (2013) *Discovering statistics using IBM SPSS statistics*. 4th Ed. London: SAGE.

Fonda, S. J., Graham, C., Munakata, J., Powers, J. M., Price, D. and Vigersky, R. A. (2016) 'The Cost-Effectiveness of Real-Time Continuous Glucose Monitoring (RT-CGM) in Type 2 Diabetes.', *Journal of diabetes science and technology*. SAGE Publications, 10(4), pp. 898–904.

Forouhi, N. G. and Wareham, N. J. (2014) 'Epidemiology of diabetes.', *Medicine*, 42(12), pp. 698–702.

Francois, M. E., Baldi, J. C., Manning, P. J., Lucas, S. J. E., Hawley, J. A., Williams, M. J. A. and Cotter, J. D. (2014) '"Exercise snacks" before meals: a novel strategy to improve glycaemic control in individuals with insulin resistance.', *Diabetologia*, 57(7), pp. 1437–45.

Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972) 'Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.', *Clinical chemistry*, 18(6), pp. 499–502.

Fritschi, C., Park, H., Richardson, A., Park, C., Collins, E. G., Mermelstein, R., Riesche, L. and Quinn, L. (2016) 'Association Between Daily Time Spent in Sedentary Behavior and Duration of Hyperglycemia in Type 2 Diabetes.', *Biological research for nursing*, 18(2), pp. 160–6.

Garg, S. K., Voelmle, M. and Gottlieb, P. A. (2010) 'Time lag characterization of two continuous glucose monitoring systems.', *Diabetes research and clinical practice*, 87(3), pp. 348–53.

Gay, J. L., Buchner, D. M. and Schmidt, M. D. (2016) 'Dose-response association of physical activity with HbA1c: Intensity and bout length.', *Preventive medicine*, 86, pp. 58–63.

Gill, J. M. R. and Cooper, A. R. (2008) 'Physical activity and prevention of type 2 diabetes mellitus.', *Sports medicine*, 38(10), pp. 807–24.

Glazer, N. L., Lyass, A., Esliger, D. W., Blease, S. J., Freedson, P. S., Massaro, J. M., Murabito, J. M. and Vasan, R. S. (2013) 'Sustained and shorter bouts of physical activity are related to cardiovascular health.', *Medicine and science in sports and exercise*. NIH Public Access, 45(1), pp. 109–15.

GOV.UK (2012) *CE marking*. Available at: https://www.gov.uk/guidance/ce-marking (Accessed: 1 March 2017).

Gray, L. J., Khunti, K., Edwardson, C., Goldby, S., Henson, J., Morris, D. H., Sheppard, D., Webb, D., Williams, S., Yates, T. and Davies, M. J. (2012) 'Implementation of the automated Leicester Practice Risk Score in two diabetes prevention trials provides a high yield of people with abnormal glucose tolerance.', *Diabetologia*, 55(12), pp. 3238–44.

Gray, L. J., Taub, N. A., Khunti, K., Gardiner, E., Hiles, S., Webb, D. R., Srinivasan, B. T. and Davies, M. J. (2010) 'The Leicester Risk Assessment score for detecting undiagnosed Type 2 diabetes and impaired glucose regulation for use in a multiethnic UK setting.', *Diabetic medicine*. Blackwell Publishing Ltd, 27(8), pp. 887–95.

Green, L., Myerson, J., Lichtman, D., Rosen, S. and Fry, A. (1996) 'Temporal discounting in choice between delayed rewards: the role of age and income.', *Psychology and aging*, 11(1), pp. 79–84.

Hall, P. A. and Fong, G. T. (2003) 'The effects of a brief time perspective intervention for increasing physical activity among young adults', *Psychology & Health*, 18(May 2015), pp. 685–706.

Hamer, M., Stamatakis, E. and Steptoe, A. (2014) 'Effects of substituting sedentary time with physical activity on metabolic risk.', *Medicine and science in sports and exercise*, 46(10), pp. 1946–50.

Hansen, B. H., Holme, I., Anderssen, S. A. and Kolle, E. (2013) 'Patterns of objectively measured physical activity in normal weight, overweight, and obese individuals (20-85 years): a cross-sectional study.', *PloS one*. Edited by N. C. Barengo. Public Library of Science, 8(1), p. e53044.

Healy, G. N., Dunstan, D. W., Salmon, J., Cerin, E., Shaw, J. E., Zimmet, P. Z. and Owen, N. (2008a) 'Breaks in sedentary time: beneficial associations with metabolic risk.', *Diabetes care*, 31(4), pp. 661–6.

Healy, G. N., Matthews, C. E., Dunstan, D. W., Winkler, E. A. H. and Owen, N. (2011) 'Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06.', *European heart journal*, 32(5), pp. 590–7.

Healy, G. N., Wijndaele, K., Dunstan, D. W., Shaw, J. E., Salmon, J., Zimmet, P. Z. and Owen, N. (2008b) 'Objectively measured sedentary time, physical activity, and metabolic risk: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab).', *Diabetes care*, 31(2), pp. 369–71.

Healy, G. N., Winkler, E. A. H., Owen, N., Anuradha, S. and Dunstan, D. W. (2015) 'Replacing sitting time with standing or stepping: associations with cardio-metabolic risk biomarkers.', *European heart journal*, 36(39), pp. 2643–9.

Heinemann, L. and Freckmann, G. (2015) 'CGM Versus FGM; or, Continuous Glucose Monitoring Is Not Flash Glucose Monitoring.', *Journal of diabetes science and technology*. SAGE Publications, 9(5), pp. 947–50.

Helmrich, S. P., Ragland, D. R., Leung, R. W. and Paffenbarger, R. S. (1991) 'Physical activity and reduced occurrence of non-insulin-dependent diabetes mellitus.', *The New England journal of medicine*. Massachusetts Medical Society, 325(3), pp. 147–52.

Henson, J., Yates, T., Biddle, S. J. H., Edwardson, C. L., Khunti, K., Wilmot, E. G., Gray, L. J., Gorely, T., Nimmo, M. A. and Davies, M. J. (2013) 'Associations of objectively measured sedentary behaviour and physical activity with markers of cardiometabolic health.', *Diabetologia*, 56(5), pp. 1012–20.

Herdman, M., Gudex, C., Lloyd, A., Janssen, M., Kind, P., Parkin, D., Bonsel, G. and Badia, X. (2011) 'Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L).', *Quality of life research*. Springer Netherlands, 20(10), pp. 1727–36.

Hill, N. (2010) *EasyGV — University of Oxford, Nuffield Department of Primary Care Health Sciences*. Available at: https://www.phc.ox.ac.uk/research/technology-outputs/easygv (Accessed: 8 October 2016).

Hill, N. R., Oliver, N. S., Choudhary, P., Levy, J. C., Hindmarsh, P. and Matthews, D. R. (2011) 'Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups.', *Diabetes technology & therapeutics*, 13(9), pp. 921–8.

Horizon Scanning Research & Intelligence Centre (2016) *New and emerging non-invasive glucose monitoring technologies*.

Hoss, U., Budiman, E. S., Liu, H. and Christiansen, M. P. (2013) 'Continuous glucose monitoring in the subcutaneous tissue over a 14-day sensor wear period.', *Journal of diabetes science and technology*, 7(5), pp. 1210–9.

Hoss, U., Budiman, E. S., Liu, H. and Christiansen, M. P. (2014) 'Feasibility of Factory Calibration for Subcutaneous Glucose Sensors in Subjects With Diabetes.', *Journal of diabetes science and technology*. SAGE Publications, 8(1), pp. 89–94.

Iliodromiti, S., Ghouri, N., Celis-Morales, C. A., Sattar, N., Lumsden, M. A. and Gill, J. M. R. (2016) 'Should Physical Activity Recommendations for South Asian Adults Be Ethnicity-Specific? Evidence from a Cross-Sectional Study of South Asian and White European Men and Women.', *PloS one*. Edited by S. Taheri, 11(8), p. e0160024.

International Diabetes Federation (2015) *IDF Diabetes Atlas 2015*, *International Diabetes Federation*.

Jeukendrup, A. and Gleeson, M. (2004) *Sport nutrition: an introduction to energy production and performance.* 1st Ed. Chapmaign, IL: Human Kinetics.

Kamada, M., Shiroma, E. J., Harris, T. B. and Lee, I.-M. (2016) 'Comparison of physical activity assessed using hip- and wrist-worn accelerometers.', *Gait & posture*, 44, pp. 23–8.

Kang, M. and Rowe, D. A. (2015) 'Issues and Challenges in Sedentary Behavior Measurement', *Measurement in Physical Education and Exercise Science*, 19(3), pp. 105–115.

Katzmarzyk, P. T. and Mason, C. (2009) 'The physical activity transition.', *Journal of physical activity & health*, 6(3), pp. 269–80.

Kirk, D., Cooke, C., Flintoff, A. and McKenna, J. (2008) *Key concepts in sport and exercise sciences*. 1st Ed. London: Sage.

Kirwan, J. P., Solomon, T. P. J., Wojta, D. M., Staten, M. A. and Holloszy, J. O. (2009) 'Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus.', *American journal of physiology. Endocrinology and metabolism*. American Physiological Society, 297(1), pp. E151-6.

Koster, A., Shiroma, E. J., Caserotti, P., Matthews, C. E., Chen, K. Y., Glynn, N. W. and Harris, T. B. (2016) 'Comparison of Sedentary Estimates between activPAL and Hip- and Wrist-Worn ActiGraph.', *Medicine and science in sports and exercise*, 48(8), pp. 1514–22.

Kovatchev, B., Anderson, S., Heinemann, L. and Clarke, W. (2008) 'Comparison of the numerical and clinical accuracy of four continuous glucose monitors.', *Diabetes care*, 31(6), pp. 1160–4.

Kropff, J., Bruttomesso, D., Doll, W., Farret, A., Galasso, S., Luijf, Y. M., Mader, J. K., Place, J., Boscari, F., Pieber, T. R., Renard, E. and DeVries, J. H. (2015) 'Accuracy of two continuous glucose monitoring systems: a head-to-head comparison under clinical research centre and daily life conditions.', *Diabetes, obesity & metabolism*, 17(4), pp. 343–9.

Lake, D. E., Richman, J. S., Griffin, M. P. and Moorman, J. R. (2002) 'Sample entropy analysis of neonatal heart rate variability.', *American journal of physiology. Regulatory, integrative and comparative physiology*, 283(3), pp. R789-97.

Lamonte, M. J. and Ainsworth, B. E. (2001) 'Quantifying energy expenditure and physical activity in the context of dose response.', *Medicine and science in sports and exercise*, 33(6 Suppl), pp. S370-8-20.

Landis, R. J. and Koch, G. G. (1977) 'The measurement of observer agreement for categorical data.', *Biometrics*, 33(1), pp. 159–74.

Langsted, A., Freiberg, J. J. and Nordestgaard, B. G. (2008) 'Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction.', *Circulation*, 118(20), pp. 2047–56.

Lavie, C. J., Johannsen, N., Swift, D., Sénéchal, M., Earnest, C., Church, T., Hutber, A., Sallis, R. and Blair, S. N. (2013) 'Exercise is Medicine - The Importance of Physical Activity, Exercise Training, Cardiorespiratory Fitness, and Obesity in the Prevention and Treatment of Type 2 Diabetes Diabetes Prevention', *US Endocrinology*, 9(2), pp. 95–100.

Ledger, D. and McCaffrey, D. (2014) *Inside Wearables: How the Science of Human Behavior Change offers the Secret to Long-Term Engagement*.

Lee, I.-M. (2007) 'Dose-response relation between physical activity and fitness: even a little is good; more is better.', *JAMA*. American Medical Association, 297(19), pp. 2137–9.

Lee, I.-M. and Shiroma, E. J. (2014) 'Using accelerometers to measure physical activity in large-scale epidemiological studies: issues and challenges.', *British journal of sports medicine*. NIH Public Access, 48(3), pp. 197–201.

Lee, I. M. and Paffenbarger, R. S. (1998) 'Physical activity and stroke incidence: the Harvard Alumni Health Study.', *Stroke*, 29(10), pp. 2049–54.

Liang, K.-Y. and Zeger, S. L. (1986) 'Longitudinal data analysis using generalized linear models', *Biometrika*, 73(1), pp. 13–22.

Little, R. R., Rohlfing, C. L., Sacks, D. B. and National Glycohemoglobin Standardization Program (NGSP) Steering Committee (2011) 'Status of hemoglobin A1c measurement and goals for improvement: from chaos to order for improving diabetes care.', *Clinical chemistry*, 57(2), pp. 205–14.

Malanda, U. L., Welschen, L. M. C., Riphagen, I. I., Dekker, J. M., Nijpels, G. and Bot, S. D. M. (2012) 'Self-monitoring of blood glucose in patients with type 2 diabetes mellitus who are not using insulin.', *The Cochrane database of systematic reviews*. Edited by U. L. Malanda. Chichester, UK: John Wiley & Sons, Ltd, 1(1), p. CD005060.

Manson, J. E., Hu, F. B., Rich-Edwards, J. W., Colditz, G. A., Stampfer, M. J., Willett, W. C., Speizer, F. E. and Hennekens, C. H. (1999) 'A prospective study of walking as compared with vigorous exercise in the prevention of coronary heart disease in women.', *The New England journal of medicine*, 341(9), pp. 650–8.

