PERSONALISING APPROACHES TO REDUCING SEDENTARY BEHAVIOUR IN THE PROMOTION OF METABOLIC HEALTH: EXTENDING THE EVIDENCE BASE

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by

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Personalising approaches to reducing sedentary behaviour in the promotion of metabolic health: extending the evidence base - Matthew McCarthy

Thesis Abstract

Background

Sedentary behaviour has emerged as an independent risk factor for unfavourable metabolic health outcomes, including Type 2 Diabetes Mellitus (T2DM). Through an acute experimental setting, it has recently been demonstrated that breaking up sitting time through short, regular light physical activity breaks (in the form of standing, walking and resistance exercise) can be an effective way to reduce postprandial glycaemia. In order to embark on a personalised approach to reducing sedentary behaviour, more information is required about how other health behaviours interact with it, and whether alternative modes of activity breaks can instigate glycaemic improvements. It is also apparent that little is known about the chronic glycaemic impacts of reducing sedentary time when accounting for other health behaviours, this warrants further exploration.

Aims

1. Design and conduct an experimental trial establishing the modifying impact of cardio-respiratory fitness (CRF) on glycaemic responses to prolonged sitting and light activity breaks.
2. Design and conduct an experimental trial establishing whether breaking up sedentary time with activity breaks, while remaining in a seated posture, is an effective way to attenuate postprandial glycaemia in those at high risk.
3. Prospectively determine whether reductions in sedentary time are associated with long-term glycaemic benefit in those at high risk when accounting for other health behaviours such as weight management and exercise.

Key Findings

1. Individuals with low CRF had worse glycaemic responses during prolonged sitting and gained the most metabolic benefit from light activity breaks.
2. Performing regular, short bouts of upper body activity during prolonged sitting effectively reduced postprandial glycaemia despite maintaining a seated posture.
3. Change in sedentary time was not significantly associated with change in HbA1c after adjustment for change in MVPA time.

Conclusions

Efforts to tackle sedentary behaviour in the promotion of metabolic health may be optimised by tailoring to an individual's CRF level. This and the notion that sitting *per se* may not be responsible for the metabolic downfalls of sedentary behaviour demonstrates potential for personalisation of strategies in those with contraindication to weight bearing activity. Future research directly extending acute trials through longer term interventions are necessary to truly elucidate whether glycaemic adaptations, such as a change in HbA1c, are evident from reductions in sedentary time over a prolonged period.

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List of Abbreviations

ADA	American Diabetes Association
ANOVA	Analysis of Variance
ARIC	Atherosclerosis Risk in Communities
AUC	Area Under the Curve
BMI	Body Mass Index
CIs	Confidence Intervals
Cpm	Counts per minute
CRF	Cardio-Respiratory Fitness
CSV file	comma separated values File
CVD	Cardiovascular Disease
EE	Energy Expenditure
FDPS	Finnish Diabetes Prevention Study
GLUT- 4	Glucose Transporter Type 4
HbA1c	Glycated Haemoglobin
iAUC	Incremental Area Under the Curve
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IMD	Index of Multiple Deprivation
IQR	Interquartile Range
Kcal	Kilocalories
KJ	Kilojoules
LPRS	Leicester Practice Risk Score
METs	Metabolic equivalents
MVPA	Moderate to Vigorous Physical Activity
NHS	National Health Service
NIHR	National Institute for Health Research
OGTT	Oral Glucose Tolerance Test
RER	Respiratory Exchange Ratio
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
T2DM	Type 2 Diabetes Mellitus
UK	United Kingdom
USA	United States of America
VO_2	Volume of Oxygen
W	Watts
WHO	World Health Organisation
β	Beta Coefficient

Chapter One - Introduction and guide to PhD thesis

The information contained in this Chapter provides a detailed overview of the study area and forms the foundations from which the aims of this PhD have been derived.

The importance of Type 2 Diabetes prevention

Prevalence and financial burden

Worldwide it is estimated that 422 million people have diabetes (**NCD Risk Factor Collaboration, 2016**), the vast majority (90 to 95%) of which is attributed to type 2 diabetes (T2DM) (**Centres for Disease Control and Prevention, 2014)**. This figure is anticipated to escalate over the next few decades, reaching approximately 642 million by 2040 (**NCD Risk Factor Collaboration, 2016**). As of 2010, it was estimated that diabetes was costing health care systems across the world $376 billion per year. As a consequence of anticipated rises in the prevalence of diabetes, this annual figure is expected to soar up to $490 billion by 2030 (**Zhang et al., 2010**). In the United Kingdom (the location in which the studies documented in this thesis were conducted), over 4.5 million individuals (one in every 16) are thought to have Diabetes, 1.1 million of which are anticipated to be living undiagnosed (**Diabetes UK, 2016**). It is estimated that this figure will rise to approximately 6.3 million by 2035, causing costs to the National Health Service (NHS) to rise dramatically from £23.7 billion in 2011 to £39.8 billion in 2035 (**Hex et al., 2012**).

Health burden of T2DM and non-diabetic hyperglycaemia

Current diagnostic cut-points for T2DM purposefully reflect the point at which health risk becomes elevated, most notably through the onset of microvascular complications. There are numerous microvascular complications that stem from diabetes, for instance, diabetic retinopathy, which is one of the leading causes of blindness (**Klein et al., 2007**), and accounts for more than 10,000 new cases of blindness each year in the United States of America (USA) alone. Nephropathy is also among the leading causes of kidney failure in those with diabetes (**National Kidney and Urologic Disease Information Clearinghouse, 2008**), and neuropathy is considered to affect more than 50% of those with diabetes at some point throughout their lifespan (**Abbott et al., 2011**), and in severe cases can lead to amputation.

There are also numerous macrovascular complications that can arise as a result of diabetes. For instance, a threefold increase in risk of myocardial infarction has been reported in those diagnosed with T2DM (**Forbes & Cooper, 2013**), and given that cardiovascular disease (CVD) is the leading cause of mortality in those with T2DM (**Haffner et al., 1998; Laing et al., 2003**), this is a concerning statistic. Furthermore, patients with T2DM are thought to be at a 150-400% higher risk of having a stroke compared to those without this condition (**Fowler, 2008**).

Based on glycaemic parameters above normal but below diabetes thresholds, one in fifteen adults are thought to have what is referred to as 'pre-diabetes' (also referred to as non-diabetic hyperglycaemia) (**International Diabetes Federation, 2016**). Although the T2DM diagnostic cut-point deliberately sets out to reflect the point at which elevated blood glucose becomes a health risk (**World Health Organisation, 2006; The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997**), individuals such as those with pre-diabetes may also be vulnerable to microvascular and macrovascular complications resulting from higher than normal blood glucose levels. For instance, research has demonstrated the presence of retinal microvascular complications preceding diabetes (**Sörensen et al., 2016; Fong et al., 2004**), and in 2012 2.2 million pre-diabetic individuals died as a result of diabetes macrovascular complications, most notably CVD (**World Health Organisation, 2016**).

It is common for pre-diabetic individuals to experience elevations in postprandial glucose levels despite maintaining well-controlled fasting glucose, a situation that can last for years (**Muller-Wieland, 2007**). Postprandial glycaemia plays a pivotal role in the pathogenesis of CVD **(Peter et al., 2009)** and reducing sub-optimal postprandial glycaemia is thought to improve inflammation and endothelial function while reducing carotid intima-media thickness (**Blaak et al., 2012**). Targeting reductions in postprandial glucose has also been speculated to hold greater potential at offsetting metabolic health risks than efforts to improve fasting levels (**Cavalot et al., 2006**), and is emerging as a legitimate therapeutic target to minimise the risk of unfavourable metabolic health outcomes (**Peter et al., 2009**).

Looking to the future

With such a high prevalence of T2DM and pre-diabetes both in the UK and globally, alongside the unfavourable health and financial implications caused by these metabolic disorders, it is vital to promote healthy glucose control (especially through improvements in postprandial glycaemia). One strategy is through lifestyle modification.

The metabolic impacts of exercise, diet and weight management have been well researched (**Colberg et al., 2010; Anderson et al., 2003**) and efforts to increase moderate to vigorous physical activity (MVPA) levels, improve diet, and reduce body weight have formed the focal point of diabetes prevention programmes worldwide (**Hamman et al., 2016; Laaksonen et al., 2005; NHS England, 2015**). Despite this, another lifestyle behaviour that has recently emerged as an important risk factor for T2DM, and that has been the focus of this PhD thesis, is sedentary behaviour.

Introducing sedentary behaviour - The overlooked lifestyle factor

The term 'sedentary' comes from the Latin 'sedere' which translated means "to sit", and has been defined by the Sedentary Behaviour Research Network as "any waking behaviour characterised by an energy expenditure of ≤1.5 METs while in a sitting, reclining or lying posture" (**Tremblay et al., 2017**). One MET is the energy cost of resting quietly and is often standardised as equal to an oxygen uptake of 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ (**Ainsworth et al., 2011**). Subsequent MET values above 1.0 symbolise the intensity of an activity as a multiple of this basal metabolic rate. Although research broadly supports this definition for categorising sedentary behaviours, certain activities such as 'playing video games' or 'typing on a computer', despite commonly featuring in sedentary behaviour questionnaires may in fact exceed the 1.5 MET threshold (**Mansoubi et al., 2015**). Given that body composition can significantly contribute to oxygen uptake, a standardised resting oxygen uptake of 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ has also faced criticism due to potential individual differences in MET levels ascribed to certain activities (**Byrne et al., 2005**). Although future research could benefit from a more nuanced discussion regarding the 1.5 MET threshold, it is still broadly accepted (**Mansoubi et al., 2015**) and succeeds in demonstrating the low energy demand of behaviours classed as sedentary.

Common examples according to the 'Compendium of Physical Activities' are: watching television, reading and writing (**Ainsworth et al., 2011**).

In recent decades, enhancement in technology and human efficiency have led to significant reductions in human movement throughout occupations, leisure time and travel (**Ng & Popkin, 2012**), resulting in a significant increase in daily sedentary time. Specifically, adults in developed countries now spend between 55 - 70 % of their waking time in sedentary behaviours (approximately 8.8–11.2 hours/day assuming 8 hours of sleep) (**Matthews et al., 2008; Colley et al., 2011; Hagstromer et al., 2010; Aresu et al., 2009**). This is not what the human body was evolved for and we are essentially becoming victims of our own efficiency.

Evolution of sedentary behaviour

A substantial chunk of human evolutionary history (3 – 4 million years) was spent as hunter-gatherers requiring large feats of daily endurance in order to withstand environmental pressures, and it was this high physical demand to survive that is thought to have led to the emergence of the bipedal gait that we possess today (**Cordain et al., 1998**). Reductions in human movement over recent decades mean that modern lifestyle has now become discordant, and is no longer conducive, to the hunter-gatherer lifestyle that we physiologically evolved for (**Katzmarzyk, 2010**). The high levels of sitting that modern humans engage in are therefore the extreme opposite of our evolutionary heritage and play a key factor in the modern epidemic of T2DM and related metabolic disorders.

Sedentary behaviour and cardio-metabolic outcomes (epidemiology research)

Over the past decade there has been an abundance of research published examining the association between sedentary behaviours and health (**Wilmot et al., 2012; Biswas et al., 2015; Edwardson et al., 2012**). For instance, a meta-analysis by Wilmot et al (2012), which incorporated data from over 794,000 participants, revealed that those who engaged in the most sedentary behaviour (compared to those who engaged in the least) were at a 112% increased risk of developing T2DM, had a 147% increased risk of cardiovascular events, a 90% increased risk of cardiovascular mortality and a 49% increased risk of all-cause mortality. Subsequent meta-analyses have confirmed these associations (**Biswas et al., 2015**).

The links between sedentary time with T2DM and CVD are further supported by Edwardson et al (2012), who incorporated data from over 21,000 participants and revealed 73% increased odds of developing metabolic syndrome (defined by a cluster of T2DM and CVD risk factors (**Kaur, 2014**)), for those with the greatest sedentary time compared to those with the least. The significant associations between sedentary time with T2DM and CVD persisted following adjustment for MVPA **(Wilmot et al., 2012; Biswas et al., 2015; Edwardson et al., 2012)**.

A major limitation of epidemiological research examining associations between sedentary behaviour and health to date, is that they predominantly stem from cross-sectional observations (**De Rezende et al., 2014**). It is thought that further prospective analyses into the role of 'changes' in sedentary behaviour on health would provide further insight (**De Rezende et al., 2014**), especially in conjunction with lifestyle interventions purposefully aimed at manipulating sedentary time. In contrast, where prospective study designs do exist, they have predominantly relied upon self-reported measures of sedentary time (**Biswas et al., 2015; Wilmot et al., 2012**), which in concordance with self-reported behaviour in general, is likely to have poor validity (**Clark et al., 2009**) and could alter true associations with health outcomes. This highlights the need for prospective study designs that investigate the associations between objective measures of sedentary behaviour with health.

<u>Regulating postprandial glycaemia through regular breaks in sedentary behaviour - (exploring the experimental evidence base).</u>

As discussed previously, in conjunction with other mediating factors such as inflammation and haemodynamic shifts (**Carter et al., 2017**), it is likely that the links between sedentary behaviour and aforementioned risk factors are likely to be mediated 'in part' by postprandial glycaemia.

As such, efforts to address sedentary time and promote glycaemic control in a cost-effective, sustainable manner have become an important feature of experimental research.

A recent review of randomised experimental trials investigating the impacts of breaking up prolonged sitting time with physical activity breaks have shown promising improvements in postprandial glycaemia (**Dempsey et al., 2016a**).

In order to ensure no relevant experimental trials had been overlooked in this thesis, a brief literature review was conducted to find relevant randomised experimental trials that had been published since those outlined in the recent review by Dempsey et al (2016a). PubMed, Medline, and Google Scholar were searched for acute experimental sedentary interventions and were subsequently included in this thesis providing that they assessed postprandial glycaemic responses to a) prolonged sitting time 'AND' b) prolonged sitting interrupted with physical activity breaks. Our search strategy involved searching article abstracts and titles (from 2016 to present [01/08/2017]) for the following terms; "sedentary time", "sedentary behaviour/behavior", "postprandial sitting" OR "prolonged sitting" AND "experimental". This review of the literature revealed seven additional randomised trials that had not been reported in the review by Dempsey et al (2016a) (**Pulsford et al., 2016; Dempsey et al., 2016c; Crespo et al., 2016; Hawari et al., 2016; Bhammar et al., 2017**; **Duvivier et al., 2017a**; **Duvivier et al., 2017b** all of which have been acknowledged within this thesis.

Table 1.1 (adapted and updated from the review of experimental trials by Dempsey et al [2016a]), summarises the population characteristics, methodology and experimental findings of all relevant acute randomised trials believed to have been published to date (01/08/2017).

As shown in **Table 1.1**, breaking up prolonged sitting time with regular bouts of light intensity walking (**Bailey et al., 2015; Dempsey et al., 2016b; Dunstan et al., 2012; Henson et al., 2016; Larsen et al., 2015; Miyashita et al., 2016; Pulsford et al., 2016; Dempsey et al., 2016c; Van Dijk et al., 2013; Crespo et al., 2016**) and moderate intensity walking (**Dunstan et al., 2012; Peddie et al., 2013; Holmstrup et al., 2014; Bhammar et al., 2017)** have shown to significantly improve postprandial glycaemia in overweight and obese adults (**Dunstan et al., 2012; Larsen et al., 2015; Bhammar et al., 2017)**, in those with dysglycaemia (**Henson et al., 2016; Holmstrup et al., 2014; Crespo et al., 2016**), diagnosed T2DM (**Dempsey et al., 2016b; Dempsey et al., 2016c; Van dijk et al., 2013**)

and in some (**Bailey et al., 2015; Peddie et al., 2013; Pulsford et al., 2016**) but not all (**Miyashita et al., 2016**) healthy non-obese populations.

In contrast, while breaking up sitting time with upright (non-seated) resistance exercise appears promising in those with T2DM (**Dempsey et al., 2016b; Dempsey et al., 2016c**), the effectiveness of standing breaks remain controversial, with overweight/obese or dysglycaemic participants experiencing glycaemic benefit (**Henson et al., 2016; Thorp et al., 2014; Crespo et al., 2016**), yet studies with relatively-young, predominantly non-obese normoglycaemic individuals failing to show support (**Bailey et al., 2015; Miyashita et al., 2013; Pulsford et al., 2016; Hawari et al., 2016**).

It is speculated that breaking up sitting time with activities of greater intensity than standing may be necessary to elicit positive glycaemic responses in relatively young normoglycaemic individuals (**Hawari et al., 2016**), though efforts to increase the intensity of activity breaks beyond that of light intensity walking have not proven to be superior for everyone (**Dunstan et al., 2012)**. For instance, Dunstan et al (2012), who used an overweight/obese population actively broke up a 5 hour prolonged sitting period with 14 x 2 minute bouts of moderate intensity walking (one bout every 20 minutes) and found no additional glycaemic benefit compared to that of implementing light intensity walking in the same manner.

Several experimental studies have also investigated whether the pattern in which total sedentary time is reduced can affect the postprandial glycaemic response to activity breaks (**Miyashita et al., 2016; Peddie et al., 2013; Holmstrup et al., 2014**). These consistently show that breaking up postprandial sitting time with regular short bouts of walking (i.e. 12 x 5mins [**Holmstrup et al., 2014**], 20 x 1min 30sec [**Miyashita et al., 2016**] or 18 x 1min 40sec [**Peddie et al., 2013**]) have a more beneficial glycaemic impact than one continuous bout of walking (i.e. 1 x 60mins (**Holmstrup et al., 2014**), 1 x 30 mins (**Miyashita et al., 2016; Peddie et al., 2013**)), despite utilising the same total break duration. These observations continue to be supported by epidemiological findings which have shown an increased number of breaks in sedentary time (independent of total sedentary time) to be beneficially associated with numerous metabolic risk markers including that of 2 hour post-meal plasma glucose (**Healy et al., 2008**). A more

recent meta-analysis also supports the favourable associations between sedentary breaks and postprandial glycaemia (**Chastin et al., 2015**).

Although it is speculated that the type, intensity, and frequency of physical activity breaks necessary to alleviate the detrimental effects of prolonged sitting may differ according to an individual's characteristics (**Benatti & Reid-Larson, 2015**), the evidence base behind light intensity walking breaks appears promising, with glycaemic improvements observed across a diverse range of individuals. This is an important milestone in the regulation of metabolic health.

The experimental investigations documented above have derived from laboratory trials. Laboratory trials to date have provided a strong proof of concept for the glycaemic potential of breaking up prolonged sedentary time, but as pointed out by the American Heart Association, investigations to reduce sedentary time in 'free-living' conditions remain scarce (**Young et al., 2016**).

Recently, it has been demonstrated in free-living conditions that reallocating approximately 5 hours/day of sitting time with 3 hours of standing and 2 hours of light walking (when divided into smaller bouts throughout the day, and over a four day period) can lead to significant improvements in 24 hour glucose levels, and improve insulin sensitivity to a greater extent than breaking up sitting with over an hour of structured exercise throughout the day in those with T2DM (**Duvivier et al., 2017a**). Similar four day regimes that have focused on breaking up sitting time with standing and light walking while in a free-living environment have also been conducted in overweight/obese (**Duvivier et al., 2017b**) as well as young, healthy-weighted individuals (**Duvivier et al., 2013**). Compared to predominantly sedentary control conditions, these have led to improvements in insulin sensitivity (**Duvivier et al., 2017b**) and reductions in insulin AUC (**Duvivier et al., 2013**) resulting from OGTT's performed the morning after each four day regime. This supports the potential translation of glycaemic improvements observed in the laboratory to free-living environments in a range of individuals.

For more detail on the experimental investigations mentioned in this section, please see **Table 1.1** below.

Table 1.1 - Summary of randomised studies examining the acute glycaemic responses to prolonged sitting and frequent light activity breaks - (*adapted and updated from Dempsey et al [2016a]*).

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Altenburg et al (2013)	11 (5 men/6women) Age: 18 – 24 years. BMI mean (range): 23 kg/m^2 (20–26). Healthy. Activity level not reported. Setting: Laboratory.	Randomised cross-over. Washout between conditions ≥ 7 days. Glycaemic outcomes: Capillary plasma glucose (fasting/postprandial – hourly).	1. *Prolonged s*itting *only* (*SIT*) – 7 h. 2. *Prolonged* sitting + moderate-intensity activity breaks (*CYCLE*) –7 h (6 × 8 min bouts of cycling at hourly intervals–total 48 min cycling–each bout 40–60 % heart rate reserve). Diet: two standardised 'high-fat' mixed meals provided at 1 and 5 h consisting of 843 and 1190 kcal, 92 and 102 g carbohydrate, 29 and 77 g fat, 16 and 28 g protein, respectively.	*CYCLE vs. SIT* No significant differences in blood glucose.
Bailey et al (2014)	10 (7 men, 3 women). Age: 24 ± 3 years. BMI: 27 ± 4 kg/m^2. Healthy. Activity level–not reported. Setting: Laboratory.	Randomised cross-over. Washout between conditions ≥ 6 days. Glycaemic outcomes: Capillary plasma glucose (fasting/postprandial–hourly).	1. *Prolonged sitting only* (*SIT*) – 5 h. 2. Prolonged *sitting + light-intensity activity breaks* (*LW*) – 5 h (14 × 2 min bouts of light walking at 20 min intervals–total 28 min walking). 3. Prolonged *sitting + standing breaks (STAND)* –5 h (14 × 2 min bouts of standing still at 20 min intervals–total 28 min standing). Diet: 2 × meal replacement beverages at start of 5 h (total of 80.3 g carbohydrate, 50 g fat, nil protein).	*LW vs. SIT* ↓ 5-h blood glucose iAUC by 15.9 %. *STAND vs. SIT* No significant differences. *LW vs. STAND* ↓ 5-h blood glucose iAUC by 16.7 %.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Bhammar et al (2017)	10 (5 men, 5 women). Age: 32 ± 5. BMI: 30 ± 5 kg/m^2. Inactive. Setting: Laboratory.	Randomised cross-over ≥72 hour washout between conditions (average washout was 7 days) Glycaemic outcomes: Continuous blood glucose monitoring over an 18.7 hour period.	1. Prolonged sitting only (SIT) – 9h. (09:00 – 18:00). 2. Prolonged sitting + continuous moderate walking (30m MOD) - 1 x 30 min of moderate walking at 12:00. 3. Prolonged sitting + short moderate walking breaks (2m MOD) – 21 x 2 min of moderate walking at 20min intervals – total 42min. 4. Prolonged sitting + short vigorous walking breaks (2m VIG) –8 x 2 min of vigorous walking at 1 hour intervals – total 16min Diet: Standardised Breakfast - 678 kcal, 77% carbohydrate, 13% fat, and 10% protein. (Provided at 09:55) Standardised Lunch - 518 kcal, 53% carbohydrate, 31% fat, and 16% protein. (Provided at 13:50)	18.7h average blood glucose: SIT – 5.6 ± 1.1 $mmol \cdot L^{-1}$ 30m MOD – 5.1 ± 0.8 $mmol \cdot L^{-1}$ 2m MOD – 5.2 ± 1.1 $mmol \cdot L^{-1}$ 2m VIG – 5.4 ± 0.9 $mmol \cdot L^{-1}$ All experimental conditions showed significant reductions in glucose compared to SIT condition.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Crespo et al (2016)	9 adults (2 men, 7 woman) BMI: 29 ± 3kg/m^2. Age: 30 ± 15 years. Dysglycaemic. Insufficiently active (< 150 mins/week of moderate-intensity physical activity per week). Setting: Laboratory.	Randomised cross-over trial. Washout between conditions was exactly 7 days Glycaemic outcomes: 24 h continuous glucose monitoring at 5 minute intervals.	1. *Prolonged sitting only (SIT)* – 8 hours For the remaining conditions, participants were instructed to do physical activity for the following durations at each given time point: - 10 min at 08:50 and 09:50 h, - 15 min at 10:45 and 11:45 h, - 20 min at 12:40 and 13:20 h, - 30 min at 14:00 and 15:30 h. 2. *Prolonged sitting + standing bouts (STAND)* – 2.5 h of standing in total throughout the 8h 3. *Prolonged sitting + cycling bouts (CYCLE)* – 2.5h of cycling at 2 METS throughout the 8h 4. Prolonged sitting + light walking bouts (WALK) - 2.5 h of light treadmill walking at 2 throughout the 8h. Diet: 3 meals on test day (breakfast, lunch and dinner). Standardised Breakfast - 479 kcal, 70% carbohydrate, 19% fat, 11% protein (08:15) Standardised Lunch - 543 kcal, 57% carbohydrate, 26% fat, 17% protein (12:00) Dinner was also standardised the night after the 8 hour lab visits. Dinner consisted of 743 kcal (66% carbohydrate, 22% fat, 12% protein.	6 h postprandial glucose: STAND vs SIT= 7% reduction (not significant). WALK vs SIT = 24% reduction CYCLE vs SIT = 44% reduction Mean 24 hour glucose: STAND, WALK and CYCLE were significantly lower than SIT.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Dempsey et al (2016b)	24 (14 men, 10 women) with T2DM Age 62 ± 6 years. BMI 33 ± 3 kg/m^2. HbA1c 7.2 ± 0.7 %. Sedentary and insufficiently active. Setting: Laboratory.	Randomised cross-over. Washout between conditions ≥ 6 days. Glycaemic outcomes: Venous plasma glucose and serum insulin (fasting/postprandial – taken every 30 – 60 minutes).	1. *Prolonged sitting only* (*SIT*) –7 h. 2. *Prolonged sitting + light-intensity walking bouts* (*LW*) –7 h (12 × 3 min bouts of light walking at 30 min intervals–total 36 min light walking). 3. Prolonged *sitting + simple resistance activity bouts* (*SRA*) –7 h (12 × 3 min bouts (body-weight half-squats, calf raises, gluteal contractions) at 30-min intervals–total 36 min). Diet: dinner standardised night before trial. Standardised breakfast and lunch meals during trial to meet estimated energy requirements (50 % carbohydrate, 35 % fat, 15 % protein, mean kcal/meal was 823 ± 124 – issued at start and 3.5 h afterwards.	*LW vs. SIT* ↓ 7 h glucose iAUC by 39 %. ↓ 7 h insulin iAUC by 36 %. *SRA vs. SIT* ↓ 7 h glucose iAUC by 39 %. ↓ 7 h insulin iAUC by 37 %. LW vs. SRA No significant differences for blood glucose or insulin.
Dempsey et al (2016c)	24 (14 men, 10 women) with type 2 diabetes. Age 62 ± 6 years. BMI 33 ± 3 kg/m^2 HbA1c 7.2 ± 0.7 % Sedentary and insufficiently active	Randomised cross-over Washout between conditions ≥ 6 days. Glycaemic outcomes: 22 h of continued blood glucose monitoring.	1. *Prolonged sitting only (SIT)* – 7 h. 2. *Prolonged sitting + light-intensity walking bouts* (*LW*) – 7 h (12 × 3 min bouts of light walking at 30 min intervals–total 36 min light walking). 3. *Prolonged sitting + simple resistance activity bouts (SRA)* – 7 h (12 × 3 min bouts (body-weight half-squats, calf raises, gluteal contractions) at 30-min intervals–total 36 min). Diet: As per the above study (Dempsey et al [2016b]).	LW and SRA compared to SIT ↓ 22 h glucose and insulin

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Dunstan et al (2012)	19 (11 men, 8 women). Age 54 ± 5 years. BMI 31 ± 4 kg/m^2. Sedentary occupation. Insufficiently active. Setting: Laboratory.	Randomised cross-over. Washout between conditions ≥ 6 days. Glycaemic outcomes: Venous plasma glucose and serum insulin (fasting/postprandial - hourly).	1. *Prolonged Sitting only* (*SIT*) –5 h. 2. *Prolonged sitting + light-intensity walking bouts* (*LW*) –5 h (14 × 2 min bouts of light walking at 20-min intervals–total 28 min light walking). 3. *Prolonged sitting + moderate-intensity walking bouts* (*MW*) –5 h (14 × 2 min bouts of moderate walking at 20-min intervals–total 28 min moderate walking). Diet: standardised test drink (75 g carbohydrate, 50 g fat, nil protein - issued at start of 5 h).	*LW vs. SIT* ↓ 5 h glucose iAUC by 24 %. ↓ 5 h insulin iAUC by 23 %. *MW vs. SIT* ↓ 5 h glucose iAUC by 30 %. ↓ 5 h insulin iAUC by 23 %. *MW vs. LW* No significant differences for blood glucose or insulin.
Hawari et al (2016)	10 males. Age: 33 ± 13 years. BMI: 28.3 ± 3kg/m^2. Normoglycaemic. Insufficiently active. Setting: Laboratory.	Randomised cross-over Washout between conditions ≥ 7 days Glycaemic outcomes: Venous plasma glucose and serum insulin (fasting and postprandial – hourly, with additional sample taken 30 minutes following each meal).	1. SIT – Prolonged sitting only for 8 h 2. SIT-STAND - Across the 8 h period, participants required to stand for 15 min of each 30 min. Total 4 h stood and 4 h seated. 3. INTERVAL-STAND – Once again participants stood for 15 min and sat for 15 min every 30 min, but the standing occurred in 10 × 90 second blocks, rather than a single 15-min block. Diet: standardised mixed meals were issued for breakfast and lunch meals (4 hours apart) containing 8 kcal per kg body mass (37% energy from fat, 49% carbohydrates, 14% protein).	No significant differences between conditions.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Henson et al (2015)	22 post-menopausal dysglycaemic (impaired glucose tolerance [IGT]) women. Age 67 ± 5 years. BMI 33 ± 5 kg/m^2. Insufficiently active. Setting: Laboratory.	Balanced incomplete block design (participants randomised to complete 2 of the 3 conditions). Day 1 (*n* = 22): experimental condition (1, 2 or 3) in laboratory. Day 2 (*n* = 17): experimental condition (prolonged sitting) in laboratory. Washout between conditions ≥ 7 days. Glycaemic outcomes: Venous plasma glucose and insulin (fasting/postprandial – hourly with additional samples 30 mins after each meal).	Day 1: 1. *Prolonged sitting only* (*SIT*) –6.5 h. 2. *Prolonged sitting + 12 standing bouts (STAND)* – 5 min every 30 min, total 60 min. 3. *Prolonged sitting + 12 light-intensity walking bouts (LW)* –5 min every 30 min, total 60 min. Day 2: *Prolonged sitting only (SIT)* – 6.5 h. Diet: standardised breakfast and lunch mixed meals during experimental conditions (both 58 % fat, 26 % carbohydrate, 16 % protein).	<u>Day 1:</u> *STAND vs. SIT* ↓ 6.5 h glucose iAUC by 34 %. ↓ 6.5 h insulin iAUC by 20 %. *LW vs. SIT* ↓ 6.5 h glucose iAUC by 28 %. ↓ 6.5 h insulin iAUC by 37 %. *LW vs. STAND* No significant differences <u>Day 2:</u> *STAND day 1 vs. SIT* ↓ 6.5 h glucose AUC by 19 %. *LW day 1 vs. SIT* ↓ 6.5 h glucose AUC by 17 %. ↓ Insulin AUC by 24 %.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Duvivier et al (2017a)	19 (13 men, 6 women with T2DM. Age 63 ± 9 years. BMI 31 ± 3 kg/m^2. HbA1c 6.7 ± 0.8 %. Inactive Setting: Free living conditions.	Randomised cross-over. Washout between conditions - 10 days. Glycaemic outcomes: 24 h continuous glucose monitoring on 4^{th} day of each experimental condition. HOMA2-IR was also established using fasting glucose and insulin the day after final treatment condition (Day 5).	Each of the below conditions lasted 4 days. 24 hour glucose was obtained on the final day (Day 4). HOMA2-IR was observed the following day (Day 5). 1. *SIT* – 14 h sitting/ 1.6 h standing/ 0.9 h light walking. Av.4415 steps/ day. 2. Exercise (EXE) – 13 h sitting/ 1.6 h standing/ 1h light walking/ with an extra 1.1 h moderate to vigorous cycling (5.9 METS). Av.4823 steps/ day. 3. SIT-LESS – 8.9 h sitting/ 4.1 h standing/ 3.1 h light walking. Av.17,502 steps/day. Diet: standardised mixed meals provided for Dinner on Day 3 and all throughout Day 4 (day of glucose testing). Meals matched to energy requirements for each individual.	<u>24 h glucose iAUC:</u> SIT 1974 ± 324 min × mmol/l. EXE 1383 ± 194 min × mmol/l. SIT-LESS 1263 ± 189 min × mmol/l. Both experimental conditions showed significantly reduced 24 h iAUC compared to SIT. <u>HOMA2-IR:</u> SIT = 2.16 ± 0.26 EXE = 2.06 ± 0.28 SIT-LESS = 1.89 ± 0.26 SIT-LESS condition significantly reduced HOMA-IR compared to SIT. EXE condition did not.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Duvivier et al (2017b)	24 (13 men, 11 women). Age 64 ± 7 years. BMI 29 ± 2 kg/m^2. Inactive Setting: Free living conditions.	Randomised cross-over. Washout between conditions - 10 days. Glycaemic outcomes: OGTT the day after each 4 day experimental condition (Day 5 - morning).	Each of the below conditions lasted 4 days. 1. *SIT* – 13.5 h sitting/ 1.4 h standing/ 0.7 h light walking. Av.3228 steps/ day. 2. SIT-LESS – 7.6 h sitting/ 4.0 h standing/ 4.3 h light walking. Av.24,626 steps/day. Diet: Energy intake and macronutrient intake was kept consistent between experimental conditions.	Glucose iAUC during OGTT did not significantly differ between conditions for the entire cohort. However, Women experienced a significant 32% reduction during the SIT-LESS compared to SIT. Insulin AUC during OGTT was 20% lower in SIT-LESS. Insulin sensitivity measured by Matsuda-Index was 16% higher during SIT-LESS.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Duvivier et al (2013)	18 (2 men, 16 women). Age 21 ± 2 years. BMI 23 ± 3 kg/m^2. Healthy Setting: Free living conditions.	Randomised cross-over. Washout between conditions - 10 days. Glycaemic outcomes: OGTT the day after each 4 day experimental condition (Day 5 - morning).	Each of the below conditions lasted 4 days. 1. *SIT* – 13.6 h sitting/ 1.0 h standing/ 0.8 h active non-exercise time. Av.4324 steps/ day. 2. Exercise (EXE) – 12.7 h sitting/ 1.1 h standing/ 1.0 h active non-exercise time. Av.6,049 steps/day. (similar to previous condition but Includes 1 h of moderate to vigorous cycling). 3. SIT-LESS - 7.4 h sitting/ 3.1 h standing/ 4.9 h active non-exercise time. Av.27,590 steps/day. Diet: Participants instructed to consume the same caloric intake during each treatment condition and to maintain their usual dietary habits during the three activity regimes.	AUC for insulin during OGTT: SIT = 7752 ± 3014 mU•min/ml EXE = 8320 ± 5383 mU•min/ml SIT-LESS = 6727.3 ± 4329 mU•min/ml SIT-LESS had significant reductions in insulin AUC compared to EXE and SIT. SIT-LESS also had borderline significant improvements in insulin sensitivity (p = 0.051).