Marschollek, M. (2013) 'A semi-quantitative method to denote generic physical activity phenotypes from long-term accelerometer data--the ATLAS index.', *PloS one*. Edited by J. P. Brody. Public Library of Science, 8(5), p. e63522.

Marschollek, M. (2014) 'Clustering physical activity phenotypes using the ATLAS index on accelerometric data from an epidemiologic cohort study.', *Studies in health technology and informatics*, 205, pp. 763–7.

Marschollek, M. (2016) 'Associations between sensor-based physical activity behaviour features and health-related parameters.', *Human movement science*, 45, pp. 1–6.

Marshall, A. L., Miller, Y. D., Burton, N. W. and Brown, W. J. (2010) 'Measuring total and domain-specific sitting: a study of reliability and validity.', *Medicine and science in sports and exercise*, 42(6), pp. 1094–102.

Mâsse, L. C., Fuemmeler, B. F., Anderson, C. B., Matthews, C. E., Trost, S. G., Catellier, D. J. and Treuth, M. (2005) 'Accelerometer data reduction: a comparison of four reduction algorithms on select outcome variables.', *Medicine and science in sports and exercise*, 37(11 Suppl), pp. S544-54.

Mather, H. M. and Keen, H. (1985) 'The Southall Diabetes Survey: prevalence of known diabetes in Asians and Europeans.', *BMJ*, 291(6502), pp. 1081–1084.

McAndrew, L., Schneider, S. H., Burns, E. and Leventhal, H. (2007) 'Does patient blood glucose monitoring improve diabetes control? A systematic review of the literature.', *The Diabetes educator*. SAGE Publications, 33(6), pp. 991-1011–3.

McKeigue, P. M., Shah, B. and Marmot, M. G. (1991) 'Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians.', *Lancet*, 337(8738), pp. 382–6.

Mekary, R. A., Lucas, M., Pan, A., Okereke, O. I., Willett, W. C., Hu, F. B. and Ding, E. L. (2013) 'Isotemporal substitution analysis for physical activity, television watching, and risk of depression.', *American journal of epidemiology*. Oxford University Press, 178(3), pp. 474–83.

Mekary, R. A., Willett, W. C., Hu, F. B. and Ding, E. L. (2009) 'Isotemporal substitution paradigm for physical activity epidemiology and weight change.', *American journal of epidemiology*. Oxford University Press, 170(4), pp. 519–27.

Metzger, J. S., Catellier, D. J., Evenson, K. R., Treuth, M. S., Rosamond, W. D. and Siega-Riz, A. M. (2008) 'Patterns of objectively measured physical activity in the United States.', *Medicine and science in sports and exercise*, 40(4), pp. 630–8.

Monnier, L., Colette, C., Boegner, C., Pham, T. C., Lapinski, H. and Boniface, H. (2007) 'Continuous glucose monitoring in patients with type 2 diabetes: Why? When? Whom?', *Diabetes & metabolism*, 33(4), pp. 247–52.

Monnier, L., Colette, C. and Owens, D. R. (2008) 'Glycemic variability: the third component of the dysglycemia in diabetes. Is it important? How to measure it?', *Journal of diabetes science and technology*, 2(6), pp. 1094–100.

NatCen, N. C. for S. R. (2009) *Health Survey for England - 2008, Physical activity and fitness: Volume 2, Methods and documentation*. (mr) Web Master, United Kingdom.

National Health Service (2015) *Can I eat and drink before having a blood test?* NHS. Available at: http://www.nhs.uk/chq/Pages/1018.aspx?CategoryID=69 (Accessed: 5 December 2016).

National Health Service (2016) *Change4Life*. Available at: http://www.nhs.uk/change4life/pages/get-going-every-day.aspx (Accessed: 26 October 2016).

Nelson, K. M., Reiber, G., Boyko, E. J. and NHANES III (2002) 'Diet and exercise among adults with type 2 diabetes: findings from the third national health and nutrition examination survey (NHANES III).', *Diabetes care*, 25(10), pp. 1722–8.

O'Gorman, D. J., Karlsson, H. K. R., McQuaid, S., Yousif, O., Rahman, Y., Gasparro, D., Glund, S., Chibalin, A. V, Zierath, J. R. and Nolan, J. J. (2006) 'Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes.', *Diabetologia*, 49(12), pp. 2983–92.

Office for National Statistics (2012) *2011 Census, Key Statistics for Local Authorities in England and Wales*. Available at: http://webarchive.nationalarchives.gov.uk/20160105160709/http://www.ons.gov.uk/ons/publications/re-reference-tables.html?edition=tcm%3A77-286262 (Accessed: 7 March 2017).

Oliver, N. S., Toumazou, C., Cass, A. E. G. and Johnston, D. G. (2009) 'Glucose sensors: a review of current and emerging technology.', *Diabetic medicine*, 26(3), pp. 197–210.

Orme, M. W., Esliger, D. W., Kingsnorth, A. P., Steiner, M. C., Singh, S. J., Malcolm, D., Morgan, M. D. and Sherar, L. B. (2016) 'Physical Activity and Respiratory Health (PhARaoH): Data from a Cross-Sectional Study', *Open Health Data*, 4(1), p. 4.

Orme, M., Wijndaele, K., Sharp, S. J., Westgate, K., Ekelund, U. and Brage, S. (2014) 'Combined influence of epoch length, cut-point and bout duration on accelerometry-derived physical activity', *International Journal of Behavioral Nutrition and Physical Activity*. BioMed Central, 11(1), p. 34.

Peddie, M. C., Bone, J. L., Rehrer, N. J., Skeaff, C. M., Gray, A. R. and Perry, T. L. (2013) 'Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial.', *The American journal of clinical nutrition*, 98(2), pp. 358–66.

Pincus, S. M. (1991) 'Approximate entropy as a measure of system complexity.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 88(6), pp. 2297–301.

Pincus, S. M. and Goldberger, A. L. (1994) 'Physiological time-series analysis: what does regularity quantify?', *The American journal of physiology*, 266(4 Pt 2), pp. H1643-56.

Plotnikoff, R. C., Taylor, L. M., Wilson, P. M., Courneya, K. S., Sigal, R. J., Birkett, N., Raine, K. and Svenson, L. W. (2006) 'Factors associated with physical activity in Canadian adults with diabetes.', *Medicine and science in sports and exercise*, 38(8), pp. 1526–34.

Prince, S. A., Adamo, K. B., Hamel, M. E., Hardt, J., Connor Gorber, S. and Tremblay, M. (2008) 'A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review.', *The international journal of behavioral nutrition and physical activity*. BioMed Central, 5(1), p. 56.

Ramdani, S., Seigle, B., Lagarde, J., Bouchara, F. and Bernard, P. L. (2009) 'On the use of sample entropy to analyze human postural sway data.', *Medical engineering & physics*, 31(8), pp. 1023–31.

Richman, J. S. and Moorman, J. R. (2000) 'Physiological time-series analysis using approximate entropy and sample entropy.', *American journal of physiology. Heart and circulatory physiology*, 278(6), pp. H2039-49.

Rodbard, D. (2009) 'New and improved methods to characterize glycemic variability using continuous glucose monitoring.', *Diabetes technology & therapeutics*, 11(9), pp. 551–65.

Rodbard, D. (2012) 'The challenges of measuring glycemic variability.', *Journal of diabetes science and technology*, 6(3), pp. 712–5.

Rodbard, D. (2016) 'Continuous Glucose Monitoring: A Review of Successes, Challenges, and Opportunities.', *Diabetes technology & therapeutics*. Mary Ann Liebert, Inc., 18 Suppl 2(S2), pp. S23-213.

Rowlands, A. V, Olds, T. S., Hillsdon, M., Pulsford, R., Hurst, T. L., Eston, R. G., Gomersall, S. R., Johnston, K. and Langford, J. (2014) 'Assessing sedentary behavior with the GENEActiv: introducing the sedentary sphere.', *Medicine and science in sports and exercise*, 46(6), pp. 1235–47.

Sattar, N. and Gill, J. M. R. (2015) 'Type 2 diabetes in migrant south Asians: mechanisms, mitigation, and management.', *The lancet. Diabetes & endocrinology*, 3(12), pp. 1004–16.

Sattelmair, J., Pertman, J., Ding, E. L., Kohl, H. W., Haskell, W. and Lee, I.-M. (2011) 'Dose response between physical activity and risk of coronary heart disease: a meta-analysis.', *Circulation*. NIH Public Access, 124(7), pp. 789–95.

Sawada, S. S., Lee, I.-M., Muto, T., Matuszaki, K. and Blair, S. N. (2003) 'Cardiorespiratory fitness and the incidence of type 2 diabetes: prospective study of Japanese men.', *Diabetes care*, 26(10), pp. 2918–22.

Sechterberger, M. K., Luijf, Y. M. and Devries, J. H. (2014) 'Poor agreement of computerized calculators for mean amplitude of glycemic excursions.', *Diabetes technology & therapeutics*, 16(2), pp. 72–5.

Sedentary Behaviour Research Network (2012) 'Letter to the editor: standardized use of the terms "sedentary" and "sedentary behaviours".', *Applied physiology, nutrition, and metabolism*. NRC Research Press, 37(3), pp. 540–2.

Service, F. J., Molnar, G. D., Rosevear, J. W., Ackerman, E., Gatewood, L. C. and Taylor, W. F. (1970) 'Mean amplitude of glycemic excursions, a measure of diabetic instability.', *Diabetes*, 19(9), pp. 644–55.

Shaw, J. E., Sicree, R. A. and Zimmet, P. Z. (2010) 'Global estimates of the prevalence of diabetes for 2010 and 2030.', *Diabetes research and clinical practice*, 87(1), pp. 4–14.

Shephard, R. J. (2003) 'Limits to the measurement of habitual physical activity by questionnaires.', *British journal of sports medicine*, 37(3), p. 197–206; discussion 206.

Shiroma, E. J., Schepps, M. A., Harezlak, J., Chen, K. Y., Matthews, C. E., Koster, A., Caserotti, P., Glynn, N. W. and Harris, T. B. (2016) 'Daily physical activity patterns from hip- and wrist-worn accelerometers.', *Physiological measurement*. IOP Publishing, 37(10), pp. 1852–1861.

Siegelaar, S. E., Holleman, F., Hoekstra, J. B. L. and DeVries, J. H. (2010) 'Glucose variability; does it matter?', *Endocrine reviews*. Endocrine Society, 31(2), pp. 171–82.

Singh, S. J., Morgan, M. D., Scott, S., Walters, D. and Hardman, A. E. (1992) 'Development of a shuttle walking test of disability in patients with chronic airways obstruction.', *Thorax*. BMJ Publishing Group Ltd and British Thoracic Society, 47(12), pp. 1019–24.

Sosnoff, J. J., Goldman, M. D. and Motl, R. W. (2010) 'Real-life walking impairment in multiple sclerosis: preliminary comparison of four methods for processing accelerometry data.', *Multiple sclerosis*, 16(7), pp. 868–77.

Squara, P., Imhoff, M. and Cecconi, M. (2015) 'Metrology in medicine: from measurements to decision, with specific reference to anesthesia and intensive care.', *Anesthesia and analgesia*, 120(1), pp. 66–75.

Stamatakis, E., Rogers, K., Ding, D., Berrigan, D., Chau, J., Hamer, M. and Bauman, A. (2015) 'All-cause mortality effects of replacing sedentary time with physical activity and sleeping using an isotemporal substitution model: a prospective study of 201,129 mid-aged and older adults.', *The international journal of behavioral nutrition and physical activity*. BioMed Central, 12, p. 121.

Staudenmayer, J., Pober, D., Crouter, S., Bassett, D. and Freedson, P. (2009) 'An artificial neural network to estimate physical activity energy expenditure and identify physical activity type from an accelerometer.', *Journal of applied physiology (Bethesda, Md.: 1985)*. American Physiological Society, 107(4), pp. 1300–7.

Strath, S. J., Kaminsky, L. A., Ainsworth, B. E., Ekelund, U., Freedson, P. S., Gary, R. A., Richardson, C. R., Smith, D. T., Swartz, A. M. and American Heart Association Physical Activity Committee of the Council on Lifestyle and Cardiometabolic Health and Cardiovascular, Exercise, Cardiac Rehabilitation and Prevention Committee of the Council on Clinical Cardiology, and C. (2013) 'Guide to the assessment of physical activity: Clinical and research applications: a scientific statement from the American Heart Association.', *Circulation*, 128(20), pp. 2259–79.

Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M., Liu, B., Matthews, P., Ong, G., Pell, J., Silman, A., Young, A., Sprosen, T., Peakman, T. and Collins, R. (2015) 'UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age.', *PLoS medicine*. Public Library of Science, 12(3), p. e1001779.

Sylvia, L. G., Bernstein, E. E., Hubbard, J. L., Keating, L. and Anderson, E. J. (2014) 'Practical guide to measuring physical activity.', *Journal of the Academy of Nutrition and Dietetics*, 114(2), pp. 199–208.

Tate, L. M., Tsai, P.-F., Landes, R. D., Rettiganti, M. and Lefler, L. L. (2015) 'Temporal discounting rates and their relation to exercise behavior in older adults.', *Physiology & behavior*, 152(Pt A), pp. 295–9.

Thomas, J. and Nelson, J. (1996) *Research methods in physical activity*. 3rd editio. Chapmaign, IL: Human Kinetics.

Thompson, P. D., Crouse, S. F., Goodpaster, B., Kelley, D., Moyna, N. and Pescatello, L. (2001) 'The acute versus the chronic response to exercise.', *Medicine and science in sports and exercise*, 33(6 Suppl), pp. S438-45–3.

Thorp, A. A., Kingwell, B. A., Sethi, P., Hammond, L., Owen, N. and Dunstan, D. W. (2014) 'Alternating bouts of sitting and standing attenuate postprandial glucose responses.', *Medicine and science in sports and exercise*, 46(11), pp. 2053–61.

Troiano, R. P. (2005) 'A timely meeting: objective measurement of physical activity.', *Medicine and science in sports and exercise*, 37(11 Suppl), pp. S487-9.

Troiano, R. P., Berrigan, D., Dodd, K. W., Mâsse, L. C., Tilert, T. and McDowell, M. (2008) 'Physical activity in the United States measured by accelerometer.', *Medicine and science in sports and exercise*, 40(1), pp. 181–8.

Troiano, R. P., McClain, J. J., Brychta, R. J. and Chen, K. Y. (2014) 'Evolution of accelerometer methods for physical activity research.', *British journal of sports medicine*. NIH Public Access, 48(13), pp. 1019–23.

Tudor-Locke, C., Camhi, S. M. and Troiano, R. P. (2012) 'A catalog of rules, variables, and definitions applied to accelerometer data in the National Health and Nutrition Examination Survey, 2003-2006.', *Preventing chronic disease*, 9, p. E113.

UK Biobank (2009) *UK Biobank Touchscreen Questionnaire*. Available at: http://www.ukbiobank.ac.uk/resources/.

Vashist, S. K. (2013) 'Continuous Glucose Monitoring Systems: A Review.', *Diagnostics*. Multidisciplinary Digital Publishing Institute (MDPI), 3(4), pp. 385–412.

Vigersky, R. A., Fonda, S. J., Chellappa, M., Walker, M. S. and Ehrhardt, N. M. (2012) 'Short- and long-term effects of real-time continuous glucose monitoring in patients with type 2 diabetes.', *Diabetes care*, 35(1), pp. 32–8.

Vigersky, R. and Shrivastav, M. (2016) 'Role of continuous glucose monitoring for type 2 in diabetes management and research.', *Journal of diabetes and its complications*.

Warburton, D. E. R., Charlesworth, S. A., Foulds, H. J. A., McKenzie, D. C., Shephard, R. J. and Bredin, S. S. D. (2013) 'Qualified exercise professionals: best practice for work with clinical populations.', *Canadian family physician*. College of Family Physicians of Canada, 59(7), pp. 759–61.

Warburton, D. E. R., Nicol, C. W. and Bredin, S. S. D. (2006) 'Health benefits of physical activity: the evidence.', *Canadian Medical Association journal*. Canadian Medical Association, 174(6), pp. 801–9.

Wei, M., Gibbons, L. W., Mitchell, T. L., Kampert, J. B., Lee, C. D. and Blair, S. N. (1999) 'The association between cardiorespiratory fitness and impaired fasting glucose and type 2 diabetes mellitus in men.', *Annals of internal medicine*. American College of Physicians, 130(2), pp. 89–96.

White, D. K., Gabriel, K. P., Kim, Y., Lewis, C. E. and Sternfeld, B. (2015) 'Do Short Spurts of Physical Activity Benefit Cardiovascular Health? The CARDIA Study.', *Medicine and science in sports and exercise*, 47(11), pp. 2353–8.

Willett, W. C., Howe, G. R. and Kushi, L. H. (1997) 'Adjustment for total energy intake in epidemiologic studies.', *The American journal of clinical nutrition*, 65(4 Suppl), p. 1220S–1228S; discussion 1229S–1231S.

Willett, W. and Stampfer, M. J. (1986) 'Total energy intake: implications for epidemiologic analyses.', *American journal of epidemiology*, 124(1), pp. 17–27.

Wolff-Hughes, D. L., McClain, J. J., Dodd, K. W., Berrigan, D. and Troiano, R. P. (2016) 'Number of accelerometer monitoring days needed for stable group-level estimates of activity.', *Physiological measurement*, 37(9), pp. 1447–55.

Wood, T. M. (2000) 'Issues and future directions in assessing physical activity: an introduction to the conference proceedings.', *Research quarterly for exercise and sport*, 71 Suppl 2(sup2), pp. ii–vii.

World Health Organization (2006) *Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia*. World Health Organization.

World Health Organization (2009) *Global health risks*. World Health Organization.

World Health Organization (2010) *Global Recommendations on Physical Activity for Health*.

World Health Organization (2011a) *Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus*.

World Health Organization (2011b) 'Waist Circumference and Waist–Hip Ratio', *WHO Expert*. World Health Organization, 64(1), pp. 2–5.

World Health Organization (2016) *Global report on diabetes*.

Yaggi, H. K., Araujo, A. B. and McKinlay, J. B. (2006) 'Sleep duration as a risk factor for the development of type 2 diabetes.', *Diabetes care*, 29(3), pp. 657–61.

Yates, T., Henson, J., Edwardson, C., Dunstan, D., Bodicoat, D. H., Khunti, K. and Davies, M. J. (2015) 'Objectively measured sedentary time and associations with insulin sensitivity: Importance of reallocating sedentary time to physical activity.', *Preventive medicine*, 76, pp. 79–83.

Yentes, J. M., Hunt, N., Schmid, K. K., Kaipust, J. P., McGrath, D. and Stergiou, N. (2013) 'The appropriate use of approximate entropy and sample entropy with short data sets.', *Annals of biomedical engineering*, 41(2), pp. 349–65.

Zaccardi, F., O'Donovan, G., Webb, D. R., Yates, T., Kurl, S., Khunti, K., Davies, M. J. and Laukkanen, J. A. (2015) 'Cardiorespiratory fitness and risk of type 2 diabetes mellitus: A 23-year cohort study and a meta-analysis of prospective studies.', *Atherosclerosis*, 243(1), pp. 131–7.

Zhao, G., Ford, E. S., Li, C. and Balluz, L. S. (2011) 'Physical activity in U.S. older adults with diabetes mellitus: prevalence and correlates of meeting physical activity recommendations.', *Journal of the American Geriatrics Society*, 59(1), pp. 132–7.

Zorn, C. J. W. (2001) 'Generalized Estimating Equation Models for Correlated Data: A Review with Applications', *American Journal of Political Science*, 45(2), p. 470.

Appendices

A. Chapter Three (Study One) - Participant Information Sheet

Loughborough University
Department of Health
University Hospitals of Leicester NHS
NHS Trust

National Institute for Health Research
Leicester Respiratory Biomedical Research Unit
Glenfield Hospital
Groby Road
Leicester
LE3 9QP

Tel: 0116 258 3370
Email: leics.respiratorybru@nhs.net
www.leicsrespiratorybru.nihr.ac.uk

PATIENT INFORMATION SHEET

Physical Activity and Respiratory Health(PhARaoH)Study

An observational study investigating the roles ofphysical activity and sedentary behaviours, and psychological and social factors in chronic obstructive pulmonary disease(COPD) patients in order to inform strategies to delay COPD development or progression.

TO TAKE PART IN THE STUDY PLEASE CALL
+44 (0) 1509 228225

Chief Investigator: Professor Mike Morgan

Co- Researchers: Professor Stuart Biddle, Dr Dale Esliger, Dr Dominic Malcolm, Professor Myra Nimmo, Dr Lauren Sherar, Professor Sally Singh, Dr Michael Steiner

You may contact:: Dr Lauren Sherar
+44 (0)1509 228225

If you have any questions regarding your health please contact your GP

Invitation

You are being invited to take part in an observational study looking at the associations among physical activity and sedentary behaviours, and psychological and social factors in the development of chronic obstructive pulmonary disease (COPD). The information obtained from this study will be used to inform strategies to delay COPD progression or development. Before you decide whether or not you want to take part, you should understand what the purpose of the study is and how you will be involved. Please take time to read this information sheet and discuss it with family and friends if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The purpose of this study is to examine the physical, psychological and social factors that are related to physical activity and sedentary behaviours (such as watching television) on respiratory health in patients diagnosed and not diagnosed with COPD. We hope that the findings of this study will enable a greater understanding of COPD and will inform strategies to improve respiratory health.

Why have I been chosen?

You have been identified as potentially eligible for the study because you are between the age of 40 and 75 years, registered with a GP practice in Leicestershire and Rutland

We hope that around 650 people will take part.

Do I have to take part?

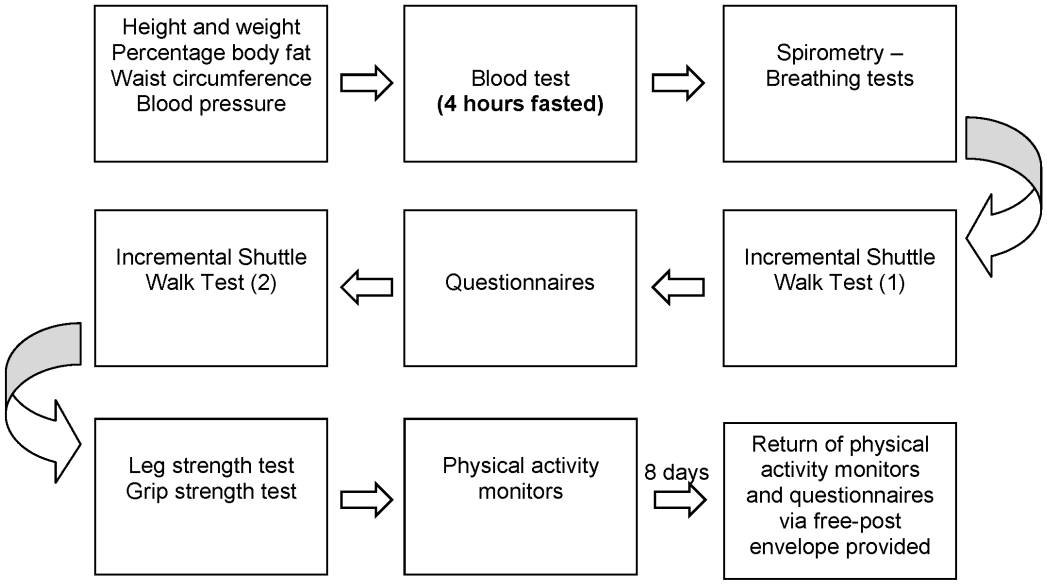
It is up to you to decide to join the study. We will describe the study and go through the information sheet with you. If you agree to take part, we will ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. If you decide not to participate or withdraw from the study, your NHS care will **not be affected** in any way. If you do decide to withdraw we may still use the data and samples collected up until the point you withdraw from the study.

What will happen to me if I take part?

We ask that you arrive to your appointment having fasted for 4 hours (not eaten or drank anything except water) and, if possible, refrain from smoking and being exposed to second-hand smoke for 24 hours prior to your appointment. We also ask that you do not take part in exercise on the day of your visit.

You will be asked to participate in a one-off visit to the Respiratory Biomedical Research Unit at Glenfield Hospital in Leicester for approximately 2 hours.

Assessment Visit Flow Chart



Description of the investigations

Height, weight, waist circumference, percentage body fat, blood pressure

Measurements of height and weight will be used to calculate your body mass index. You will be asked to remove your shoes, socks and personal belongings during the measurements. Weight and percentage body fat will involve standing on a weighing scale device. Waist circumference is measured using a tape measure and is an important indicator of abdominal or central obesity. Blood pressure will be measured whilst you are seated at rest. Immediate feedback of these results will be provided to you.

Blood sample

We kindly ask that you do not eat or drink (except water) 4 hours prior to your appointment time as blood samples will need to obtained with you in a fasted state. A small blood sample will be taken by a trained health professional in order to examine your cardiovascular and metabolic health status including blood sugar, cholesterol and blood chemistry. These results will be sent to your GP if consented, who will contact you directly if the results suggest that you may require treatment or advice.

Breathing Tests

We kindly ask that, if possible, you do not smoke and avoid second-hand smoke for 24 hours prior to your appointment time. An opportunity to have a cigarette will be provided during the visit. These will involve several breathing tests on different machines by breathing out as hard as you can several times separated by recovery periods. These breathing tests may cause some temporary light headedness, and coughing.

Incremental Shuttle Walk Test (ISWT)

The ISWT is a fitness test requiring you to walk only. You will walk up and down a 10meter (11 yard) course in an empty corridor with the speed of the walk dictated by a CD player. The time limit to walk the 10m course will be signalled by bleeps, with the time between bleeps shortening as the test progresses. You will be asked to continue walking until you feel unable to maintain the necessary speed without becoming unduly breathless. A researcher will help with pacing by walking alongside you at the beginning of the walk. This test will be performed twice, with a 20-30 minute rest period in between. Portable oxygen or walking aids can be used if this is what you are used to. You will also be asked to wear a chest strap which monitors heart and breathing rate during the test.

Questionnaires

The rest period between the shuttle walk tests provides the ideal time to complete the questionnaires. The questionnaires cover a variety of factors important for understanding the effects of lifestyle (e.g. physical activity and smoking); psychological factors (e.g. anxiety and perceived health); social factors (e.g. demographics); and physical influences (e.g. breathlessness) on respiratory health. Researchers will be on hand to answer any questions you may have whilst filling them in.