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Holmstrup et al (2014)	11 (8 men, 3 women). Age 25 ± 3 years. BMI 36 ± 3 kg/m^2. All had IFG. Activity level– All report light to moderate walking ≤5 times per week as main exercise. Setting: Laboratory.	Randomised cross-over. Washout between conditions: not reported. Glycaemic outcomes: Venous serum glucose and insulin (fasting/postprandial–10 min intervals).	1. *Prolonged sitting only* (*SIT*) –12 h 2. *Prolonged sitting + regular-activity breaks* (*MW*) – 12 h (5 min moderate walking bout at 60–65 % VO_2 max every hour–total of 60 min walking, 660 min sitting). 3. *Prolonged sitting + one continuous physical activity bout (EX)* –12 h (60 min moderate walking bout at 60–65 % VO_2 max, then sit at 660 min). Diet: six small liquid meals consumed every 2 h (~1046 kJ/meal; 65 % carbohydrate, 20 % fat, 15 % protein) across each condition.	*LW vs. SIT* ↓ 12 h insulin iAUC by 15 %. ↓ 2 h insulin iAUC across all time blocks. *LW vs. EX* ↓ 12 h glucose iAUC by 15 %.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Larsen et al (2015)	19 (11 men, 8 women). Age 57 ± 2 years (SE). BMI 33 ± 1 kg/m^2. Sedentary (>5 h/day sitting) and insufficiently active. Setting: Laboratory.	Randomised cross-over. 3 consecutive days per condition with assessment on days 1 and 3. Washout between conditions ≥ 12 days. Glycaemic outcomes: Venous plasma glucose and insulin (fasting/postprandial)	1. *Prolonged sitting only* (*SIT*) – 6 h/day x 3 consecutive days. 2. *Prolonged Sitting + light-intensity activity breaks* (*LW*) –17 × 2 min bouts of light walking at 20 min intervals (total 34 min light walking) x 3 consecutive days. Diet: 4 h meal tolerance test completed on days 1 and 3 of each condition (75 g carbohydrate, 50 g fat, protein – nill) issued after 1 hour of steady state sitting.	*LW vs. SIT* <u>Day 1</u> ↓ 4 h glucose iAUC by ∼32 %. ↓ 4 h insulin iAUC by ∼15 %. <u>Day 3</u> ↓ 4 h glucose iAUC by ∼31 %. ↓ 4 h insulin iAUC by ∼15 %.
Miyashita et al (2013)	15 men. Age 27 ± 2 years. BMI 23 ± 3 kg/m^2. Insufficiently active. Setting: Laboratory.	Randomised cross-over. Washout between conditions ≥7 days Glycaemic outcomes: Venous plasma glucose and insulin (fasting, postprandial)	1. *Prolonged Sitting only* (*SIT*) –7.5 h. 2. *Prolonged sitting + standing* (*STAND*) –7.5 h (6 × 45 min standing bouts–total standing duration 4.5 h). 3. *Prolonged sitting + one continuous walking bout* (*EX*) –7.5 h (30-min brisk walk at approximately 60% of their maximum heart rate). Diet: weighed food diaries completed before and on day 1 and replicated on subsequent trials (amounted to approximately 8.4 MJ/day; 59 % carbohydrate, 29 % fat, 12 % protein).	*STAND vs. SIT* No significant differences *EX vs. SIT* ↓ 6 h glucose AUC by 7 % *EX vs. STAND* No significant differences

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Miyashita et al (2016)	15 post-menopausal Japanese women. Age 69 ± 2 years. BMI 24 ± 3 kg/m^2. Healthy and physically inactive (self-reported). Setting: Laboratory.	Randomised cross-over. Washout between conditions ≥ 7 days. Glycaemic outcomes: Venous plasma glucose and insulin (fasting/postprandial – every 2 hours).	1. *Sitting* (*SIT*) –8 h 2. *Sitting + one continuous exercise bout* (*EX*) –8 h (first 1 h sit, then 30 min walking bout, then sit 6.5 h). 3. *Sitting + regular-walking bouts* (*LW*) –8 h [(first 1 h sit, then 20 × 1.5 min walking bouts at ∼15 min intervals (7× post breakfast, 13× post lunch)–total of 30 min walking. Diet: standardised breakfast (08:00) and lunch (11:00) mixed meals during experimental conditions (both 50 % carbohydrate, 35 % fat, 15 % protein).	*EX vs. SIT* No significant differences *LW vs. SIT* No significant differences *LW vs. EX* ↓ 8 h glucose iAUC and AUC
Peddie et al (2013)	70 (28 men, 42 women). Age 25 ± 5 years. BMI 24 ± 4 kg/m^2. Sedentary occupation. Insufficiently active. Setting: Laboratory.	Randomised cross-over. Washout between conditions > 6 days. Glycaemic outcomes: Venous plasma glucose and insulin (fasting/postprandial–hourly between baseline and 9 h; additional samples 30 and 45 min after each meal)	1. *Prolonged sitting* only (*SIT*) – 9 h 2. *Prolonged sitting + one continuous exercise bout* (*EX*) – 9 h (first 15 min sit, then 30 min walking bout, then sit 8.25 h) 3. *Prolonged sitting + regular-walking bouts* (*LW*) – 9 h [first 15 min sit, then 18 × 1min.40 seconds moderate walking (60 % maximal aerobic capacity) equally spaced over the 9 h period–total of 30 min walking]. Diet: meal replacement beverages provided at 1, 4 and 7 h (each meal consisting of 1.12 g carbohydrate, 0.46 g fat, 0.54 g protein per kg body mass).	*EX vs. SIT* No significant differences *LW vs. SIT* ↓ 9 h glucose iAUC by 39 % ↓ 9 h insulin iAUC by 26 % *LW vs. EX* ↓ 9 h glucose iAUC by 37 % ↓ 9 h insulin iAUC by 18 %

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Pulsford et al (2016)	25 males. Age 40 ± 12 years. BMI 26.1 ± 4 kg/m^2. Healthy and physically inactive. Setting: Laboratory.	Randomised cross-over. Washout between conditions ≥ 6 days. Glycaemic outcomes: Venous plasma glucose and serum insulin (fasting and postprandial – taken every 30 mins following the OGTT test (Meal 1), additional samples also taken at 10 and 20mins following meal. Blood samples were taken at the same time intervals following meal 2 [(with addition of 210 and 240 minutes post mixed test meal (Meal 2)].	1. *Prolonged sitting only* (*SIT*) –7 h. 2. *Prolonged sitting + standing (STAND)*– 7 h (21 × 2 min bouts of standing still at 20-min intervals–(total 42 min standing). 3. *Prolonged sitting + light-intensity walking bouts (LW)* – 7 h (21 × 2 min bouts of light walking at 20-min intervals–total 42 min light walking). Diet: Participants provided with two meals (10:00 and 13:00). Meal 1 – OGTT test with 435 ml of a standardised glucose drink containing 75g glucose. Issued at 10:00. Meal 2 - Mixed test meal with 0.3 g protein, 0.4 g fat, 1.2 g carbohydrate, and 39 kJ per kilogram body mass. Issued at 13:00.	*LW vs. SIT* ↓ 7 h glucose AUC by 9 %. ↓ 7 h insulin iAUC by 21 %. *STAND vs. SIT* No significant differences.

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Thorp et al (2014)	23 (17 men, 6 women). Age 48 ± 8 years. BMI 29.6 ± 4 kg/m^2. Sedentary (desk-bound occupation). Insufficiently active. Setting: laboratory designed to simulate office work conditions.	Randomised cross-over. Each experimental condition was performed for 5 days. Days 1 and 5: testing in laboratory after overnight fast. Washout between conditions >7 days. Glycaemic outcomes: Venous plasma glucose and serum insulin (fasting, postprandial)– 0, 60, 120, 180 and 240 min post-meal).	1. *Seated only work* (*SIT*) –instructed to sit to perform computer-based work tasks for 8 h (480 min) each day for 5 days. 2. *Seated and standing work* (*STAND*) –instructed to perform computer-based work tasks using a height-adjustable workstation, systematically interchanging between sitting and standing postures every 30 min over each 8 h workday–sitting 240 min, standing 240 min Diet: participants are provided with standardised energy-balanced mixed meals (breakfast, lunch, snack) daily (∼55 % carbohydrate, ∼30 % fat, ∼15 % protein).	*STAND vs. SIT* ↓ 4 h glucose iAUC by 11.1 % (average reduction observed between day 1 and day 2). No significant differences for insulin

Author's	Population/setting	Study design/outcomes	Experimental conditions	Glycaemic findings
Van Dijk et al (2013)	20 men with T2DM. Age 64 ± 1 years. BMI 30 ± 1 kg/m^2. HbA1c 6.9 ± 0.1 %. Activity level–not reported. Setting: Laboratory.	Randomised cross-over. Washout between conditions ≥ 7 days. Glycaemic outcomes: Venous plasma glucose and insulin collected 5 minutes before, and 90 and 150 minutes after each of the 3 meals (9 samples). 24 h continuous glucose monitoring also in place.	1. Prolonged *sitting only* (*SIT*) –12 h 2. Prolonged *sitting + ADL bouts* (*ADL*) –11.25 h (3 × 15 min bouts of slow paced strolling at 3 METS, 45 min after each meal–total 45 min) 3. Prolonged *sitting + exercise bout* (*EX*) –11.25 h (1 × 45 min bouts of moderate intensity cycling at 6 METS, 45 min after breakfast meal–total 45 min moderate-intensity exercise) Diet: standardised mixed meals provided at 08:30, 12:30 and 17:00 h (~9.8 MJ/day; 50 % carbohydrate, 35 % fat, 15 % protein).	*ADL vs. SIT* ↓ 10.5 h glucose iAUC by 19 %. ↓ 10.5 h insulin positive iAUC by 14 %. *EX vs. SIT* ↓ 10.5 h glucose iAUC by 36 %. ↓ 10.5 h insulin positive iAUC by 32 %. ↓ 24 h prevalence of hyperglycaemia by 30 %.

Chronic behaviour change

Despite the recent abundance of experimental research demonstrating the acute glycaemic benefits that can be achieved through reductions in sedentary time (as displayed in **Table 1.1**), little is known about whether these acute benefits would transfer to chronic improvements if implemented long term.

Workplace interventions that have successfully reduced sitting time through predominantly standing (**Healy et al., 2017; Alkhajah et al., 2012; Graves et al., 2015**) or ambulating (**John et al., 2011; Koepp et al., 2013**) over a 2 month (**Graves et al., 2015**), 3 month (**Healy et al., 2017; Alkhajah et al., 2012**), 6 month (**Koepp et al., 2013**) 9 month (**John et al., 2011**) and 12 month (**Healy et al., 2017; Koepp et al., 2013**) period, have only assessed blood glucose as an outcome measure while in a fasted state, and have failed to show improvement. Given that the glycaemic benefits of breaking up sedentary behaviour appear to be the most pronounced while in a postprandial state (**Dempsey et al., 2016a)**, it makes it difficult to distinguish whether the beneficial impacts observed throughout the acute experimental investigations can be translated to chronic changes and warrants further research.

Despite experiencing no improvements in fasting glucose *per se*, one study did report that fasting glucose was 'maintained' in those who significantly reduced their sitting time through standing, compared to the control group who experienced natural increases in fasting glucose while maintaining their current sitting habits (**Healy et al., 2017**). The ability to mitigate or prevent deterioration of fasting glucose through the displacement of sitting time with standing does have its merits, especially given the rapid escalation in global rates of diabetes (**NCD Risk Factor Collaboration, 2016**).

Although research to date assessing the chronic effects of reducing sedentary behaviour are generally discouraging, it may be that the displacement of sitting with standing and ambulation are not an intense enough stimuli to significantly reduce fasting blood glucose levels. This should not be used to dismiss potential chronic effects of reducing/ breaking up sedentary time, but should highlight the need for further investigation that focuses more on extending the acute research, with particular focus on postprandial (rather than fasting) glycaemic outcomes.

Sedentary behaviour guidelines

In light of the growing body of epidemiological and experimental evidence into the deleterious metabolic impacts of sedentary behaviour, countries worldwide have started to acknowledge the importance of reducing and breaking up sitting time in their physical activity guidelines (**Australian government – Department of Health, 2014; Canadian Society for Exercise Physiology, 2012; United Kingdom Government – Department of Health, 2011**). In July 2011, the United Kingdom joined Australia in providing public health guidelines encouraging adults (aged 19 – 64) to "minimise the amount of time spent being sedentary (sitting) for extended periods" (**United Kingdom Government – Department of Health, 2011**). Australian physical activity guidance expands upon this further by advising adults to break up prolonged bouts of sitting "as often as possible" **(Australian government – Department of Health, 2014)**. The acknowledgement of sedentary behaviour within physical activity guidelines is a good step towards raising awareness of this independent modifiable lifestyle risk factor, however, research revolving around sedentary behaviour still remains in its infancy compared to that of MVPA, and inevitably lacks detail in comparison. Guidance regarding MVPA specifies exactly how much is recommended (a minimum of 150 minutes of moderate or 75mins vigorous physical activity accumulated in minimum bout lengths of ≥10 minutes [**World Health Organisation, 2010]**), shows global consistency (**World Health Organisation, 2011**), and has been adapted and personalised to those with T2DM or pre-diabetes, who inevitably have different requirements (**Hodern et al, 2012**).

Although sedentary guidance currently lacks detail in adults, efforts have been made to quantify the exact amount of sedentary behaviour that is harmful to health in children and youth, urging individuals aged between 5-17 years old to limit screen time or general electronic media usage to no more than 2 hours per day (**Australian government – Department of Health, 2014; Canadian Society for Exercise Physiology, 2012**). Although this guidance is informative and goal driven, it has received criticism for being based upon expert opinion as opposed to experimental evidence (**Ekelund et al., 2010**), hence why such parameters are yet to be applied in adults. It is anticipated that as the evidence continues to accumulate, sedentary guidance will become more

widespread and consistent around the world and that it may become possible to refine guidance and provide more precise recommendations on par with those set for MVPA (**British Heart Foundation, 2012**). As it stands, there still remains limited data on precisely how much sedentary behaviour is too much and how sedentary behaviour is optimally reduced and broken up (**Lewis et al., 2016**).

An international group of experts have recently convened to form guidance specifically focused on addressing sitting time in the workplace (**Buckley et al., 2015**). Due to the rapid improvements in technology through the development of robots and machinery over recent decades, many manual labour jobs have become surplus to requirements. This has caused an inevitable shift away from physically active manual jobs towards predominantly sedentary service jobs. Precisely, service jobs now account for 80% of modern day occupations, a statistic that was as low as 50% in 1960 (**Church et al., 2011**). Given that a large portion of an individual's waking hours can be spent in the workplace, this has made a significant contribution to the high prevalence of sedentary behaviour. Guidance by Buckley et al (2015) specifies that predominantly desk based workers should aim to initially progress towards accumulating 2 h/day of standing and light activity (light walking) during working hours, eventually progressing to a total accumulation of 4 h/day. It is anticipated that this rough guidance will be refined in upcoming years as more evidence is published (**Buckley et al., 2015**) (I.e. through advances in chronic intervention research as mentioned in the previous section of this thesis).

A personalised approach to sedentary behaviour

It is anticipated that detailed guidelines (on par with those established for MVPA) are on the horizon (**British Heart Foundation, 2012**) and that efforts to reduce/regularly interrupt sedentary time will join MVPA and body weight as primary targets for diabetes prevention programmes. Given that it is unlikely that the 'one size fits all' guidance that is emerging to date will be appropriate for all individuals, it is important to embark on a personalised approach to sedentary guidance.

Introducing Primary Aim 1:

In order to embark on a personalised approach to sedentary guidance, research needs to establish the interaction that sedentary behaviour has with other health behaviours

and outcomes. One health outcome that is predominantly dictated by MVPA level is cardio-respiratory fitness (CRF) (**Bouchard et al., 1994**). CRF is one of the strongest determinants and predictors of overall health status, alongside future risk of morbidity and mortality (**Kodama et al., 2009**) and its modifying influence on sedentary behaviour is therefore an important consideration.

Although associations between sedentary time and metabolic health outcomes have shown to be 'independent' of habitual MVPA engagement (**Wilmot et al., 2012; Biswas et al., 2015; Edwardson et al., 2012**), this does not imply that MVPA has no 'modifying' influence over these associations. A recent epidemiological study comprising data from an international cohort in excess of one million people found that when stratifying their findings by quartiles of habitual MVPA, associations between sedentary time and all-cause mortality were significantly attenuated in those engaging in very high levels of MVPA (typically in the region of ≥ 60 mins/day), who are likely to have higher CRF levels (**Ekelund et al., 2016**).

This is consistent with other cross-sectional research that has shown the influence of sedentary time on a cluster of cardio-metabolic issues to be significantly less pertinent in those with higher fitness levels (**Cooper et al., 2014; Nauman et al., 2016; Shuval et al., 2014**), and shown significantly weaker associations between sedentary time with HbA1c (**Bakrania et al., 2016**) and inflammatory markers (**Henson et al., 2013**) in 'active', compared to 'inactive', individuals. A previous review of experimental interventions has also speculated that the effectiveness of interrupting sitting time with physical activity breaks may differ by the level of habitual physical activity an individual engages in (and consequently their CRF level) (**Benatti & Ried-Larsen, 2015**).

Despite strong emerging indications that CRF may play a protective role in offsetting the deleterious metabolic impacts of sedentary behaviour, this is yet to be tested through experimental design. Accordingly, the first aim of this PhD thesis is:

"to design and conduct an experimental trial establishing the modifying impact of cardio-respiratory fitness on glycaemic responses to prolonged sitting and light activity breaks".

If CRF offers protection against the metabolic impacts of high sitting time, this could have important practical implications. For instance, those who have 'unavoidable' sedentary occupations (i.e. long haul drivers) could potentially engage in MVPA outside of working hours to build CRF and reduce the harms of their occupational sitting time. Findings from this investigation also have the potential to inform diabetes prevention programmes and upcoming guidance regarding sedentary behaviour, which may benefit more from targeting lower fitness, less active populations.

Introducing Primary Aim 2:

In order to embark on a personalised approach to sedentary guidance, research also needs to establish more about the nature of efforts to break up sedentary behaviour. All successful experimental investigations that have broken up prolonged sitting time and reduced postprandial glycaemia have come in the form of upright (non-seated) strategies, namely standing, walking and upright resistance activity. Consequently, it is currently unknown whether or not these favourable postprandial improvements can be emulated through seated upper body activity breaks while maintaining a seated posture.

The mechanisms responsible for the observed metabolic improvements stemming from intermittent activity breaks (such as those detailed in **Table 1.1**) are not completely clear. It is speculated that increased substrate utilisation and the contraction of large muscle groups, especially during activity breaks involving ambulation, may play a key role. Muscular contraction increases blood flow and upregulates glucose transporter type 4 (GLUT-4) expression in a dose dependant manner, which helps to restore homeostasis of postprandial glycaemia (**Bauman et al., 2013; Sylow et al., 2016**). Skeletal muscle is also the largest insulin sensitive organ in the body, accounting for approximately 80% - 90% of insulin stimulated blood glucose disposal (**DeFronzo et al., 1981**). Consequently, the quiescent nature of major lower body musculature during seated upper body activities may negate the effectiveness of such strategies. Although greater upper body movement during sedentary time has been associated with better metabolic outcomes such as BMI and waist circumference (**Van Der Burg et al., 2014**), the effectiveness of seated upper body activity breaks at regulating postprandial

glycaemia has yet to be tested through experimental design. Accordingly, the second aim of this PhD thesis is:

"to design and conduct an experimental trial establishing whether breaking up sedentary time with upper body activity breaks, while remaining in a seated posture, is an effective way to attenuate postprandial glycaemia in those at high risk".

The findings from this research could have important clinical implications for those with weight bearing difficulty (i.e. wheelchair users or those with severe peripheral neuropathy) who may be able to regulate postprandial glycaemia while remaining seated, rather than simply reducing sitting time as guided to do so in physical activity guidelines as they stand at present (**Australian government – Department of Health, 2014; Canadian Society for Exercise Physiology, 2012; United Kingdom Government – Department of Health, 2011)**. Findings from this experimental investigation may also help to establish whether or not efforts to reduce sedentary behaviour should focus solely on breaking up the posture of sitting, or look more towards targeting muscular inactivity that co-exists with traditional sitting time.

Introducing Primary Aim 3:

As mentioned previously, a limitation of epidemiological research to date is that they predominantly use cross-sectional study designs to determine the associations between sedentary behaviour and health (**De Rezende et al., 2014**), making it difficult to infer direct causality. Where prospective study designs have been utilised, they have predominantly relied upon self-reported measures of sedentary time (**Biswas et al., 2015; Wilmot et al., 2012**) which also leads to questionable validity (**Clark et al., 2009**). Prospective epidemiological studies that investigate the associations between objectively measured sedentary behaviour with important health markers (such as HbA1c – as discussed below) are therefore warranted.

Since the inclusion of HbA1c within the diagnostic framework for T2DM (**World Health Organisation, 2011**), there has been a migration towards HbA1c in the classification of diabetes risk and in the assessment of diabetes prevention programmes run within routine care (**International Expert Committee, 2009; National Institute for Health and Clinical Excellence, 2016; NHS England, 2015**). HbA1c therefore acts as an important

health marker to assess the effectiveness of lifestyle modification. To date, the majority of research quantifying the metabolic impacts of lifestyle change have used fasting and 2 hour glucose as their glycaemic markers of interest (**Gong et al., 2015**), however with the emerged popularity of HbA1c, these are increasingly becoming outdated and no longer reflect clinical reality. It is also worth noting that sedentary behaviour has been broadly overlooked as a lifestyle component throughout research, with the main focus residing with changes in body weight, diet and MVPA levels (**Colberg et al., 2010; Anderson et al., 2003**).

Accordingly, the third aim of this PhD is:

"to prospectively determine whether reductions in sedentary time are associated with long-term glycaemic benefit in those at high-risk when accounting for other health behaviours such as weight management and exercise".

As mentioned previously, given that HbA1c is used in the classification of diabetes risk and used to determine the effectiveness of lifestyle interventions, an ability to manipulate this marker through reductions in sedentary time would provide an important rationale for targeting sedentary behaviour in future and existing diabetes prevention programmes.

Please see below a summary of all the primary experimental and epidemiological aims of this PhD thesis.

Summary of the Primary Research Aims

(Experimental):

1) Design and conduct an experimental trial establishing the modifying impact of cardio-respiratory fitness on glycaemic responses to prolonged sitting and light activity breaks.

2) Design and conduct an experimental trial establishing whether breaking up sedentary time with upper body activity breaks, while remaining in a seated posture, is an effective way to attenuate postprandial glycaemia in those at high-risk.

(Epidemiological):

3) Prospectively determine whether reductions in sedentary time are associated with long-term glycaemic benefit in those at high-risk when accounting for other health behaviours such as weight management and exercise.

The next Chapter of this thesis (Chapter Two) addresses the first aim of this programme of work, providing details of an experimental investigation that was conducted to determine whether an individual's cardio-respiratory fitness modified their glycaemic response to prolonged sitting and light activity breaks.

A publication that has stemmed from this research is referenced below. Full text of this publication is showcased later on in the thesis (**Appendix Five** – *Page 207*);

- McCarthy, M., Edwardson, C. L., Davies, M. J., Henson, J., Bodicoat, D. H., Khunti, K., Dunstan, DW., James, JA. and Yates, T., 2017. Fitness Moderates Glycemic Responses to Sitting and Light Activity Breaks. ***Medicine and Science in Sports and Exercise***. DOI: 10.1249/MSS.0000000000001338.

Chapter Two: Does cardio-respiratory fitness modify an individual's glycaemic response to prolonged sitting and light activity breaks? A randomised crossover trial.

ABSTRACT

Purpose: This study aimed to experimentally determine whether CRF modifies postprandial glycaemia during prolonged sitting and investigated the potentially blunting influence this may have upon the benefits of interrupting postprandial sitting time with light activity breaks.

Methods: Thirty-four adults (18 female; 16 male; mean ± SD age: 40 ± 9 years, BMI: 24.5 ± 3 kg/m^2) undertook two 7·5 hour experimental conditions in a randomised order: 1) Prolonged sitting; 2) Sitting interspersed with 5 min light walking bouts every 30 min. Blood samples were obtained while fasting and postprandially following ingestion of two identical meals. Incremental Area Under the Curve (iAUC) was calculated for glucose and insulin throughout experimental conditions. Maximal exercise testing quantified VO_2 peak as a measure of CRF. A repeated measures ANOVA investigated whether VO_2 peak modified glucose and insulin iAUC between conditions.

Results: Breaking sedentary time with light walking breaks reduced blood glucose iAUC from 3.89 ± 0.7 to 2·51 ± 0.7 mmol·L^{-1}·h (p = 0.015) and insulin iAUC from 241 ± 46 to 156 ± 24mU·L^{-1}·h (p = 0.013) after adjustment for VO_2 peak and sex. A significant interaction between treatment response and VO_2 peak was observed for glucose (p=0.035), but not insulin (p = 0.062), whereby the treatment effect reduced with higher CRF. Average blood glucose iAUC responses for a man at the 25th centile of CRF within our cohort (42.5 mL·kg^{-1}·min^{-1}) went from 5.80 to 2.98 mmol·L^{-1}·h during the prolonged sitting and light walking break conditions respectively, whereas average responses for a man at the 75th centile of CRF (60.5 mL·kg^{-1}·min^{-1}) went from 1.99 to 1.78 mmol·L^{-1}·h. Similar trends were observed for women.

Conclusion: Individuals with low CRF gained the most metabolic benefit from breaking prolonged sitting with regular bouts of light walking. In contrast, individuals with high CRF demonstrated healthier glycaemic responses during prolonged sitting and did not benefit as much from the implementation of light walking breaks.

INTRODUCTION

Given that sitting time encompasses a large portion of the average adults waking hours, (**Matthews et al., 2008; Colley et al., 2011; Hagstromer et al., 2010; Aresu et al., 2009**)), sedentary behaviour is the new reference of modern living. As highlighted previously, this is of great concern given that greater time spent in sedentary behaviours has been associated with an increased likelihood of; metabolic syndrome (**Edwardson et al., 2012**), diabetes (**Biswas et al., 2015; Wilmot et al., 2012**) , CVD and all-cause mortality (**Biswas et al., 2015; Wilmot et al., 2012**).

Recent epidemiological evidence however suggests that physical activity levels and CRF may moderate these associations, such that the association between sedentary time and markers or outcomes of health may be weaker in those with higher fitness levels (**Cooper et al., 2014; Nauman et al., 2016; Shuval et al., 2014**), or those undertaking greater physical activity (**Ekelund et al., 2016**). This suggests that sedentary behaviour may be a less important determinant of health in those with adequate CRF or those that are physically active. While experimental evidence largely confirms that breaking prolonged bouts of sitting with light-intensity walking has the potential to significantly reduce postprandial glycaemic outcomes (**Dempsey et al., 2016a**) in healthy non-obese individuals (**Bailey et al., 2015; Peddie et al., 2013; Pulsford et al., 2016**), in those who are overweight and obese (**Dunstan et al., 2012; Larsen et al., 2015; Bhammar et al., 2017**), and in those with dysglycaemia (**Henson et al., 2015; Holmstrup et al., 2014; Crespo et al., 2016**), no previous experimental trials have investigated whether these responses are modified by CRF or habitual physical activity levels.

CRF in particular is an important candidate for further investigation, as it is one of the strongest predictors of morbidity and mortality (**Kodama et al., 2009**). CRF has been shown to moderate the deleterious impacts of other exposures such as body mass index (BMI), whereby obese individuals with moderate to high CRF levels have a lower risk of morbidity and mortality outcomes compared to normal weighted individuals with low CRF levels (**Fogelholm, 2010**). It is therefore plausible that high levels of CRF may also protect against the deleterious impacts of prolonged sedentary behaviour**.** Therefore, we hypothesised that CRF would modify the postprandial glucose response to breaking

prolonged sitting with light walking breaks, whereby lower CRF levels would be associated with greater reductions to postprandial plasma glucose.

METHODS

Study Design

All participants attended the Leicester Diabetes Centre - UK, on three separate occasions between September 2014 and September 2015. The first visit involved consent, familiarisation and a fitness assessment which was followed by two experimental condition visits that were a minimum of seven days apart. This was a randomised cross-over trial whereby each participant took part in two experimental treatment conditions in a random order, thereby acting as their own controls. Order randomisation was conducted by a statistician using an online tool. Due to the nature of the trial, participants were not blinded to their randomised order, however all outcomes including blood assays were analysed blinded to the experimental condition that they derived from. Prior to commencing, this study received ethical approval from the University of Leicester - Health Sciences department and from the local NHS Research and Development committee.

This trial was also registered with ClinicalTrials.gov (NCT0493309).

Participants

Thirty six non-obese adults (BMI <30 kg/m^2) aged between 25 – 55 years old (inclusive) who worked in a predominantly seated environment were recruited from the general public via study-specific information distributed in the community, around the University of Leicester campus and University Hospitals of Leicester NHS Trust. Two individuals were withdrawn following enrolment in the study due to a change in personal circumstances (n = 2). This left 34 participants who went on to complete the remaining experimental conditions. This recruitment progression is detailed in **Figure 2.1.**

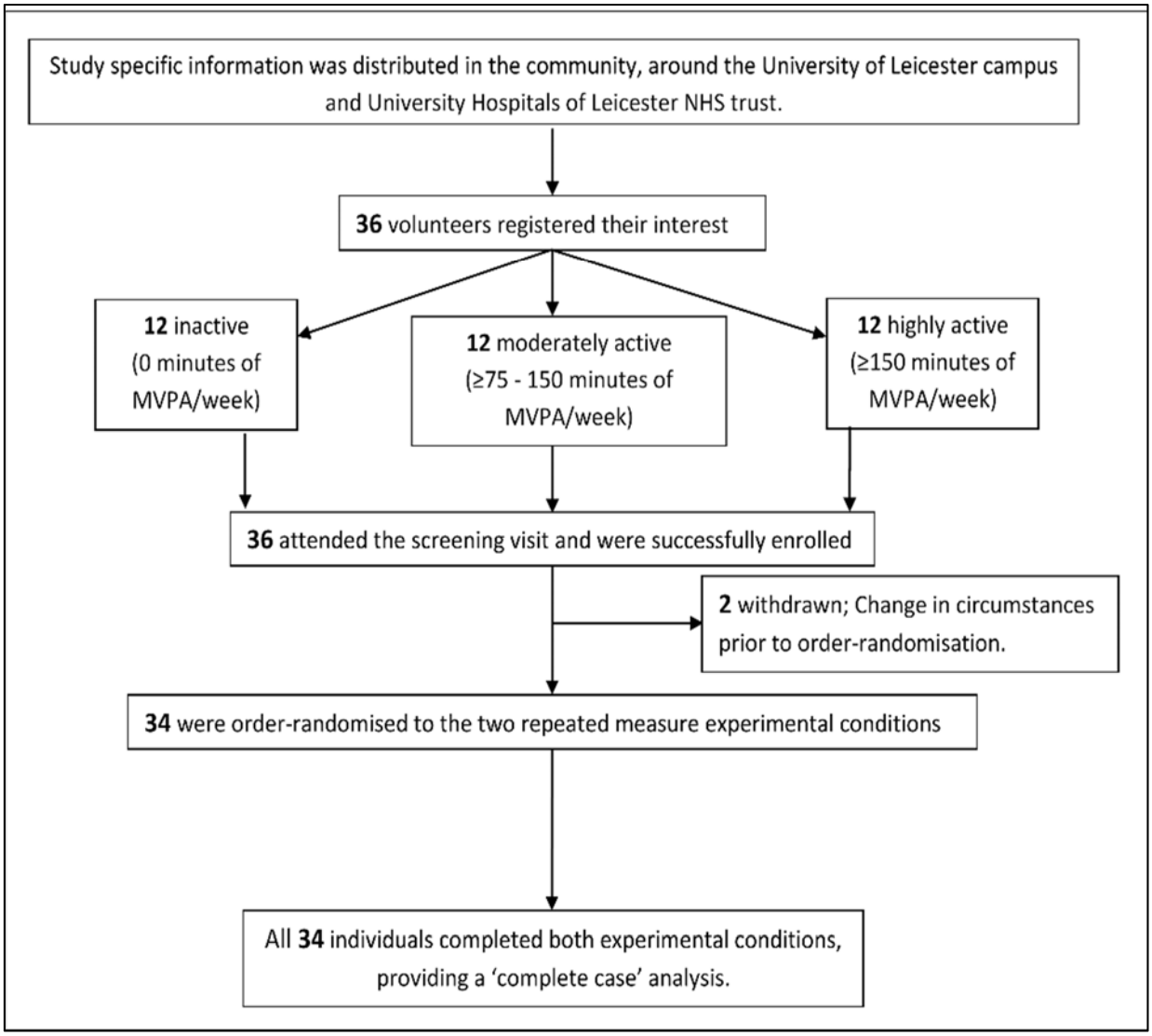


Figure 2.1 – Trial CONSORT Profile showing participant flow

Exclusion from taking part in this study came under the following circumstances; an inability to communicate in spoken English, a BMI ≥30 kg/m^2, pregnancy, steroid usage, regular smoking habits, diagnosed T2DM, CVD or psychotic illness. As our study was predicated on having a broad range of fitness levels, and considering that most of the variance in CRF is explained by habitual physical activity levels (**Bouchard et al., 1994**), we stratified recruitment by self-reported leisure time physical activity *(please see '**Appendix Three**' – page 162 for a copy of the Physical Activity Questionnaire that was used to determine this)*. Consequently, we enrolled 12 inactive (reporting 0 minutes of MVPA/week), 12 moderately active (reporting ≥75 minutes - <150 minutes of MVPA/week), and 12 highly active (reporting ≥150 minutes of MVPA/week) individuals. (*Please see **Table 2.1** for the scope of CRF levels captured in this cohort*).

Table 2.1: Relative VO_2 peak results for each participant in ascending order

Participant Number	VO_2 Peak ($mL \cdot kg^{-1} \cdot min^{-1}$)
1	25.07
2	26.45
3	31.48
4	31.58
5	32.21
6	32.4
7	32.58
8	32.92
9	33.31
10	33.85
11	34.23
12	34.29
13	37.64
14	38.98
15	39.5
16	39.82
17	41.26
18	41.42
19	41.96
20	44.15
21	47.25
22	48.39
23	48.5
24	48.97
25	50.48
26	52.18
27	53.55
28	57.03
29	57.6
30	61.53
31	62
32	68.1
33	73.39
34	76.85

Consent, familiarisation and fitness assessment visit

On arrival, a researcher described in detail all study procedures, systematically went through the participant information sheets, and obtained written informed consent Participants were then shown the designated experimental area for the study *(please*

*see '**Appendix Three**' for a copy of the participant information sheets (page 154) alongside the informed consent form (page 163) that was used here).*

A venous blood sample was taken to assess HbA1c and confirm absence of T2DM (<6·5% [<47·5 mmol/mol]) (**World Health Organisation, 2011**). Body weight (Tanita TBE 611: Tanita, West Drayton, UK), waist circumference (midpoint between lower costal margin and iliac crest) and height were measured to the nearest 0·1 kg, 0·5 cm and 0·5 cm, respectively.

In order to assess CRF, participants undertook a maximal incremental exercise test on a motor driven treadmill (Technogym Excite® 700). Following a three minute warm-up at 4 km/h (0% incline), participants would walk or jog at a constant speed that they felt comfortable with (from 6, 8, 10, or 12 km/h) while elevations in treadmill gradient occurred at a rate of 0·5% every 30 seconds. All participants received encouragement to continue this exercise for as long as possible. The test was terminated upon volitional exhaustion. Throughout the test, gas was sampled continuously and analysed using a Metalyser 3B gas analyser (Cortex 3B, Cortex Biophysik, Leipzig, Germany). Peak oxygen consumption (VO_2 peak) was calculated using the highest ten second average throughout the testing period. Before each test, the gas analyser was calibrated according to the manufacturer's recommendations. As a safety precaution, a 12 lead electrocardiogram was performed by a cardiac nurse for each participant at rest and during the exercise test.

Finally, participants were issued with two activity monitors; an ActiGraph GT3X+ accelerometer (Pensacola, FL) worn on the right anterior axillary line, and an activPAL3 physical activity monitor (PAL Technologies, Glasgow, UK) worn on the midline anterior portion of the right thigh. ActiGraph monitors were required to be worn continuously during 'waking' hours only, whereby ActivPAL monitors were required to be worn at all times of day. In total, participants were required to wear these monitors for seven consecutive days, allowing insight into their habitual sitting and physical activity levels.

<u>Experimental procedure</u>

Participants were asked to avoid alcohol and caffeine for the 48 hours preceding experimental treatment conditions. As the influence of an acute bout of physical activity

on insulin sensitivity can persist for 48 hours (**Holtz et al., 2008**), avoidance of MVPA for this timeframe was also instructed. Continuation in this study was subject to participants being able to confirm their compliance with these restrictions. Following an ethical amendment to the protocol during the course of this study, a subset of participants were asked to wear an accelerometer in the two days leading up to each experimental condition in order to confirm adherence to the MVPA restriction (See **Table 2.2** for activity data leading up to experimental conditions).

Participants fasted from 10pm the evening before each visit and were asked to keep a record of all food eaten during the day leading up to their first experimental condition. This could then be replicated prior to their second experimental condition in an attempt to eliminate the potentially confounding influence of pre-experimental food intake.

Participants underwent two separate 7·5 hour experimental treatment conditions:

1) Prolonged sitting - participants sat in a designated room (occupied with a desk, books, and laptop with internet services) while minimising excessive movement. Lavatory breaks were permitted using a wheelchair to and from the lavatory in order to further reduce unnecessary movements that could otherwise confound the study.

2) Light walking breaks - participants emulated the above, but interrupted sitting time with 5 minute bouts of walking at a light intensity of 3 km·hr on the treadmill (Technogym Excite® 700) every 30 minutes. These bouts were performed 12 times, totalling one hour of light activity and 6·5 hours of sitting throughout the course of the experimental day.

On arrival, participants had a cannula fitted into an accessible vein from which 10 mL samples were obtained throughout the day. Immediately following the two fasting samples (depicted at timepoints -1 and 0 in **Figure 2.2**), participants were given a standardised meal consisting of 8 kcal per kilogram of their body weight, with a macronutrient composition reflective of co-ingestion in modern western diets (14% protein, 51% carbohydrate and 35% fat). Once consumed (within ≤15 minutes), blood sampling commenced at 30, 60, 120, and 180 minutes thereafter, enabling us to capture the postprandial period. An identical meal was then issued (time point 3 in **Figure 2.2**) and sampling continued in a similar fashion at 30, 60, 120, 180, and 210 minutes

following this. It is worth noting that throughout the study, participants were supervised by study staff to ensure compliance with the protocol. Participants were also asked to wear an activPAL monitor to objectively confirm sitting and walking times during each experimental condition (See **Table 2.3** for sitting and walking data during experimental conditions). *Ad libitum* water consumption was also noted and made consistent between experimental conditions.

Biochemical analysis

Glucose (the primary outcome of this investigation) was analysed on the day of collection by the University Hospitals of Leicester pathology department, using standard enzymatic techniques with commercially available kits (Beckman, High Wycombe, UK).

Centrifuged (4^{o}C) plasma samples were stored in -80^{o}C freezers and insulin was analysed from these collectively at the end of the trial using an electrochemiluminescence assay (Meso Scale Discovery, Maryland, USA). Each sample was run in duplicate to ensure reliability of readings. Duplicate sample values with ≥20% variability were reanalysed. Ambient conditions of the laboratory were kept consistent in order to reduce undesired variability between assays.

Free-living activity monitor processing

ActivPAL data were downloaded using the manufacturers software (activPAL Professional Research Edition, PAL technologies, Glasgow, UK) and 'Event' csv files were processed using a validated automated algorithm in STATA (StataCorp LP, Texas, USA) which has been described in detail elsewhere (**Winkler et al., 2016**).

Actigraph data (100Hz) were downloaded using the manufacturer's software (ActiLife version 6·10·4, Lite Edition), reintegrated into 60 second epoch files and processed using a bespoke tool (KineSoft, version 3·3·76; KineSoft, New Brunswick, Canada [www.kinesoft.org]). Freedson cut points were used to categorise activity intensities (**Freedson et al., 1998**). Non-wear time was defined as a minimum of 60 minutes of continuous zero counts, and when assessing habitual activity levels, days with at least 10 hours of wear time were required in order to be considered valid.

The minimum amount of valid days utilised for both ActivPAL and ActiGraph data was three days.

Statistical analysis

Descriptive characteristics of those who completed this study are summarised overall (n = 34) and stratified by sex for descriptive purposes (please see **Table 2.4**).

Missing glucose and insulin data during the experimental conditions accounted for roughly 2% of overall required samples (34 out of 1,496) (please see **Table 2.5** for a summary of missing glucose and insulin data). These 34 missing data points were imputed using a regression model that used key predictors (BMI, ethnicity, age, fasting values and treatment condition) to derive a regression equation for the glucose and insulin values at each individual time point. This is a method that has been used previously in acute experimental research into sedentary behaviour (**Henson et al., 2015**).

The iAUC of glucose and insulin was calculated for each experimental condition. Total AUC was calculated by applying the trapezium rule and further subtraction of fasting levels gave a single value of iAUC for each participant. To be precise, the calculation of iAUC was computer generated in Microsoft Excel using a pre-validated formula used in previous research (**Henson et al., 2015**), this ensured accurate and consistent calculations throughout. Utilising iAUC as opposed to total AUC is common practice in acute interventions where fasting levels should remain unaffected by the intervention (**Le Floch et al., 1990**). Glucose iAUC was defined as the primary outcome prior to conducting this investigation. The effect of light walking breaks compared to continuous sitting on outcomes (glucose and insulin iAUC) and whether CRF modified this response was assessed using a repeated measures ANOVA. Treatment was entered as a within-person variable, with CRF (as a continuous variable) entered as a between-subjects covariate. Sex was also entered as a between-subjects factor. 'Treatment by CRF' and 'treatment by sex' interaction terms were investigated to assess the modifying effect of fitness and sex respectively. Sex was included in the model given that it is a strong determinant of fitness and an important potential confounder. Treatment by CRF interactions were further explored by calculating the linear regression coefficients within each treatment condition. To highlight the direction of significant interactions, derived average glucose iAUC values for men and women at the 25^{th}, 50^{th} and 75^{th} centile of the CRF distribution are shown in **Figure 2.3**.

A two-tailed p-value of ≤ 0.05 was considered significant. Analyses were performed with SPSS (version 24). Results are presented as mean ± SE or regression coefficient (95% CI) unless stated otherwise.

RESULTS

The key characteristics of those who successfully completed all three study visits are displayed in **Table 2.4** (n = 34). Stratification of these characteristics for both males and females are also presented here.

Table 2.4: Metabolic, demographic, and anthropometric characteristics taken at baseline

Baseline characteristics	Overall (n = 34)	Male (n = 16)	Female (n = 18)
Age (years)	41 (15)	35 (17)	43 (13)
BMI (kg/ m^2)	23·8 (6·1)	25·9 (5·1)	22·7 (4·6)
Body weight (kg)	66·5 (23·5)	82·2 (19·6)	59·9 (9·0)
Waist circumference (cm)	78·5 (13)	83 (12·8)	75 (10·0)
HbA1c (%)	5·3 (0·3)	5·3 (0·3)	5·3 (0·4)
HbA1c (mmol/mol)	34 (3)	34 (3)	34 (3)
Total cholesterol (mmol/l)	5 (1·2)	5·1 (1·2)	5·0 (1·2)
Ethnicity			
White European	26 [76·5]	13 [81]	13 [72]
Black and minority ethnic	8 [23·5]	3 [19]	5 [28]
ActiGraph derived accelerometer variables			
Habitual sedentary time (Av.mins/day)	564 (92)	547 (164)	595 (126)
Habitual MVPA time (Av.mins/day)	35 (28)	43 (47)	32 (21)
Accelerometer wear time (Av. mins/day)	892 (117)	899 (118)	890 (97)
Habitual Step counts (Av/day)	8048 (4551)	9048 (7030)	7451 (3941)
Fitness test			
Vo2 max ($mL \cdot kg^{-1} \cdot min^{-1}$)	41·3 (18·9)	50·3 (19·6)	34·0 (7·9)

Data are presented as median (Interquartile Range) or n [%]

Overall treatment condition effect

The average postprandial concentrations of glucose and insulin witnessed throughout the 7·5 hour testing periods for both experimental conditions ('prolonged sitting' and 'light walking breaks') are depicted in **Figure 2.2.** There was a significant main effect of treatment for both glucose ($F(1, 31) = 6.67$, $p = 0.015$) and insulin ($F(1, 31) = 7.00$, $p = 0.013$) iAUC after adjustment for fitness and sex. Interrupting prolonged sitting time with light walking breaks reduced blood glucose iAUC by 35% (from 3.89 ± 0.7 to 2·51 ± 0.7 mmol·L^{-1}·h) and insulin iAUC by 35% (from 241 ± 46 to 156 ± 24 mU·L^{-1}·h).

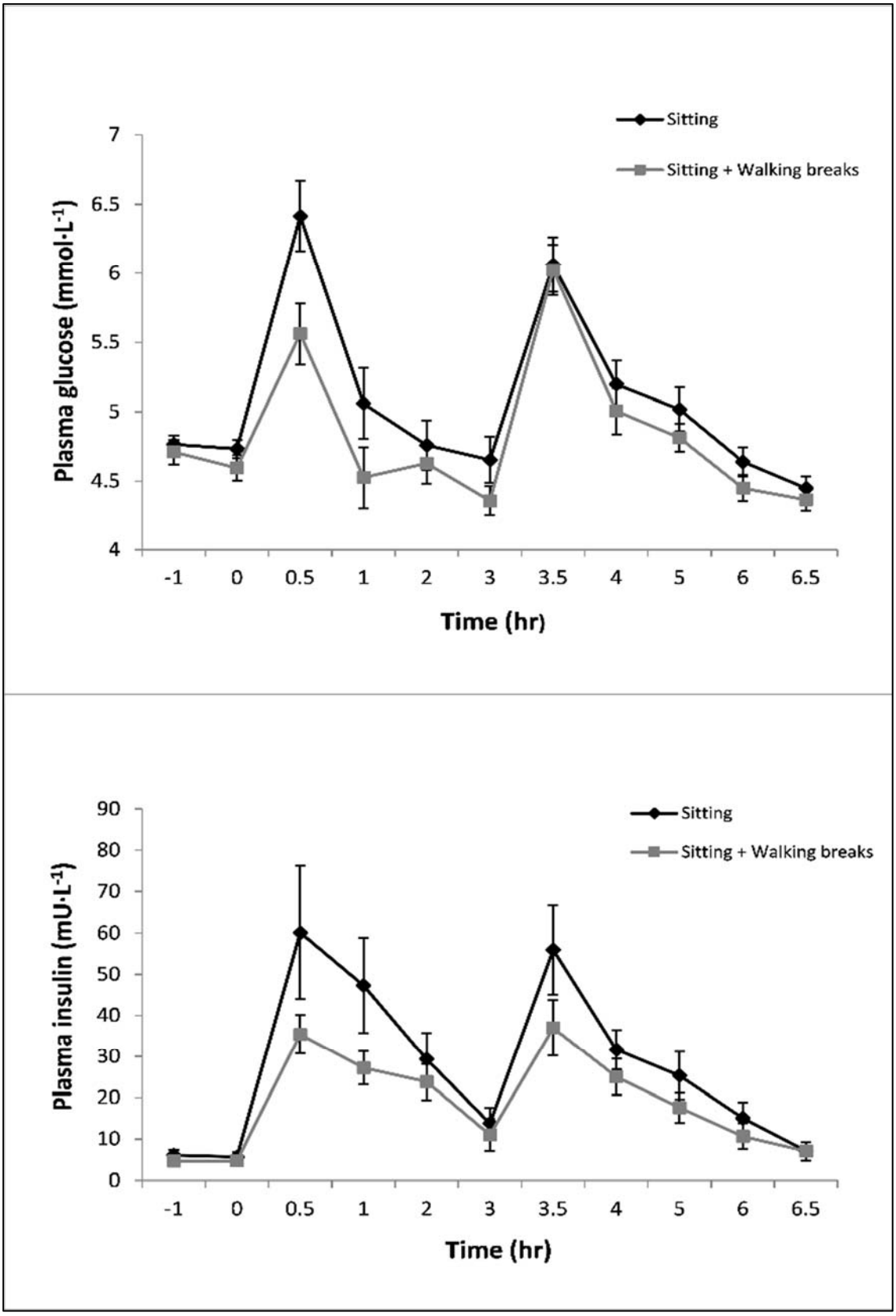


Figure 2.2 - Effect of treatment condition on average Blood Glucose and Insulin (*error bars represent standard deviations around the mean*)

Impact of CRF and sex

There was a significant treatment by CRF interaction for glucose iAUC (F (1, 31) = 4.89, p = 0.035). The treatment by CRF interaction for insulin iAUC failed to reach significance (F (1, 31) = 3.76, p = 0.062). There was no treatment by sex interaction for glucose (F (1, 31) = 1.77, p = 0.194) or insulin (F (1, 31) = 1.54, p = 0.223) iAUC.

Stratified analysis revealed that each unit increment in CRF (per $mL \cdot kg^{-1} \cdot min^{-1}$) was associated with a lower glucose iAUC (-0.21 $mmol \cdot L^{-1} \cdot h$; 95% CI -0.38, -0.05) (p = 0.013) in the prolonged sitting condition, whereas there was no association between CRF and glucose iAUC during the light walking breaks condition (-0.07 $mmol \cdot L^{-1} \cdot h$; -0.21, 0.07) (p = 0.335). In contrast, each unit increment in CRF was associated with a lower insulin iAUC (-10.93 $mU \cdot L^{-1} \cdot h$; -19.48, -2.37) (p = 0.014) in the prolonged sitting condition and a lower insulin iAUC (-6.35 $mU \cdot L^{-1} \cdot h$; -10.90, -1.83) (p = 0.007) in the light walking breaks condition.

For more insight into the spread of cardio-respiratory fitness levels and treatment response for this cohort, please see Supplementary figure 1 on page 119 of Appendix One.

Figure 2.3 uses the derived regression coefficients to show how the predicted average difference between conditions for glucose iAUC changes as CRF increases for males and females. This demonstrates that average blood glucose iAUC response for a man at the 25^{th} centile of CRF within our cohort went from 5.80 to 2.98 $mmol \cdot L^{-1} \cdot h$ (from prolonged sitting to light walking breaks, respectively), whereas average responses for a man at the 75^{th} centile went from 1.99 to 1.78 $mmol \cdot L^{-1} \cdot h$. Similar trends were observed for women.

Despite no significant treatment by age interaction (p = 0.303), Supplementary figure 2 shows derived regression coefficients demonstrating the predicted average difference between conditions for glucose iAUC changes as age increases for males and females. Consistent with the non-significant interaction, this shows less steep changes in treatment effect throughout the age tertiles for both males and females compared to figure 2.3.

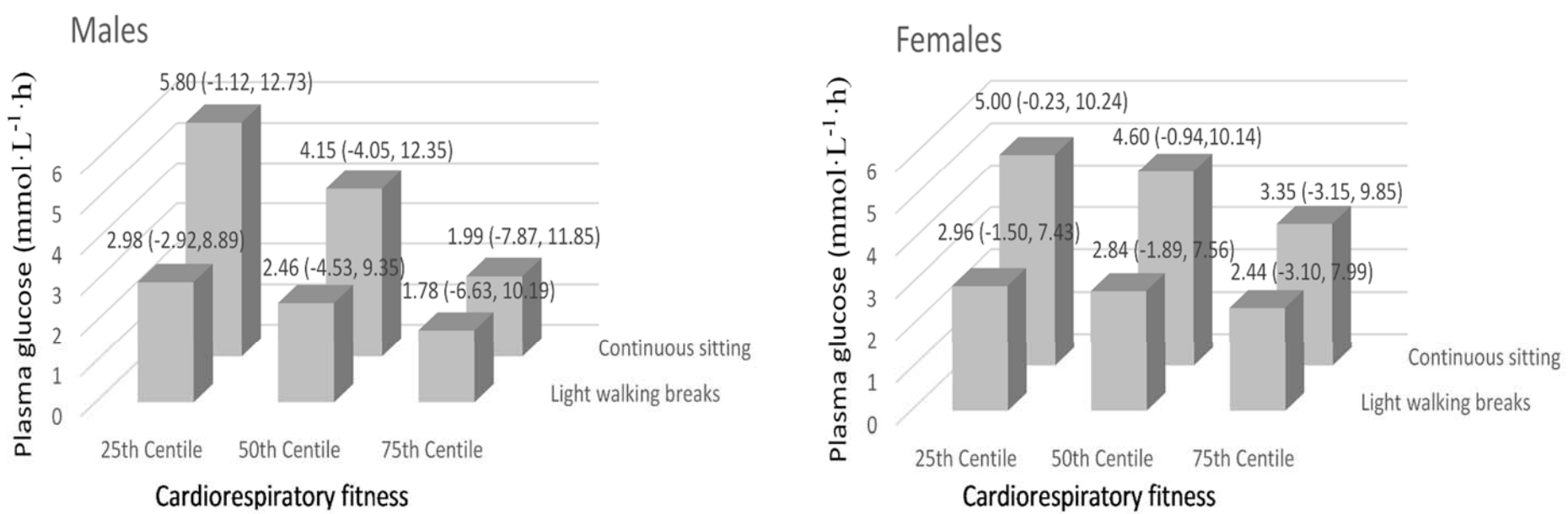


Figure 2.3 – Predicted glucose values between treatment conditions across sex-specific centiles of CRF.

25th centile of CRF corresponds to 42.5 mL·kg^{-1}·min^{-1} for Males, and 32.1 mL·kg^{-1}·min^{-1} for Females.

50th centile of CRF corresponds to 50.3 mL·kg^{-1}·min^{-1} for Males, and 34.0 mL·kg^{-1}·min^{-1} for Females.

75th centile of CRF corresponds to 60.5 mL·kg^{-1}·min^{-1} for Males, and 39.9 mL·kg^{-1}·min^{-1} for Females.

Predicted glucose iAUC values were derived from the below equations gained from linear regression models entering glucose iAUC within each condition as the dependant variable with CRF and sex entered as independent variables. 95%CI values show the variability around the derived estimates; negative values represent postprandial glucose concentrations that are suppressed below fasting levels. The derived glucose iAUC values and 95% CIs are within the range observed in this study (minimum observed glucose iAUC = -9.73 mmol·L^{-1}·h, maximum observed glucose iAUC = 16.50 mmol·L^{-1}·h)

Equations: Glucose iAUC during prolonged sitting condition = 11.81 - (0.21; 95% CI 0.05, 0.38) x CRF + 3.00 if male.

Glucose iAUC during walking breaks condition = 5.12 + (-0.07; 95% CI -0.21, 0.08) x CRF + 0.72 if male.

Table 2.2: Physical activity data before each experimental condition

	Mean ± SD						
	No. days accelerometer worn	Wear time (mins)	Sedentary (mins)	MVPA (mins)	Light activity (mins)	Steps	Movement counts
2 days prior to sitting condition (n=8)	2	707 ± 148	483 ± 106 68% of wear time	6 ± 8 1% of wear time	215 ± 89 30% of wear time	4002 ± 2503	130343 ± 84488
2 days prior to light breaks condition (n=8)	2	779 ± 106	498 ± 101 64% of wear time	8 ± 6 1% of wear time	273 ± 41 35% of wear time	5020 ± 1454	153823 ± 42594

Table 2.3: ActivPAL results during each experimental condition

	Average hours ± SD		
	Sitting	Standing	Walking
Sitting condition	7.9 ± 0.3	0.1 ± 0.1	0.00 ± 0.01
Light breaks condition	6.6 ± 0.5	0.2 ± 0.1	1.1 ± 0.02

Table 2.5: Missing data imputed via regression predictions

Participant Number	Sitting treatment condition		Light breaks treatment condition	
	No. missing Glucose samples (out of 11)	No. missing Insulin samples (out of 11)	No. missing Glucose samples (out of 11)	No. missing Insulin samples (out of 11)
1	-	1	-	-
2	-	-	-	-
3	-	-	1	2
4	1	1	-	-
5	1	1	-	-
6	-	-	-	-
7	-	-	1	-
8	-	-	-	-
9	-	-	-	1
10	-	-	-	-
11	1	-	-	-
12	-	-	-	-
13	-	-	-	-
14	-	-	-	-
15	-	-	1	2
16	-	1	-	-
17	-	-	-	-
18	-	-	-	-
19	-	-	-	-
20	-	-	-	-
21	1	1	1	1
22	1	-	-	-
23	-	-	-	-
24	1	1	-	-
25	-	-	-	-
26	-	-	-	-
27	-	-	-	-
28	-	-	1	2
29	-	-	-	-
30	-	-	-	-
31	-	-	-	-
32	1	1	1	1
33	2	2	-	-
34	1	-	-	-
Total	10	9	6	9

DISCUSSION

This study found that interrupting prolonged sitting with regular light walking breaks reduced postprandial glucose and insulin levels in a healthy cohort. However, CRF modified the response for glucose such that individuals with lower levels of fitness received incrementally greater reductions in postprandial glucose. For example, the average response for a man at the 25th centile of CRF within our population (VO_2 peak of 42.5 $mL \cdot kg^{-1} \cdot min^{-1}$) demonstrated relatively high postprandial glucose levels during prolonged sitting (5.80 $mmol \cdot L^{-1} \cdot h$) but was able to almost half this level through employing regular light walking breaks. In contrast, the average response for a man at the 75th centile of fitness (VO_2 peak of 60.5 $mL \cdot kg^{-1} \cdot min^{-1}$) demonstrated relatively low levels of postprandial glucose during prolonged sitting (1.99 $mmol \cdot L^{-1} \cdot h$) but only reduced this by a further 11% through employing regular light walking breaks. The same pattern was demonstrated for women. These results were supported by further analysis which demonstrated that CRF was inversely associated with postprandial glucose during prolonged sitting, whereby every unit increment in VO_2 peak (per $mL \cdot kg^{-1} \cdot min^{-1}$) was associated with an average reduction of 0.21 $mmol \cdot L^{-1} \cdot h$ in glucose iAUC values. Taken together, our results suggest that having high CRF or employing regular light walking breaks in those with low CRF can both reduce postprandial levels of glucose during periods of prolonged sitting. Elevated postprandial glucose levels are implicated with the development of T2DM and CVD (**Blaak et al., 2012**) and therefore strategies to promote healthy glycaemic responses when sedentary are of high importance.

Our observation that those with higher CRF demonstrate less metabolic benefit from light activity breaks is consistent with previous experimental research that has tended to show relatively lower metabolic benefits of light activity breaks in healthy cohorts (**Altenburg et al., 2013; Miyashita et al., 2016**) compared to those at high risk of chronic disease (**Dunstan et al., 2012; Henson et al., 2016**). Our findings also correspond to cross sectional research that has shown the influence of sedentary time on a cluster of cardio-metabolic issues to be significantly less pertinent in those with higher fitness levels (**Cooper et al., 2014; Nauman et al., 2016; Shuval et al., 2014**). The concept that fitter individuals may gain less pronounced health benefits from lower levels of sitting time is supported by cross-sectional research that have stratified data by habitual MVPA

level, finding that individuals with higher MVPA levels display significantly weaker associations between sedentary time with HbA1c (**Bakrania et al., 2016**), inflammation markers (**Henson et al., 2013**) and all-cause mortality (**Ekelund et al., 2016**).

In contrast, a recent meta-analysis found that the association between sedentary time and health outcomes persisted in sufficiently active individuals (**Biswas et al., 2015**). However, this pooled analysis was predominantly derived from self-reported measures of sedentary time and MVPA which are prone to bias and consequently may have been insensitive to detecting true interactions. It should also be noted that although observational research linking sedentary behaviour to health is plentiful, the vast majority have investigated the confounding rather than the modifying influence of physical activity (**Biswas et al., 2015; Wilmot et al., 2012**) or fitness (**Shuval et al., 2014**). Although associations between sedentary behaviour and health may persist when statistically controlling for the physical activity or fitness (when treated as a potential confounder), this overlooks the potential that physical activity and fitness may still be changing (modifying) associations.

The growing observational and experimental data that has come to light over recent years has supported new guidance and recommendation calling for reductions in sitting time (**United Kingdom Government – Department of Health, 2011; Australian government – Department of Health, 2014; Canadian Society for Exercise Physiology, 2012**). However, if the findings of the current study continue to be supported by further research, there may be reasonable grounds to embark upon a more personalised/tailored approach to T2DM prevention. Precision medicine is important given that a one size fits all recommendation is rarely effective. For example, interventions to reduce sitting time may be optimised by targeting those with poor CRF, whereas those with high CRF may be better served by interventions aimed at maintaining CRF and physical activity levels across the lifespan. However, it should be noted that median levels of CRF within our population for men and women were 50.3 and 34.0 $mL \cdot kg^{-1} \cdot min^{-1}$ respectively, and that the average reductions in postprandial glucose at this level of CRF was 41%. As the majority of the general population within the age range included in this study are estimated to fall below the median levels of fitness within our population (**Cooper Institute, 2006**), the importance of interrupting

sitting time with light activity breaks is likely to remain generalisable to the majority of the population.

This research also suggests that increasing CRF levels may be a viable way to protect against the potential harms of prolonged sitting. Although there are genetic contributions to fitness, the largest contributor to an individual's fitness is their time spent in MVPA (**Bouchard et al., 1994**). Participation in regular MVPA outside of seated hours may therefore offer some protection, particularly in unavoidable seated occupations.

Our observation that fitter individuals experienced less pronounced postprandial glycaemic excursions during prolonged sitting may result from favourable physiological adaptations stemming from regular engagement in MVPA (one of the main determinants of fitness (**Bouchard et al., 1994**)), such as increased skeletal muscle GLUT-4 protein expression (**Hawley & Lessard, 2008**). This would also leave less scope for further improvement, potentially explaining why the benefits of interrupting sitting time with light activity breaks appear to be blunted in those with higher CRF. However, given that CRF is determined by a mixture of both MVPA engagement and genetics (**Bouchard et al., 1994**), we cannot distinguish between behavioural and genetic mechanisms driving the results of the current study.

This study has some important limitations. Although this study provides an initial proof-of-concept from which future research can tailor to alternative study cohorts, findings should not be generalised outside the population investigated. In particular, given that the population utilised in this study were healthy, the extent to which CRF modifies responses in high risk or clinical population remains to be investigated. Our second limitation is that despite instructions to standardise food intake, and refrain from caffeine and alcohol consumption leading up to treatment conditions, we did not objectively test participant compliance and relied on self-reported adherence. In addition, fitness assessments were only conducted at one time-point, thus direct causality cannot be inferred. Future interventions that actively set out to manipulate fitness levels and assess prospective change in experimental data are required to elucidate direct causality. Another concern was that those with higher fitness in this study were predominantly men and conversely, those with lower fitness were

predominantly women. However, our results were adjusted for sex and it was not found to modify the treatment effect for glucose which was in contrast to CRF. Therefore the correlation between sex and CRF is unlikely to be confounding the results of this study.

In conclusion, participants with lower fitness had worse postprandial glucose and insulin responses during prolonged sitting, and were able to gain greater metabolic benefit through breaking their sitting time with light activities compared to individuals with higher fitness. Future interventions aimed at alleviating the deleterious metabolic impacts of sedentary behaviour may therefore be optimised by tailoring to cardio-respiratory fitness levels of the general population.

The next Chapter of this thesis (Chapter Three) addresses the second aim of this PhD providing details of an experimental investigation that was conducted to determine whether high risk individuals can regulate their metabolic health by breaking up sedentary time with seated upper body activity.

A publication that has stemmed from this research is referenced below. Full text of this article is showcased later on in the thesis *(please see '**Appendix Five**'- page 214)*;

McCarthy, M., Edwardson, C.L., Davies, M.J., Henson, J., Rowlands, A., King, J., Bodicoat, D.H., Khunti, K. and Yates, T., 2017. Breaking up sedentary time with seated upper body activity can regulate metabolic health in obese high risk adults: A randomised crossover trial. ***Diabetes, Obesity and Metabolism***.
DOI: 10.1111/DOM.13016.

Chapter Three – Can breaking up sedentary time with seated upper body activity regulate metabolic health? A randomised crossover trial

ABSTRACT

Purpose: To investigate the impact of performing short bouts of seated upper body activity on postprandial blood glucose and insulin levels during prolonged sitting.

Methods: Participants undertook two 7·5 hour experimental conditions in a randomised order: 1) prolonged sitting only 2) sitting interspersed with 5 minutes of seated arm ergometry every 30 minutes. Blood samples were obtained while fasting and throughout the postprandial period following ingestion of two standardised meals. iAUC's for glucose and insulin were calculated for both experimental conditions. Paired samples t-test assessed the difference in iAUC data between conditions for glucose (primary outcome) and insulin (secondary outcome).

Results: Thirteen obese adults (7 female; 6 male; age: 66 ± 6 years, BMI: 33.8 ± 3.8 kg/m^2 (mean ± SD) completed this investigation. Compared with the prolonged sitting only condition, the implementation of seated arm ergometry every 30 minutes significantly reduced mean [95% CI] blood glucose iAUC (from 7.4 [5.2, 9.5] mmol·L^{-1}·h to 3.1 [1.3, 5.0] mmol·L^{-1}·h, p = 0.001). Significant reductions in mean insulin iAUC (from 696 [359, 1032] mU·L^{-1} ·h to 554 [298, 811] mU·L^{-1} ·h, p = 0.047) were also observed.

Conclusion: Performing short bouts of arm ergometry during prolonged sitting attenuated postprandial glycaemia despite maintaining a seated posture. This may have clinical significance for those with weight bearing difficulty who may struggle with postural change.

INTRODUCTION

As mentioned in the previous chapters, greater time spent sedentary is increasingly being recognised as an independent risk factor for morbidity (especially type 2 diabetes) (**Biswas et al., 2015; Edwardson et al., 2012; Wilmot et al., 2012; Dempsey et al., 2016a**) and mortality (**Biswas et al., 2015; Wilmot et al., 2012; Ekelund et al; 2016**), associations that persist after controlling for MVPA levels (**Biswas et al., 2015; Edwardson et al., 2012; Wilmot et al., 2012).**

Epidemiological findings have been strengthened by recent experimental evidence demonstrating beneficial effects of interrupting prolonged sitting on markers of metabolic health, particularly postprandial glycaemia. For example, as demonstrated in **Table 1.1**, interrupting sitting time with regular bouts of light intensity walking (**Bailey et al., 2015; Dempsey et al., 2016b; Dunstan et al., 2012; Henson et al., 2016; Larsen et al., 2015; Pulsford et al., 2016; Dempsey et al., 2016c; Van Dijk et al., 2013; Crespo et al., 2016**) and moderate intensity walking (**Dunstan et al., 2012; Peddie et al., 2013; Holmstrup et al., 2014; Bhammar et al., 2017)** have shown to be effective at reducing postprandial blood glucose levels in overweight and obese adults (**Dunstan et al., 2012; Larsen et al., 2015; Bhammar et al., 2017)** in those with dysglycaemia (**Henson et al., 2016; Holmstrup et al., 2014; Crespo et al., 2016**), diagnosed type 2 diabetes (**Dempsey et al., 2016b; Dempsey et al., 2016c; Van dijk et al., 2013**), and in healthy, normal-weight populations (**Bailey et al., 2015; Peddie et al., 2013; Pulsford et al., 2016**). Breaking up prolonged sitting time with standing (**Henson et al., 2016; Thorp et al., 2014; Crespo et al., 2016; Buckley et al., 2014**) or light resistance activities (while in a standing posture) (**Dempsey et al., 2016b**) **Dempsey et al., 2016c)**, have also proven to be effective.

Interrupting sitting time with upright (non-seated) physical activities therefore appear to be a viable way of attenuating postprandial glucose. Whether or not these improvements can be replicated through the introduction of upper body muscle activity while maintaining a seated posture is currently unknown. Addressing this question will help clarify whether it is the posture of sitting that is driving the association with poor health or whether it is the resulting generalised muscular inactivity. Importantly, investigating non-weight bearing strategies for reducing sedentary behaviour will also

have important clinical implications for individuals who have restricted mobility or find standing difficult. In addition, strategies for breaking sedentary behaviour that have been investigated to date not only overlook those with weight bearing difficulty, but have also been criticised for being disruptive and non-conducive to the working day (**De Cocker et al., 2015**). Given that seated strategies would not require vacating the desk area, this could pose as a more appealing option for sedentary workers.

METHODS

Study design

As with the previous study reported in Chapter Two, each participant attended the Leicester Diabetes Centre - UK on three separate occasions (this time between May and August 2016). The first visit involved consent, familiarisation and energy expenditure measurement. This was followed by two experimental condition visits that were at least 7 days apart. A randomised cross-over design was used whereby each participant took part in two experimental treatment conditions in a random order, thereby acting as their own controls. Order randomisation was conducted by a statistician using an online tool. Due to the nature of the trial, participants were not blinded to their randomised order, however all outcomes including blood assays were analysed blinded to the experimental condition that they derived from. Prior to commencing, this study received ethical approval from the NHS East Midlands - Leicester South Research Ethics Committee.