Leg and Grip Strength Tests

We will measure the strength of your leg muscles by asking you to sit on a specially designed strength testing machine and push with your leg as hard as you can against a pad, while we measure the force you produce. The grip strength test involves you squeezing a measuring device with maximal force on three occasions with a rest period in between.

Cardiovascular Health

These quick, non-invasive measurements will be taken whilst you are seated at rest in order to measure the function and health of your blood vessels (arteries). The first involves a sensor being placed on your finger for a few seconds. The second involves resting your forearm on a device which assesses, using light, blood vessel heath.

Physical activity

You will be given a wrist-worn activity monitor and a waist-worn activity monitor to take home after the assessment. These are to measure your physical activity and sitting time over an 8 day period. You are asked to take the waist-worn device off during water-based activities e.g. bath, swimming, etc. and overnight. The wrist-worn monitor can be kept on continuously. We do not wish you to change your normal day-to-day routine. These devices will be returned after the 8 days using the free-post envelope we provide you with. An information sheet about the monitors and verbal instructions will be provided at the visit. **We ask that you do not change your usual activity habits.**

Interview Sub-study

In addition to the visit conducted at Glenfield Hospital, approximately 50 COPD patients will take part in a smaller-scale qualitative study which will involve one-on-one interviews, at the participant's chosen location, to obtain responses to developing COPD; particularly regarding physical activity. Evidence will be used to inform tailored interventions to increase physical activity in an effort to reduce the rate of decline in lung function and improve health-related quality-of-life. Each participant will complete one interview lasting no longer than 1 hour. Participation in this sub-study is also entirely voluntary with interviews scheduled to suit you. As only a sub-sample of individuals will be recruited, consenting to be contacted does not mean you will definitely be asked to take part. More information can be found in the enclosed information sheet.

Expenses

We will reimburse you for your travel expenses (petrol and parking) from home to Glenfield Hospital and if required we can arrange taxis for you. Refreshments will also be provided during study visits.

What will I have to do?

We kindly ask that you do not eat or drink (except water) 4 hours prior to your appointment time.

If you are a smoker, we kindly ask that you do not smoke for 24 hours or as long as possible prior to your appointment time.

We also ask that you do not take part in exercise on the day of your visit.

If you currently take prescription medication, please bring a copy of your prescription or medication packaging with you to the visit.

Please wear light clothing for the visit. A snack will be provided after your blood sample has been taken. We **do not** ask that you make any other changes to your lifestyle.

What are the possible benefits of taking part?

By taking part in this study, you will receive a comprehensive health check sent to both yourself and your GP as well as written information and explanation of your physical activity levels. Any clinically important results will be passed on to your GP. Ultimately, taking part in the research will help to facilitate better care for COPD patients and those at risk of developing COPD.

What are the possible disadvantages and risks of taking part?

As with all physical activity, there is a very small risk of accident or injury during the exercise tests (walking and strength tests). All the exercise will be supervised by trained research staff and will take place on NHS premises with resuscitation equipment available and trained staff on hand to use it. Taking blood samples from your arm may cause slight pain or bruising afterwards. There are no specific disadvantages other than the time commitment for the visit.

What will happen to the samples that I have donated?
The blood samples will be processed at Glenfield Hospital by the research team. If you do not complete the whole study we will still use the samples that you have donated up until the point you withdrew unless you request otherwise.

What happens when the research study ends?
The results of this study will be circulated in medical journals, professional publications and presentations made at relevant conferences. Results will be reported in such a way that you or your information will not be identifiable. You will receive a summary of the results and will be invited to a presentation of the findings.

What if there is a problem?
If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you. Advice can also be sought from the Patient Information and Liaison Service, contact 0808 178 8337 (free number).

Will my taking part in this study be kept confidential?
When you enter this study you will be given a unique study number. This number will be used in place of any identifiable information, such as your name. All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital, for example for data analysis or monitoring, will have your name and address removed so that you cannot be recognised from it. Your medical records may also be looked at by the regulatory authorities, the sponsor or the NHS Trust to check that the study is being carried out correctly. All information resulting from you taking part in the study will be stored both in paper and computerised form, and will be treated confidentially.

Who is organising and funding the research?
The study is sponsored by University Hospitals of Leicester NHS Trust. The study is being funded by the Department of Health, UK after thorough review by leading scientists in this field. The study is supported by NIHR Leicester Respiratory Biomedical Research Unit.

Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be reviewed by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision. The NHS Research Ethics Committee(East Midlands Nottingham 2) approved the study on (11th November 2013).The UHL Trust Research Management Governance team has also reviewed and given permission for the study to be carried out.

The study has also been passed through a public and participation group who have had an input into the study design.

Contact for further information

If you require any further information or have any concerns please do not hesitate to contact us on the details at the beginning of the information sheet or contact the lead researcher Dr Lauren Sherar and her team, +44 (0) 1509 228225.

Thank you for reading this.

NHS
National Institute for Health Research

B. Chapter Three (Study One) - Additional Methods

All study measurements were taken by trained research personnel at the Respiratory Biomedical Research Unit located at Glenfield Hospital, Leicester from March to September 2014.

Blood pressure was measured in the upper right arm after a minimum of 10 minutes of seated rest (Omron 705IT, Omron, UK). The appropriately sized cuff was selected by the researcher by measuring the circumference of the upper arm using a tape measure. Three blood pressure measurements were taken (averaged) in 1 minute intervals with the measurement arm rested on a clinic table at the level of the heart. Participants were asked to place their feet flat on the floor, not to speak during the measurement and to not look at the measurement device whilst the reading was being taken.

A fasting blood test was obtained by the study team during the first part of the visit by Leicester Hospital NHS Trust trained study personnel. Due to consecutive appointments which could either be scheduled early morning or evening, a decision was made to ask participants to only fast for $\geq$ 4 hours. Confirmation of fasted status was ascertained by asking individuals 'What time did you last eat/drink anything other than water including chewing gum?'. Any that had eaten < 4 hours were excluded in the analysis. Blood samples were analysed on site by the pathology laboratory at Glenfield Hospital.

Blood biomarkers included: total cholesterol, low-density lipoprotein chlesterol (LDL), high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, HbA1c, sodium, potassium, urea, creatine, glomerular filtration rate, albumin, adjusted calcium, inorganic phosphate, alkaline phosphatase, aspartate transaminase, alanine transaminase, mean cell volume, mean cell haemoglobin, platelet count, neutrophil count, total lymphocyte count, monocytes, eosinophils, basophils, nucleated red blood cells, total bilirubin, free thyroxine, chloride, c-reactive protein, thyroid stimulating hormone, white blood cells, red blood cells, haemoglobin and haematocrit.

Height was measured using a portable stadiometer (SECA 213, SECA, Germany) and weight, total body fat percentage and calculated body mass index (BMI) was obtained using a bio electrical impedance device (Tanita MC780MA, Tanita, The Netherlands). Waist circumference was measured using a tape measure (HaB International Ltd, UK), around the mid-point between the lowest rib and iliac crest (World Health Organization, 2011b). Waist

circumference was taken twice and if the difference was greater than 3cm, a third was conducted. Mean waist circumference was calculated as the average of two measurements (mean of closest two if three measurements were taken).

A test of exercise capacity, the Incremental Shuttle Walk Test (ISWT) was conducted by asking the participant to walk up and down a 10m course in time to auditory beeps played by an audio device (Singh et al., 1992). As a progressive test, both the time in-between beeps and the number of shuttles increase level by level. The test has 12 levels at a maximum distance of 1020m. The test was stopped either by the participant, if they indicated that they are unable to continue, or by the study staff. If the participant failed to complete a shuttle in time with the beep (more than 0.5m away) or if the participant was deemed unfit to continue. ISWT tests were conducted twice, with a minimum of 30 minutes rest in-between attempts. Those with two attempts were carried forward for analysis and participants that completed the test in the first instance were not required to complete the test again. Peak VO_2 was derived using the following equation:

Peak VO_2 = 257 + (0.038 X ISWT distance X body mass (kg))

Equation B.1. Formula to calculate Peak VO_2 from the Incremental Shuttle Walk Test (ISWT) (Dourado et al., 2013).

Scores were then divided by body weight (kg) to calculate VO_2 in ml/kg/min. Prior to the ISWT, the Physical Activity Readiness Questionnaire (PAR-Q) (Warburton et al., 2013) was used as a suitability check for participation. A positive answer to the PAR-Q required a health practitioner to sign off the individual case.

Demographic, childhood, employment, general health, family history, medication and chronic disease information, smoking, lung health, breathlessness and physical activity and sitting were collected using sections from the Health Survey for England 2008 and UK Biobank questionnaires (NatCen, 2009; UK Biobank, 2009). The EuroQol (EQ-5D-5L) was answered by participants to assess their perceived health status (Herdman et al., 2011).

Physical activity and self-reported weekday and weekend domain-specific sitting time was recalled using the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003) and the domain-specific sitting questionnaire (Marshall et al., 2010). Frequency and context

of lifetime physical activity history was obtained using a sport inventory checklist and was broken down into the following age categories: < 18, 18-29, 30-39, 40-75, 'doing regularly' and 'could see yourself doing in the next year'. Questionnaires were checked for completeness and the participant had a chance to ask questions if anything was unclear.

C. Chapter Three (Study One) - Additional Results

Table C.1. Additional accelerometry cut-points split by diabetes risk score groupings for Kamada 2 cut-points.

	Whole group n = 251	Low risk n = 28	Increased risk n = 92	Moderate risk n = 90	High risk n = 41	
Behaviour						
Sed Kam minutes PD	527.1 ± 98.4	497.1 ± 71.2	494.2 ± 99.1	542.1 ± 98.6	588.3 ± 75.0	***
Li Kam2 minutes PD	423.3 ± 89.2	440.6 ± 55.0	447.5 ± 94.0	410.7 ± 91.6	384.8 ± 74.2	***
MV Kam2 minutes PD	22.6 ± 21.1	26.5 ± 15.9	27.9 ± 26.7	19.5 ± 17.2	14.7 ± 12.7	**

Reported as means and standard deviations unless otherwise stated; diabetes risk groups calculated according to Gray et al. (2010); abbreviations: PD, Per Day / Sed, Sedentary / Kam, Kamada / Li, Light / MV, moderate to vigorous physical activity; *p < 0.05, **p < 0.005, ***p < 0.0005

Table C.2. Additional associations between diabetes risk score and physical activity intensity variables for Kamada 2 cut-points.

	Unstandardised Coefficients		**Standardised Coefficient**		**95% Confidence Intervals**		
	b	**SE**	**β**	**Sig.**	**Lower**	**Upper**	**R^2**
MODEL ONE							
SedKam PD	0.028	0.005	0.361	< **0.0005**	0.019	0.037	0.130
LiKam2 PD	−0.023	0.005	−0.266	< **0.0005**	−0.033	−0.013	0.071
MVKam2 PD	−0.101	0.022	−0.276	< **0.0005**	−0.145	−0.057	0.076
MODEL TWO							
SedKam PD	0.024	0.005	0.303	< **0.0005**	0.015	0.033	0.243
LiKam2 PD	−0.024	0.005	−0.281	< **0.0005**	−0.034	−0.014	0.233
MVKam2 PD	−0.373	0.062	−0.336	< **0.0005**	−0.502	−0.245	0.196

Model one represents unadjusted associations and model two is adjusted for Peak VO_2 derived from the Incremental Shuttle Walk Test (ISWT); regression coefficients represent the change in diabetes risk score per 1 unit change (1 minute) in behavioural variables; abbreviations: B, unstandardised regression coefficient / sig, significance / PD, Per Day / Sed, Sedentary / Kam, Kamada / Li, Light / MV, moderate to vigorous physical activity; significant results are in bold; R^2 = explained variance.

Table C.3. Associations between diabetes risk score and Isotemporal substitution replacement into intensity variables of sedentary, light and moderate to vigorous physical activity (MVPA) for Kamada 2 cut-points.