This trial was registered with ClinicalTrials.gov (NCT02909894).

Participants

Fourteen obese adults (BMI ≥ 30kg/m^2) deemed to be inactive (failing to meet global physical activity guidelines for MVPA, defined as 150 minutes per week of self-reported moderate intensity physical activity or 75 minutes per week of self-reported vigorous intensity physical activity (**World Health Organisation, 2010**)) and at high-risk of type 2 diabetes according to the Leicester Practice Risk Score (LPRS) (**Gray et al., 2012**) were identified and recruited from a database (**Yates et al., 2012**). The LPRS calculates risk of T2DM based on six variables (age, sex, ethnicity, BMI, family history of the disease and antihypertensive drug usage), all individuals eligible for this study scored within the top 10% for risk within their respective GP surgery.

Obese and inactive individuals identified as being at high risk distinguish those most vulnerable to T2DM and given the rapid increase in co-morbidities when overstepping into T2DM territory, it is vital to intervene in this population (especially when dealing with diabetes prevention research). Utilising an obese, inactive and high risk population in the previous chapter would have been problematic given that a broad range of fitness

levels were a fundamental part of the study design. However, there were no such contraindications in the current investigation, and I was able to recruit from this cohort.

Exclusion criteria were as follows; an inability to communicate in spoken English, diagnosed T2DM, CVD, psychotic illness, pregnancy, steroid usage, regular smoking habit or an inability to walk without an assistive device.

One individual was withdrawn due to having an HbA1c indicative of T2DM. This left thirteen participants who went on to complete the trial. This process is detailed in **Figure 3.1**

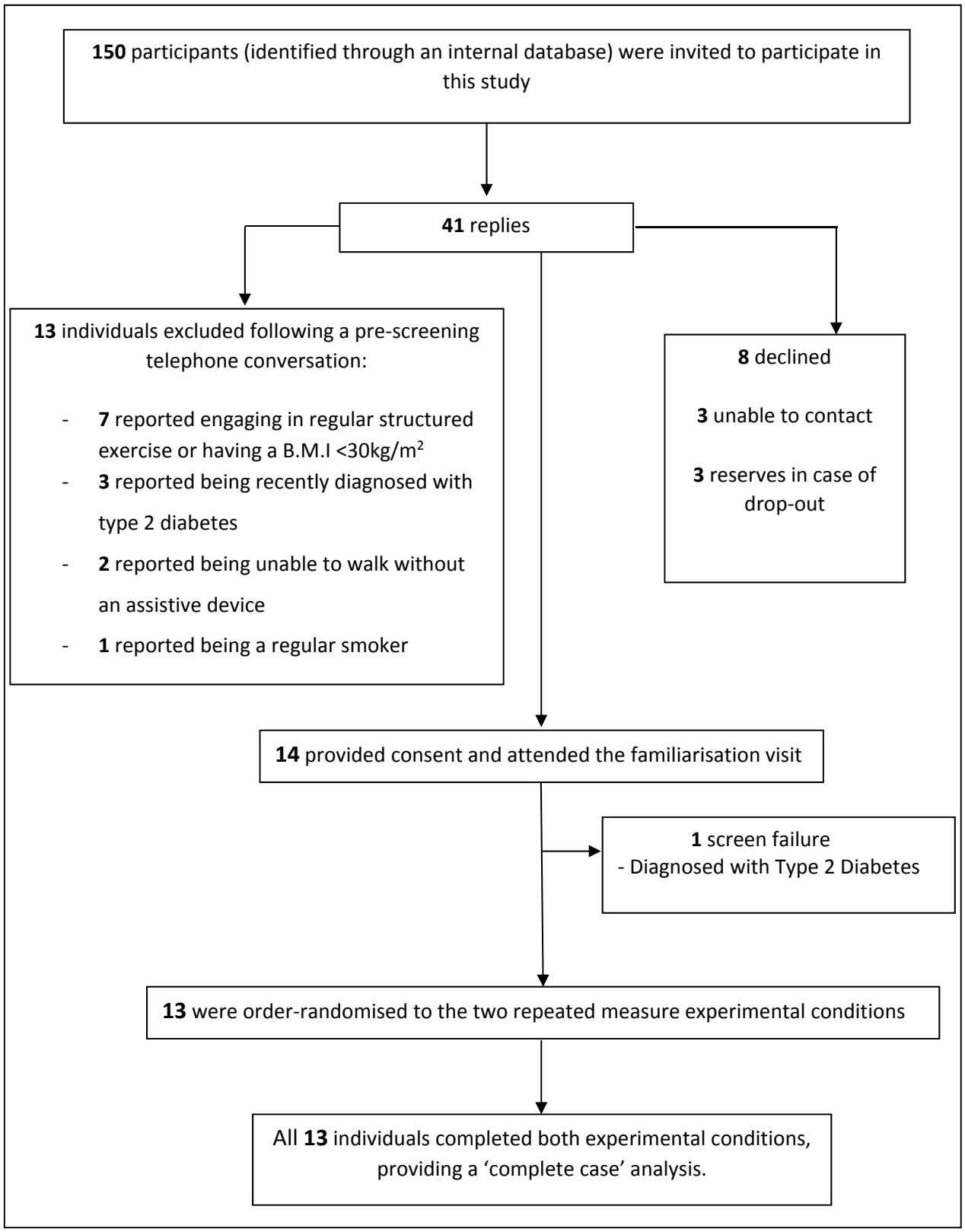


Figure 3.1 – Trial CONSORT Profile showing participant flow

Consent, familiarisation and energy expenditure assessment visit

On arrival, a researcher described in detail all study procedures and systematically went through the study specific participant information sheets allowing the participant to ask questions. Written informed consent was then obtained. (*please see '**Appendix Four**' for a copy of both the participant information sheets [page 195] and informed consent form [page 203] that was used here*).

As a part of the screening process, a venous blood sample was taken to assess HbA1c levels, and confirm absence of T2DM (<6·5% [<47·5 mmol/mol]) (**World Health Organisation, 2011**). Body weight (Tanita TBE 611: Tanita, West Drayton, UK), waist circumference (midpoint between lower costal margin and iliac crest) and height were measured to the nearest 0·1 kg, 0·5 cm and 0·5 cm, respectively.

During this first visit, we also undertook arm ergometry energy expenditure (EE) testing. Specifically, we sought to identify the power output (watts) necessary to elicit the desired EE during the main experimental condition. To allow comparison of metabolic responses to arm ergometry with previous findings that have examined the impact of light walking at 3 km/h) (**Henson et al., 2016; Dunstan et al., 2012; Bailey et al., 2015; Dempsey et al., 2016**), we aimed to match participants' arm ergometry EE to their 3 km/h walking EE. To achieve this, EE was captured: a) while at rest b) while walking at 3 km/h and c) while performing arm ergometry at various power outputs. In order for EE to be derived throughout each of these three domains, participants wore a face-mask that was directly attached to a breath-by-breath gas-analysis system (Metalyser 3B, Cortex Biophysik, Leipzig, Germany). Herein, oxygen uptake and carbon dioxide production were used to calculate EE via indirect calorimetry (**Weir, 1990**). Before undertaking each testing occasions (detailed below), the gas analyser was calibrated according to the manufacturer's recommendations.

In order to assess EE while at rest (phase a), each participant sat quietly (refraining from movement) for 30 minutes. Expired gas data was collected over the final 15 minutes of this 30 minute period once values had stabilised.

In order to assess EE while walking at 3 km/h (phase b), participants wore the face mask while walking on a motor driven treadmill (Technogym Excite® 700) for 10 minutes. Expired gas data was collected in the latter 5 minutes.

In order to assess EE during seated arm ergometry (phase c), participants wore the face-mask while pedalling at various wattages on an arm ergometer (Monark Rehab Trainer 881 E, HaB International Ltd, Warwickshire, UK). Participants performed three 5 minute bouts of arm ergometry, with the first bout standardised to a wattage of 15 W for 5 minutes. For the remaining bouts, investigators manipulated the resistance of the arm ergometer and/or the speed at which the participants pedalled until the wattage of arm ergometry initiated an EE that matched that of light walking (this ranged from 15 – 35 W). Expired gas data was collected in the 2nd 3rd and 4th minute, discarding both the first and last minute from each bout. The face-mask was removed for 5 minutes in between each bout in order for EE outputs to return to their resting level prior to the next measurement. From these three bouts, the wattage of arm ergometry that most closely resembled the average EE of light walking was prescribed in the subsequent experimental condition (which took place during a separate visit).

Finally, participants were issued with a GENEActiv accelerometer (ActivInsights Ltd, Cambridgeshire, UK) to wear on their non-dominant wrist for 24 hours/day for 7 consecutive days, allowing quantification of habitual physical activity and sedentary behaviour levels.

Experimental procedure

Participants were asked to avoid alcohol and caffeine for 48 hours preceding experimental conditions and to replicate their diet in the 24 hours before main trials. Given that the influence of an acute bout of physical activity on insulin sensitivity can persist for 48 hours (**Holtz et al., 2008**), avoidance of MVPA for this timeframe was also instructed. GENEActiv accelerometers were worn in the 2 days leading up to each experimental condition to confirm compliance with the MVPA restriction. Participant's fasted from 10pm on the evening before main trials with only water permitted to drink.

The two experimental treatment conditions that formed this repeated measures crossover trial were as follows:

1) Prolonged sitting only - participants sat in a designated room for 7.5 hours (occupied with a desk, books, and laptop with internet services) while minimising excessive movements. Lavatory breaks were permitted using a wheelchair to and from the lavatory to further reduce unnecessary movements that could confound the study.

2) Arm ergometry breaks - participants emulated the 7.5 hour prolonged sitting condition, but every 30 minutes they performed 5 minutes of arm ergometry. These bouts were performed 12 times, totalling one hour of seated upper body activity and 6·5 hours of sedentary time throughout the course of the experimental day. As mentioned previously, the intensity of arm ergometry performed was dictated by phase b) and c) of the EE testing performed during visit one. The selected arm ergometry intensities closely resembled the EE achieved during that of the 3 km/h light intensity walk for each participant.

On arrival at the research centre, participants had a cannula fitted into an accessible vein from which 10 mL samples were obtained throughout the day. Immediately following the two fasting samples (depicted at time points -1 and 0 in **Figure 3.2**), participants were given a standardised breakfast meal consisting of 8 kcal per kilogram of body weight, with a macronutrient composition reflective of co-ingestion in modern western diets (14% protein, 51% carbohydrate and 35% fat). Once breakfast had been consumed (within ≤15 minutes), blood sampling commenced at 30, 60, 120, and 180 minutes thereafter, enabling us to capture the postprandial period. An identical lunch meal was then issued (time point 3 in **Figure 3.2**) and sampling continued in the same fashion at 30, 60, 120 and 180 minutes afterward. Participants were supervised by study staff to ensure compliance with the protocol and were asked to wear an activPAL monitor to objectively confirm sitting time during both experimental conditions. *Ad libitum* water consumption was made consistent between conditions.

Measuring mood during experimental conditions

The Feeling Scale (**Hardy & Rejeski, 1989**) was used to quantify mood/affect prior to each blood sample (10 times in total) for both experimental conditions. Participants were asked to estimate their current mood state on an 11-point scale (very good= +5, 0 = neutral and very bad= –5) throughout the day.

Safety

Incidences of hypoglycaemia (defined as glucose levels below 4 mmol/L) during the final measurement period before lunch (3 hours post breakfast) and in the final measurement period of the day (3 hours post lunch) were also investigated during each experimental condition.

Free-living activity monitor processing

ActivPAL proprietary software (activPAL Professional V5.9.1.1) was used to create processed csv event files in order to quantify postural data collected during the 7.5 hour experimental conditions. GENEActiv .bin files were analysed with R-package GGIR version 1.2- 11 (http://cran.r-project.org) (**Rowlands et al., 2016; Van Hees et al., 2014**). Habitual data were included if participants had over 16 hours of wear-time recorded during the 24 hour day of interest, and providing they had ≥3 valid days of data collected. MVPA was calculated using an acceleration threshold of 100 mg (**Da-Silva et al., 2014**). MVPA bouts were identified as ≥ 10 min of consecutive 5 second epochs where 80% of epochs were equal to, or higher than, the 100 mg threshold. Time spent in 0- 50 mg and 50 – 100 mg was used to establish sedentary (minus sleep time) and light activity, respectively.

A summary of all GENEActiv data collected at each phase of the study is detailed in **Table 3.2**. ActivPAL data collected during experimental conditions is detailed in **Table 3.3.**

Biochemical analysis

Glucose (primary outcome measure) was analysed on the day of collection by the University Hospitals of Leicester pathology department using standard quality controlled enzymatic assays with commercially available kits (Beckman, High Wycombe, UK).

Centrifugated plasma samples (spun at 3,000g for 10 minutes immediately following extraction) were stored in -80·C freezers. Insulin (secondary outcome measure) was analysed from these collectively at the end of the trial using an electrochemiluminescence assay (Meso Scale Discovery). Each sample was ran in duplicate to ensure reliability of readings. Duplicate sample values with ≥20% variability were reanalysed. Ambient conditions of the laboratory were kept consistent.

Sample size

The primary aim of this study was to assess the difference in postprandial glucose levels between the two experimental treatment conditions. Assuming a population standard deviation of 2.5 mmol·L^{-1}·h in glucose iAUC and a within-person correlation of 0.5, 13 participants were required to complete the study in order to detect a difference of 1.8mmol·L^{-1}·h in blood glucose iAUC between the experimental conditions with 90% power (alpha=0.05).

Statistical analysis

Missing glucose and insulin data during the experimental conditions (highlighted in **Table 3.4**) resulted from an inability to draw enough blood from the cannula at given time points and accounted for roughly 3.7% of required samples (19 out of 520). These 19 missing data points were imputed via a regression model used previously (**Henson et al., 2015**). The iAUC of glucose and insulin was calculated for each experimental condition. Total AUC was calculated by applying the trapezium rule. Subtraction of the fasting area from this total then gave a single value representing incremental AUC for each participant. Utilising iAUC as opposed to total AUC is common practice in acute interventions where fasting levels should be unaffected by the intervention (**Le Floch et al., 1990**). Each outcome (glucose and insulin iAUC) was compared between treatments using a paired samples t-test. Data from the feeling scale were averaged across each condition and also analysed using a paired samples t-test. All statistical analyses were performed using IBM SPSS Statistics (Version 22·0) and statistical significance was set to $p < 0{\cdot}05$ throughout. Data distribution was interpreted by visual inspection and through the Shapiro-Wilk test. Normally distributed descriptive data and experimental data are presented as Mean ± SD and Mean (95% CI), respectively, while all non-parametric data is reported as Median (Interquartile Range [IQR]) unless specified otherwise. For the experimental data, the unstandardised residuals were checked for normality.

RESULTS

Descriptive characteristics of those who completed this study are summarised in **Table 3.1** (n = 13). The study characteristics show that the energy expenditure of arm ergometry breaks conducted in the experimental condition was similar to that achieved through a light intensity walk at 3km/h (4.5 vs 4.6 kcal/min, respectively), however the average Respiratory Exchange Ratio (RER) was higher during arm ergometry compared to light intensity walking (1.00 vs 0.84, $p < 0.001$).

Overall treatment condition effect

Biochemical results collected during each experimental condition are presented in **Figure 3.2.**

The mean (95% CI) glucose iAUC response during the arm ergometry breaks condition (3.1 [1.3, 5.0] mmol·L^{-1}·h) was significantly lower than the mean glucose iAUC response to the prolonged sitting only condition (7.4 [5.2, 9.5] mmol·L^{-1}·h), $p = 0.001$. This was also the case for mean insulin iAUC (554 [298, 811] mU·L^{-1}·h vs 696 [359, 1032] mU·L^{-1}·h, $p = 0.047$).

For more insight into the spread of glucose iAUC responses for each experimental treatment condition throughout this cohort (n = 13), please see Supplementary Table 1 on page 121 of Appendix One.

Physical activity and sedentary time data

Physical activity and sedentary behaviour data is displayed in **Table 3.2**. Free-living accelerometer data collected after the familiarisation visit (*n* = 13), showed that participants spent on average 644 ± 106 min/day sedentary and only engaged in 2 [0, 13] min/day of purposeful MVPA, thus confirming the inactive nature of this study cohort.

MVPA data collected in the two days leading up to the prolonged sitting only condition (0 [0, 10] min/day) and in the two days leading up to the arm ergometry breaks condition (0 [0, 7] min/day) confirm adherence to the standardised MVPA restriction.

Mood, tolerance and safety

Mean ± SD self-reported feelings throughout the day were 3.1 ± 1.1 and 2.7 ± 1.2 for the prolonged sitting only and arm ergometry breaks conditions, respectively ($p = 0.101$ for difference), demonstrating positive mood states during both conditions. All participants completed the required number of arm ergometry bouts, and none reported musculoskeletal pain or discomfort.

Two participants did have asymptomatic hypoglycaemia during the final measurement of the day during the arm-ergometry breaks condition with no incidences reported during the prolonged sitting condition.

Table 3.1: Metabolic, demographic, and anthropometric characteristics taken at baseline alongside important in-study characteristics

Characteristics	Overall (n = 13)
Age (years)	66 ± 6
Female	7 [54]
BMI (kg/ m^2)	33.8 ± 3.8
Body weight (kg)	93.2 ± 13.2
Waist circumference (cm)	105 ± 16
HbA1c (%)	5.5 ± 0.4
HbA1c (mmol/mol)	37 ± 4
Total cholesterol (mmol/L)	4.6 ± 0.6
Resting heart rate (bpm)	60 ± 6
Systolic blood pressure (mm Hg)	140 ± 13
Diastolic blood pressure (mm Hg)	79 ± 9
White European	13[100]
Experimental characteristics	
Energy intake per experimental meal (kcal/meal)	746 ± 106
Prescribed power output of arm ergometry (Av.Watts)	20 ± 4
Energy expenditure while walking at 3km/h (Av.kcal/min)	4.6 ± 1.0
Energy expenditure at prescribed wattage of arm ergometry (Av.kcal/min)	4.5 ± 0.9
Av. Respiratory Exchange Ratio while walking at 3km/h (VCO_2/VO_2)	0.84 ± 0.07
Av. Respiratory Exchange Ratio at prescribed power output of arm ergometry (VCO_2/VO_2)	1.00 ± 0.07

Data are presented as Mean ± SD or n [%]

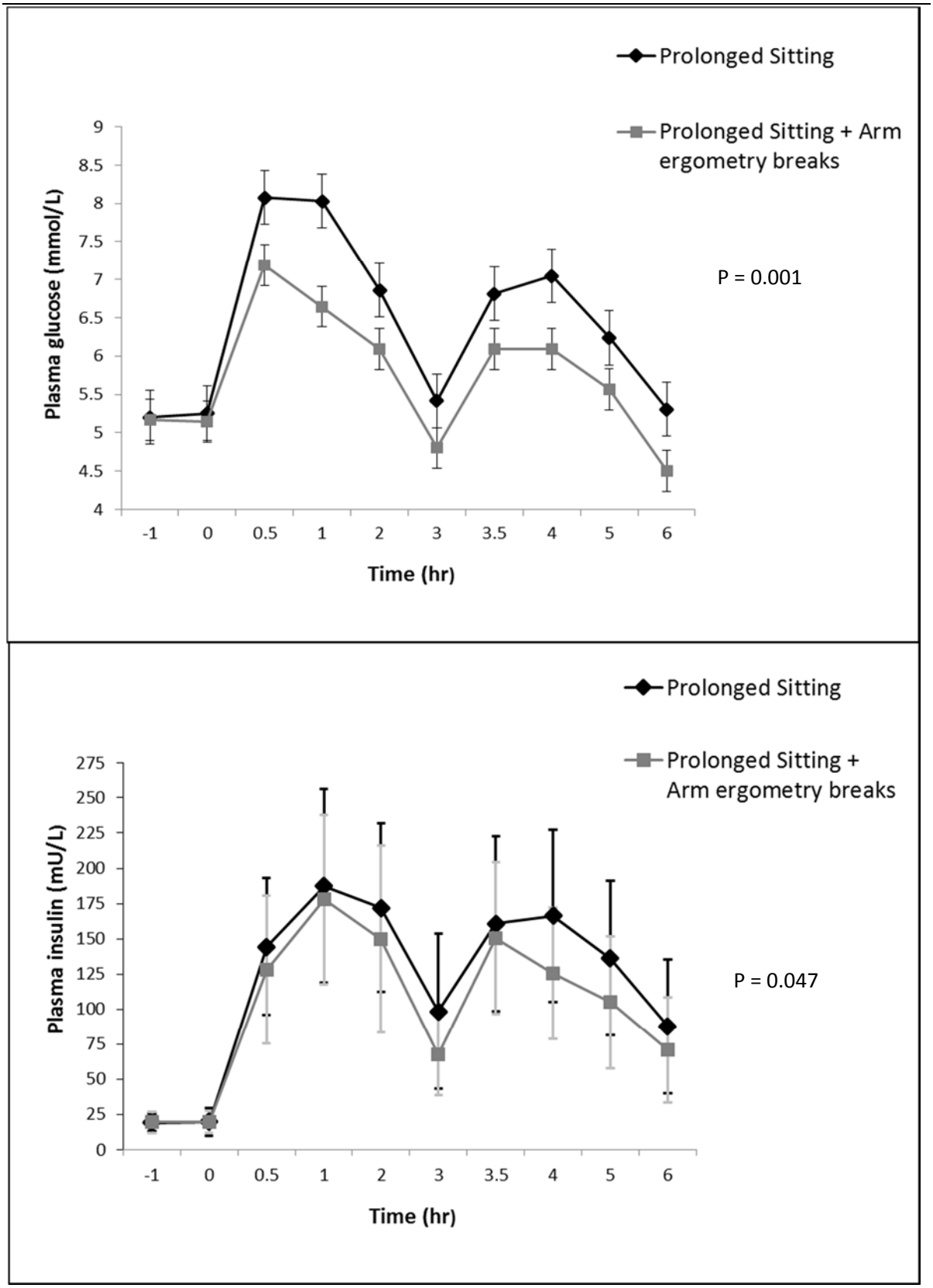


Figure 3.2 – Effect of treatment condition on average Blood Glucose and Insulin (*error bars represent standard deviations around the mean ; p-values represent the significance of difference between experimental conditions for glucose/ insulin iAUC's*)

Table 3.2: Physical activity data collected in each phase of the trial (GENEActiv)

* MVPA minutes accumulated in bouts ≥ 10 minutes.

	Mean (range)	Mean ± SD	Median (IQR)	
Phase of study	No. days accelerometer worn	Sedentary (min)	MVPA (min)	Light activity (min)
Familiarisation (n = 12) *'Habitual monitoring'*	5 (3 – 6)	644 ± 106	2 (0, 13) *	143 (141, 163)
2 days prior to the 'prolonged sitting only' condition (n = 11) *'While under MVPA restriction'*	2	747 ± 92	0 (0, 10) *	133 (112, 145)
2 days prior to the 'arm ergometry breaks' condition (n = 11) *'While under MVPA restriction'*	2	683 ± 172	0 (0, 7) *	138 (127, 169)

Table 3.3: ActivPAL results during each experimental condition

	Mean hours ± SD		
	Sitting	Standing	Walking
Prolonged sitting only condition	7.8 ± 0.1	0.1 ± 0.1	0.01 ± 0.01
Arm ergometry breaks condition	7.6 ± 0.5	0.3 ± 0.2	0.03 ± 0.01

Table 3.4: Missing data imputed via regression predictions

Participant Number	Prolonged sitting only condition		Arm ergometry breaks condition	
	No. missing Glucose samples (out of 10)	No. missing Insulin samples (out of 10)	No. missing Glucose samples (out of 10)	No. missing Insulin samples (out of 10)
1	-	-	1	1
2	-	-	-	-
3	1	1	2	-
4	1	-	4	4
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	
10	-	-	-	-
11	-	-	-	-
12	1	1	1	1
13	-	-	-	-
Total	3	2	8	6

DISCUSSION

This study is the first to investigate the metabolic impact of interrupting postprandial prolonged sitting time with regular bouts of upper body activity while remaining seated. Our results show that introducing 5 minutes of arm ergometry every 30 minutes while remaining in a seated posture is well tolerated and can attenuate postprandial blood glucose and insulin levels by approximately 57% and 20% respectively compared to that of prolonged sitting only. The fact that the observed reductions in glucose coincided with reductions in insulin concentration is suggestive of improved insulin sensitivity during the seated activity breaks condition using upper body muscle activation.

Our findings are consistent with the majority of experimental research to date. For example, experimental studies that have interrupted prolonged sitting with 3 km/h walking breaks have led to clinically significant reductions in postprandial blood glucose by 28% (**Henson et al., 2016**) when implemented for 5 minutes every 30 minutes; by 39% when implemented for 3 minutes every 30 minutes (**Dempsey et al., 2016**) and by 16% (**Bailey et al., 2015**) to 24% (**Dunstan et al., 2012**) when implemented for 2 minutes every 20 minutes post-meal. Our findings add to this evidence by demonstrating that regular bouts of seated arm ergometry may also be a viable method of improving postprandial glycaemia. Moreover, despite closely matching the energy demand of arm ergometry breaks to that of the 3km/hr walking bouts used in previous studies, we achieved a larger reduction in postprandial glucose iAUC than observed in these studies, even compared to those operating activity breaks at the same time intervals (**Henson et al., 2016**).

Given that arm ergometry breaks were implemented while maintaining a seated posture, our findings could not have been driven by postural change, and benefits to postprandial glycaemia may be attributed to other factors. For instance, physical activity breaks are accompanied by increases in muscle activation. These increases in muscle activation not only raise energy expenditure but also increase blood flow and upregulate GLUT-4 expression in a dose dependant manner, which helps to restore homeostasis of postprandial glycaemia (**Bauman et al., 2013; Sylow et al., 2016**). Greater intensity of muscle activation in the smaller muscle mass during arm ergometry may have been necessary to achieve the same energy expenditure elicited by a 3 km/hr walk. In turn,

this greater muscle activation may have compensated for the limited muscle mass involved, and may explain the enhanced blood glucose utilisation observed here. This was supported in the present study by the higher RER observed during the arm ergometry compared to the energy matched walking, suggesting a greater relative intensity. Previous research has shown that enhanced postprandial blood glucose regulation is observed following higher intensity physical activity bouts compared to energy matched lower intensity physical activity bouts (**Urland et al., 2016; Rynders et al., 2013; Blaak et al., 2012**). Thus, the higher intensity of arm ergometry, compared to light walking, may have helped augment reductions in postprandial glucose. Further research is therefore needed to assess whether reductions in postprandial glucose are also observed when using arm ergometry at a perceived light intensity.

The current study suggests an alternative strategy to help regulate postprandial glycaemia while sitting in a population at high risk of T2DM. Not only are arm ergometry breaks an alternative strategy, but they may even act as a sole strategy for individuals with weight bearing difficulty such as wheelchair users and those with severe peripheral neuropathy, which is thought to affect up to half of all people diagnosed with T2DM (**Cavalot et al., 2006**). Given the disruptive nature of alternative strategies such as frequent walking breaks, seated activity may also appeal to office workers who find it difficult to leave their desk or office space at regular intervals throughout the day. Portable lightweight desktop arm ergometers may also be of use in a hospital environment to improve postprandial glycaemia of patients who are bed bound yet able to sit upright.

The main strength of this study lays in the exploration of a novel strategy to alleviate the deleterious impacts of prolonged sitting bouts on postprandial glycaemia in a population at high risk of developing T2DM recruited through a primary care setting. However, it is important to acknowledgement some limitations.

Although comparing our findings to those observed when introducing 3 km/hr walking breaks (**Henson et al., 2016; Dunstan et al., 2012; Bailey et al., 2015; Dempsey et al., 2016**), we did not include a third experimental walking condition which may have strengthened our conclusions. In addition, this study was not designed to elucidate

potential mechanisms underpinning the acute reductions in postprandial glucose and insulin concentrations observed when employing seated activity breaks. However, this study was specifically designed to establish proof-of-concept for the efficacy of employing seated arm ergometry breaks as a method of acutely reducing postprandial glucose concentrations during prolonged sedentary behaviour. This is clinically important given that exaggerated postprandial glucose oscillations are associated with the development of T2DM (**Sasso et al., 2004**), CVD (**Sasso et al., 2004; O'Keefe & Bell, 2007; Leiter et al., 2005**) and obesity (**Sasso et al., 2004**). Even small elevations in postprandial glycaemia are thought to contribute to the development of atherosclerosis and subsequent coronary heart disease events (**Tesfaye & Selvarajah, 2012**).

While a sample size of 13 provided adequate power for comparison between experimental conditions, the small sample makes it harder to generalise findings beyond the specific subject population recruited to this study. Given that efforts to manipulate blood glucose control are thought to be more pronounced in those with worse glycaemia (**Livesey et al., 2008**), the potential of such interventions in a diagnosed T2DM population would also be intriguing and warrants further investigation. Future intervention studies observing the impacts of seated activity breaks using more ecologically valid regimes in settings outside of the laboratory (such as the home, or in a hospital environment) would also be of interest. The ability to emulate reductions in postprandial glycaemia through regular bouts of electro-stimulated muscular contractions would also be an interesting focal point for future research given recent links to improved insulin sensitivity (**Joubert et al., 2015**) and its potential application to non-weight bearing populations. Likewise, given that arm ergometers are not easily accessible to all, engaging in seated upper body resistance band exercises could also pose as an intriguing alternative for future research. Future research exploring the minimal time, frequency and intensity that activity breaks can be implemented to bring about clinically significant improvements in postprandial glycaemia is warranted to promote more attractive, feasible and sustainable strategies. In addition, given that two subjects were found to be over the threshold for asymptomatic hypoglycaemia at the end of the arm ergometer condition, the safety of the current regime needs further investigation in those with a high risk or diagnosed T2DM, particularly in the 24 hours

following the intervention. Further research utilising hyperinsulinaemic-euglycaemic clamp techniques could also be used to give more detailed insight into the dynamics of glucose metabolism when employing seated upper body breaks during prolonged sedentary behaviour.

In conclusion, this study demonstrates that seated arm ergometry breaks are a viable way to attenuate postprandial glycaemia. This suggests that breaking up the posture of sitting may not be necessary to elicit glycaemic benefit and that interventions to reduce sedentary behaviour should not focus solely on postural change.

The next Chapter of this thesis (Chapter Four) addresses the third and final aim of this PhD, providing details of an epidemiological analysis that was conducted to determine the associations between changes in sedentary behaviour with HbA1c over a 3 year period in those deemed to be at high risk of T2DM. This Chapter also acknowledges the associations of other key lifestyle behaviour changes.

A publication that has stemmed from this research is referenced below. The full text of which is showcased later on in this thesis (please see '**Appendix Five'** – page 222);

McCarthy, M., Edwardson, C.L., Davies, M.J., Henson, J., Gray, L., Khunti, K. and Yates, T., 2017. Change in Sedentary Time, Physical Activity, Body weight, and HbA1c in High-Risk Adults. ***Medicine and Science in Sports and Exercise***, *49*, 1120-1125.

Chapter Four - Prospective change in sedentary time, physical activity and body weight with Hba1c in high-risk adults: An epidemiological analysis.

ABSTRACT

Purpose*:* In recent years, there has been a migration towards the use of HbA1c in determining glycaemic control. This study aimed to quantify the associations between changes in sedentary time, MVPA time, and body weight with HbA1c levels over a three year period among adults at high risk of T2DM.

Methods: This study reports baseline and three year follow-up data from the Walking Away from Type 2 Diabetes study. ActiGraph GT3X accelerometers captured sedentary time and MVPA. Linear regression examined the independent associations of changes in sedentary time, MVPA and body weight with HbA1c between baseline and three year follow-up.

Results: The sample comprised of 489 participants (mean age 64.2 ± 7.3 years, BMI 31.7 ± 5.1, 63.4% male) with valid baseline and follow-up accelerometer, body weight and HbA1c data. Following adjustment for known confounders, an increase in MVPA time (per 30 mins/day) was associated with a decrease in HbA1c percentage

(β = -0.11 (-0.18, -0.05), p=0.001) and an increase in body weight (per 6 kg) was associated with an increase in HbA1c percentage (β = 0.08 (0.04 ,0.12), p<0.001). Presence of dysglycaemia at baseline (HbA1c ≥6.0%) strengthened these associations (p<0.001 for interactions). Change in sedentary time was not significantly associated with change in HbA1c after adjustment for change in MVPA time.

Conclusion: Increases in MVPA and body weight were associated with a reduction and increase in HbA1c respectively, particularly in those with dysglycaemia. Quantifying the impact that health behaviour changes have on HbA1c can be used to inform prevention programmes. Further research is necessary to fully determine whether the acute glycaemic impacts of reducing sedentary behaviour, that have been shown throughout experimental research, can translate to chronic glycaemic adaptation.

INTRODUCTION

T2DM is one of the most prevalent chronic diseases and accounts for between 7 to 14% of health care expenditure globally (**Zhang et al., 2010**). Both the prevalence and cost of T2DM in the UK are projected to rise in the future with 17% of the NHS budget required for its treatment by 2035 (**Hex et al., 2012**). Given this current and projected increase in burden, health care policies and recommendations targeting prevention are gaining national and international traction with defined budgetary commitments (**NHS England, 2015; Schwarz et al., 2012**).

Lifestyle interventions have consistently been shown to reduce the risk of, and slow progression to T2DM in high risk populations, and form the cornerstone of diabetes prevention recommendations and programmes (**Gillies et al., 2007; National Institute for Health and Clinical Excellence, 2012**). There has been a wealth of good quality interventional and epidemiological evidence quantifying the combined and individual impact of lifestyle factors in improving glucose regulation and reducing the risk of T2DM based on outcomes from an oral glucose tolerance test (fasting and 2-hour post-challenge glucose levels) (**Gong et al., 2015**). However, such data no longer reflects clinical reality and decision making processes. Since the inclusion of HbA1c within the diagnostic framework for T2DM (**World Health Organisation, 2011**), there has been a migration towards HbA1c in the classification of diabetes risk and assessment of diabetes prevention programmes run within routine care (**International Expert Committee, 2009; National Institute for Health and Clinical Excellence, 2012; NHS England, 2015**). This change is reflective of greater clinical utility of HbA1c compared to plasma glucose derived from an oral glucose tolerance test. For example, HbA1c does not need to be measured fasting, is a better indicator of chronic hyperglycaemia, is less affected by any short term, illness related changes in plasma glucose levels and shows lower inter-test variability (**International Expert Committee, 2009**). Given the abundant shift in focus towards HbA1c in recent years, there is a requirement to extend prevention research by quantifying the impact of lifestyle change on this metabolic marker.