	Sedentary			**Light**			**MVPA**		
	b	**95% Confidence Intervals**		**b**	**95% Confidence Intervals**		**b**	**95% Confidence Intervals**	
		Lower	**Upper**		**Lower**	**Upper**		**Lower**	**Upper**
MODEL ONE									
SedKam PD		**Reference**		**−0.221*****	−0.330	−0.112	**−0.740****	−1.185	−0.295
LiKam2 PD	**0.221*****	0.112	0.330		**Reference**		**−0.518***[a]	−1.008	−0.029
MVKam2 PD	**0.740****	0.295	1.184	**0.518***[a]	0.029	1.008		**Reference**	
MODEL TWO									
SedKam PD		**Reference**		**−0.211*****	−0.314	−0.108	**−0.460***[b]	−0.889	−0.031
LiKam2 PD	**0.211*****	0.108	0.314		**Reference**		−0.249	−0.718	0.221
MVKam2 PD	**0.460***[b]	0.031	0.890	0.249	−0.220	0.719		**Reference**	

Model one represents unadjusted associations, model two is adjusted for Peak VO_2 derived from the Incremental Shuttle Walk Test (ISWT); regression coefficients represent the substitution of 10 minutes of one behaviour into another of a different intensity; abbreviations: B, unstandardised regression coefficient / PD, Per Day / Sed, Sedentary / Kam, Kamada / Li, Light / MV, moderate to vigorous physical activity; reference represents the behaviour being 'dropped' from Isotemporal analysis; significant results are in bold. * < 0.05, ** < 0.005, *** < 0.0005, [a] p = 0.038, [b] p = 0.036

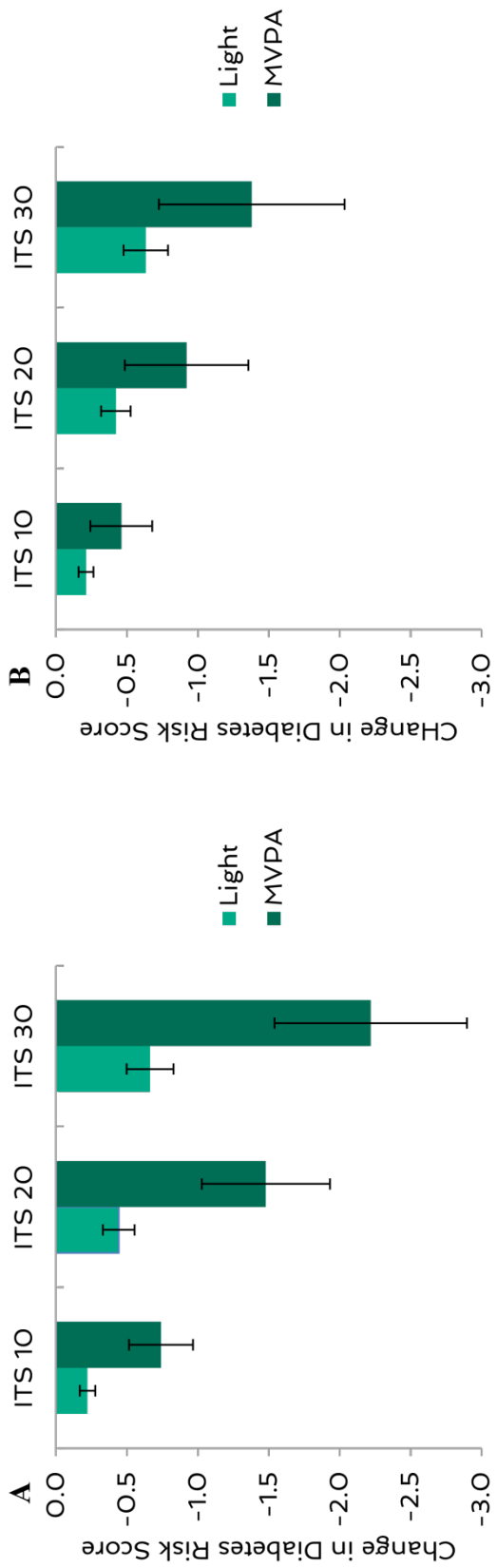


Figure C.1. Bar graphs representing the change in diabetes risk if sedentary time was substituted with light or moderate to vigorous physical activity (MVPA) in blocks of 10 (ITS 10), 20 (ITS 20) or 30 (ITS 30) minutes per day.
Error bars = standard error of coefficients. All relationships are < 0.05.
A = unadjusted Kamada threshold 2 variables (Sedentary: 0-1999, Light: 2000-8249, MVPA: ≥8250)
B = adjusted Kamada threshold 2 variables.

D. Chapter Four (Study Two) - Additional Methods

As the present study is only interested in calculating entropy for behaviour not labelled as sleep, it must be removed from each file. In the methods section, it was described how sleep was detected. However after detection, sleep was coded as '0' to enable the runtime algorithm to remove sleep up until the first epoch that was above '0' in the file (OUTBED). If a consecutive range of zeros was left within each file, the number of matches would be high for each file and sleep removal would be coded as self-similar or 'inactive' in this interpretation. The next following section will discuss the methods used to remove sleep data points for each accelerometry file.

ActiGraph accelerometry files are commonly stored in either a .gt3x (raw) or .agd (processed) formats. Whilst conducive to being analysed by software, to be able to edit the content directly, they must be transferred into a different file format first before editing. Sleep data points were removed using the INBED and OUTBED times were used in a semi-automated process using Excel (Microsoft, Washington, USA). This process is described in the following section:

File processing

- Removal of sleep data points required a temporal anchor so that the correct date and time point was removed from the data array. Accelerometry data files are typically processed in 'whole' days and does not cross over midnight without file manipulation. As INBED times commonly occurred past midnight, a date was required in addition to a time to ensure the correct sequence of sleep was detected. A document was drafted that matched the ID number of the participants with the generated INBED and OUTBED times. Additionally a batch output file from the quality control process in KineSoft (KineSoft, Loughborough, UK) generated the wear dates for all participants from the original .agd files. The dates and times were matched and concatenated to create a date relative time point. Due to the nature of the processing techniques used, the date and time was then converted into a decimal time for sleep filtering to take place.
- Files were converted from .agd to .csv using ActiLife (ActiGraph Corp, Pensacola, USA) and then into .xlsx using Excel as this is the format the entropy script requires in the MATLAB (MathWorks, Cambridge, UK).

- A macro script was developed to insert a decimal date/time column at the right of the data table in the .xlsx (working) files. This was to ensure filtering could take place using the date and times generated from the quality control report.
- INBED and OUTBED times were checked again for consistency and accuracy. The following rules were followed throughout the process:
    - During the sleep detection process, periods of non-wear were identified where it was unclear whether it was sleep or non-wear followed by sleep e.g. if a person took off the device at 6pm and didn't put it back on until 8am the next morning. If sleep could not be determined, physical activity information was removed until a corroborated date and time point was determined and the time points were edited so that the data for the day was removed.
    - Any INBED or OUTBED times before 9pm or after 11pm were visually inspected for accuracy. Any time points found to be erroneous were corrected.
    - Corrupt or 'short' files (containing less days than expected) were amended so that all data points were removed for the whole file.
    - Any files with a day 7 INBED time of 23:59 (either through actual sleep detection or by being the end of the file) was amended to 00:00 of the next day so that the macro did not cut off the last epoch of the file.

- A macro was developed to add a 'wear tab' onto each file that detailed the generated INBED and OUTBED times (Figure D.1). They were reordered so that a subsequent macro could filter between the INBED and OUTBED times.
- Once each .xlsx (working file) was prepared, another macro was developed to remove sleep using the time points on the wear tab. All data between INBED and OUTBED times were removed and replaced with blank data. A process was undertaken to assess how the entropy programme handled missing data. There was no difference between removing rows entirely and just the individual data points therefore the latter process was used.
- A selection of files (5) was assessed for accuracy at random. Due to using a macro, the processes were identical each time and therefore the number of files chosen for quality control was small.
- Any 'short' files were amended to contain seven day sequence in the array as they would not be processed through the MATLAB program otherwise.
- Files were then ready to be processed for Entropy analysis.

Participant ID FC002

	Wear dates	Out (< timepoint)	In (> timepoint)
Day 1	13/03/2014	13/03/2014 07:37	13/03/2014 23:05
Day 2	14/03/2014	14/03/2014 07:25	14/03/2014 23:47
Day 3	15/03/2014	15/03/2014 07:07	15/03/2014 21:02
Day 4	16/03/2014	16/03/2014 08:35	16/03/2014 23:12
Day 5	17/03/2014	17/03/2014 07:39	17/03/2014 22:38
Day 6	18/03/2014	18/03/2014 07:38	18/03/2014 23:10
Day 7	19/03/2014	19/03/2014 06:36	19/03/2014 21:52

		Timepoint 1	Timepoint 2	Timepoint 1	Timepoint 2
Removal 1	Out	13/03/2014 07:37		<	
Removal 2	In / Out	13/03/2014 23:05	14/03/2014 07:25	>=	<
Removal 3	In / Out	14/03/2014 23:47	15/03/2014 07:07	>=	<
Removal 4	In / Out	15/03/2014 21:02	16/03/2014 08:35	>=	<
Removal 5	In / Out	16/03/2014 23:12	17/03/2014 07:39	>=	<
Removal 6	In / Out	17/03/2014 22:38	18/03/2014 07:38	>=	<
Removal 7	In / Out	18/03/2014 23:10	19/03/2014 06:36	>=	<
Removal 8	In	19/03/2014 21:52		>=	

Figure D.1. A wear tab was generated using the INBED and OUTBED times for each participant. This was used to place a filter in the data and to remove the sleep data points.

E. Chapter Four (Study Two) - Additional Results

Table E.1. Additional descriptive statistics (Mean ± SD) for the whole sample (Kamada 2 cut-points).

	Sample n = 290		
Behaviour			
Sedentary Kam minutes per day	531.1	±	104.6
Light Kam2 minutes per day	423.2	±	94.6
MVPA Kam2 minutes per day	21.9	±	21.0

Means and standard deviations unless otherwise stated; abbreviations: Kam, Kamada / MVPA, moderate to vigorous physical activity.

Table E.2. Additional entropy multiple linear regressions associations with intensity variables for Kamada 2 cut-points.

	Unstandardised Coefficient (x10,000)		**Standardised Coefficient**		**95% Confidence Intervals (x10,000)**		
	b	**SE**	**β**	**Sig.**	**Lower**	**Upper**	**R^2**
MODEL ONE							
Sedentary Kam PD	1.41	1.85	0.045	0.447	−2.24	5.06	0.002
Light Kam2 PD	−0.34	2.05	−0.010	0.867	−4.39	3.7	< 0.000
MVPA Kam2 PD	−8.63	9.19	−0.055	0.348	−26.72	9.46	0.003
MODEL TWO							
Sedentary Kam PD	2.34	2.11	0.074	0.269	−1.82	6.51	0.016
Light Kam2 PD	−2.13	2.28	−0.061	0.352	−6.62	2.37	0.015
MVPA Kam2 per day	−11.28	9.86	−0.072	0.253	−30.69	8.13	0.017

Model one represents a univariate association and model two is adjusted for wear minutes, age, sex and body mass index (BMI); abbreviations: SE, standard error / Sig, significance / PD, per day / MVPA, moderate to vigorous physical activity; regression coefficients represent the change in diabetes risk score per 1 unit change (1 minute) in behavioural variables; R^2 = coefficient of determination; unstandardised coefficients and standard errors have been divided by 10,000.

F. Chapter Five (Study Three) - Participant Information Sheet

PAPHRG
Physical Activity & Public Health Research Group

Loughborough University

National Centre for Sport & Exercise Medicine
Working for Health & Wellbeing

Investigating the influence of acute health feedback for behaviour change using Continuous Glucose Monitoring (CGM) and fMRI in adults

Participant information sheet

Investigators

The investigators of the current study are all part of the Physical Activity and Public Health Research Group which is based at the National Centre for Sport & Exercise Medicine at Loughborough University. The team and their contact details are outlined below;

Mr Andrew Kingsnorth
PhD Research Student
a.kingsnorth@lboro.ac.uk

Miss Maxine Whelan
PhD Research Student
m.e.whelan@lboro.ac.uk

Dr Dale Esliger
Senior Lecturer in the Measurement of Physical Activity
d.esliger@lboro.ac.uk
01509 223280

What is the purpose of the study?

Already advocated as a preventive and treatment therapy for diabetes, physical activity has been shown to reduce the amount of glucose in your blood and support the return of glucose levels towards a beneficial target range. Additionally, sedentary behaviours such as sitting has recently been shown to have a detrimental effect on glucose levels, with many researchers implementing interventions that actively break up lengthy periods of sedentary behaviour. Adults in the UK are currently recommended to undertake 150 minutes of moderate-to-vigorous physical activity a week to reduce the risks of developing chronic disease later in life such as obesity, hypertension and diabetes. However, studies assessing physical activity levels in England have found that only a small percentage of individuals are completing the recommended levels of activity.

We believe that if individuals could see how physical activity influences their health after each 'bout', they may be more inclined to modify their behaviour to undertake activities that have been linked with more positive lifestyle choices as the benefits are more apparent to them. Before we can support glucose being used as "health feedback", the relationship between physical activity, sedentary behaviour and glucose must be established. Consequently, this study will collect physical activity, sedentary behaviour and glucose information using current sensing technologies, in order to explore the interactions over time; to find out what types of behaviour are most effective at controlling glucose levels.

The continuous measurement of glucose levels (or sugar levels) in real time has only recently been made possible by the relatively recent developments in continuous glucose monitoring (CGM) technology. Able to measure glucose levels at regular intervals up to a number of days at a time, a CGM consists of a small disposable sensor that is inserted under the skin and calculates a reading by measuring the interstitial fluid (the fluid between your cells). Commonly used in Type 1 diabetic patients to help them manage their glucose levels, researchers are now applying the devices in non-diabetic populations to illuminate the complex relationships between lifestyle related behaviours and health. Newer technologies such as the Freestyle Libre device have also brought the field of CGM forward by eliminating the need for calibrations and can continuously read glucose levels up to 14 days. Providing this level of information at the available frequency opens up an exciting amount of possibilities to investigate the role that behaviour has on acute health.

In parallel to identifying the relationship between physical activity, sedentary behaviour and glucose, there is a need for a resonant health message to be developed that will engage individuals to change their behaviour. This feedback is more commonly provided via smartphone applications or wrist-worn devices (with a screen) with the recent surge in commercial technology. This feedback could for instance, reveal current number of steps, total time spent sitting on this day or average glucose levels across the past week. Understanding how to optimise this feedback is pivotal to enhance the chance of behaviour change at the individual and population level. The relevance of feedback is commonly assessed via focus groups or questionnaires where individuals are requested to comment on the visuals presented to them. However, these methods can present issues such as inaccurate reporting as individuals often present themselves in the best possible light. Researchers have started to look at levels of brain activation via functional magnetic

resonance imaging (fMRI) to understand whether messages relating to health will be effective in changing behaviour; identified via increased levels of activation in the region associated with behaviour change/self-processing.