Increased physical activity and weight loss have consistently been shown to independently reduce the risk of T2DM and are key behavioural targets for prevention

programs that have been translated into real world settings (**Dunkley et al., 2014; Laaksonen et al., 2005**). The importance of these factors on change in HbA1c needs further elucidation, although recent research is encouraging. For example, obese individuals with currently normal HbA1c levels (5.2-5.6%) have a greater chance of developing early onset T2DM than a lighter individual with currently higher levels (5.7 - 6.4%) (**Nakajima & Suwa, 2015**).

In addition to physical activity and body weight, high levels of sedentary time have also been associated with poor metabolic health (**Edwardson et al., 2012**), increased risk of T2DM (**Biswas et al., 2015; Edwardson et al., 2012; Wilmot et al., 2012**), CVD and mortality (**Biswas et al., 2015; Wilmot et al., 2012**). Recent cross sectional links between sedentary time and insulin sensitivity have also emerged which further support the potentially detrimental impact of sedentary time upon glycaemic control (**Brocklebank et al., 2015; Yates et al., 2015**).

To date, epidemiological research investigating the associations of sedentary time with health outcomes have predominantly used cross-sectional study designs (**De Rezende et al., 2014**), making it difficult to infer direct causality. Where prospective study designs have been utilised, they have tended to rely upon self-reported measures of sedentary time (**Biswas et al., 2015; Wilmot et al., 2012**) which have disputable validity (**Clark et al., 2009**).

The aim of this paper is therefore to use a prospective dataset to quantify the association between objective changes in sedentary time, MVPA and body weight with changes in HbA1c using a population at high risk of T2DM recruited from primary care over a three year period.

METHODS

Research Design

This study performed an observational cohort analysis utilising baseline and three year follow up data from the Walking Away from Type 2 Diabetes trial, the design and results of which are described elsewhere (**Yates et al., 2012; Yates et al., 2016**). In brief, this was a randomised controlled trial that evaluated the effectiveness of a pragmatic structured education program aimed at increasing physical activity and promoting healthy lifestyles over three years among those who were at high risk of T2DM.

Participants

Individuals taking part in the trial were recruited through 10 primary care practices in Leicestershire - UK, in 2010. Individuals were recruited based on having a high risk of T2DM defined using the LPRS (**Gray et al., 2012**). As mentioned previously, this score calculates risk based on six variables (age, sex, ethnicity, BMI, family history of the disease and antihypertensive drug usage) and individuals ranked within the top 10% within their GP surgery were invited to take part in the study. Those with T2DM diagnosed at baseline, with established T2DM or currently taking steroids were excluded.

Informed consent was obtained from all eligible participants and full ethical approval from the local ethics committee was granted for the trial.

Demographic data

Information regarding medication, ethnicity, smoking status and home postcode (used to calculate index of multiple deprivation [IMD] score) was obtained following an interview administered protocol conducted by healthcare professionals. The IMD scores are publically available continuous measures of compound social and material deprivation which are calculated using a variety of data including current income, employment, health, education and housing.

Anthropometric data

Body weight, body fat percentage (Tanita TBE 611, Tanita, West Drayton, UK) and height were measured to the nearest 0.1 kg, 0.1 % and 0.1 cm respectively.

Bio-chemical data

Venous blood samples were obtained following a 12 hour overnight fast. All assays were measured in the same laboratory using stable methodologies and conducted by individuals blinded to the patient's identity. HbA1c was analysed using the Bio-Rad Variant II HPLC system (Bio-Rad Clinical Diagnostics, Hemel Hempstead, UK). All venepuncture was undertaken by trained phlebotomists. Data collection procedures between baseline and follow-up were standardised.

Accelerometer data

Participants were asked to wear an accelerometer (Actigraph GT3X, Pensacola, Florida, USA) on the right anterior axillary line above the hip for seven consecutive days during waking hours at both baseline and three year follow up. Data were collected in 60 second epochs. Freedson cut-points, using counts in the vertical axis only, were used to categorise sedentary time (<100 counts per min) and MVPA time (≥1952 counts per min) (**Freedson et al., 1998**). In addition, MVPA time accumulated in bouts ≥ 10min (allowing for a two minute exception in the intensity threshold) were also derived. Non-wear time was defined as a minimum of 60 minutes of continuous zero counts and days with at least 600 minutes of wear time were considered valid (**Healy et al., 2008**). In order to be included in the analysis, a minimum of any three valid days was required (**Trost et al., 2005**). Accelerometer files were processed using KineSoft V3.3.76, a commercially available analytical software (KineSoft, Loughborough, UK).

Statistical analysis and data inclusion

From the 808 individuals randomised into the Walking Away from Type 2 Diabetes trial at baseline, 489 (61%) had valid measures of accelerometer data, body weight and HbA1c at both baseline and three years, and were subsequently included in this analysis. Of the 319 participants who were not included in this analysis, 289 were excluded on the basis of failing to meet the minimum accelerometer wear time requirements, while a further 30 did not provide biochemical data at both time points. The results of this intervention are reported elsewhere (**Yates et al., 2016**). All analyses were conducted using IBM SPSS Statistics (version 22.0) and statistical significance was set to $p < 0.05$. Only participants with valid measures of accelerometer data, body weight and HbA1c at both baseline and three years were included in the following analyses.

Linear regression models examined the independent associations between changes in; MVPA, sedentary time and body weight with a change in HbA1c over the three year period. Changes in all variables were calculated as three year follow-up data minus baseline data. Beta-coefficients representing changes in 'HbA1c %' reflect absolute changes in HbA1c units and not relative statistical percentage changes. Change data for MVPA and sedentary time were displayed in 30 minute/day unit increments for ease of interpretation. The lifestyle intervention arm of the Diabetes Prevention Program targeted a 7% reduction in body weight (**American Diabetes Association, 1999**), change data for body weight in the current study was therefore displayed in 6kg unit increments, as this represents a 7% difference in the average body weight of our cohort. Analyses were adjusted for the following variables: age, sex, ethnicity, beta-blocker use for hypertension, IMD score, change in accelerometer wear time and baseline measures of; HbA1c, body weight, sedentary time and MVPA. Smoking status was also added as a measure of deprivation. Additional models simultaneously added change in all variables (MVPA, sedentary time and body weight) into the same model to establish the extent to which associations with HbA1c were independent of each other. A sensitivity analysis was conducted to see whether using MVPA time accumulated from bouts lasting ≥10 minutes (in line with global public health physical activity guidelines (**World Health Organisation, 2010**)) influenced the findings.

In addition, we also set out to investigate whether glycaemic status at baseline independently modified associations through adding interaction terms to the model. Interaction significance was set to $p<0.10$. Glycaemic status was defined as having dysglycaemia (HbA1c ≥ 6.0% at baseline) or normal glycaemia (<6% at baseline). Significant interactions were followed up with stratified analyses. A threshold of 6.0% was chosen to make the analysis consistent with recommendation for UK populations (**National Institute for Health and Clinical Excellence, 2012**) and with international guidance (**International Expert Committee, 2009**).

Although commonly used, a cut-off value of <100 counts per minute (cpm) to categorise sedentary time may be too high, particularly in older adults (**Aguilar-Farias et al., 2014; Koster et al., 2016**). We therefore ran a further sensitivity analysis to address whether similar results were yielded if sedentary time was categorised at a lower cut-off value

of <50 cpm. Similarly, Freedson cut-points for MVPA (≥1952 cpm) may underestimate time spent in MVPA (**Gorman et al., 2013**), therefore we conducted a sensitivity analysis to determine whether a lower cut-point (≥1041 cpm) influenced our findings.

RESULTS

Those included in this analysis had a similar ethnic breakdown and baseline sedentary time compared with those who were excluded. There were also no significant differences in sex between those included and excluded. However, those excluded had a higher social deprivation score (22.7 vs. 17.6; p<0.001) were more likely to be; younger (61.8 ± 9.1 vs 64.2 ± 7.3 years, p<0.001) have a higher BMI (33.6 ± 6 vs. 31.7 ± 5.1 kg/m2, p<0.001) and engage in less MVPA at baseline (32.7 ± 25.1 vs 40.3 ± 27.6 mins/day, p<0.001).

Table 4.1: Demographic, anthropometric, and cardiometabolic characteristics of those included in the study analysis

Characteristics	Walking Away participants (n = 489)
Age (years)	64.2 ± 7.3
Male	310 (63.4)
Current smokers	30 (6.1)
Family history of diabetes (first degree)	169 (34.5)
B.M.I (kg/ m^2)	31.7 ± 5.1
<u>Cardiometabolic variables</u>	
Total cholesterol (mmol/l)	5.2 (4.5 - 6)
HDL – cholesterol (mmol/l)	1.4 (1.2 - 1.6)
<u>Ethnicity</u>	
White European	441 (90.2)
South Asian	31 (6.3)
Other	17 (3.5)
<u>Diagnosis</u>	
Normal glycaemic function (HbA1c <6%)	319 (65.2)
Dysglycaemia (HbA1c ≥6%)	170 (34.8)
Continuous parametric results displayed as Mean ± SD, number (percentage) and continuous nonparametric results displayed as median (interquartile range)	

Table 4.2: Baseline and 3 year follow-up data for key anthropometric, cardio-metabolic and accelerometer derived measures

Characteristics	Baseline	3 years
HbA1c (%)	5.8 (5.6 - 6.1)	5.7 (5.4 - 5.9)
HbA1c (mmol/mol)	39.9 (37.7 - 43.2)	38.8 (35.5 – 41.0)
Body weight	87.6 (79.3 - 98.9)	87.1 (78.1 - 98.1)
Accelerometer variables		
Wear time (h/day)	14.4 (13.5 – 15.2)	14.3 (13.5 – 15.1)
Sedentary time (mins/day)	542 (477 - 597)	566 (499 - 632)
Total MVPA (mins/day)	21 (12 – 41)	16 (7 - 33)
MVPA (mins/day accumulated in bouts ≥ 10mins)	4 (0 - 10)	3 (0 – 10)
Results displayed as median (interquartile range)		

Table 4.3 - Multiple linear regression models for changes in sedentary time, MVPA and body weight with HbA1c

	Sedentary time change (per 30mins/day) [a]	MVPA time change (per 30mins/day) [b]	Body weight change (per 6 kilograms) [c]
Model 1			
HbA1c Change (%)	0.02 (0.01, 0.03), p = 0.021	-0.14 (-0.2, -0.08), p < 0.001	0.09 (0.06, 0.13), p < 0.001
~HbA1c Change (mmol/mol)	0.2 (0.03, 0.38), p = 0.021	-1.5 (-2.2, -0.88), p < 0.001	1.0 (0.62, 1.4), p < 0.001
Model 2			
HbA1c Change (%)	0.01 (-0.01, 0.02), p = 0.402	-0.13 (-0.2, -0.07), p < 0.001	0.09 (0.05, 0.12), p < 0.001
~HbA1c Change (mmol/mol)	0.1 (-0.11, 0.26), p = 0.402	-1.4 (-2.15, -0.74), p < 0.001	1.0 (0.56, 1.34), p < 0.001
Model 3			
HbA1c Change (%)	0.004 (-0.01, 0.02), p = 0.615	-0.11 (-0.18, -0.05), p = 0.001	0.08 (0.04, 0.12), p < 0.001
~HbA1c Change (mmol/mol)	0.04 (-0.13, 0.22), p = 0.615	-1.2 (-1.93, -0.53), p = 0.001	0.9 (0.49, 1.26), p < 0.001

Data are unstandardised regression coefficients (95% CI), p-value.

Model 1: adjusted for age, sex, smoking status, ethnicity, beta-blocker use for hypertension, change in accelerometer wear time, IMD score, baseline HbA1c, baseline body weight, baseline sedentary time and baseline MVPA time.

Model 2: adjusted for all covariates in Model 1 and **[a]** MVPA time change, **[b c]** sedentary time change

Model 3: adjusted for the same covariates as Model 2 and **[a b]** body weight change **[c]** MVPA time change

Sedentary time: Following adjustment for known confounders, greater sedentary time (per 30mins/day) was associated with an increase in HbA1c (*β* = 0.02% (0.01, 0.03)**,** p = 0.021). This association disappeared after further adjusting for a change in MVPA (*β* = 0.01% (-0.01, 0.02), p = 0.402).

MVPA time: An increase in MVPA time (per 30 mins/day) was significantly associated with a decrease in HbA1c (*β* = -0.14% (-0.20, -0.08), p < 0.001) after adjustment for potential confounding variables. This remained significant after further adjustment for changes in both sedentary time and body weight (*β* = -0.11% (-0.18, -0.05), p = 0.001*).*

Body weight: When adjusting for all covariates, including change in MVPA, an increase in body weight (per 6 kg) was associated with significantly greater HbA1c levels *(*β = 0.08*%* (0.04, 0.12), p<0.001).

Sensitivity analyses revealed that these results were largely unaffected when using MVPA accumulated in bouts ≥10 minutes (**Table 4.5**), when using lower cut-points for sedentary time (**Table 4.6**) or when utilising lower MVPA cut-points (**Table 4.7**).

When interaction terms were added to the model, they revealed that glycaemic status at baseline significantly modified the independent associations between a change in MVPA (p < 0.001) and a change in body weight (p < 0.001) with a change in HbA1c. Following-up on this interaction, stratification by glycaemic status showed that those with dysglycaemia had stronger associations compared to those with normal glycaemia (**Table 4.4**). For individuals with dysglycaemia, each 30 minute increase in MVPA per day was associated with a 0.17% (0.04, 0.29) decrease in HbA1c in the fully adjusted model (including change in sedentary time and body weight, p = 0.012), and each 6 kg increase in body weight was associated with a 0.19% (0.11, 0.27) increase in HbA1c (p < 0.001). Glycaemic status did not significantly modify the association between sedentary time and HbA1c, and therefore did not warrant further stratification.

Table 4.4 - Associations between change in MVPA and body weight with a change in HbA1c stratified by glycaemic status.

	MVPA time change (per 30mins/day) [a]	Body weight change (per 6 kilograms) [b]
Impaired glycaemic function (HbA1c ≥ 6%)		
HbA1c Change (%)	-0.17 (-0.29, -0.04), p = 0.012	0.19 (0.11, 0.27), p < 0.001
~ HbA1c Change (mmol/mol)	-1.8 (-3.19, -0.4), p = 0.012	2.1 (1.17, 2.98), p < 0.001
Normal glycaemic function (HbA1c < 6%)		
HbA1c Change (%)	-0.07 (-0.13, -0.01), p = 0.031	0.04 (0.01, 0.08), p = 0.012
HbA1c Change (mmol/mol)	-0.8 (-1.47, -0.07), p = 0.031	0.5 (0.1, 0.82), p = 0.012

Adjusted for age, sex, smoking status, ethnicity, beta-blocker use for hypertension, baseline body weight, change in accelerometer wear time, IMD score, baseline HbA1c, baseline MVPA, baseline sedentary time, changes in sedentary time and [a] changes in body weight [b] changes in MVPA.

Table 4.5 - Multiple linear regression models for changes in sedentary time, MVPA and body weight with HbA1c

(Showing adjusted associations when using MVPA time accumulated in bouts ≥10 minutes).

	Sedentary time change (per 30mins/day) [a]	MVPA time change (per 30mins/day) [b]	Body weight change (per 6 kilograms) [c]
Model 1			
HbA1c Change (%)	0.02 (0.01, 0.03), p = 0.021	-0.14 (-0.21, -0.07), p < 0.001	0.09 (0.06, 0.13), p < 0.001
~HbA1c Change (mmol/mol)	0.2 (0.03, 0.38), p = 0.021	-1.6 (-2.34, -0.79), p < 0.001	1 (0.62, 1.4), p < 0.001
Model 2			
HbA1c Change (%)	0.02 (0, 0.03), p = 0.056	-0.14 (-0.21, -0.06), p < 0.001	0.09 (0.05, 0.12), p < 0.001
~HbA1c Change (mmol/mol)	0.2 (-0.01, 0.34), p = 0.056	-1.5 (-2.26, -0.7), p < 0.001	1 (0.58, 1.36), p < 0.001
Model 3			
HbA1c Change (%)	0.01 (-0.01, 0.03), p = 0.16	-0.12 (-0.19, -0.05), p = 0.001	0.08 (0.05, 0.12), p < 0.001
~HbA1c Change (mmol/mol)	0.1 (-0.05, 0.29), p = 0.16	-1.3 (-2.04, -0.51), p = 0.001	0.9 (0.5, 1.28), p < 0.001

Data are unstandardised regression coefficients (95% CI), p-value.

Model 1: adjusted for age, gender, smoking status, ethnicity, beta-blockers, change in accelerometer wear time, IMD score, baseline HbA1c, baseline body weight, baseline sedentary time and baseline MVPA time.

Model 2: adjusted for all covariates in Model 1 and [a] MVPA time change, [b c] sedentary time change

Model 3: adjusted for the same covariates as Model 2 and [a b] body weight change [c] MVPA time change

Table 4.6 – Multiple linear regression models for changes in sedentary time with HbA1c showing adjusted associations when using lower sedentary time cut-points (<50cpm as opposed to <100cpm)

	Sedentary time change (per 30mins/day)
Model 1	
HbA1c Change (%)	0.02 (0.01, 0.03), p = 0.016
~HbA1c Change (mmol/mol)	0.2 (0.04, 0.37), p = 0.016
Model 2	
HbA1c Change (%)	0.01 (-0.01, 0.02), p = 0.293
~HbA1c Change (mmol/mol)	0.1 (-0.08, 0.27), p = 0.293
Model 3	
HbA1c Change (%)	0.005 (-0.01, 0.02), p = 0.491
~HbA1c Change (mmol/mol)	0.06 (-0.11,0.23), p = 0.491

Data are unstandardised regression coefficients (95% CI), p-value.

Model 1: adjusted for age, sex, smoking status, ethnicity, beta-blocker use for hypertension, change in accelerometer wear time, IMD score, baseline HbA1c, baseline body weight, baseline sedentary time and baseline MVPA time.

Model 2: adjusted for all covariates in Model 1 and MVPA time change,

Model 3: adjusted for the same covariates as Model 2 and body weight change.

Table 4.7 – Multiple linear regression models for changes in MVPA time with HbA1c showing adjusted associations when using lower MVPA time cut-points (>1041cpm as opposed to >1952cpm)

	MVPA time change (per 30mins/day)
Model 1	
HbA1c Change (%)	-0.07 (-0.1, -0.03), p < 0.001
~HbA1c Change (mmol/mol)	-0.7 (-1.11, -0.37), p < 0.001
Model 2	
HbA1c Change (%)	-0.07 (-0.1, -0.03), p = 0.002
~HbA1c Change (mmol/mol)	-0.8 (-1.28, -0.29), p = 0.002
Model 3	
HbA1c Change (%)	-0.07 (-0.1, -0.02), p = 0.003
~HbA1c Change (mmol/mol)	-0.7 (-1.21, -0.24), p = 0.003

Data are unstandardised regression coefficients (95% CI), p-value.

Model 1: adjusted for age, sex, smoking status, ethnicity, beta-blocker use for hypertension, change in accelerometer wear time, IMD score, baseline HbA1c, baseline body weight, baseline sedentary time and baseline MVPA time.

Model 2: adjusted for all covariates in Model 1 and sedentary time change

Model 3: adjusted for the same covariates as Model 2 and body weight change.

DISCUSSION

Whilst the effect of physical activity and weight loss interventions on HbA1c have been well established in those with T2DM, (**Avery et al., 2012; Umpierre et al., 2011**) the impact of individual lifestyle components on HbA1c in non-diabetic populations is not as well defined. This study helps to address this evidence gap by quantifying the relative importance of changes to; sedentary time, MVPA and body weight in the regulation of HbA1c levels in those at high risk of T2DM.

The current study demonstrated that both change in MVPA and body weight were independently associated with change in HbA1c whereby every 30 minute increase in MVPA per day was associated with a 0.11% (1.2 mmol/mol) decrease in HbA1c and every 6 kg increase in body weight was associated with a 0.08% (0.9mmol/mol) increase. Of note, we found there was a significant interaction with glycaemic status whereby those with dysglycaemia at baseline had stronger associations of MVPA and body weight with HbA1c, further supporting the importance of lifestyle change in those with non-diabetic hyperglycaemia.

Using linear scaling from data published for the large Atherosclerosis Risk in Communities (ARIC) study (**Selvin et al., 2010**), it is suggested that each 0.1 absolute percentage increase in HbA1c (1.1 mmol/mol), is associated with a 6.1% increased risk of diabetes, a 1.8% increased risk of coronary artery disease, a 3% increased risk of stroke and a 1.1% increased risk of all-cause mortality in non-diabetic populations. Therefore the change in HbA1c associated with a 30 minute change in MVPA or a 6 kg change in body weight is likely to be clinically meaningful in a non-diabetic population.

Our findings are consistent with that of the Finnish Diabetes Prevention Study (FDPS), which achieved around a 0.2% reduction in HbA1c over three years with an intervention aimed at achieving a 5% body weight loss, at least 30 minutes of MVPA per day and a healthy diet in those with impaired glucose tolerance (**Lindstrom et al., 2003**).

However, results from the Look Ahead study, which focused on achieving similar parameters to the FDPS, observed a reduction in HbA1c of 0.36% (3.9 mmol/mol) over a four year period (**Look AHEAD Research Group, 2010**). This supersedes both the results of the FDPS mentioned above and the associations that attaining such

parameters would have in the current study, however their use of overweight and obese diabetic participants may have steepened the gradient for improvement beyond that observed in non-diabetic populations.

Results of the current analysis are also consistent with cross sectional analyses from national surveys which have reported associations of MVPA, but not sedentary time, with HbA1c (**Hamer et al., 2014; O'Donovan et al., 2013; Stamatakis et al., 2012**) and extend previous research that has demonstrated the effect of physical activity and body weight with risk of T2DM based on fasting or 2-h glucose values (**Gong et al., 2015**).

The current study supports MVPA and body weight as key targets for the prevention of T2DM when assessed by HbA1c. However, the results for change in sedentary time were more equivocal. Although sedentary time has been associated with an increased risk of T2DM (**Biswas et al., 2015; Wilmot et al., 2012**) and metabolic syndrome (**Edwardson et al., 2012**), the degree to which this is independent of MVPA or total physical activity level remains controversial (**Maher et al., 2014**). This study found that although change in sedentary time was associated with change in HbA1c, the findings were attenuated when adjusted for MVPA. This finding is in contrast to studies which have found associations between sedentary time and 2-h post challenge glucose and levels of insulin sensitivity (**Healy et al., 2008; Henson et al., 2013; Yates et al., 2015**), in addition to experimental interventions which have found improved postprandial glucose responses with reductions to sitting time (**Dempsey et al., 2016a**). This discrepancy in findings could result from the properties of HbA1c which reflect 'average' glucose concentration and may therefore be less sensitive to the more subtle effects on postprandial responses and peripheral insulin sensitivity. Furthermore, experimental investigations showing improved glucose outcomes with reductions in sitting time have been generated by protocols that are designed to break up prolonged sedentary time with short regular bouts of light activity while in a postprandial state (**Dempsey et al., 2016a**), and the cohort from which our current analysis derived from were under no such instruction. Whether or not a change in sedentary time would have been independently associated with a significant change in HbA1c if this cohort were instructed to frequently break up 'postprandial' sitting time rather than simply reduce 'total' sitting time is unknown. Given that research has demonstrated that advice to

walk ‘after meals’ is more effective at lowering postprandial glycaemia than general advice to walk more (**Reynolds et al., 2016**), this would be an interesting focal point for future research to explore. It is also worth noting that activity data from our study cohort was generated by hip-worn accelerometers, these are currently unable to differentiate between static seated and standing postures, and are often prone to the misclassification of standing (a light intensity activity) as a sedentary behaviour (**Kozey-Keadle et al., 2011; McMahon et al., 2010; Skotte et al., 2014**). This may also have influenced our associations between sedentary behaviour with HbA1c. In light of the above, our current findings should not therefore be used to dismiss the role of reducing sedentary behaviour in promoting reduced HbA1c, but should be viewed in the context of the wider available evidence and highlights the need for more research in this area.

The ‘Walking Away from Type 2 Diabetes’ randomised control trial (**Yates et al., 2012**), from which the data in this analysis derived from, experienced no differences between control and lifestyle intervention groups, with a small decrease in activity levels for the entire cohort (**Yates et al., 2016**). A wide range of variation in both directions allowed the current analysis to be undertaken, but demonstrates the challenging nature of initiating and sustaining the amount of physical activity required to elicit clinically significant results.

The main strength of this study is that it provides novel prospective evidence in a high-risk primary care population using objective measures of sedentary behaviour, physical activity and body weight. Despite the prospective nature of this study, direct causality cannot be inferred and it is possible that unmeasured lifestyle factors were confounding relationships. In addition, whilst the study population is likely to be broadly representative of those referred into diabetes prevention pathways within primary care, their high risk nature means the results are not be generalisable to the general population.

In conclusion, although further research into the chronic glycaemic effects of sedentary behaviour change is warranted, increasing MVPA and reducing body weight both appear to have favourable influences on HbA1c levels in those identified as being at high risk of T2DM through a primary care setting. Through the use of regression modelling, this study is able to quantify the impact that manipulating important behavioural targets

would have on HbA1c levels, this addresses an important limitation and can be used to inform future diabetes prevention interventions within primary care. Given the observational nature of this study, further research is needed to confirm these results.

Having addressed the main aims of this PhD, the next Chapter of this thesis (Chapter Five) summarises all the main findings, identifies ways in which the current research could be extended, and discusses key areas for future research that have emerged as a result of this programme of work.

A summary table of the main findings for each chapter, alongside their strengths and limitations, are also presented in **Table 5.1.**

Chapter Five: Thesis discussion and future directions

Thesis summary of findings

In Chapter Two of this thesis, I set out to experimentally investigate whether an individual's CRF level (which is known to be predominantly determined by habitual engagement in MVPA) modified their postprandial glycaemic responses to both sedentary time and light activity breaks. From this investigation, it was found that individuals with higher fitness had healthy glycaemic profiles in response to prolonged sitting and subsequently efforts to strategically break up sitting time with regular light activity breaks had limited effect. In contrast, those with lower fitness experienced exaggerated rises in postprandial glycaemia during prolonged sitting time and in turn experienced far greater benefit from the implementation of light walking breaks. This was the first experimental investigation to explore whether CRF protectively modified the relationship between sedentary time and postprandial glycaemia.

In Chapter Three of this thesis, I set out to experimentally investigate whether introducing upper body physical activity breaks, while maintaining a seated posture, could emulate postprandial improvements witnessed throughout upright (non-seated) strategies that have emerged throughout experimental research to date. This investigation was conducted in obese adults at high risk of T2DM and it was found that introducing regular bouts of seated arm ergometry was an effective way of improving postprandial glycaemia (marked by significant reductions in both glucose and insulin) compared to that of uninterrupted sitting alone. In light of this, it appears there is no direct requirement for postural adaptation from that of sitting in order to elicit significant glycaemic improvements. Given that all experimental research to date has focused on physical activity breaks that alter the posture of sitting (from sitting to upright), this is an important insight into the nature of how physical activity breaks act to regulate postprandial glycaemia. Seemingly, muscle activation during activity breaks appears far more crucial than postural manipulation.

In Chapter Four of this thesis, I conducted a prospective secondary data analysis to determine how much a change in objectively measured sedentary time over a 36 month period was associated with a change in long-term glycaemic control (measured by HbA1c). In a group of individuals deemed to be at high risk of T2DM, we found that

although change in sedentary time was significantly associated with a change in HbA1c, this did not persist following adjustment for a change in MVPA levels. This finding is in contrast to experimental interventions (including those highlighted in Chapters Two and Three), which have found improved postprandial glucose responses through physical activity break induced reductions in sedentary time. This discrepancy in findings could result from the properties of HbA1c which reflect average glucose concentration and may therefore be less sensitive to the more subtle effects of postprandial reductions. Furthermore, the way in which sedentary behaviour was measured (ie. via hip worn accelerometers) may have led to misclassification of standing as a sedentary behaviour, further contributing to the discrepancy in our findings. Nonetheless, the other lifestyle changes (body weight and MVPA) both showed significant independent associations. The ability of this study to quantify the amount of change in lifestyle behaviours required to elicit a given amount of change in HbA1c is an important finding. If these novel findings continue to be supported in the literature, this may contribute to tailored parameters being set by diabetes prevention programmes depending on the severity of an individual's current HbA1c level.

Future directions for personalised experimental research and clinical implications

Extending Chapter Two

In light of the results shown in Chapter Two, it is anticipated that individuals with unavoidable sedentary occupations may be able to increase their fitness outside of working hours to protect themselves from the unfavourable glycaemic impacts of their prolonged sitting time. Furthermore, it appears that guidance targeting reductions and interruptions in sedentary time may be better suited for those with lower fitness levels, whom subsequently reflect the majority of society. This reinforces the demand for targeting reductions, or more importantly breaks, in sedentary time.

Given that this investigation was undertaken in normoglycaemic individuals, the protective effects of fitness in those with pre-diabetes can not be generalised and further experimental research in this cohort is warranted to extend diabetes prevention research. Given that people with pre-diabetes are likely to be unfit, they are likely to gain more benefit from sedentary breaks than the general population. This may even

help to explain why standing breaks, despite their low intensity stimulus, have shown to be sufficient in this population (**Henson et al., 2016**).

To further determine direct cause and effect for the modifying influences of fitness, it would be necessary to instigate a lifestyle intervention aimed at prospectively increasing or decreasing fitness levels, and monitoring postprandial glycaemic responses to both a prolonged sedentary condition and a light activity breaks condition both before and after this intervention. Providing other lifestyle behaviours such as diet are kept constant, this would certainly help to further elucidate the cause and effect of higher fitness in the protection of glycaemic profiles while engaging in sedentary behaviours.

Having investigated and demonstrated the modifying effects that fitness may have, it would also be interesting to see how sedentary behaviour interacts with other risk factors. For example, whether BMI or ethnicity can modify an individual's glycaemic response to sedentary behaviour and light activity breaks is currently unexplored and warrants further investigation. The South Asian phenotype in particular has been hypothesised to respond differently to physical activity interventions (**Chapman et al., 2013; Bhopal et al., 2014**), although it is unclear whether this extends to sedentary behaviour research. Greater insight into the modifying effect of different risk factors will allow greater tailoring for sedentary behaviour interventions in the future.

Our results could be used to help inform current diabetes prevention programmes (**NHS England, 2017**) which should now consider promoting reductions in sedentary time through regular light intensity physical activity breaks in those with low CRF levels.

<u>Extending Chapter Three</u>

A clear priority and area of focus is in translating the findings from Chapter Three into longer term interventions in clinical populations. The ability to regulate postprandial glycaemia while remaining in a seated posture has received large amounts of clinical interest from podiatrists and foot clinics across the East Midlands (UK) given its potential applicability in populations who are unable to stand or weight bear, for instance, those with diabetes related foot ulceration who are advised against this. I am currently working with leading clinicians and academics to initiate my first post-doctoral research

project whereby we plan to roll out the use of seated arm ergometry to individuals with diabetic foot complications.

It is thought that up to 25% of individuals with diabetes will develop a foot ulcer at some point during their lifetime (**Singh et al., 2005**). This is a shocking statistic given that up to 80% of individuals identified with having a diabetes induced foot ulcer will require amputation (**Pandian et al., 2001**), and 50% will die within 5 years of its occurrence (**National Institute for Health and Care Excellence, 2015**). The prognosis for those with diabetic foot ulcers as it stands is discouraging.

Postprandial hyperglycaemia plays a pivotal role in the pathology of diabetic foot ulcers (please refer to figure 5.1 adapted from citation (**Prakash, 2011**)). The recurrence, chronicity and healing time of diabetic foot ulcers are also strongly influenced by postprandial hyperglycaemia (**Prakash, 2011**). It has been shown that better glycaemic control leads to far greater wound healing rates (**Marston, 2006**). The ability to regulate or control hyperglycaemia in those with diabetic foot ulcers, is therefore of paramount importance as demonstrated in **Figure 5.1**.

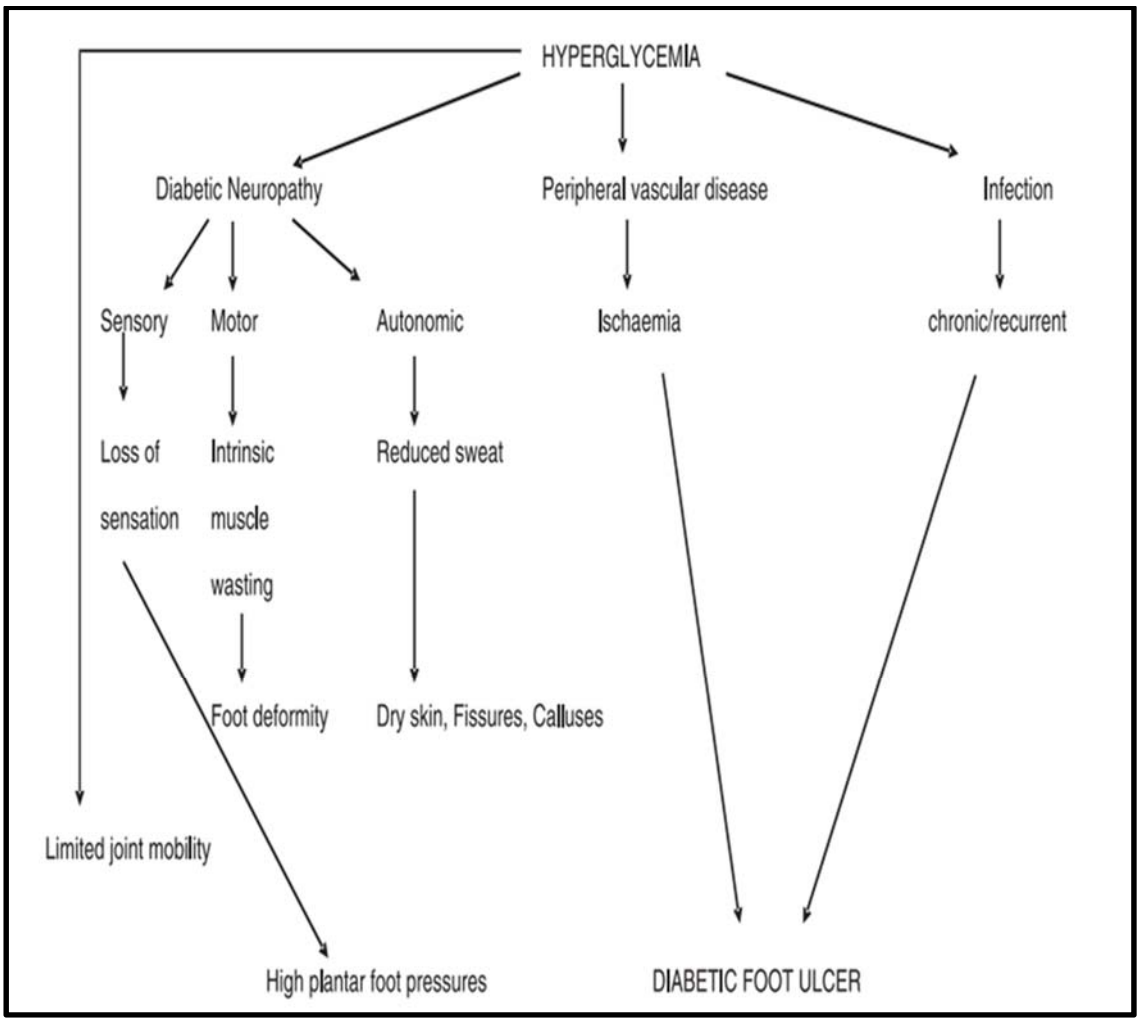


Figure 5.1 – The role of hyperglycaemia in the pathogenesis of diabetic foot ulcers (adapted from Prakash, [2011]).

One way to improve glycaemic control is to regularly engage in exercise. However, guidance from the American Diabetes Association (ADA) state that non-weight bearing (otherwise known as offloading) is vital to offset risk of diabetic foot ulcers and aid in their treatment, specifically quoting "It is not what one puts on a wound that heals it, it is what one takes off" (**Armstrong et al., 2014**). This guidance is also supported by a recent consensus statement by the American Podiatric Association (2014), who also emphasise the importance of offloading to heal diabetic foot ulcers (**Snyder et al., 2014**). Despite the undeniable benefits of offloading, this too can be problematic. For instance, the simplest way to offload an affected limb is via bed rest or sedentary behaviour, and as discussed in Chapter One, we know how detrimental this can be for postprandial glycaemic regulation, especially in those with diagnosed T2DM.

The benefits of offloading have been repeatedly weighed up against the metabolic downfalls of low physical activity levels that stem from it. This has often lead to mixed messages regarding the management of diabetic foot ulcers, some experts endorsing exercise and others in favour of completely offloading (**Dhatariya & Fox, 2014**). However, regardless of the theoretical argument, promoting purposeful weight bearing exercise in those with diabetic foot conditions is unlikely to be feasible or clinically supported.

An aspect that seems to have been overlooked when discussing the management of diabetic foot ulcers is that offloading and physical inactivity do not have to be mutually exclusive. Introducing seated arm ergometry breaks is a novel initiative whereby an individual can be non-weight bearing and still engage in physical activity. Not only this, but breaking up sedentary behaviours with short bouts of light activity also overcomes many of the common barrier of exercise participation such as change of clothing, sweating and time restraints. Light intensity physical activity breaks may therefore pose as a more appealing way to improve glycaemic regulation. My findings from Chapter Three demonstrate around a 50% reduction in postprandial glucose levels when implementing 5 minutes of seated arm ergometry every 30 minutes following a meal. Although this investigation was conducted in those at high risk of T2DM, we anticipate that those with diagnosed T2DM will gain similar benefit.

Although research has acknowledged the potential of arm ergometry as a non-weight bearing alternative to upright exercise, our current study was the first to show the glycaemic impacts of using this mode of activity to break up prolonged sedentary time. Given the anticipated benefits in individuals with T2DM, it appears seated upper body activity may provide a hybrid approach to satisfying non-weight bearing instruction and remaining physically active, which may in turn alleviate the discouraging prognosis for individuals diagnosed with diabetic foot ulceration. This is certainly an area that needs further exploration, particularly in investigating whether acute changes to postprandial glycaemia can be translated into clinically meaningful outcomes in this population, and is already in the pipeline for my postdoctoral research.

Not only does the implementation of seated arm ergometry breaks provide an alternative strategy to break up sedentary time in those with contraindications to

weight bearing, it may also provide a more appealing and feasible strategy to regulate postprandial dysglycaemia in those who would simply rather remain seated. For instance, other effective modes of activity that have emerged to date such as walking breaks could be criticised for being disruptive to the working day due to the necessity of leaving the workspace and desk area (**De Cocker et al., 2015**).

If the current findings continue to be supported by experimental research, future diabetes prevention programmes targeting reductions in sedentary time should not primarily intend to manipulate the posture of sitting, but should be aimed more at manipulating the low levels muscle activation associated with traditional sitting. Continuing to provide alternative strategies to break up sedentary time will allow for more personalised guidance which would logically lead to better adherence and consequently better clinical outcomes.

Given that arm ergometers are not readily available to the public, future research would also benefit from investigating alternate ways to instigate upper body contractile activity while remaining seated, (i.e. seated resistance band movements). It is also worth considering that those with weight bearing contraindications as a result of severe diabetes induced peripheral neuropathy may also exhibit upper body nerve damage that could inhibit their ability to grip, this could make upper body muscle contraction harder to instigate. Research has begun to suggest that isometric electro-stimulation of muscle contractions are a plausible way of reducing blood glucose levels (**Joubert et al., 2015**), whether or not the implementation of electro-stimulation at similar time intervals as the activity breaks highlighted in Chapters Two and Three could bring about significant glycaemic improvements would certainly be of great interest and increase the personalisation of efforts to tackle sedentary behaviour in those least able to counteract it.

<u>Other factors to consider when extending experimental research</u>

The experimental research reported in both Chapters Two and Three utilised an extensive ≥8 hour prolonged sitting condition while refraining from excessive movements. Although this has become common practice for experimental research in this field, it is undoubtedly extreme in nature. Likewise, although completely feasible, breaking up prolonged sedentary time for 5 minutes as often as once every half an hour

is a large commitment. Further research into the minimal frequency, duration and intensity of physical activity breaks that are required to instigate clinically significant reductions in postprandial glycaemia are necessary in order to embark on more attractive and sustainable goals in future. A population of inactive, obese, pre-diabetic individuals are the most predisposed to T2DM, conducting optimal strategies for targeting sedentary time within this cohort would therefore be pivotal in the progression of diabetes prevention research.

Although investigations into the chronic effects of long term reductions in sedentary behaviour have already been undertaken (**Healy et al., 2017; Alkhajah et al., 2012; Graves et al., 2015; John et al., 2011; Koepp et al., 2013**), these overlook postprandial glucose as an outcome measure. Given that the successes of breaking up sedentary time in an acute setting stem from improvements in postprandial glycaemia (**Dempsey et al., 2016a**), future research into the chronic effects of sedentary breaks on postprandial glycaemia would be of clinical interest. It is also important that chronic interventions promoting sedentary breaks enforce the importance of physical activity in metabolically sensitive timeframes in order to emphasise their true potential to manipulate glycaemic profiles.

It is also important to ensure that all work instigated in a laboratory environment can be generalised to a more ecologically valid setting, as such it is crucial that the feasibility of rolling out experimental research to workplaces, homes and hospital settings are explored, especially given that this is where the majority of sedentary behaviours take place.

<u>Extending Chapter Four</u>

Our findings update previous research, suggesting that increasing MVPA and reducing body weight to levels that are commonly promoted in diabetes prevention programmes are likely to lead to clinically meaningful reductions in HbA1c, thereby further highlighting these behavioural targets as key ingredients for diabetes prevention programmes in real world settings. However, our neutral findings for sedentary behaviour require a more nuanced interpretation, particularly in light of the recent abundance in experimental evidence (including the findings outlined in Chapters Two and Three) linking reductions in sedentary behaviour to improved postprandial glucose

profiles. The discrepancy of our current findings may reflect the properties of HbA1c which reflect 'average' glucose concentration and may therefore be less sensitive to the more subtle effects on postprandial responses, especially given that the largest contributor to our average glycaemic control are fasting glucose levels. Furthermore, whether a change in sedentary time would have been independently associated with a significant change in HbA1c if this cohort were instructed to frequently break up sitting time following food intake, rather than simply to reduce 'total' sitting time is unknown, and would provide further insight. Our research should not therefore be used to dismiss the role of reducing sedentary behaviour in promoting reductions in HbA1c, but should be viewed in the context of the wider available evidence and highlights the need for more research in this area. In particular, this study should act as a prompt for epidemiologists to analyse accelerometer data in more sophisticated ways helping to generate new hypotheses around the optimal pattern of reducing sedentary behaviour (duration, intensity, frequency and timing of breaks) to improve metabolic health. The use of new generations of accelerometers in large cohorts, such as UK Biobank, will provide researchers with the perfect tools to progress knowledge in this area over coming years.

Overall conclusions

Sedentary behaviour has become ubiquitous in modern society and has contributed to the epidemic of T2DM. With accumulating experimental and epidemiological evidence continually emerging, it is anticipated that detailed sedentary guidelines (on par with those established for MVPA) are on the horizon and that efforts to reduce/regularly interrupt sedentary time will join MVPA and body weight as primary targets within diabetes prevention programmes.

The programme of research documented in this thesis has assisted in bridging the gaps in our existing knowledge surrounding sedentary behaviour, allowing for a more personalised interpretation. Specifically, efforts to promote reductions in sedentary time through regular light intensity physical activity breaks appear to be of more benefit if aimed at those with low CRF levels (whom are likely to reflect the majority of the population). Furthermore, findings from this programme of work demonstrate that sedentary guidance should not solely focus on breaking up the posture of sedentary

time but rather the muscular inactivity that co-exists with it. The notion that sitting *per se* is not responsible for the metabolic downfalls of sedentary behaviour is an important insight, one of which could be ideal for the personalisation of strategies for those with contraindication to weight bearing, who may employ activity breaks while remaining in a seated posture. These findings may also be used to update current physical activity guidelines which focus on reducing 'sitting' time to tackle the harms of sedentary behaviour, this completely overlooks the merits of 'seated' activity breaks.

The level of postprandial blood glucose reductions witnessed when implementing frequent walking breaks *in those with low CRF* (Chapter Two) and when implementing upper body contractile activity breaks (Chapter Three) would certainly be expected to influence HbA1c if these acute findings were sustained chronically through long term lifestyle intervention. Although no chronic glycaemic improvements (when gauged by associations with HbA1c) were witnessed with general reductions in sedentary time over a three year period within our epidemiological analysis (Chapter Four), this should not be used to dismiss the role of reducing sedentary behaviour in promoting reduced HbA1c, but highlights the need for research in this area to build long term interventions based on the acute experimental methodologies (as far as can be feasibly transferred).

A summary of the main findings for each chapter of thesis, alongside their strengths and limitations, are presented in **Table 5.1**.

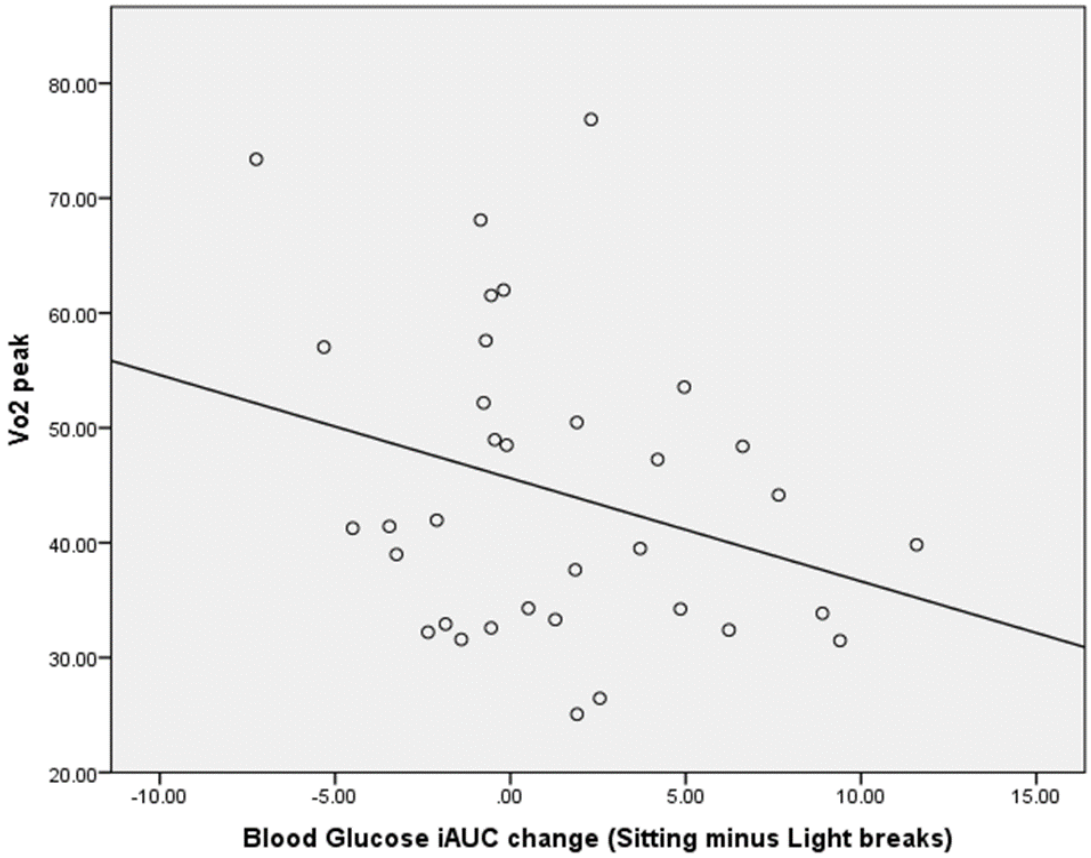
Table 5.1 – Main findings, strengths and limitations of each chapter

Chapter	Main findings & real world application	Strengths	Limitations
Two **The 'FIT 2 SIT' experimental investigation**	**Main findings:** • CRF significantly modified treatment response for the primary outcome of glucose iAUC. • Participants with lower CRF had worse postprandial glycaemic responses during prolonged sitting, and were able to gain greater metabolic benefit through breaking their sitting time with light intensity activity compared to individuals with higher fitness. **Real-world application:** • Guidance to break up sedentary behaviour would be more appropriately aimed at those with lower CRF and therefore remains appropriate for the majority of the population. • Those with unavoidable sedentary occupations may build CRF outside of working hours to negate the deleterious metabolic impacts of their sitting time.	• The first experimental investigation to determine whether; a) CRF modifies metabolic responses to prolonged bouts of sitting. b) CRF modifies the metabolic benefits elicited by introducing frequent light activity breaks. • Using an objective measurement of VO_2 peak, we were able to capture a broad spectrum of CRF levels within the study cohort. • Glycaemic response to a) prolonged sitting and b) light walking breaks were tested under standardised, order randomised, controlled laboratory conditions. • Supports epidemiological research indicating that high levels of MVPA (the strongest determinant of CRF) may negate deleterious impacts of prolonged sitting time.	• CRF levels were only assessed at one time-point. Direct causality of CRF on treatment effect may have been further elucidated by an intervention designed to manipulate CRF levels and observe prospective changes in glycaemic response to experimental conditions. • Can not be generalised to alternate cohorts. The modifying effect of CRF may differ in those at high risk or clinically diagnosed T2DM populations compared to that of the healthy individuals utilised here. • Did not objectively test whether instructions to standardise diet and refrain from alcohol/caffeine prior to experimental conditions was adhered to, relying on self-reported adherence only.

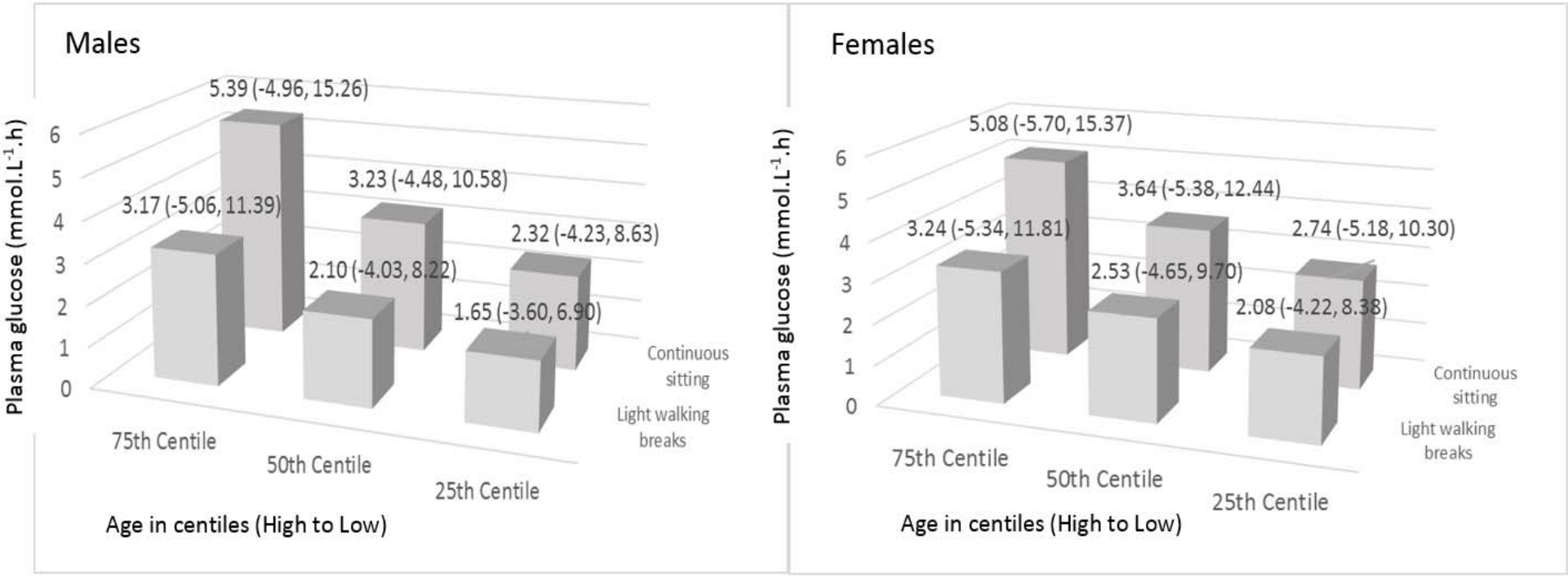
Chapter	Main findings & real world application	Strengths	Limitations
<u>Three</u> **The 'Arming your Health' experimental investigation**	**Main findings:** • Compared to the prolonged sitting only condition iAUC (7.4 ± 3.5 mmol/L · h), seated arm ergometry breaks significantly reduced glucose iAUC by 57% (3.1 ± 3.1 mmol/L · h). • Compared to the prolonged sitting only condition iAUC (696 ± 557 mU/L · h), seated arm ergometry breaks significantly reduced insulin iAUC by 20% (554 ± 425 mU/L · h). • Performing short bouts of arm ergometry during prolonged sitting attenuated postprandial glycaemia despite maintaining a seated posture. **Real-world application:** • Individuals may be able to regulate their metabolic responses to prolonged sitting without necessarily breaking up their seated posture. This could be of particular interest to those with weight baring difficulty who can not engage in the upright strategies that have emerged to date. • Seated activity breaks may be more appealing to those who find it difficult to leave the desk space/ work area. This overcomes a common criticism of alternative strategies such as walking breaks. • Sedentary behaviour guidance would be better aimed at regulating the low levels of muscle activation that are commonly associated with sedentary time rather than aiming to manipulate the posture.	• This is the first experimental investigation to observe whether glycaemic responses during prolonged sitting time can be attenuated while remaining in a seated posture. • Utilised a population at high-risk of T2DM recruited through a primary care setting. • Glycaemic response to a) prolonged sitting and b) light walking breaks were tested under standardised, order randomised, controlled laboratory conditions.	• Small sample size limits generalisability beyond the cohort utilised. • Despite purposefully matching the energy expenditure of arm ergometry breaks to that of light intensity walking (to allow for better comparison to previous experimental research), we did not include a third experimental condition of light intensity walking, this would have allowed for more accurate comparison. • The self-perceived intensity of the seated breaks necessary to match to the energy expenditure of light walking was not explored. • Arm ergometers are not readily available to the public.

Chapter	Main findings & real world application	Strengths	Limitations
Four **The epidemiological analysis**	**Main findings:** • Change in sedentary time was not significantly associated with change in HbA1c after adjustment for change in MVPA time. • An increase in MVPA time (per 30mins/day) was associated with a clinically significant decrease in HbA1c percentage (β = -0.11 (-0.18, -0.05), p=0.001). • An increase in body weight (per 6 kg) was associated with a clinically significant increase in HbA1c percentage (β = 0.08 (0.04, 0.12), p<0.001). • Associations above were strengthened in those with dysglycaemia at baseline. **Real-world application:** • Increasing MVPA (by ≥ 30min) and reducing body weight (≥ 6 kg) represent key targets for the prevention of T2DM when assessed by HbA1c. • The role of reducing sedentary behaviour in promoting reductions in HbA1c should not be dismissed in light of this research, but highlights the need to build longer term interventions that directly extend acute experimental methodologies used to date.	• Provides novel prospective evidence in a large high risk primary care population using objective measures of sedentary behaviour, physical activity and body weight. • First to quantify associations of lifestyle behaviour change (including sedentary behaviour) with HbA1c in those at high risk of T2DM. • Builds upon associations derived from oral glucose tolerance testing (OGTT) which are becoming outdated due to the recent migration towards HbA1c usage.	• Little is known about how sedentary time was accumulated: - Was it in prolonged bouts? - What were the number of activity breaks? - How were reductions in sitting time distributed, were they distributed strategically around meals? • As this information was unknown, it may have overlooked true potential associations between sedentary time change and HbA1c. • Activity data was generated by hip-worn accelerometers that are often prone to the misclassification of standing (a light intensity activity) as a sedentary behaviour, which may have influenced associations.

Appendix One – Supplementary Tables and Figures



Supplementary Figure 1 – Spread of treatment response against VO_2 peak during the 'FIT 2 SIT' study (documented in Chapter 2).



Supplementary Figure 2 – Sensitivity analysis showing predicted glucose values between treatment conditions across sex-specific tertiles of age

25th tertile of age corresponds to 30 for Males, and 36 for Females. 50th tertile of CRF corresponds to 35 for Males, and 41 for Females.
75th tertile of CRF corresponds to 47 for Males, and 49 for Females.

Predicted glucose iAUC values were derived from the below equations gained from linear regression models entering glucose iAUC within each condition as the dependant variable with age and sex entered as independent variables. 95%CI values show the variability around the derived estimates; negative values represent postprandial glucose concentrations that are suppressed below fasting levels. The derived glucose iAUC values and 95% CIs are within the range observed in this study (minimum observed glucose iAUC = -9.73 mmol·L-1·h, maximum observed glucose iAUC = 16.50 mmol·L-1·h)

Equations: Glucose iAUC during prolonged sitting condition = -3.74 + (0.18; 95% CI 0.04, 0.39) x Age + 0.665 if male.

Glucose iAUC during walking breaks condition = -1.123 + (0.089; 95% CI -0.086, 0.264) x Age + 0.106 if male.

Supplementary Table 1 – Individual glucose iAUC values across treatment conditions

Participant Number	Glucose iAUC during prolonged sitting	Glucose iAUC during light activity	Change in Glucose iAUC between conditions (sitting minus light breaks)
1	1.55	-0.9	2.45
2	1.4	-0.1	1.5
3	11.55	6.35	5.2
4	9.85	4.45	5.4
5	4.8	-0.2	5
6	13.8	3.7	10.1
7	6.35	7.05	-0.7
8	8.3	0.5	7.8
9	5.9	7.75	-1.85
10	8.45	1.6	6.85
11	8	4.25	3.75
12	7.2	5.85	1.35
13	8.4	0.5	7.9

Appendix Two - Author contribution to overall programme of work:

The author of this thesis, Matthew McCarthy, performed the following activities in relation to Chapters Two, Three and Four contained within this thesis.

Chapter Two: **Fitness modifies glycaemic responses to sitting and light activity breaks: A randomised crossover trial (FIT 2 SIT Study).**

- Obtained University of Leicester ethical approval alongside Research Development ethical approval.
- Processed several ethical amendments to the study prior to enrolment.
- Developed the study protocol and all study documents (included in **Appendix Three**).
- Responsible for recruitment from the general public. This involved promoting research at numerous public lectures and distributing approved study material at the University of Leicester, Loughborough University, local community leisure centres, sports clubs, and supermarket/newsagent notice boards.
- Performed a wide range of administrative duties: including booking laboratory time and nursing staff for experimental visits; checking biochemical results using iLAB software; presenting screening results to participants (and medics where necessary); arranging/ re-arranging appointments and sorting transport for participants.
- Liaised with nurses and administrative staff at the Leicester Diabetes Centre to ensure adequate nurse and laboratory availability.
- Undertook informed consent for all participants (n = 36).
- Initialised, downloaded and analysed activity monitors (ActiGraphs and ActivPALs) for all participants across all visits.
- Prepared standardised meals for all visits (n = 180).
- Carried out Vo2 maximal fitness testing procedure for all participants enrolled in the study (n = 36).
- Labelled blood bottles, centrifuged whole blood samples to seperate plasma in preparation for insulin analysis (n = 748).

- Analysed plasma samples for insulin using a Meso-Scale Discovery electrochemiluminescence technique collectively at the end of the study.
- Arranged all study visits (n = 104) and monitored participants throughout each visit to ensure compliance and tolerance to the procedures (> 650 hours in total).
- Communicated study results to participants.
- Organised travel expense reimbursements.
- General maintenance of laboratory conditions throughout study visits and during insulin analysis.
- Analysed and interpreted study data (under the guidance of a senior statistician).
- Wrote up official manuscript for publication consideration and responded to reviewer comments (under the guidance of supervisors).
- Site-File maintenance.

Chapter Three: **Breaking up sedentary time with seated upper body activity can regulate metabolic health in obese high-risk adults: A randomised crossover trial (Arming your Health Study).**

- Obtained 'NHS Research Ethics Committee' and 'Research Development' ethical approval.
- Identified and contacted all eligible participants from existing databases.
- Developed the study protocol and all study documents (included in **Appendix Four**).
- Performed a wide range of administrative duties: including developing and sending invitation/appointment/results letters to participants; checking biochemical results using iLab software; re-arranging appointments; arranging transport for participants where necessary.
- Undertook informed consent for all participants (n = 14).
- Initialised, downloaded and analysed activity monitors (GENE-Activ's and ActivPALs) for all participants across all study visits.

- Prepared standardised meals for all visits (n = 70).
- Performed all energy expenditure testing during the familiarisation visit using a breath-by-breath gas-analysis system (Metalyser 3B, Cortex Biophysik, Leipzig, Germany).
- Labelled blood bottles, centrifuged whole blood samples to separate the plasma in preparation for insulin analysis (n = 260).
- Analysed insulin samples using a Meso-Scale Discovery electrochemiluminescence technique collectively at the end of the study.
- Arranged all study visits (n = 40) and monitored participants throughout each visit to ensure compliance/tolerance to the procedures (>250 hours in total).
- Communicated study results to participants (and GP's where necessary).
- Organised payments for participation and travel expenses.
- General maintenance of laboratory conditions throughout study visits and during insulin analysis.
- Analysed and interpreted study data.
- Wrote up official manuscript for publication consideration and responded to reviewer comments (under the guidance of my supervisors).
- Site File maintenance.

Chapter Four: **Change in sedentary time, physical activity, body weight and Hba1c in high risk adults (prospective secondary data analysis).**

- Retrieved all necessary data/ variables from the Walking Away from Diabetes database.
- Merged data in Microsoft Excel.
- Conceived and designed the research question.
- Analysed all data using IBM SPSS Statistics Version 22.
- Wrote up official manuscript for publication consideration and responded to reviewer comments (under the guidance of my supervisors).
- Responded to journal editor queries following acceptance of manuscript in the 'Medicine & Science in Sports & Exercise' journal

Appendix Three – Supporting documents related to the 'FIT 2 SIT' study

This appendix contains the following documents in the order that they are presented:

- University of Leicester Ethics Approval
- Research and Development Ethical Approval
- Research Protocol
- Participant Information Sheet
- Pre-screening Questionnaire
- Physical Activity Questionnaire
- Informed Consent Form

University of Leicester Ethics Approval

University of Leicester Ethics Review Sign Off Document

To: **MATTHEW MCCARTHY**

Subject: Ethical Application Ref: **mm636-e61c**

(Please quote this ref on all correspondence)

04/07/2014 18:20:09

Health Sciences

Project Title: **The impact of cardio-respiratory fitness on an individual's metabolic response to prolonged sitting and light activity breaks**

Thank you for submitting your application which has been considered.

This study has been given ethical approval, subject to any conditions quoted in the attached notes.

Any significant departure from the programme of research as outlined in the application for research ethics approval (such as changes in methodological approach, large delays in commencement of research, additional forms of data collection or major expansions in sample size) must be reported to your Departmental Research Ethics Officer.

Approval is given on the understanding that the University Research Ethics Code of Practice and other research ethics guidelines and protocols will be compiled with

- http://www2.le.ac.uk/institution/committees/research-ethics/code-of-practice
- http://www.le.ac.uk/safety/

The following is a record of correspondence notes from your application **mm636-e61c.** Please ensure that any proviso notes have been adhered to:-

Jun 17 2014 1:38PM Hi Matthew

Thank you for this well-thought out application. I have just a couple of queries/suggestions before I give approval.

1. My main concern is recruitment. You are targeting people in full-time work yet are not providing payment. Some office-based staff may be able to work during the visits, but lorry drivers (as mentioned in your documentation) will not. Do you have a back-up plan if you struggle to recruit?

2. The poster looks a bit busy - you may wish to consider reducing the text (not a condition of approval, just my personal opinion).

Please reply to point 1 (and point 2 if you wish) using the Notes field, and resubmit. I'll then approve asap.

With best wishes
Emma

Jul 1 2014 1:09PM Hi Emma,

Many thanks for reviewing my University Ethics application for the 'FIT 2 SIT' study it is much appreciated and I shall try to address your comments as best as possible.

Point 1: I appreciate that without payment full time workers may struggle to attend my study and subsequently this will make recruitment much more difficult. In response to this, as a back up plan, I have been assured by my supervisor (Dr.Tom Yates) and also Prof.Melanie Davies that people working here at the Leicester

Diabetes Centre (a large workforce of over 200 people) will be allowed time off their normal duties to take part in my study while receiving their normal daily salary. Furthermore, to cover all angles, I am currently in discussion with colleagues here at the Leicester Diabetes Centre to see if it is applicable to run my study out of hours (weekends for instance), this would certainly reduce the availability barrier for many potential subjects.

Also thank you for your comment on the poster, this is definitely something I shall consider.

Many thanks for your time and I look forward to your response (and hopefully approval).

Kind Regards,

Matthew McCarthy.

Jul 4 2014 6:20PM Thanks for your response. Good luck with your project.
Emma

--- END OF NOTES ---

Research and Development Ethical Approval

University Hospitals of Leicester NHS
NHS Trust

DIRECTORATE OF RESEARCH & DEVELOPMENT

Research & Development Office
Leicester General Hospital
Gwendolen Road
Leicester
LE5 4PW

Director: **Professor Nigel Brunskill**
Assistant Director: **Dr David Hetmanski**
Head of Research Operations: **Carolyn Maloney**

Direct Dial: (0116) 258 8351
Fax No: (0116) 258 4226

Prof Melanie Davies
Professor of Diabetes Medicine
Leicester Diabetes Centre
Leicester General Hospital

21.07.2014

Dear Prof Melanie Davies

Ref: **CSP 151741**
Title: **The impact of cardiorespiratory fitness on an individual's metabolic response to prolonged sitting and light activity breaks – FIT 2 SIT Study**
Project Status: **Approved**
End Date: **18.08.2015**

Date of Valid Application: 17.07.2014
Days remaining to recruit first patient: 66

I am pleased to confirm that with effect from the date of this letter, the above study has Trust Research & Development permission to commence at University Hospitals of Leicester NHS Trust. The research must be conducted in line with the Protocol and fulfil any contractual obligations agreed between UHL & the Sponsor. If you identify any issues during the course of your research that are likely to affect these obligations you must contact the R&D Office.

In order for the UHL Trust to comply with targets set by the Department of Health through the 'Plan for Growth', there is an expectation that the first patient will be recruited within 70 days of receipt of a Valid Application. The date that a Valid application was received is detailed above, along with the days remaining to recruit your first patient. **It is essential that you notify the UHL Data Management Team as soon as you have recruited your first patient to the study either by email to RDData@uhl-tr.nhs.uk or by phone 0116 258 4573.**

If we have not heard from you within the specified time period we will contact you not only to collect the data, but also to record any issues that may have arisen to prevent you from achieving this target. It is essential that you get in touch with us if there is likely to be a problem in achieving this target so that we can discuss potential solutions. The Trust is contractually obliged to meet the 70 day target and an adequate reason acceptable to the NIHR has not been submitted to explain the issues preventing the recruitment of your first participant, the Trust will be financially penalised.

In addition, we are required to publish the Title, REC Reference number, local target recruitment and actual recruitment as well as 70 days data for this study on a quarterly basis on the UHL public accessed website.

All documents received by this office have been reviewed and form part of the approval. The documents received and approved are as follows:

Document Title	Version	Date	REC Approval
FIT 2 SIT Activity monitor instruction sheet		03.04.2014	N/A
FIT 2 SIT Informed Consent form	1.0	03.04.2014	N/A
FIT 2 SIT Participant Info Sheet	1.0	03.04.2014	N/A
FIT 2 SIT Physical activity Questionnaire	1.0	03.04.2014	N/A
FIT 2 SIT Protocol	1.0	03.04.2014	N/A
FIT 2 SIT Recruitment Poster	1.0	03.04.2014	N/A
Fit 2 Sit. Pre-screening questionnaire	1.0	03.04.2014	N/A

Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments should be followed. Failure to do so may invalidate the approval of the study at this trust.

Undertaking research in the NHS comes with a range of regulatory responsibilities. Please ensure that you and your research team are familiar with, and understand the roles and responsibilities both collectively and individually.

Documents listing the roles and responsibilities for all individuals involved in research can be found on the R&D pages of the Public Website. It is important that you familiarise yourself with the Standard Operating Procedures, Policies and all other relevant documents which can be located by visiting www.leicestershospitals.nhs.uk/aboutus/education-and-research

The R&D Office is keen to support and facilitate research where ever possible. If you have any questions regarding this or other research you wish to undertake in the Trust, please contact this office. Our contact details are provided on the attached sheet.

This study has been reviewed and processed by the Clinical Research Network: East Midlands using the Coordinated System for gaining Trust Permission (CSP). If you require any further information on the approval of this study please contact the CRN: East Midlands Leicester office on 0116 258 6185 making reference to the CSP number which is located at the top of this letter.

We wish you every success with your research.

Yours sincerely

Carolyn Maloney
Head of Research Operations

Encs: .R&D Office Contact Information

cc. Matthew McCarthy – PhD Student
Jayne Hill – Research Governance Manager
CRN: East Midlands CSP Generic Inbox

Research Protocol

FIT 2 SIT

Research Protocol:

Title

The impact of cardio-respiratory fitness on an individual's metabolic response to prolonged sitting and light activity breaks.

Chief Investigator

Dr Thomas Yates

Study Co-ordinator

Matthew McCarthy

Co-investigators

Dr Charlotte Edwardson
Prof. Melanie Davies
Prof. Kamlesh Khunti

Sponsor: The University of Leicester

Funder: Leicester–Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit

Chief Investigator Signature:

Confidentiality Statement:

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host NHS Trust (s), regulatory authorities, and members of the Research Ethics Committee.