Functional MRI (fMRI) is a technique that directly measures blood flow in the brain using an MRI scanner. We aim to use fMRI to assess the effectiveness of personalised feedback commonly presented to individuals by smartphone applications. This feedback will focus on physical activity (e.g. number of steps), sedentary behaviour (e.g. hours spent sitting) and glucose levels (e.g. percentage time spent in target range). We can map brain activity whilst these images are presented to individuals; helping identify activated regions of the brain. Understanding which materials cause activation in the brain region related to behaviour change is vital to better understand how we can help individuals change their behaviour. The findings will inform an intervention to change activity levels, sedentary behaviour and/or tighten glucose levels in the near future.

Who is doing this research and why?

This study is part of a student research project supported by Loughborough University and will form part of a PhD thesis

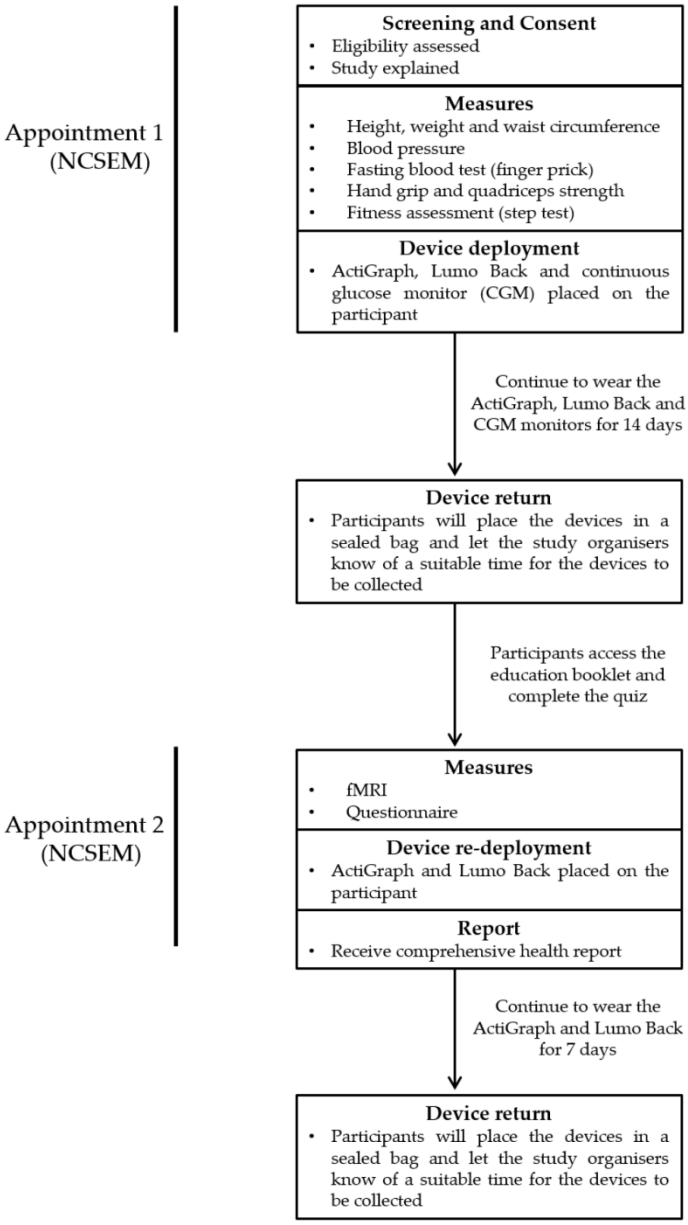
Are there any exclusion criteria?

Unfortunately if you fulfil any of the following criteria then you will not be allowed to take part in the study:

• Younger than 30 or older than 60	• Diagnosed with diabetes (Type 1 or Type 2)
• Are pregnant	• Taking diabetes medication (e.g. insulin therapy)
• Left-handed (fMRI standardisation)	• Have any permanent metal items in their body e.g. pacemaker, shrapnel
• Mobility related musculoskeletal problems	• Not willing to give signed consent
• Has any psychological disorders e.g. claustrophobic	• Cannot adhere to the study protocol
• Currently involved in any structured exercise training	

What will I be asked to do? Will I be required to attend any sessions and where will these be? How long will it take?

You will be asked to attend 2 sessions at the National Centre for Sport and Exercise Medicine (NCSEM) and be fitted with devices to be worn during your daily routine. The flow diagram below outlines all procedures with the subsequent text explaining the measures in more detail.



5

Consent, health measures, deployment of sensors
On arrival at your first appointment, you will have your eligibility confirmed using a health screen questionnaire and if considered eligible, we will provide you with a verbal recap of the study in its entirety (i.e. measurements, expectations and time commitments). If you are happy to proceed, you will receive a consent form to sign and date to confirm participant at this stage. Following consent, you will then be taken through a number of measurements including height, weight, waist circumference, percentage body fat, blood pressure and a fasting finger prick blood sample measuring cholesterol, glucose and triglycerides. Once these have been completed, you will then be asked to conduct a few fitness assessments, more specifically a grip strength assessment (hands, 3 grip assessments on each hand), a leg strength assessment (seated, 3 assessments on each leg) and a submaximal step test (conduct steps in time to an audio track, maximum of 20 minutes). This information will be provided at your second appointment in the form of a comprehensive health report.

Important: If a health issue is picked up at the visit, we will refer you onto your normal GP for further discussions.

Once these measurements have been completed, you will be provided with three wearable devices; a physical activity monitor (ActiGraph GT3x-BT), a posture sensor (LUMO) and a continuous glucose monitor (Freestyle Libre). Once deployed, you will be asked to wear these three devices for 14 days. An overview of these 3 devices is provided below.

Physical activity monitor
Size: 4.6 x 3.3 x 1.5cm
Weighs: 19g

We will ask you to wear this monitor under your clothing, on an elasticated belt around the waist, middle top of the right leg. You only need to wear this during waking hours and should only be removed before bedtime or before engaging in water-based activities (e.g. bathing or swimming). We will ask you to note these removal times down in a diary.

Posture sensor
Size: 4.15 x 10 x 0.8cm
Weighs: 25g

We will ask you to wear this monitor under your clothing, on an elasticated belt around the waist, around the lower back. You only need to wear this during waking hours and should only be removed before bedtime or before engaging in water-based activities (e.g. bathing or swimming). We will ask you to note these removal times down in a diary.

Continuous glucose monitor
Size: size of a £2 coin: 3.5 x 0.5cm
Weighs: 5g

We will ask you to wear this monitor under your clothing attached to the back of your upper arm. A small (5mm), thin and flexible fibre is inserted just below the skin in a relatively painless procedure. The sensor stays in place via an adhesive patch and tape will be placed on top to secure it in place. We will ask you to carry a handheld reader that will capture your glucose levels (by hovering the device over the glucose sensor) and ask you to try and scan the sensor at least once every 7 hour period. As sleep may last for longer than 7 hours, we will ask that you try to scan prior to falling asleep and another scan as soon as awake, where possible. The monitor is to be worn 24 hours a day (no removal) and does not require removal for water-based activities (it is water-resistant up to 1 meter for a maximum of 30 minutes).

During your appointment, the 3 devices will be explained in more detail to you and you will be informed of who to contact if any problems arise. You will also be given an education booklet providing some information about physical activity, sedentary behaviour and glucose levels. This appointment is expected to last 1.5-2 hours.

Free-living period – no feedback provided
During the 14 days of wear for the three devices, you will not receive any feedback on your physical activity, sedentary behaviour or glucose levels. We kindly ask that you maintain your usual daily activities during this period and to complete a dairy of food intake on 4 out of the 14 days using a mobile phone application. This is important as glucose levels are affected by a number of factors including food.

We will schedule appointment 2 to happen shortly after the 14 days has ended, at a convenient time. However, before this appointment happens, we will collect the devices from you. You will be provided a biohazard bag (at the first appointment) to dispose of the sensor appropriately (once removed from your arm after the 14 days) and an envelope for the physical activity and posture sensors.

Before the second appointment, you will be asked to read an education booklet and complete a brief quiz relating to its content. A link to access the quiz will be sent to you via email, it should take no longer than approximately 20 minutes. The reason we are asking you to complete a very short quiz is it ensure that all participants have the same knowledge about physical activity and sedentary behaviour prior to the second appointment. This will help us in our analysis of the fMRI.

fMRI – feedback provided
At the second appointment, you will receive feedback relating to your physical activity, sedentary behaviour and glucose levels. This information will cover data obtained from the devices that you each wore in the 14 day period. You will be presented this personalised feedback during an fMRI session. Whilst in the fMRI, your levels of brain activation will be

measured to provide information as to whether these messages will be effective in activating regions of the brain associated with behaviour change.

Before entering the MRI, the radiographers will request that you to complete a health screen questionnaire and a consent form. Participants must remove all metal items from their person before entering the scanner and you will be provided with earplugs to help minimise noise.

Figure – participant lying down inside the MRI scanner with headphones.

You will remain in the scanner for up to 40 minutes. Visual stimuli including your personal feedback will be presented to you on a monitor which you will be able to see via a mirror from a lying position. The visual stimuli will be presented in blocks of images (approximately 5 in one block). In between these blocks of visual stimuli, there will be rest periods (approximately 40 seconds) to return brain activation to normal; during which a fixation cross will be presented on the screen. Each visual stimuli will be presented for approximately 8 seconds, before the next stimuli is shown and so on in a cycle. You will not need to do anything except look at the monitor throughout this period. All participants will be guided through the scans via a microphone to maintain communication with the Radiographers. If at any time you do not want to continue with the scan, we ask you to press an emergency button and you will be removed from the scanner.

Following the fMRI, we will conduct a short 6 minute body scan to assess % body fat more accurately. We will then compare this information against % body fat measured by usual methods.

Important: a MRI scan will be obtained but please note that it will not be reviewed by a medical practitioner.

You will then be requested to assess the same visuals observed in the fMRI but via a pen and paper Likert scale. These results will allow comparisons to be made between the objective fMRI data and the subjective (Likert scale) data. Once completed, participants will be asked to complete a short questionnaire which focuses on your physical activity, sedentary behaviour and education history; following which you will receive a comprehensive health report (containing results from the first appointment). This appointment is scheduled to last 1.5 hours.

You will then be asked to wear the physical activity monitor and posture sensor (only) for a further 7 days. Following these 7 days, the devices will be collected from you at a convenient time.

Once I take part, can I change my mind?

Yes! After you have read this information and asked any questions you may have we will ask you to complete an Informed Consent Form, however if at any time, before, during or after the sessions you wish to withdraw from the study please just contact the main investigator. If you do decide to withdraw from the study your data will not be used for further analysis or publication, however withdrawal may not be possible if publication has already occurred.

What personal information will be required from me?

We will only require basic demographic information that will characterise the sample. Included within this is a questionnaire about your education history and physical activity/sedentary behaviour. We understand that this questionnaire may contain sensitive information. However, your responses will be kept confidential. Your questionnaire responses, alongside basic demographic details, will only be used to characterise the sample and in analysis.

We will also ask during the consenting process whether you would be willing to be re-contacted about other research that is being conducted by the research group. This is **optional** and will not stop you from taking part in the current study. If you would like to discuss this further then please ask one of the team who will be able to clarify this point further.

Are there any risks in participating?

On the first appointment a fasting blood test will be conducted. This will be completed by a trained individual; however there is a small chance of localised bruising. The continuous glucose monitor comprises of a thin flexible sterile fibre (5mm long) and a sensor pod. As the fibre will need to be inserted just below the skin, there is a very small chance of discomfort; however research conducted by the manufacturer states that 93.4% of users strongly agree or agree that while wearing the sensor, they did not feel any discomfort under their skin. You will also be asked to complete a small number of fitness assessments including a grip strength, leg strength and a step test. This may get you out of breath, however the procedure is conducted by an exercise scientist and should not cause you to worry. As you are required to be in the fasted state for this appointment, a small snack will be provided to allow you to be comfortable whilst completing these fitness assessments. The fMRI scan is considered an invasive procedure that could cause discomfort as it can be considered loud and claustrophobic inside the scanner. If participants have not been screened for metal objects such as a pacemaker, the MRI scan would cause significant harm. To minimise this risk, all participants will be screened before entering the MRI using the standardised MRI safety questionnaire.

Will my taking part in this study be kept confidential?

All results from the study will be anonymised by assigning a project code and will only be analysed by the research team. Blood samples will be taken, processed immediately and then disposed of. No blood samples will be stored for future use. All data will be treated in confidence and held securely in line with University guidance on collection and storage of data and the Data Protection Act 1998. Any paper records will be scanned and shredded after data inputting has taken place and all data will be held in an electronic encrypted format for 10 years. Only the primary researchers will have access to the data at any time.

What will happen to the results of the study?

The data collected will form part of a PhD thesis and has the potential to be published within an academic journal.

Is there anything I need to do before the sessions?

We kindly ask that you **do not eat or drink (except water) 8 hours prior to your appointment** time as blood samples will need to be obtained in a fasted state. We also ask that you **drink a glass of water at least 1 hour** before your appointment as this will help with us acquiring an accurate measurement from the fingerstick blood test. Finally, to ensure the submaximal fitness assessments are as accurate as possible, we ask that you **refrain from any strenuous activity 24 hours before** your appointment. If any of the above are going to be a problem then please contact the researchers at the beginning of this document.

What type of clothing should I wear?

Please can you wear light clothing for all appointments that are comfortable and you do not mind perspiring in (visit 1). If possible, please wear a short-sleeved top for appointment 1 to allow the researcher to access your arm to apply the glucose monitoring device.

What do I get for participating?

As a thank you for taking part, you will receive a full comprehensive health check assessment report detailing the results from any tests completed and your physical activity and glucose levels during the free-living component of the protocol. You will also have the chance to win an activity monitor for your participation.

What if I am not happy with how the research was conducted?

If you are not happy with how the research was conducted, please contact Ms Jackie Green, the Secretary for the University's Ethics Approvals (Human Participants) Sub-Committee:

Ms J Green, Research Office, Hazlerigg Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU. Tel: 01509 222423. Email: J.A.Green@lboro.ac.uk

The University also has a policy relating to Research Misconduct and Whistle Blowing which is available online at: http://www.lboro.ac.uk/committees/ethics-approvals-human-participants/additionalinformation/codesofpractice/

I have some more questions; who should I contact?

If you have any more questions, please do not hesitate to contact any of the investigators using the contact details at the beginning of the document.

G. Chapter Five (Study Three) - Participant Health Report

Study ID		**Visit date**	
DOB		**Gender**	**Female**
Height	**171.8** cm	**Weight**	**99.2** kg

All measurements in this report were obtained for research purposes and do not represent medical diagnoses.

Body composition

Body composition analyses are a good indication of the amount of body fat and lean tissue (muscle) you have. Body mass index (BMI) is a well known measure of body composition based on a calculation between height and weight. As a high BMI alone only suggests that you may be carrying too much weight for your height and not solely fat, we combine BMI with % body fat (% BF) and waist circumference (WC) to get a better picture of your overall health. These are listed below:

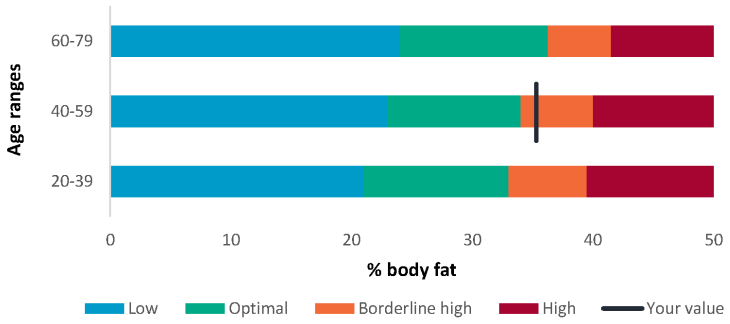
BMI: 33.6 kg/m2

- **Low < 18.5**
- **Optimal 18.5 - 24.9**
- **Borderline high 25.0 - 29.9**
- **High > 30**

WC: 113.2 cm

- **Low**
  Male <102cm - Female <88cm
- **High**
  Male >102cm - Female >88cm

% BF: 35.3 %



If your BMI is greater than 25 (and you are 'borderline high' - see above for gender specific ranges), you have an increased risk of developing health problems such as heart disease, diabetes, and cancers. If your BMI, WC or % BF have been recorded as above their respective healthy ranges, evidence suggests you should consider losing weight.

Cardiovascular health

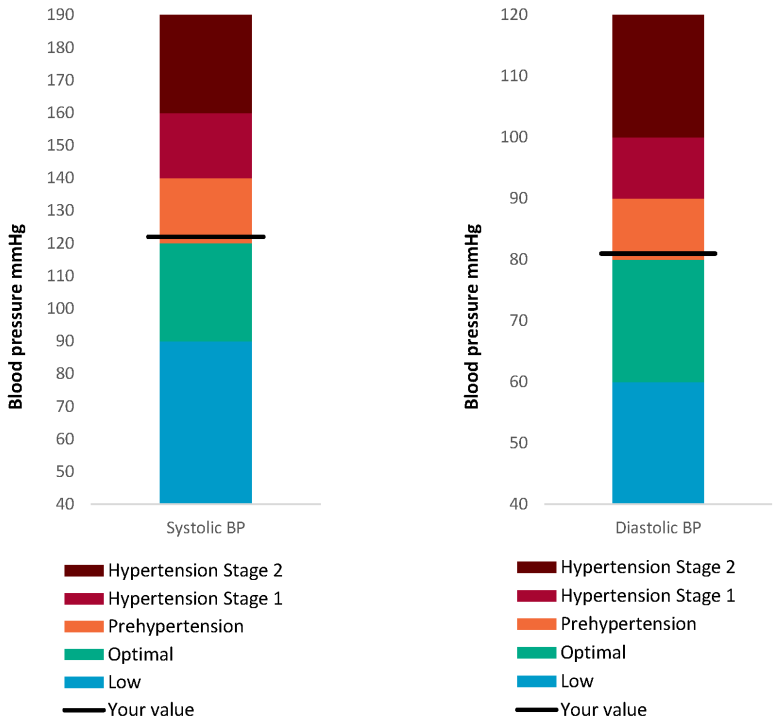
Maintaining good cardiovascular health is important. Measures such as resting heart rate, blood pressure and blood biomarkers provide insight into an individual's cardiovascular health. Each are provided below.

Your resting heart rate while sitting was 64 beats per minute

Normal resting heart rate ranges from 60-80 beats per minute, however the fitter you are, the lower your resting heart rate.

Blood pressure is recorded with two numbers: The **systolic pressure (SBP)** (higher number) is the force at which your heart pumps blood around your body. The **diastolic pressure (DBP)** (lower number) is the resistance to the blood flow in the blood vessels. Both are measured in millimetres of mercury (mmHg).

Your blood pressure was SBP 122 over DBP 81 mmHg

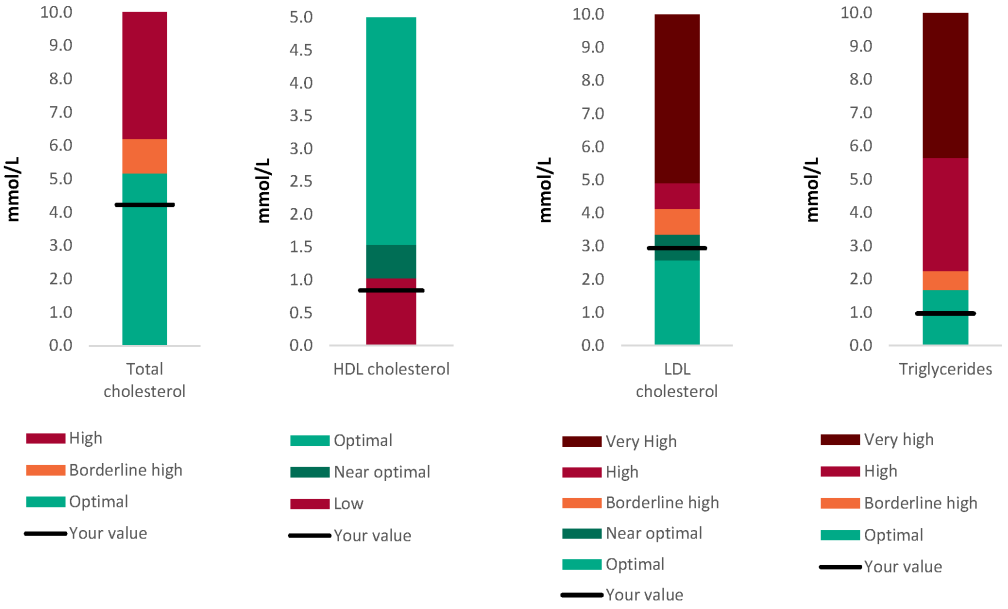


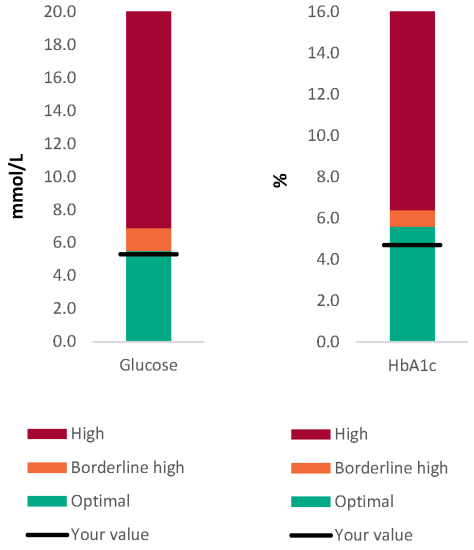
A high blood pressure reading can be dangerous as it puts extra strain on your blood vessels, heart and other organs, such as the brain, kidneys and eyes. Having a continuously high blood pressure can lead to the development of conditions such as heart disease. If your blood pressure has been recorded as above 140 SBP or 90 DBP then we advise you to visit your GP / practice nurse to have another blood pressure reading taken.

Your cholesterol, triglycerides, and glucose values were as follows:

Total cholesterol:	4.23	mmol/L	Triglycerides:	0.97	mmol/L
HDL cholesterol:	0.84	mmol/L	Glucose:	5.31	mmol/L
LDL cholesterol:	2.94	mmol/L	HbA1c	4.70	%

If a value is missing from the report (labelled -99), the sample was deemed outside the reference range of the machine.
If you would like more information on this, then please get in contact with us.





Broadly speaking, there are two types of cholesterol: good and bad. The bad cholesterol that clogs-up the arteries is known as LDL-cholesterol. The good cholesterol that helps remove fatty deposits from the arteries is called HDL-cholesterol.

Blood sugar or plasma glucose levels is the amount of glucose in the blood and it usually stays within narrow limits when you are fasted. HbA1c is a measure of your blood glucose levels over the last 2-3 months.

Higher sugar levels than normal over time can lead to the multiple health problems associated with diabetes. If your glucose is outside of normal range, it would be advisable to visit a clinic and/or your GP and have your glucose re-checked.

Generally speaking high levels of HDL and low levels for LDL, triglycerides and glucose is better for your health. Poor cardiovascular health, such as consistently high glucose levels, can lead to the development of conditions such as Type 2 diabetes. In order to achieve this, consider the following lifestyle changes: become more active, lose weight, exercise, reduce intake of saturated fat and cholesterol, eat oily fish and eat more fruit and vegetables.

Aerobic Fitness

Aerobic fitness reflects the level of function of the lungs, heart, blood vessels and muscles in your body.

You managed to get to stage: 3 and based on this value we can work out your health benefit zone.

Your health benefit zone: 280

Rating	Female Health Benefit Zones			
	30-39	40 - 49	50 - 59	60 - 69
Excellent	454 +	400 +	366 +	358 +
Very good	401 - 453	351 - 399	340 - 365	328 - 357
Good	360 - 400	319 - 350	310 - 339	296 - 327
Fair	330 - 359	271 - 318	246 - 309	235 - 295
Needs improvement	< 330	< 271	< 246	< 235

Low aerobic fitness is a strong predictor of heart disease risk, even in healthy middle-aged people.

Note that being in the 'Good' category is not necessarily optimal; it is important to consider that these norms are based on a population that is largely sedentary. If you want to improve your longevity, your best bet is to aspire to the 'Very good or Excellent' categories. Occasionally, sedentary people score well on aerobic fitness tests; however, naturally fit people are rare, and are not protected from heart disease.

Upper body strength

Muscular strength refers to the maximum force that can be generated by a specific muscle or muscle group. The most common field measure is grip strength which is related to independent living and disability as we age.

Your grip strength was: 65 Kg (largest left + right hand values combined)

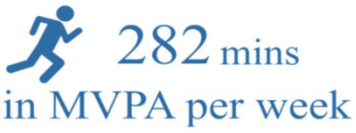
Rating	Female Grip Strength Kg			
	30-39	40 - 49	50 - 59	60 - 69
Excellent	≥ 71	≥ 69	≥ 61	≥ 54
Very good	63 - 70	61 - 68	54 - 60	48 - 53
Good	58 - 62	54 - 60	49 - 53	45 - 47
Fair	51 - 57	49 - 53	45 - 48	41 - 44
Needs improvement	≤ 50	≤ 48	≤ 44	≤ 40

You can look up your score in the table to help interpret your results based on your age. A minimal level of muscular strength is needed to perform certain daily tasks. Additional strength above the minimum provides a reserve capacity and offers protection from functional limitations and increases your capacity to live independently.

Grip strength generally decreases with age and can be used for early screening of populations to determine those at higher risk of physical disability related to low muscle strength.

Physical activity

We have analysed the data from the activity monitor that you wore for 14 days. Please find below an overview of your results. We have broken down your data into the amount of time spent in 3 intensity categories: sedentary (sitting), light physical activity and moderate-to-vigorous physical activity (MVPA). Generally speaking, the more MVPA and less Sedentary time, the better your activity profile.



The above data will not be accurate if you removed the device during waking hours and/or participated in non-ambulatory activities, such as swimming and cycling. Also, your MVPA guideline tile is for time spent in MVPA of bouts lasting 10 minutes or more.