Background and Rationale

Sedentary Behaviour – Definition, prevalence and health outcomes

Sedentary behaviour is defined by the Sedentary Behaviour Research Network as "any waking behaviour characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture" [1]. Studies that have objectively assessed population movement patterns (via accelerometry) have shown that adults in developed countries spend typically 55 % to 70 % of their waking time in sedentary behaviours (about 8.8–11.2 h/d assuming 8 h/d of sleep) [2] [3] [4] [5]. This is not surprising given the rapid increase in television/computer usage, motorised transport and a shift from manual to office based occupations in recent decades [6][7].

Research consistently demonstrates the negative impact that prolonged sedentary behaviour has on metabolic health. Recent meta-analyses have revealed that greater time spent in sedentary behaviour was associated with a 73% increased likelihood of developing metabolic syndrome [8], alongside a 112% increased likelihood of developing Type 2 Diabetes when comparing highest sedentary time to the lowest [9]. Interestingly, these findings were largely unchanged when adjusting for moderate to vigorous physical activity (MVPA), implying that sedentary behaviour has an unfavourable influence on metabolic health regardless of how much MVPA an individual engages in.

Light activity breaks from prolonged sitting

Several studies have assessed the impact of interrupting prolonged sedentary time with light activity breaks. Light activity breaks such as standing or lightly ambulating provide muscular contraction that up regulates GLUT-4 [10] and Lipoprotein Lipase [11] activity, this offsets processes such as hyperglycemia, hyperinsulinemia and hyperlipidemia, allowing for normal levels of glucose, insulin and triglycerides to circulate the blood stream, restoring homeostasis. Support of this favourable impact comes from Dunstan et al [12], they found that interrupting sitting time with two minutes of light intensity walking every 20 minutes lowered postprandial glucose and positively influenced insulin levels in an overweight/ obese population. Postprandial light activity has also been

associated with improved 2hr blood and plasma glucose [13] [14]. These associations are supported by intervention based studies, whereby a dose response between postprandial light activity duration and 2hr blood glucose lowering was evident [15] [16]. The strong, independent dangers of Postprandial Glucose are well documented [17] [18] [19] and these findings suggest that light activity breaks may be a valuable way of countering this.

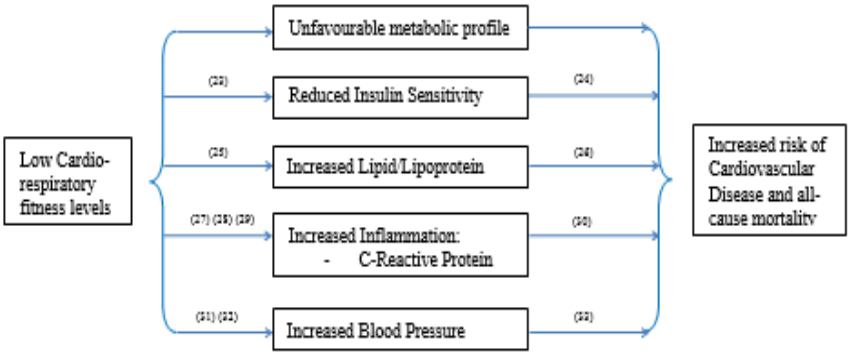
Cardio-respiratory fitness as a potential moderator

Absence of randomised control trials assessing the extent to which cardio-respiratory fitness (CRF) moderates the relationship between sedentary behaviour and metabolic health mean that its influence is yet to be verified and warrants further investigation.

CRF is a health-related component of physical fitness defined as the ability of the circulatory, respiratory, and muscular systems to supply oxygen during sustained physical activity. Data from the Aerobics Centre Longitudinal Study has revealed the importance of CRF on overall health [20]. From a long term follow-up analysis of 40,842 men and 12,943 women there were 3,824 deaths, ~ 16.5% of these deaths were attributed to having low CRF levels. This accounted for more deaths than obesity (~2.5%), tobacco Use (~8.5%), and diabetes (~3%) put together, for both men and women.

According to the World Health Organization, in 2011 [21], Cardiovascular Disease (CVD) was the number one cause of worldwide death, accounting for ~17 million of 55 million fatalities. (Approximately 3/10 deaths). An individual's cardiorespiratory fitness level has been shown to strongly protect against the risk of CVD, to the extent where obese individuals with moderate/high CRF have less than half the risk of CVD mortality than a normal weighted individual with low CRF [20] [22]. Given the strong link between obesity and CVD risk, this demonstrates the power that improving CRF possesses, supporting its role as a vital modifiable factor in disease mortality prevention. Regardless of body mass index (B.M.I), as fitness declines the risk of CVD increases, the gradient of this impact is exacerbated in obese individuals but the trend stays the same.

This relationship between CRF and CVD/all-cause mortality risk is strongly mediated by the influence that CRF has on metabolic health. This domino effect is depicted below:



Despite the strong ability of Cardio-respiratory fitness to manipulate metabolic profiles, only one study to my awareness has documented its potential ability to help offset the negative metabolic responses associated with prolonged sedentary behavior. Cooper and Berge et al [34], found that when grouping participants by below and above median CRF levels, results were suggestive of a stronger association between sedentary time and subcomponents of metabolic risk among individuals below the median. This cross-sectional, observation based association suggests that sedentary time has less influence on metabolic risk in individuals with higher CRF. Further lab based randomised control trial investigations are required to confirm the notion that CRF can help protect against the deleterious impact of sedentary behaviour.

MVPA and its relationship with cardiorespiratory fitness

Although being highly active and having high cardio-respiratory fitness are terms not to be used interchangeably, their strong relationship with each other is well documented as physical activity is the primary determinant of CRF [35]. Consistent with this, many randomized control trials have demonstrated a dose–response relationship between physical activity engagement and CRF improvement [36]. Although genetics also play a crucial role in the modification of CRF [37], physical activity is undoubtedly the strongest contributor.

Given the limited recognition of fitness level in sedentary behaviour research, we can use studies investigating the impact of MVPA on sedentary behaviour outcomes to gain a potential insight into the impact CRF would have, given its strong connection to physical activity. Statistical adjustments have shown the impact of sedentary behaviour on metabolic health to be independent of the amount of MVPA an individual engages in [8] [9]. However, the use of statistical adjustment may have dismissed/overlooked the potential role of MVPA. When stratifying findings by high and low active groups, the amplitude of influence is exacerbated and true influences are revealed. This was a strategy adopted by Henson et al [38].

Henson et al [38] cross-sectionally investigated the relationship between sedentary time and markers of low grade inflammation. Low grade inflammation is thought to be a strong pathological contributor to the onset of metabolic disturbances and disorders such as Type 2 Diabetes Mellitus [39] [40]. Henson et al categorised their participants into two groups (active and inactive), these were classified by dichotomisation into high and low MVPA levels around the median. Results show that sedentary time had a larger detrimental impact on Interlukin-6 (marker of inflammation) in individuals classed as inactive. They also found that breaks in sedentary time had a stronger favourable association with markers C-Reactive Protein (CRP) and Adiponectin (HADP) for the inactive group. From this it can be postulated that the deleterious impact of sedentary time may be particularly less pertinent in individuals that engage in sufficient amounts of MVPA (with assumedly higher fitness) and that breaking this sedentary time is more beneficial in those that do not engage sufficiently (assumedly lower fitness).

Studies that have stratified by "Inactive and active" [38] and by "high and low fitness" levels [34] have revealed the potential protective role of both MVPA and CRF in offsetting the deleterious impact of sedentary behaviour. Despite this, no randomised control trials have identified the 'extent' to which these variables can be attributed to the outcome. Given that CRF is stable over time/ easily determined and is not exposed to daily fluctuation or reactivity bias, a more conclusive finding may be drawn from assessing the moderating effect of CRF.

Overall, it is hypothesised that in individuals with high CRF, the deleterious impact of sedentary behaviour will not be as substantial, nor will light activity breaks be as advantageous, compared to individuals with lower CRF as they have a smaller scope for metabolic improvement. However, the absence of randomised control trials assessing the 'extent' to which fitness moderates the relationship between sedentary behaviour and metabolic health markers is yet be verified, warranting further interventional investigation.

Practicalities of this research

Many individuals have unavoidable sedentary occupations (lorry drivers), if it is found that high fitness levels can substantially protect against the negative impacts of daily prolonged sitting this will be of great benefit, as engagement in time efficient exercise to improve fitness around working hours will suffice. For example High Intensity Interval Training (HIIT).

Study Aim

The aim of this study is to determine the extent to which an individual's metabolic response to prolonged sitting and light activity breaks is moderated by their CRF level.

Primary Objective

- To investigate whether an individual's cardio-respiratory fitness influences the impact of prolonged sitting and light ambulatory breaks on blood glucose area under the curve (AUC).

Secondary Objective

- To investigate whether an individual's cardio-respiratory fitness influences the impact of prolonged sitting and light ambulatory breaks on triglyceride concentration area under the curve.
- To investigate whether an individual's cardio-respiratory fitness influences the impact of prolonged sitting and light ambulatory breaks on insulin concentration area under the curve.

Methods

Study Design

This study is a randomised cross-over trial whereby each participant will take part in two treatment conditions in a random order, acting as their own controls. Randomising the order of conditions is an essential way to eliminate any potential unforeseen order effects.

Please refer to appendix 1 for a flow chart of the study sequence and its time frame.

Participants

Recruitment

Healthy volunteers will be recruited from the general public (does **not** require NHS patients or service users). Posters advertising for willing volunteers that fit the inclusion criteria will be distributed around the University of Leicester and Loughborough University in staff rooms/ lifts/ stair wells and billboards. Posters will also be displayed at local community leisure centres/ sports clubs, and supermarket and newsagent notice boards. The study may also be included on NHS Trusts and other websites. E.g Leicester Diabetes Centre, Leicestershirediabetes.org or Facebook. This may include a link to the study poster. People will register their interest by phoning or e-mailing the research team on the contact details provided on the poster. They will then receive more information on the study and be sent a participant information sheet, alongside a pre-screening/ physical activity questionnaire that can be completed and e-mailed/posted back to us. The individual will then receive a follow up phone call from the research team to arrange a date for their familiarisation and screening visit.

Individuals with a range of fitness levels will be recruited in order to observe an effect of this moderating variable in the investigation. Given that vigorous physical activity is the strongest contributor of fitness, equally distributing the recruitment of participants into the following groups should provide a wide spread of fitness levels throughout the sample;

Group 1 = 0 minutes of vigorous physical activity per week.

Group 2 = 0 – 75 minutes of vigorous physical activity per week.

Group 3 = 75+ minutes of vigorous physical activity per week.

This information will be attained from the vigorous physical activity questionnaire as a part of the pre-screening process. The pre-screening questionnaire will allow the researchers to gather demographic information such as; age, gender and smoking habits etc.) Medical history checks for history of diseases or current health issues that may affect participation in this study will be highlighted here.

Withdrawal and discontinuation from the study

If a participant withdraws their consent during the study and requests for their data not to be used, all samples will be destroyed and data will be deleted. The participant will be withdrawn from the study. If a participant withdraws from the study, but not their consent, because they are no longer able to take part in future visits, data already obtained will be used for the study. The investigator may discontinue a participant from the study at any time if they consider it necessary to do so. Reasons for this might include, but is not restricted to:

Significant non-compliance with treatment regime or study requirements

Ineligibility (may arise during the study).

Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study
- Body Mass Index: 20 - 30 kg/m^2
- Male and Female
- Aged: ≥ 25 to ≤ 55 years of age.
- Occupation: Work full-time in a predominantly sedentary occupation.

Exclusion Criteria

Due to the nature of the trial, our exclusion criteria are as follows:

The participant may not enter the study if ANY of the following apply:

- Aged <25 or >55 years of age.
- Physical condition which limits full participation in the study
- Active psychotic illness or other significant illness which, in the view of the investigators, would prevent full participation
- Inability to communicate in spoken English
- Steroid use
- Known Type 2 Diabetes
- Pre-existing Cardio-vascular Disease (disease of the heart or blood vessels at present or in the past) For example: Heart attack, Stroke or Angina.
- Pregnancy
- Smoker
- Terminal illness

*In the circumstance that an individual is not sure whether they meet they are a part of the exclusion criteria, for instance they are not sure if they have pre-existing CVD, this will be reviewed/ assessed by a named medic on the delegation of authority log for a clinical decision to be made.

Setting

The study will be co-ordinated within the Bio-medical Research Unit (Leicester Diabetes Centre) at the Leicester General Hospital.

Informed Consent

Before any study related procedure can take place, the participant must sign and date the latest approved version of the informed consent form. Participant information will be presented verbally and in writing, with full details of what will be expected from the participants, potential risks involved, and their right to withdraw at any time throughout the study. The consent form will be signed and dated based upon an informed decision from this information. They will have as much time as they feel necessary to consider this the participant information and will not be rushed into a decision. Consent will be taken by someone suitably experienced that has received generic consent training, this will either be the study co-ordinator or a nurse involved with the study, these people will be authorised by the Chief investigator and included in the delegation of authority log. A copy of this will be given to the participant.

Familiarisation screening visit

Before participating in the study, all participants will visit the exercise laboratory for a familiarisation and screening visit where they will be shown the designated experimental area, provide written informed consent and have various measurements taken.

All participants will be required to fast from 10pm onwards the evening prior to this first visit (drinking water is allowed). Participants will then be provided with a standardised breakfast on arrival. This meal will be prescribed according to body mass and will provide approximately 8 kcals per kg of bodyweight made up of 13-14% protein, 51-52% carbohydrate and 35% fat.

It is also important that participants avoid general exercise and drinking alcohol or coffee in the 48 hours (2 days) leading up to this first visit (and the remaining two visits), this will be made clear in the participant information sheet. Very strenuous exercise must be avoided 72 hours **(3 days)** prior.

Participants will be asked to record all food and drink consumed the day before the first visit. They will then be asked to replicate this diet the day before the remaining visits using their dietary record to guide them. Therefore, meals will be standardised across conditions and will not confound the results.

The measurements taken during this visit include; basic anthropometry (body mass, waist circumference, body fat % and blood pressure); biochemical variables (HbA1c and blood lipids); Energy expenditure (at rest and during slow steady state treadmill walking); and measures of objectively determined free-living physical activity, sedentary behaviour and posture. They will also undergo a treadmill ramp test to calculate V02 max, from this each individual's cardiorespiratory

fitness can be determined. If participants have a substantial amount of cycling experience then a tailored cycle ergometer ramp V02 max test will be implemented.

Anthropometry

Arterial blood pressure will be measured in the sitting position. Three measurements will be obtained and the average of the last two measurements will be used.

Body mass, body fat percentage, waist circumference and stature will also be measured.

Biochemical variables

This study will measure relevant markers of metabolic health including: measures of HbA1c and lipid profile. All venepuncture will be undertaken by trained health care professionals and all biochemical analyses will be conducted blinded to treatment condition.

Energy Expenditure

The amount of energy people expend while walking (in comparison to their resting energy expenditure) differs between individuals. Each participant will be asked to sit quietly (refraining from movement) for 30 minutes while wearing a gas mask. Following this, the gas mask will be attached to a gas analyser that will be switched on to give values of stabilized energy expenditure (EE) for a further 15 minutes while sitting at rest. Once EE has stabilized for 15 minutes (after the 30 minutes of rest), the participants average EE at rest will be quantified. Following this, the participant will be required to perform a 10 minute walk on the treadmill at 3km.h (also wearing the gas mask), this will allow the researchers to calculate the percentage change in EE between sitting and lightly ambulating for each participant. Following the 10 minutes of light treadmill walking, participants will be required to sit back down (while wearing the gas analyser) for a further 15 minutes to allow researchers to observe how long it takes for participants to return to their resting EE post walking.

Objective measure of physical activity

Participants will wear an Actigraph accelerometer to measure levels of time spent sedentary and in moderate to vigorous physical activity under free-living conditions. Participants will be asked to wear the accelerometer (placed on right anterior axillary line) for 7 days after the familiarisation visit, 7 days leading up to the treatment conditions and throughout the experimental conditions in order to monitor any potential changes in physical activity levels and make sure participants have avoided exercise in the days leading up to the treatment conditions. (see Appendix 2). These accelerometers are one of the most extensively validated and accurate on the market and one of the few commercially available accelerometers to correlate with energy expenditure as measured by double-

labelled water [42]. Outputs will include steps per day, total body movement (counts per day), and time in sedentary, light, moderate and vigorous-intensity physical activity as determined by counts per minute cut points proposed by Freedson et al. [43]

Posture and sedentary time

Postural allocation (sitting and standing) and walking will be quantified using an *activ*PAL physical activity monitor (PAL Technologies, Glasgow, Scotland). The *activ*PAL is a single-unit monitor based on a triaxial accelerometer that is worn midline on the anterior aspect of the thigh and attached directly onto the skin using medical dressing. The monitor produces a signal related to thigh inclination and has been shown to be a valid and reliable measurement tool for determining posture during activities of daily living in a healthy population [44]. Outputs from this device include; time spent lying, sitting, standing, stepping and numerous sit-to-stand transitions. The device will be worn for 7 days after the familiarisation visit, (alongside the Actigraph) and throughout experimental conditions (see appendix 2).

Cardio-respiratory fitness level

Cardio-respiratory fitness will be quantified by measuring each individuals V02 max, based on a maximal test to exhaustion of the Bio-medical Research Unit (BRU)'s best practice Treadmill ramp test. If participants have a substantial amount of cycling experience and little running experience, then a tailored cycle ergometer ramp V02 max test will be implemented. With a ramp test, each participant will begin at an intensity/speed on the treadmill (or cycle ergometer) that is tailored to their ability and physical activity levels. The gradient will then be gradually increased until exhaustion with speed remaining stable throughout.

Treatment Regimens

One to two weeks following this initial familiarization visit, participants will be assigned to receive the following two treatment conditions (A: prolonged sitting, B: light activity breaks) in a random order (see Figures 1 and 2 for schematic representations of the study design and timeline). Each treatment condition will be carried out on one single day. In total, the study requires three separate visits (see appendix 1 and 2) to the Leicester Diabetes Centre, Leicester General Hospital, University Hospitals of Leicester.

Where participants have limited access to motorised transport, we will provide taxis to and from their home address to reduce ambulatory activity involved in the commute to and from each visit.

For male participants, there will be a minimum of 7 days between visit 2 and 3. For females, there will be a gap of exactly one month (or near as possible) between visit 2 and 3. Reasoning for this is

due to the potential that different phases of a women's menstrual cycle affect their blood glucose response to a meal [48]. By testing at similar times of the month, we avoid this unwanted variability.

Experimental conditions

Treatment Condition A: Prolonged sitting

During the sitting condition, participants will be restricted from walking and standing. They will be in a designated room equipped with a chair/desk and have access to a computer with internet services, books and magazines or movies throughout the day. Lavatory breaks will be permitted.

The day before each treatment condition, participants will be asked to consume the same type and quantity of food and drink that they recorded prior to Visit One. Participants will also be asked to avoid alcohol, caffeine and moderate intensity exercise for 2 days prior to each treatment condition (as done prior to Visit One) as reproducibility data has shown that insulin and triglyceride responses to meal ingestion are good under these conditions [45]. Furthermore, there is evidence that the effect of a single exercise session on insulin sensitivity and glucose tolerance may last up to 48 hours [46]. Very strenuous exercise should be avoided for 3 days prior to each treatment condition, as this has been found to increase insulin sensitivity for up to 72 hours [49]

Participants will be asked to fast the night before all three visits to the laboratory from 10pm onwards. On the morning of the test, participants will have a cannula inserted into an accessible vein by a trained health care professional and the first of the blood samples will be taken (time point: -1hr). Blood pressure will also be measured immediately before each blood sample as replacing sedentary behaviour with light activity breaks has been suggested to lower Blood pressure (especially in diastolic state)[47]. Participants will then be asked to sit quietly for 60 minutes in order to achieve a steady state. Following this, participants will have another blood pressure measurement and blood sample taken and then be provided with a standardised mixed meal breakfast (09:00am) (time point: 0h). The meal will be prescribed according to body mass and will provide approximately 8kcals per kg of bodyweight made up of 13-14% protein, 51-52% carbohydrate and 35% fat. A mixed-meal of this nature will be used to ensure ecological validity as fat and carbohydrates are usually co-ingested in real-life situations. Blood sampling and blood pressure measurements will continue at 30, 60, 120 and 180 minutes following breakfast. A second, lunch meal (12:00pm) (with identical nutrient composition to breakfast), will then be consumed over 15 minutes. Blood sampling and blood pressure measurements will continue at 30, 60, 120, 180 and 210 minutes following lunch (Figure 1). Participants will remain sitting throughout the test period whilst undertaking typical sedentary pursuits such as watching TV/DVDs/reading.

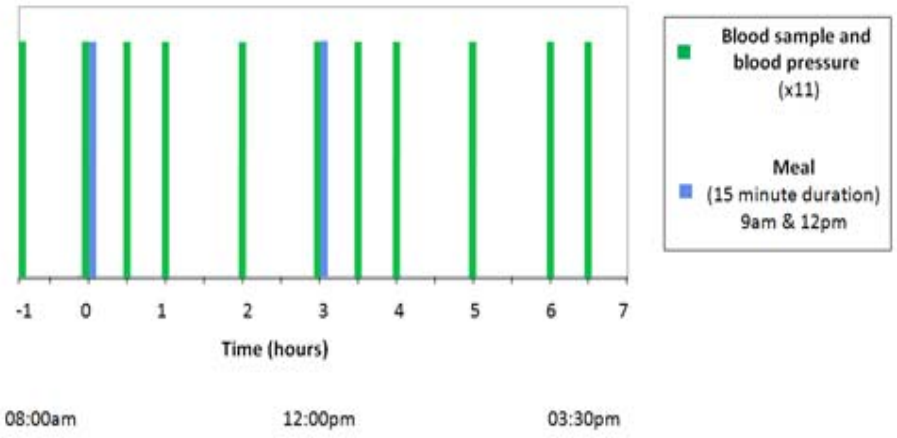


Figure 1. Timeline for Treatment Condition A

Treatment Condition B: Light activity breaks

This is identical to the sitting condition (A), but will introduce breaks in sitting time. This will consist of 5 minute bouts of light-intensity treadmill walking (equivalent to around 3.0 $km \cdot h^{-1}$). In total, individuals will accumulate 12 bouts (60 minutes) of light-intensity activity throughout the test period. The light-intensity walking activity undertaken here replicates the low-grade ambulatory activity associated with everyday life.

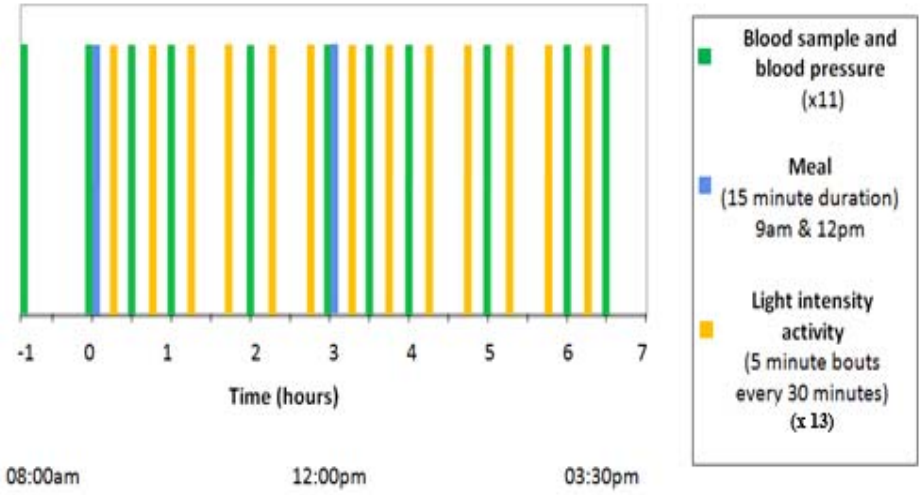


Figure 2. Timeline for Treatment Condition B

(Appendix 1 and 2 show the progress of the participants through the study)

Defining the end of the trial

The end of the trial is marked by the date at which the participant finishes their last visit to the laboratory.

Primary Outcome

Glucose area under the curve (AUC); Plasma glucose will be measured using a glucose oxidase method undertaken in University Hospitals of Leicester laboratory.

Secondary Outcomes

This study will also measure Insulin AUC and triglyceride AUC, as they are directly involved in the underlying physiological mechanism of sedentary behaviour [11].

Serum triglycerides will be analysed using the glycerol phosphate oxidase (GPO) assay.
The Mercodia Insulin ELISA will be used to provide a quantitative determination of insulin. Triglycerides may be measured in the University Hospitals of Leicester or at another certified laboratory.

In addition to traditional markers of metabolic health, we will also store samples for analysis of inflammatory proteins (including interlukin-6). Samples will be stored in secure -80oC freezers located within the Leicester Diabetes Centre and analysed at the end of the study.

Sample Size

In this regression analysis, in order to attribute 20% of the change in blood glucose AUC to fitness level with 80% power, we estimated that we would require 34 participants to complete this study. As mentioned under the 'recruitment' section, I will be splitting my sample equally into 3 groups based on their vigorous activity engagement per week. I will therefore create a quota of 12 people to be allocated into each group. In total, 36 participants will be required to complete this study.
It is also worth noting that data will not be compared between these 3 groups, they have simply been formulated to filter a large range of fitness levels required for this study.

Data Analysis

Each condition will assess the primary outcome (glucose AUC), a direct comparison of this will be made between Treatment A and B. This process also applies to the secondary outcomes (Insulin AUC and Triglyceride AUC) that will be assessed in this study. Descriptive statistics (mean values and frequencies) will be calculated. Histograms will be used to identify any outliers and to test for normality. Extreme outliers (more than 4 standard deviations from the mean) will be removed from the analysis. Regression analyses will be used to assess how much of the variation in outcome variables in each treatment condition can be attributed to both cardiorespiratory fitness and percentage change in EE between rest and light walking. All models will be run both unadjusted and adjusted for potentially important covariates explaining residual outcome variance (age, sex, and body mass). Carryover effects will not be formally tested, given the 7-day washout between treatment groups.

The calculation of the AUC is critical for analysing the response to a standardised meal. Analysis will involve calculating the entire area under the curve (using the trapezium rule) and the results will be represented as the total area under the curve. The main effects of each treatment condition will also be analysed.

Safety Issues

We do not foresee any adverse events over and above those associated with everyday life and routine health care that could be attributable to the study. However, all participants will undergo venepuncture and cannulation, which occasionally results in bruising, swelling and temporary discomfort. Baseline safety checks will also include blood pressure (to detect underlying hypertension), reporting of drugs which may increase the risk of bleeding (warfarin, aspirin) and a measurement of triglycerides (to detect hypertriglyceridaemia).

Regarding routine measurements collected in the FIT 2 SIT study, in the case of urgent circumstances (e.g. blood pressure 170/95, raised potassium) the individual will be strongly advised to visit their General Practitioner. In Emergencies (e.g. BP> 220/ 120 or K+ >7.0, blood sugar > 20.0mmol/l with diabetes symptoms) the individual will be referred to A+E / MAU / UHL services as considered appropriate by a doctor depending on the nature and history of the condition.

Reporting of SAE and timelines

All SAEs will be reported internally to the R+D office and the sponsor (University of Leicester) using appropriate reporting forms, within 24 hours of the study team becoming aware of the event. The immediate report may be made orally or in writing and shall be followed by a detailed written report of the event. Additional information can be provided if requested to the sponsor, University of Leicester Departmental Research Ethics Committee (REC), or R+D (e.g. in the event of a death). The principal investigator is responsible for the review and sign off the SAE, or in their absence, another appropriately qualified member of the research team.

The sponsor and/or R+D personnel will ensure that all relevant information about a SUSAR which occurs during the course of the FIT 2 SIT study and is fatal or life-threatening is reported as soon as possible to the sponsor not later than seven calendar days after they were first aware of the reaction. Any additional relevant information will be sent within eight days of the report. The sponsor and/ or R+D will ensure that a SUSAR which is not fatal or life-threatening is reported to the main REC no later than 15 calendar days after they were first aware of the reaction.
The investigator site file will contain documentation for:

- SAE, SAR and SUSAR reports
- Evidence of submission of SAEs to the sponsor within 24 hours of the team becoming aware of an event
- Evidence of timely SUSAR submission to the main REC.

Access to documents and Source Data

Direct access to information gathered in this study will only be available to individuals who have been granted access. The sponsor, host institution and regulatory authorities can permit trial related monitoring, audits and inspections.

Participant Confidentiality

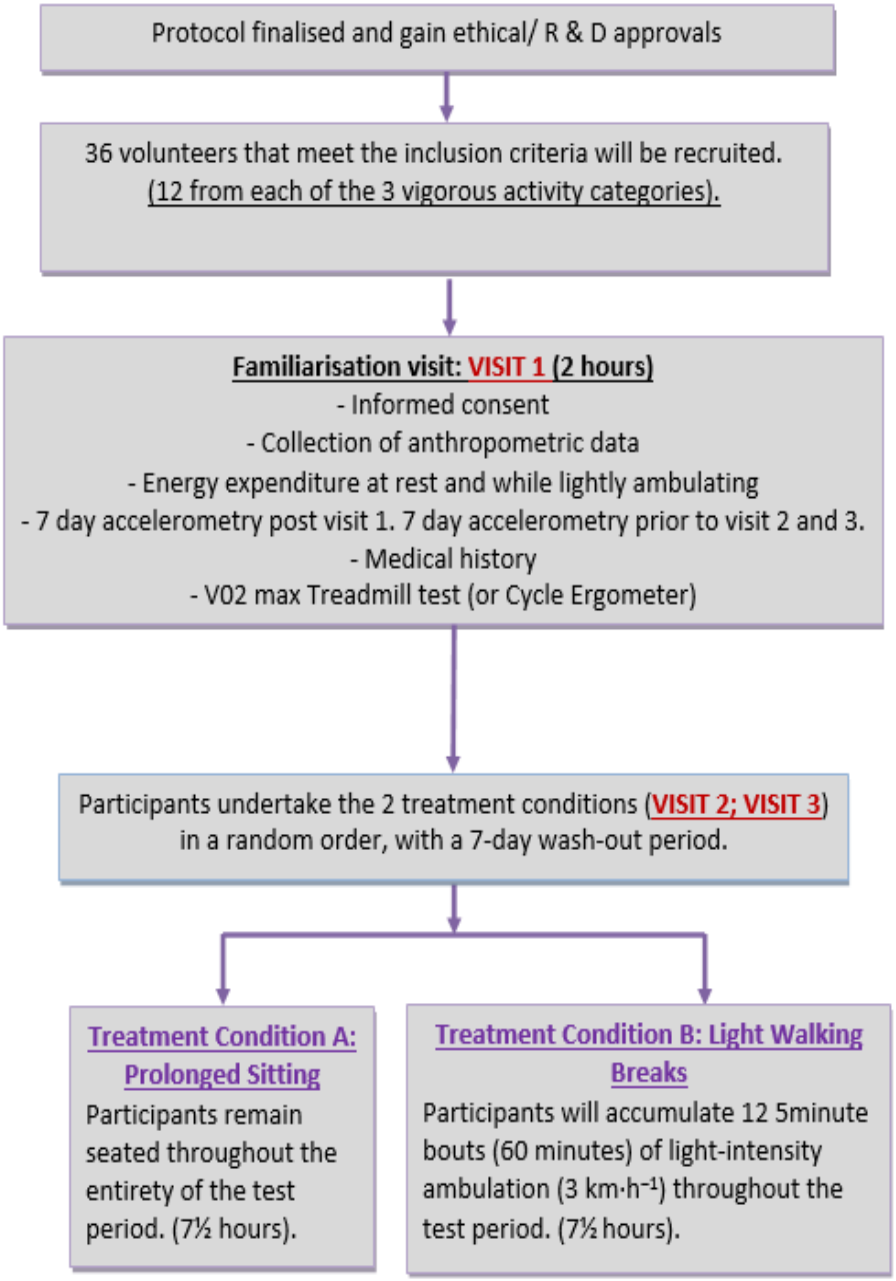
All staff involved in this study will make sure that the participant's anonymity is maintained. Participants will only be identified by participant ID number. Documents will be stored securely and be assessed by trial staff and authorized personnel. This procedure abides by the Data Protection Act which requires data to be anonymized as soon as possible.

Ethical Issues

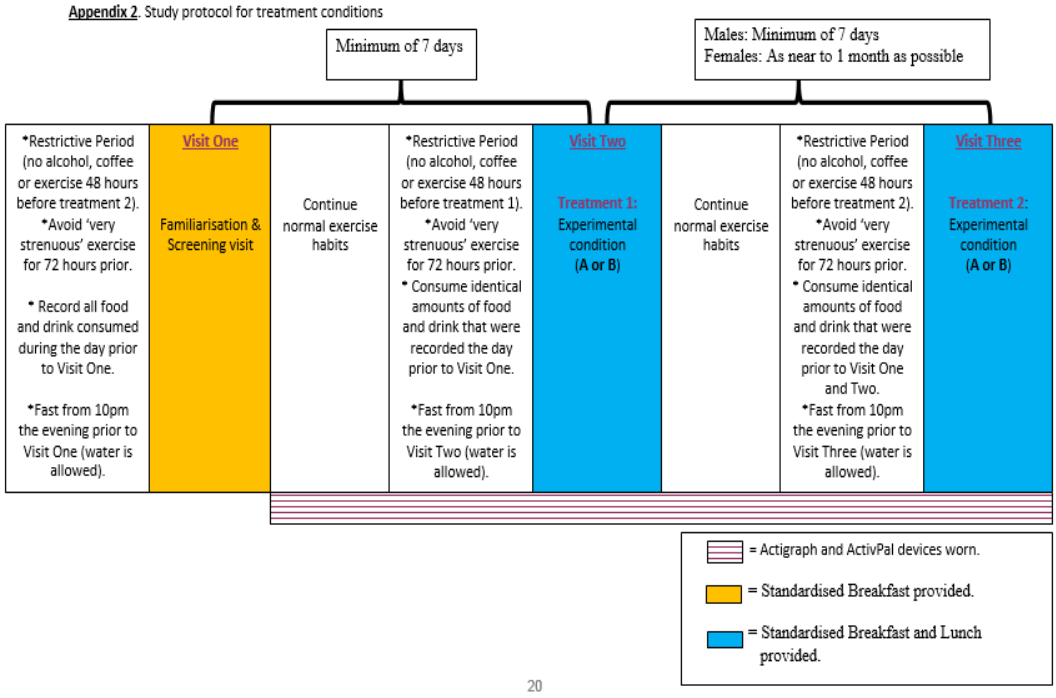
University of Leicester departmental Research Ethics Committee approval and University Hospitals of Leicester Trust R&D Approval will be sought for the study before it commences. This will ensure that all ethical and indemnity issues are dealt with.