In general, we recommend that you minimise time spent sedentary (sitting) and become more physically active (e.g. brisk walking). Try and follow the notion of "some is good, and more is better" in order to increase your activity levels!

On behalf of the research team, thank you very much for your valued participation in this study. If you have any comments, questions, and/or concerns regarding this health report or your recent involvement in the study, please do not hesitate to contact me.

Sincerely,

Dale Esliger, PhD (Principle Investigator)
E-mail: paphrg@lboro.ac.uk

H. Chapter Five (Study Three) - Additional Methods

After arrival at the appointment, participants were reminded of the procedures and informed consent was taken. A health screen and Physical Activity Readiness Questionnaire (PAR-Q) (Warburton et al., 2013) was completed to assess whether the participant was safe to undertake the fitness test. Any positive answers required a qualified Exercise is Medicine qualified practitioner to screen the participant. After a period of at least 10 minutes rest, three blood pressure readings (Omron 705IT, Omron, UK) were taken in the upper left arm with at least 1 minute in-between readings. The correct cuff size for the monitor was chosen by placing a tape measure around the circumference of a supported flexed upper arm. Readings were taken with the participants feet flat, without talking and with the measurement readings hidden. The mean of the three measurements was used to describe systolic and diastolic blood pressure.

A hand-held dynamometer measured grip strength in both the dominant and non-dominant hands (Takeii analogue dynamometer, Takei Scientific Instruments Co., LTD, Japan). After taking in a breath, participants were instructed to squeeze the dynamometer by their side as hard as they could. When the needle ceased to move, a reading was taken by the study staff and then reset for the next measurement. A combined score of maximum attempts from both hands were used as a measure of grip strength.

A fasting (≥ 8 hour) capillary blood test was then taken to measure total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides and glucose using a point of care device (Lipid Profile•Glucose Cartridge, Cholestech LDX® Analyzer). Glycated haemoglobin (HbA1c) (mmol/mol and %) was also obtained using a separate device (Afinion HbA1c, Afinion Analyzer, Alere, Massachusetts, USA). Both have been tested for accuracy and against venous whole blood and has sufficient agreement against the reference method (serum) for total cholesterol (−1%), HDL cholesterol (−2%), triglycerides (0%), glucose (0%) (Alere, 2016b), and International Federation of Clinical Chemistry (IFCC) HbA1c Network laboratory secondary reference methods for HbA1c (95% Confidence intervals 0.97 to 1.02) (Alere, 2016a). LDL was estimated using the Friedewald formula (Friedewald et al., 1972):

LDL cholesterol = Total cholesterol – HDL cholesterol – Triglycerides/5

Equation H.1. The Friedewald formula that calculates low-density lipoprotein cholesterol (LDL) (Friedewald et al., 1972).

The hands of the participants were warmed using water prior to measurement and both measurements were taken from one draw site if possible. If sufficient blood had not been collected within 60 seconds, another site was used. Relevant optical/quality control checks were performed prior to testing and in accordance with the guidance from Alere. Height was measured by portable stadiometer (SECA 213, SECA, Germany) and weight, total body fat percentage and calculated body mass index (BMI) was obtained using a bio electrical impedance machine (Tanita MC780MA, Tanita, The Netherlands). Waist circumference was measured twice using a tape measure (HaB International Ltd, UK), around the mid-point between the lowest rib and iliac crest (World Health Organization, 2011b). A third measurement was taken if the difference was above 3cm and mean waist circumference was derived using the mean of two measurements or the closest two if an additional measurement was taken.

Quadriceps maximal voluntary contraction was measured using a leg extension machine (DAVID Health Solution Ltd., Finland) in a fixed 90 degree angle against a stationary ankle pad. Participants were secured by a lap belt and asked to not rise out of the seat whilst the measurement was undertaken. Both dominant and non-dominant legs were measured by asking the participants to place their hands in a cross fashion on their shoulders, to take a deep breath and to push as hard as they could on the pad for 5-6 seconds. A brief pause was taken in-between measurements and the highest score for both legs was recorded. The highest attempt from the dominant leg was used as the measure of leg strength. The Modified Canadian Aerobic Fitness Test (mCAFT) was used to assess submaximal aerobic capacity during the visit (CSEP, 2003). Participants were required to step up and down on a set of double steps (20.3cm each) for one or more 3 minute stages, keeping their time to a beat played on a laptop. Starting stages were predetermined based on age and sex and the end of the test was if their heart rate met a predefined range of 85% age predicted maximum (220−age). If they did not meet this level, the next stage was then started. Predicted maximum VO_2 was converted into an 'Aerobic fitness score' using the following equation and the oxygen cost for the last stage achieved:

Aerobic fitness score = 10*[17.2 + (1.29*O_2 Cost) – (0.09*Weightkg) – (0.18*age)]

Equation H.2. The equation that calculates an aerobic fitness score from the The Modified Canadian Aerobic Fitness Test (mCAFT).

This corresponds to a health benefit zone that corresponds to age and gender specific categories of 'Needs improvement', 'Fair', 'Good', 'Very Good' and 'Excellent' (Figure H.1).

Health Benefit Zone from Aerobic Fitness Score

Age 15–19		
Zone	Males	Females
Excellent	574+	490+
Very Good	524–573	437–489
Good	488–523	395–436
Fair	436–487	368–394
Needs improvement	<436	<368
Age 20–29		
Zone	Males	Females
Excellent	556+	472+
Very Good	506–555	420–471
Good	472–505	378–419
Fair	416–471	350–377
Needs improvement	<416	<350
Age 30–39		
Zone	Males	Females
Excellent	488+	454+
Very Good	454–487	401–453
Good	401–453	360–400
Fair	337–400	330–359
Needs improvement	<337	<330
Age 40–49		
Zone	Males	Females
Excellent	470+	400+
Very Good	427–469	351–399
Good	355–426	319–350
Fair	319–354	271–318
Needs improvement	<319	<271
Age 50–59		
Zone	Males	Females
Excellent	418+	366+
Very Good	365–417	340–365
Good	301–364	310–339
Fair	260–300	246–309
Needs improvement	<260	<246
Age 60–69		
Zone	Males	Females
Excellent	384+	358+
Very Good	328–383	328–357
Good	287–327	296–327
Fair	235–286	235–295
Needs improvement	<235	<235

Figure H.1. The corresponding health benefit zones from the Modified Canadian Aerobic Fitness Test (mCAFT).

Glucose data processing

After the devices were returned, the glucose information was downloaded using the Freestyle Libre standard software Version 1 (Abbott Laboratories, Illinois, USA). The data had to be then processed manually using a combination of excel (Microsoft, Washington, USA) workbooks before being in a suitable format for analysis. In its raw format (.txt) the data matrix consists of unique scan ID number, a combined date and time column, record type and

the glucose value. The Freestyle Libre further divides the data points by record type and most commonly they are either a 0 which indicates an automatic ('Historic') scan or a 1 which indicates a user ('Scan') glucose reading. As each user scan could be at varying time points, only the automatic scans were kept within each file to leave a dataset that had data points occurring at equal time points (15 minutes).

If the participant had not scanned their device within the 8 hour time window, data would be missing in the data file. When the device was scanned again, data from the last 8 hours would then be transferred onto the reader. The missing data represents the time between the last download information and the first data point in the 8 hour time window. Missing data were coded within the dataset under the following rules:

- The data point was confirmed missing from the raw .txt file and if there was a corresponding code 'user' scan point (scan type 1) within ± 3 minutes of the data point, this was replaced in the dataset.
- If missing values were below or equal to two adjacent data points, data were replaced using linear interpolation in SPSS (IBM, NY, USA). A decision was made that two points represents 30 minutes in the data and over 45 minutes glucose could introduce too much error.
- If two data points were missing in succession, the points were coded as missing.

A 'wear tab' was generated for each file that described the detail of each file (Figure H.2). This allowed for the generation of a valid day statistic. There is no current definition for a valid day of CGM and therefore the approach taken in this study was conservative to reduce potential variability. As CGM produces a continuous signal, any breaks in the data may not reflect the true variability of biological data. To ensure any missing data did not influence the results, only data points in continuous blocks (after missing data processing) were carried forward for evaluation. In the below example (Figure H.3), the data from block B would be carried forward into the analysis as there is a gap of 4 missing values and the block is larger than block A.

For a day to be valid the file had to have ≥ 90% of data in the largest block to be included. This decision was made in order to be sure that a true representation of glucose parameters were evaluated against physical activity. The largest block of data points were then analysed

using EasyGV software to calculate measures of glycaemic variability which included mean glucose (MGluc), StDevG and mean amplitude of glycaemic excursions (MAGE) (Hill, 2010).

Dates	Day Datapoints (n)	Day Datapoints (%)	Code 1 replacements (n)	Missing (n)	Interpolated (n)	Total points	Largest block (n)	Largest block %	First largest continuous block data point	Last largest continuous block datapoint	Coding	Day	Comments
26/05/2016	48	50	0	48	0	96	48	50.0	12:04:00	23:50:00	H	Day 1	First day
27/05/2016	96	100	0	0	0	96	96	100.0	00:05:00	23:49:00	F	Day 2	
28/05/2016	76	79	0	20	0	96	76	79.2	00:04:00	18:50:00	F	Day 3	Did not scan for 8 hours
29/05/2016	91	95	0	5	0	96	91	94.8	01:19:00	23:49:00	F	Day 4	Did not scan for 8 hours
30/05/2016	96	100	0	0	0	96	96	100.0	00:04:00	23:49:00	F	Day 5	
31/05/2016	96	100	0	0	0	96	96	100.0	00:04:00	23:48:00	F	Day 6	
01/06/2016	96	100	0	0	0	96	96	100.0	00:03:00	23:48:00	F	Day 7	
02/06/2016	96	100	0	0	0	96	96	100.0	00:03:00	23:48:00	F	Day 8	
03/06/2016	96	100	0	0	0	96	96	100.0	00:03:00	23:47:00	F	Day 9	
04/06/2016	96	100	0	0	0	96	96	100.0	00:02:00	23:46:00	F	Day 10	
05/06/2016	96	100	0	0	0	96	96	100.0	00:01:00	23:46:00	F	Day 11	
06/06/2016	96	100	0	0	0	96	96	100.0	00:01:00	23:45:00	F	Day 12	
07/06/2016	97	101	0	0	0	97	97	101.0	00:00:00	23:59:00	F	Day 13	Additional is how time falls
08/06/2016	96	100	0	0	0	96	96	100.0	00:14:00	23:58:00	F	Day 14	
09/06/2016	44	46	0	52	0	96	44	45.8	00:13:00	10:58:00	H	Day 15	Last day

Possible comments:
Additional is how time falls
Did not scan for 8 hours
First day
Last day
Missing is how time falls
New sensor started
Sensor removed

Figure H.2. A weartab was created that outlined how many data points were missing, the largest block of data present for each day and the time points this occurred at.

Daily glycaemic variables were then matched with temporally ordered physical activity data and only if a day had valid glucose data (≥ 90%) and physical activity data (≥ 600 minutes), this information was then averaged to create 'per valid day' analysis. Additionally, to ensure a fair comparison, physical activity was recoded into new variables if there were a corresponding valid glucose values. These new variables were then used for regression analyses.

ID	Date	Time	Record Type	Historic Glucose (mmol/L)	Time difference	
194175	29/05/2016	01:19:00	0	5.6	00:14:00	
194176	29/05/2016	01:34:00	0	5.8	00:15:00	
194177	29/05/2016	01:49:00	0	6	00:15:00	
194178	29/05/2016	02:04:00	0	5.9	00:15:00	
194179	29/05/2016	02:19:00	0	6.1	00:15:00	A
194180	29/05/2016	02:34:00	0	6.3	00:15:00	
194181	29/05/2016	02:49:00	0	6.1	00:15:00	
194182	29/05/2016	03:04:00	0	6.1	00:15:00	
194183	29/05/2016	03:19:00	0	6.3	00:15:00	
194184	29/05/2016	03:34:00	0	6.5	00:15:00	
		03:49:00	-99		00:15:00	
		04:04:00	-99		00:15:00	
		04:19:00	-99		00:15:00	
		04:34:00	-99		00:15:00	
194189	29/05/2016	04:49:00	0	5.4	00:15:00	
194190	29/05/2016	05:04:00	0	5.6	00:15:00	
194191	29/05/2016	05:19:00	0	5.8	00:15:00	
194192	29/05/2016	05:34:00	0	5.3	00:15:00	
194193	29/05/2016	05:49:00	0	4.7	00:15:00	
194194	29/05/2016	06:04:00	0	4.6	00:15:00	
194195	29/05/2016	06:19:00	0	4.7	00:15:00	
194196	29/05/2016	06:34:00	0	4.8	00:15:00	B
194197	29/05/2016	06:49:00	0	4.7	00:15:00	
194198	29/05/2016	07:04:00	0	4.6	00:15:00	
194199	29/05/2016	07:19:00	0	4.8	00:15:00	
194200	29/05/2016	07:34:00	0	5.2	00:15:00	
194201	29/05/2016	07:49:00	0	5.2	00:15:00	
194202	29/05/2016	08:04:00	0	4.8	00:15:00	
194203	29/05/2016	08:19:00	0	4.7	00:15:00	

Figure H.3. The largest blocks of data were highlighted in each file.
In this example, Block B contains more data points than block A and is separated by 4 missing time points. Time block B would therefore be used to calculate valid days.