Appendices

Appendix 1. Design and flowchart for the participants included in the study



Appendix 2. Study protocol for treatment conditions



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Participant Information Sheet

FIT 2 SIT

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Leicester,
LE5 4PW

Mob: 0116 2588686
Email: mm636@le.ac.uk

Chief Investigator: Dr. Thomas Yates

PARTICIPANT INFORMATION SHEET

You are being invited to take part in a research study but before you make a decision, it is important that you understand why the research is being carried out and what it involves. The study is being conducted by a student as part of their research degree (PhD) and this information sheet is designed to help you decide whether you would like to take part. You can talk it over with your family or friends, and if anything is not clear, or you would like to know more, we have put a contact number at the end of this leaflet allowing you to talk to us directly.

What is the purpose of the study?

Research shows that sitting for long periods of time is bad for our health. We want to see if breaking up long periods of sitting time with short, frequent bouts of slow walking can help to protect against this harmful effect. We would also like to find out if a person's fitness can also provide protection against the harmful effects of prolonged sitting.

Why have you chosen to invite me?

You have registered your interest by responding to one of our research advertisements and feel you meet the inclusion criteria to take part in this study.

What if I do not meet the inclusion criteria?

We will see whether you meet our inclusion criteria on your first visit to the Leicester Diabetes Centre. If it is found that you do not meet the criteria for this study we will give you general feedback on any measurements taken from your first visit, reimburse any travel costs and thank you for your time. Unfortunately you would no longer be allowed to continue in this study.

Do I have to take part?

It is your right to decide whether or not you would like to take part in this study. If you decide to take part, you can withdraw your consent from the study at any time by contacting the study team. If you do this we will ensure that personal details held are carefully destroyed. You can also withdraw from this research at any point without having to give any reason for doing so. Any future medical care will not be affected in any way.

If I decide to take part, what happens then?

If you would like to take part, please fill in the pre-screening and physical activity questionnaires that are attached alongside this participant information sheet and send them back to us. We will then contact you to arrange your first appointment. Taking part in the study will involve **three** separate visits to the Leicester Diabetes Centre, at the Leicester General Hospital. For your first visit we will describe the study and go through this information sheet step by step, allowing you to ask any questions you may have. We will then ask you to sign a consent form to show that you have agreed to take part (this is not a contract and does not mean you definitely have to take part, you can still stop taking part at any point during the study). This is called 'informed consent'. This first visit will take around 4 hours and you will have the chance to meet the friendly members of our team who will fully familiarise you with what is expected on your remaining visits to the centre.

How can I prepare for my first visit?

You will be required to fast from 10pm onwards the evening prior to this first visit. (drinking water is allowed). You will then be provided with a standardised breakfast on arrival. It is also important to avoid exercise and drinking alcohol or coffee in the 48 hours (2 days) leading up to this first visit. Vigorous/ very strenuous exercise should be avoided 3 days prior. You should also record all food and drink consumed the day before your first visit, this will then be replicated the day before both Visit Two and Visit Three.

Will you be measuring anything at the first visit?

During this visit we will check your blood pressure, height, weight, waist measurement and body fat percentage. Blood tests will also be taken to measure the levels of sugar and cholesterol in your blood.

We will also measure the amount of energy you use per minute while at rest and during a slow walking task (3km.h on a treadmill) using a gas mask technique. Energy responses to sitting and walking differ between individuals, therefore it is of interest to observe how each participant in this study responds to these conditions as it may help explain any potential findings of this study.

You will also be introduced to two types of activity monitors known as the 'Actigraph' and 'ActivPAL'. These are small devices worn on the waist and thigh that record movement patterns, allowing researchers to get information on your activity levels and how much time you spend sitting. After your first study visit we would like you to wear these devices for 7 days (1 week). We will demonstrate how to use the devices and provide you with written instructions. These devices will be returned at your next visit. Also, 1 week prior to both your second and third visit, we would like you to wear the 'Actigraph' activity monitor to give us further information on your activity levels leading up to the treatment conditions.

At the end of this first visit you will be asked to perform an exercise test to assess your fitness level, this will involve running on a treadmill. The speed of the treadmill will be kept the same throughout the exercise test but the treadmill will slowly become steeper, making the exercise harder. Whilst running, a special mask covering your nose and mouth will be worn to analyse your breathing, this will be connected to a machine (gas analyser) that gives information on the amount of oxygen you breathe in and out. The point at which the amount of oxygen you breathe in remains stable despite increases in exercise difficulty is the point that we will stop the exercise test because this tells us that you have reached your exercise capacity (called V02max), and from this we can determine your fitness level. For safety reasons the electrical activity of your heart may be continuously monitored during the exercise test by electrodes (small plastic patches) placed at numerous locations across the upper body. Electrodes would then be attached to a devise known as an Electrocardiography machine to give continuous data regarding the electrical activity of your

heart allowing the research team to determine how well your heart is responding to the exercise test. This is predominantly used as a safety precaution in those that have low physical activity levels.

This exercise test will take around 10 – 15 minutes.

Please bring clothes that you feel comfortable running in. We have shower facilities if you want to have a shower afterwards. Remember to bring a towel and change of clothes if you do so.

What happens next?

Approximately seven days after you have been for your first visit, you will be asked to return to the Leicester Diabetes Centre for your first of the two treatment conditions, (condition A or B, detailed on the next page). Each condition lasts 7 ½ hours (8am until 3:30pm). Following this, females will require a 1 month gap between Treatment 1 and Treatment 2 (visit 2 and 3) and males will require a minimum of 7 days.

What are the two treatment conditions?

- **Condition A** is referred to as the '**sitting'** condition. Here you will remain seated throughout the whole of the 7 ½ hour test period (8am – 3:30pm) whilst watching TV/DVD's, reading, using the internet, doing paperwork etc. at your will. The day before 'Visit Two', you will be asked to consume the same food and drink as recorded before 'Visit One'. Following this, you will be required to fast from 10pm onwards, (drinking water is allowed). It is also important to avoid exercise in the 2 days leading up to both condition A and B (very strenuous exercise should be avoided **3 days** prior). On the morning of the test you will come into the Leicester Diabetes Centre and have a cannula (a small tube that allows us to take blood) inserted, this will stay in throughout the day allowing us to take regular blood samples without the need for multiple needles. After we take the first blood sample, you will then sit quietly for an hour. We will then provide you with breakfast. Whilst you are sitting, we will take blood samples (using the cannula) and your blood pressure at 30, 60, 120 and 180 minutes after breakfast. You will then eat a lunch meal that we will provide and we will continue taking blood samples and your blood pressure at 30, 60, 120, 180 and 210 minutes after this lunch meal. In total, we will take 11 blood samples over the 7 ½ hour testing period. Although this may sound like a lot, it is equivalent to merely 8-9 teaspoons of blood over the course of the day.

- **Condition B** is the **'light activity breaks'** condition. You would go through exactly the same process as condition A but you will also be asked to do 5 minute bouts of slow walking on a treadmill every 30 minutes following breakfast and lunch. In total you will do 12 five minute walks on the treadmill throughout the 7 ½ hour test period (60 minutes of walking in total). In total, we will take 11 blood samples on the day.

Please see the next page for a breakdown of the study

Breakdown of the study

Visit One:

- Informed Consent.
- Receive a standardised breakfast on arrival
- Measurement of height, weight, body fat percentage, waist circumference, blood pressure, blood tests (e.g. sugar and cholesterol) and energy requirements for sitting and light walking using the gas mask technique.
- Medical History check.
- Exercise test on the treadmill for 10 -15minutes.
- Provided with activity monitors.

Visit Two:

- Remain seated for 7 ½ hours
- Have breakfast at 9am and lunch at 12pm.
- Frequent blood pressure and blood samples taken

Visit Three:

- Replicate visit two but interrupt this sitting time with 5 minutes of slow treadmill walking every 30 minutes after breakfast and lunch.
- Frequent blood pressure and blood samples taken

Study complete

Additional Reminders

Do not consume any food from 10pm onwards the evening prior to each visit. (Water is allowed).	Wear both activity monitors provided (ActiGraph and ActivPAL) for 7 days after 'visit one' and return them to the researchers on 'visit two'.
Do not engage in exercise and do not drink alcohol or coffee in the 48 hours (2 days) leading up to each visit. Avoid very strenuous exercise for 3 days.	Make a note of all food eaten on the evening prior to 'visit one' and replicate this the evening before 'visit two and visit three'. Eat the exact same food that you recorded eating.

What is a cannula and are there any risks?

A cannula is a small flexible tube that is inserted into a vein to allow blood samples to be taken. As with any object that punctures the skin there is a risk of infection but using a clean technique when putting it in will substantially reduce this risk.

Where will the cannula be put and how is it inserted?

Your cannula will usually be placed in a vein in the lower arm/hand. The doctor/nurse will try to avoid the hand you use for writing; however this may not be possible. The healthcare worker will clean their hands using soap and water or alcohol gel/hand sanitizer and wear disposable gloves. The area around a suitable vein will be cleaned using a recommended product. A tight strap called a tourniquet will be placed around your arm to help identify the best vein to use. The cannula will be inserted through the skin into a vein, using a needle and when it is correctly in position the needle will be removed leaving only the cannula in the vein. The cannula will then be flushed through with sterile salty water (saline) to ensure it is working.

Is it painful and can it fall out?

There may be a small amount of pain or discomfort as the cannula is inserted and should pass very quickly once the cannula is in place. The cannula will be secured with a see-through dressing and there is usually no need for the cannula to be bandaged. A cannula may fall out if the dressing becomes loose. Please inform staff if the dressing becomes loose.

When will the cannula be taken out?

The cannula will be removed at the end of each day, or earlier if a problem occurs.

What happens after removal of the cannula?

When the cannula has been taken out, the place where it has been may feel slightly bruised. This sensation can last for up to one week and is quite normal. The dressing which is put over the site after removal can usually be taken off within a couple of hours.

Will I get any refreshment whilst I attend the study visits?

Yes, we will provide breakfast and lunch for your second and third visit. At your first visit we will provide breakfast on arrival.

What are the possible benefits of taking part?

By taking part in the study you will find out information about; your risk of developing diabetes, the fat levels in your blood, body fat percentage, current physical activity levels and your overall fitness level using the latest technology. You will also find out how vulnerable you are to the health problems associated with sitting and advised of ways to reduce your risk. This study itself may not be of direct benefit to you but it will contribute to the ongoing work aimed at the prevention and management of Type 2 diabetes.

What are the risks of taking part?

Taking part involves minimal risk for you, just the inconvenience of taking the time to participate in the study. A small amount of bruising from having bloods taken is possible but will be minimal.

The tests in the study are not designed for clinical diagnosis, but in the unlikely event that we may find an abnormality with the blood results this will be discussed directly with you and you may be advised to see your GP.

What if something goes wrong?

It is very unlikely that you would be harmed by taking part in this type of research study. However, if you wish to complain or have any concerns about the way you have been approached or treated in connection with the study, you should ask to speak to Dr. Thomas Yates on **0116 258 7453** who will do his best to answer your questions. If you remain unhappy and wish to address your concerns or complaints on a formal basis, you should contact Patient Information & Liaison Service at pils.complaints.compliments@uhl-tr.nhs.uk. The Firs, c/o Glenfield Hospital, Groby Road, Leicester. LE3 9QP Freephone: 0808 1788337. Your legal rights to claim compensation for injury where you can prove negligence are not affected.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Data will be stored either in locked filing cabinets or in password protected databases which are only accessible by members of the research team. Any information that is shared will have your name and address removed so that you cannot be recognised from it. Information collected will not be used for any other purpose than that explained here. Study data and procedures may also be looked at by authorized people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant.

What will happen to the results of the research study?

The results of the study may be published in a professional journal, but you will not be identified by name in any publications. You will be informed about the results of the study when it has finished.

Who is organising and funding the research?

This study is being organised and co-ordinated by the Diabetes Research Centre, University of Leicester. It is funded by the Leicester-Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit.

Who has reviewed the study?

This study has been reviewed by the University of Leicester Committee for Research Ethics and approved by the University Hospitals of Leicester Trust Research & Development.

Will I get study and travelling expenses?

Travelling expenses (up to £10 per visit) can be reimbursed. We are also able to issue a ticket so that you do not have to pay to park your car at the Leicester General Hospital.

What do I do if I decide to volunteer?

We are pleased that you are considering taking part in our research study. For the next step please fill in the pre-screening questionnaire and physical activity questionnaire attached to this e-mail and simply send them back to us on the e-mail address below. If it has already been established that you do not have access to e-mail and you have received this participant information sheet by post, please fill in the questionnaires by hand and return them to us in the pre-paid envelope attached to this paper copy. A member of the study team will then contact you. We look forward to welcoming you for your first visit.

Contact for further information

In the meantime, the researchers involved in this study will be pleased to discuss any questions or concerns that you may have. If you have any further questions about this research please e-mail the team at mm636@le.ac.uk or call us on 07740049606, (if unavailable, leave a message and we will get back to you as soon as possible).

Thank you for taking the time to read this participant information sheet

Pre-screening Questionnaire

FIT 2 SIT

Pre-screening Questionnaire

(Please cross (☒) the options that apply to you by clicking the relevant boxes).

Name:
Address:

I have read the information sheet provided and **would like to take part** in the 'FIT 2 SIT' Study.

Signed: ___ **Date:**

Date of Birth:

Please complete the section below if you would like to take part in the 'FIT 2 SIT' study.

Do you have diabetes? ☐ Yes ☐ No ☐ Not sure

Have you had a stroke? ☐ Yes ☐ No

Are you a smoker? ☐ Yes ☐ No

Have you ever had Cardio-vascular disease?
(Disease of the heart or blood vessels) ☐ Yes ☐ No ☐ Not sure

Do you have any food allergies? ☐ Yes ☐ No
If so, please state here: Click here to enter text.

Has your doctor ever told you that you have heart trouble? ☐ Yes ☐ No

Do you have any injuries that may prevent you from exercise? ☐ Yes ☐ No
If YES, give details below:
Click here to enter text.

List any medication you are currently taking?

Your approximate height: and weight:

Please include your telephone number and e-mail address so we can contact you

Tel: ☎

Please call me: ☐ Morning (9am-12pm)
☐ Afternoon (12pm-5pm)
☐ Evening (5pm-7pm)

E-mail:

Leicester Diabetes Centre University of Leicester Loughborough University

Physical Activity Questionnaire

FIT 2 SIT

Physical Activity Questionnaire

Instructions: Please click the boxes to cross ☒ all activities in the left hand column that are relevant to you. Select the days (Monday - Sunday) that you take part in the activity to help with your recall. Then use the right hand column to write how many minutes per week you engage in each activity that you have crossed. Only report physical activity that is; very demanding, leaves you out of breath/struggling to hold conversation and sweating). Only acknowledge bouts of physical activity that are above or equal to 10 minutes in duration at a time.

Activity	M	T	W	T	F	S	S	Minutes per week
Transport related physical activity								
☐ Cycling	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐Running	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐Brisk Walking	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
Sport and Leisure Time physical activity								
☐ Jogging/running	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Cycling	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Rugby	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Tennis	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Badminton	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Squash	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Water Aerobics	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Walking for exercise	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Volleyball	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Weight Training	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Swimming	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Tai Chi	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Skating	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Martial Arts (Judo/Taekwondo)	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
☐ Other (Specify) Click here to enter text.	☐	☐	☐	☐	☐	☐	☐	Click here to enter text.
								Total minutes per week: Click here to enter text.

Informed Consent Form

FIT 2 SIT

FIT 2 SIT STUDY

Study ID ☐

CONSENT FORM: Version 3 20/10/2014

Title of project: FIT 2 SIT Study: The impact of cardio-respiratory fitness on an individual's metabolic response to prolonged sitting and light activity breaks.

Chief Investigator: Dr Thomas Yates

Please Initial Every Box

1) I confirm that I have read and understand the FIT 2 SIT participant information sheet, version 3.0, dated 20/10/2014. I have had the opportunity to ask questions and have had them answered satisfactorily. ☐

2) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without any future care or legal rights being affected. ☐

3) I understand that relevant sections of my medical notes and/or study data may be looked at by responsible individuals from the study team, the sponsor, or from regulatory authorities where it is relevant to my taking part in the research. I give permission for these individuals to access my records. ☐

4) I agree to being contacted with details of future research and for my details to be stored on the University of Leicester database. ☐

5) I agree to take part in the above study. ☐

Name of participant	Date	Signature
Researcher taking consent	Date	Signature

Appendix Four – Supporting documents related to the 'Arming your Health' study

This appendix contains the following, in the order that they are listed:

- NHS Research Ethics Committee approval
- Research and Development ethical approval
- Research Protocol
- Participant Information Sheet
- Pre-screening questionnaire
- Informed consent form
- Individual screening results letter
- GP screening results letter

NHS Research Ethics Committee approval

NHS
Health Research Authority
East Midlands - Leicester South Research Ethics Committee
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0207 104 8077

01 February 2016

Dr Thomas Yates
Reader in Physical Activity, Sedentary Behavior and Health
University of Leicester
Leicester Diabetes Centre (Origin)
Leicester General Hospital
LE5 4PW

Dear Dr Yates,

Study title:	**Investigating whether breaking up sedentary behaviour with seated upper body contractile activity can regulate an individual's metabolic health.**
REC reference:	15/EM/0563
Protocol number:	0548
IRAS project ID:	191419

Thank you for your letter of 20 January 2016, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Rebecca Morledge, NRESCommittee.EastMidlands-LeicesterSouth@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper [REC response letter]	N/A	20 January 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Arming your Health - Sponsor Indemnity certificate]		06 November 2015
GP/consultant information sheets or letters [Arming your Health - GP results letter (no diabetes)]	V1	19 January 2016
GP/consultant information sheets or letters [Arming your Health - GP results letter (pre diabetes)]	V1	19 January 2016
GP/consultant information sheets or letters [Arming your Health - GP results letter (diabetes)]	V1	19 January 2016
Instructions for use of medical device [Arming your Health - Activity monitor instruction sheet]	V2	02 November 2015
IRAS Checklist XML [Checklist_21012016]		21 January 2016
Letter from funder [Arming your Health - Confirmation of BRU funding]		23 November 2015
Letters of invitation to participant [Arming your Health - Letter of invitation]	V2	19 January 2016
Non-validated questionnaire [Arming your Health - Sleep diary]	V2	02 November 2015
Non-validated questionnaire [Arming your Health - Pre-screening questionnaire]	V3	19 January 2016
Other [Arming your Health - Senior Investigator Award funding confirmation correspondence]		12 November 2015
Other [Arming your Health - Confirmation of Senior Investigator Award]		
Other [Po9sitive affect, mood and sleepiness scale]	2	02 November 2015
Other [Arming your Health - Individual results letter (no diabetes)]	V1	19 January 2016
Other [Arming your Health - Individual results letter (pre-diabetes)]	V1	19 January 2016
Other [Arming your Health - Individual results letter (diabetes)]	V1	19 January 2016
Participant consent form [Arming your Health - Informed consent form]	V3	19 January 2016
Participant information sheet (PIS) [Arming your Health - Participant information Sheet]	V3	19 January 2016
REC Application Form [REC_Form_27112015]		27 November 2015
Referee's report or other scientific critique report [Arming your Health - External scientific peer review of protocol]		22 October 2015
Research protocol or project proposal [Arming your Health - Protocol]	V3	19 January 2016
Summary CV for Chief Investigator (CI) [Dr.Yates CV]		01 October 2015

Summary CV for student		
Summary CV for supervisor (student research) [Dr.Yates CV]		01 October 2015
Summary CV for supervisor (student research) [Dr C. Edwardson]		10 November 2015

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

15/EM/0563	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely,

pp

Mr John Aldridge
Chair

Research and Development ethical approval

RESEARCH. & INNOVATION

University Hospitals of Leicester NHS
NHS Trust

DIRECTORATE OF RESEARCH & INNOVATION

Director: **Professor Nigel Brunskill**
Assistant Director: **Dr David Hetmanski**
Head of Research Operations: **Carolyn Maloney**

Research & Innovation Office
Leicester General Hospital
Gwendolen Road
Leicester
LE5 4PW

Direct Dial: (0116) 258 8351
Fax No: (0116) 258 4226

29.02.2016

Professor Melanie Davies
Professor of Diabetes Medicine
University Hospitals of Leicester
Leicester Diabetes Centre
Leicester General Hospital
Leicester
LE5 4PW

Dear Professor Melanie Davies

Ref: **UHL 191419**
Title: **The 'Arming your Health' study**
Project Status: **Approved**
End Date: **01/09/2016**

Date of Valid Application: 25.02.2016
Days remaining to recruit first patient: 66

I am pleased to confirm that with effect from the date of this letter, the above study has Trust Research & Development permission to commence at University Hospitals of Leicester NHS Trust. The research must be conducted in line with the Protocol and fulfil any contractual obligations agreed between UHL & the Sponsor. If you identify any issues during the course of your research that are likely to affect these obligations you must contact the R&I Office.

In order for the UHL Trust to comply with targets set by the Department of Health through the 'Plan for Growth', there is an expectation that the first patient will be recruited within 70 days of receipt of a Valid Application. The date that a Valid application was received is detailed above, along with the days remaining to recruit your first patient. **It is essential that you notify the UHL Data Management Team as soon as you have recruited your first patient to the study either by email to RIData@uhl-tr.nhs.uk or by phone 0116 258 4573.**

If we have not heard from you within the specified time period we will contact you not only to collect the data, but also to record any issues that may have arisen to prevent you from achieving this target. It is essential that you get in touch with us if there is likely to be a problem in achieving this target so that we can discuss potential solutions. The Trust is contractually obliged to meet the 70 day target and if an adequate reason acceptable to the NIHR has not

been submitted to explain the issues preventing the recruitment of your first participant, the Trust will be financially penalised.

In addition, we are required to publish the Title, REC Reference number, local target recruitment and actual recruitment as well as 70 days data for this study on a quarterly basis on the UHL publicly accessed website.

All documents received by this office have been reviewed and form part of the approval. The documents received and approved are as follows:

Document Title	Version	Date	REC Approval
REC favourable opinion letter	N/A	01.02.2016	N/A
GP/consultant information sheets or letters [Arming your Health - GP results letter (no diabetes)]	1	19.01.2016	01.02.2016
GP/consultant information sheets or letters [Arming your Health - GP results letter (pre diabetes)]	1	19.01.2016	01.02.2016
GP/consultant information sheets or letters [Arming your Health - GP results letter (diabetes)]	1	19.01.2016	01.02.2016
Instructions for use of medical device [Arming your Health - Activity monitor instruction sheet]	2	02.11.2015	01.02.2016
Letters of invitation to participant [Arming your Health - Letter of invitation]	2	19.01.2016	01.02.2016
Non-validated questionnaire [Arming your Health - Sleep diary]	2	02.11.2015	01.02.2016
Non-validated questionnaire [Arming your Health - Pre-screening questionnaire]	3	19.01.2016	01.02.2016
Other [Po9sitive affect, mood and sleepiness scale]	2	02.11.2015	01.02.2016
Other [Arming your Health - Individual results letter (no diabetes)]	1	19.01.2016	01.02.2016
Other [Arming your Health - Individual results letter (pre-diabetes)]	1	19.01.2016	01.02.2016
Other [Arming your Health - Individual results letter (diabetes)]	1	19.01.2016	01.02.2016
Participant consent form [Arming your Health - Informed consent form]	3	19.01.2016	01.02.2016

RESEARCH. & INNOVATION

University Hospitals of Leicester NHS
NHS Trust

Participant information sheet (PIS) [Arming your Health - Participant information Sheet]	3	19.01.2016	01.02.2016
Research protocol or project proposal [Arming your Health - Protocol]	3	19.01.2016	01.02.2016

Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments should be followed. Failure to do so may invalidate the approval of the study at this trust.

Undertaking research in the NHS comes with a range of regulatory responsibilities. Please ensure that you and your research team are familiar with, and understand the roles and responsibilities both collectively and individually.

Documents listing the roles and responsibilities for all individuals involved in research can be found on the R&I pages of the Public Website. It is important that you familiarise yourself with the Standard Operating Procedures, Policies and all other relevant documents which can be located by visiting www.leicestershospitals.nhs.uk/aboutus/education-and-research

The R&I Office is keen to support and facilitate research where ever possible. If you have any questions regarding this or other research you wish to undertake in the Trust, please contact this office. Our contact details are provided on the attached sheet.

This study has been reviewed and processed by the East Midlands Clinical Research Network (EM CRN) (Leicester Office) using the Coordinated System for gaining Trust Permission (CSP). If you require any further information on the approval of this study please contact the EM CRN office on 0116 258 6185 making reference to the CSP number which is located at the top of this letter.

We wish you every success with your research.

Yours sincerely

Carolyn Maloney

Carolyn Maloney
Head of Research Operations

Encs: .R&I Office Contact Information
CC: Mr Matthew McCarthy, PhD student

Research Protocol

ARMING your HEALTH

Research Protocol:

Title

Investigating whether breaking up sedentary behaviour with seated upper body contractile activity can regulate an individual's metabolic health.

Chief Investigator

Dr Thomas Yates

Study Co-ordinator

Matthew McCarthy

Co-investigators

Dr Charlotte Edwardson

Prof. Melanie Davies

Prof. Kamlesh Khunti

Sponsor: The University of Leicester

Funded by: Leicester–Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit & Professor Melanie Davies senior investigator award.

Chief investigators signature:

Confidentiality Statement:

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host NHS Trust (s), regulatory authorities, and members of the Research Ethics Committee.

Contents Page

Background and Rationale

An introduction into sedentary behaviour

Sedentary behaviour has been defined by the Sedentary Behaviour Research Network as "any waking behaviour characterized by an energy expenditure of ≤1.5 METs while in a sitting or reclining posture" [1]. It is this low energy requirement (ascribed to ≤1.5MET's) that allow us to distinguish sedentary behaviours from other seated activities such as cycling.

Common sedentary behaviours in modern society include: Watching television, using a computer, playing video games, driving a vehicle and reading. Constant engagement in activities such as these mean that people can sit for many hours at a time on a daily basis. However this is not what the human body is designed for.

Evolutionary origins, prevalence and consequences of sedentary behaviour

A substantial period of human evolutionary history (around 3-4million years) was spent in a hunter-gatherer lifestyle that required large feats of daily endurance in order to withstand environmental pressures. An unprecedented increase in lifestyle efficiency at the end of the 20th century, characterised by reductions in human movement, meant that our previous highly active lifestyles have become discordant from modern day living. This occurred in such a relatively short period of time (compared to the millions of years spent being highly active) that modern day humans had no time to evolve. It is for this reason that we are so metabolically sensitive to sedentary living, as we possess the physiology of our far more active ancestors.

The consequences of our inherited physiology extend down the physical activity intensity spectrum to sedentary behaviour. Research has revealed that those who engage in the most sedentary behaviour (compared to those who engage in the least) exhibit a cluster of biochemical and physiological abnormalities associated with the development of cardiovascular disease and type 2 diabetes [2]. Consequently, these individuals are considered to be at a 147% and 112% increased risk of these outcomes, respectively [3]. Importantly, these findings were shown to be independent of moderate to vigorous physical activity (MVPA), suggesting that the association between sedentary behaviour and metabolic disorders may still remain regardless of regular engagement in exercise.

Sitting is now dominating three main domains of modern day living. Firstly our occupations, in America sedentary based service jobs account for over 80% of US occupations, a statistic that was 50% in 1960.[4] Secondly transport, there has been over a 10 fold increase in motor vehicle traffic between 1949 and 2012 (from 28.9 to 302.6 billion vehicle miles/year) in Great Britain.[5] Lastly,

sitting time dominates our leisure time. With a sharp rise in game consoles, televisions, laptops and mobile phones [6] people do not feel the need to ascend the comfort of their chair to have fun or socialise. With this in mind it is not surprising that adults in developed countries spend typically 55 % to 70 % of their waking time in sedentary behaviours (about 8.8–11.2 h/d assuming 8 h/d of sleep) [7] [8] [9] [10].

Introducing light activities to reduce sitting time and regulate metabolic health

Frequently interrupting sitting time with brief bouts of light activity such as walking [11,12,13], standing [14] or both [15] [38] has been shown to be a simple and effective approach to alleviate postprandial hyperglycemia. For instance Dunstan et al (2012) found that interrupting sitting time with 2 minutes of light walking every 20 minutes (a total of 14 times over the 5 hour period) positively ameliorated postprandial blood glucose (PPG) levels in overweight/obese individuals [11]. This finding is consistent with the work of Peddie et al (2014)[12] and Bailey et al (2015)[13] who interrupted sedentary time with 1 minute 40 seconds of walking every 30 minutes (over a 9 hour period) [12] and with 2 minutes of walking every 20 minutes (over a 5 hour period) [13] in normal weighted individuals. Substantial reductions in PPG, comparable to that of light walking breaks, have also been witnessed when breaking up sitting time with 5 minutes of standing every 30 minutes [38]. The potential ability of standing breaks alone to regulate PPG has also been observed when alternating 30 minutes of sitting with 30 minutes of standing throughout an 8 hour day [14]. These findings are important because postprandial hyperglycemia is a powerful risk factor for cardiovascular disease in both people with Type 2 diabetes [16] and non-diabetics. [17] Reducing postprandial hyperglycemia improves inflammation and endothelial function [18] and reduces carotid intima-media thickness [19]. This is associated with a considerably reduced risk of cardiovascular events in individuals with impaired glucose tolerance [20].

The above data provide compelling support for the interruption of sitting time with light intensity physical activity. However, all studies to date have utilised weight bearing strategies, namely 'standing' and 'walking'. As a result of this, we are unable to elucidate whether it is the increased energy expenditure or the change in posture (from sitting to upright) of these activities that is predominantly fuelling their favourable metabolic associations.

Hypothetically if energy expenditure is solely responsible, then introducing light upper body activities, while remaining seated, should replicate the beneficial effects of light walking breaks if similar energy expenditures are used. Addressing this question will help clarify whether interventions to reduce sedentary behaviour should focus on interrupting the actual posture of

sitting or emphasise more on increasing the low energy expenditure associated with sitting. This will also have important implication for individuals who are unable to engage in upright, weight baring activities.

Given the sedentary nature of modern day occupations [4], the workplace has become an ideal target for introducing light activity breaks. Cognitive performance, mood state and sleepiness of employees are important factors to consider within the workplace. By investigating the impact that light breaks have on these factors we can begin to address the feasibility of implementing them in to a workplace environment.

Study Aim

The aim of this study is to compare an individual's metabolic response to a meal when a) interrupting postprandial sitting time with seated upper body activity breaks (using arm ergometry) and b) sitting for a prolonged period of time with no activity breaks.

Primary Objective

- To investigate whether interrupting postprandial sitting time with seated upper body contractile activity can reduce blood glucose area under the curve (AUC).

Secondary Objectives

- To investigate whether interrupting postprandial sitting time with seated upper body contractile activity can reduce blood triglyceride area under the curve (AUC).
- To investigate whether interrupting postprandial sitting time with seated upper body contractile activity can reduce insulin area under the curve (AUC).
- To investigate whether interrupting sitting time with seated upper body contractile activity improves psychological health (mood/positive affect/ sleepiness), cognitive performance and sleep quality.

Methods

Study Design

This study is a randomised cross-over trial whereby each participant will take part in two treatment conditions in a random order, acting as their own controls. Randomisation will be implemented using an online randomisation tool and conducted by a trained statistician named on the delegation of authority log.

Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study
- Body Mass Index: ≥ 30 kg/m^2
- Male and Female
- Aged: ≥ 30 to ≤ 75 years of age.
- Inactive (I.e. no habitual structured exercise).
- Participant is able to walk (without any assistive devices and not requiring assistance from another person).

Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- Aged < 30 or > 75 years of age.
- Physical condition which limits full participation in the familiarisation visit or treatment visits.
- Active psychotic illness or other significant illness which, in the view of the investigators, would prevent full participation
- Inability to communicate in spoken English
- Steroid use
- Known Type 2 Diabetes Mellitus
- Pre-existing Cardio-vascular Disease including a previous heart attack, stroke, angina or coronary artery bypass surgery of cardiac stents.
- Pregnancy
- Smoker
- Terminal illness
- Contraindication to gas mask procedure (I.e. Claustrophobia).
- Chronic Obstructive Pulmonary Disease. (I.e. Chronic Bronchitis or Emphysema).

*In the circumstance that an individual is not sure whether they meet the inclusion/ exclusion criteria, (I.e. they are not sure if they have pre-existing CVD) this will be reviewed by a named medic on the delegation of authority log for a clinical decision to be made during familiarisation.

Setting

The study will be co-ordinated within the Leicester- Loughborough Biomedical Research Unit (Leicester Diabetes Centre) at the Leicester General Hospital.

Participant Recruitment

Individuals identified from other studies conducted by our group, who have provided consent to be contacted about other research (and who meet the inclusion criteria), will be primary targets for recruitment in to this study. Only study staff on the original research studies will have access to participant's information initially. Following this, identified individuals will be invited to participate and will be sent a participant information sheet alongside a pre-screening questionnaire. This initial study information will be sent from the Principal Investigator of the screening study database used for initial identification. If participants are interested in the study, the pre-screening questionnaire will be returned directly to the research team in a pre-paid envelope and used to assess their eligibility to take part. A member of the team will then contact the interested participant in order to arrange a familiarisation visit (providing they are eligible).

Volunteers will also be recruited from the general public via links to ethically approved study material such as the Participant Information Sheet included on NHS Trust websites. e.g, leicesterdiabetescentre.org.uk and Leicestershirediabetes.org or on other occupational e-mail distribution lists. If responding to study recruitment material in this way, potential participants can register their interest by phoning or e-mailing the research team on the contact details provided. They will then receive a pre-screening questionnaire that can be completed and e-mailed/posted back to us. Once we have received an individual's pre-screening questionnaire, providing they have not been deemed ineligible to take part, they will receive a follow up phone call from the research team to arrange a date for their familiarisation and screening visit.

The pre-screening questionnaire will allow the research teams to gather demographic information such as; age, gender and smoking habits etc. Medical history checks for history of diseases or current health issues that may affect participation in this study will also be highlighted here.

Informed Consent

Before any study related procedure can take place, the participant must sign and date the latest approved version of the informed consent form. Participant information with full details of procedures, expectations, potential risks and withdrawal rights will be sent to the participants a minimum of 48 hours prior to their first visit in order to give them adequate time to read through it. It will then be presented both in writing and verbally on arrival at their first study visit prior to consent. The consent form will be signed and dated based upon an informed decision from this information. Consent will be taken by someone suitably experienced that has received generic consent training and has been authorised by the Chief investigator to do so, they will also be included in the delegation of authority log. A copy of the consent form will be given to the participant.

Familiarisation screening visit - (Visit 1)

Before participating in the study, all participants will visit the exercise laboratory for a familiarisation and screening visit where they will be shown the designated experimental area, provide written informed consent and have various measurements taken.

All participants will be required to fast from 10pm onwards the evening prior to this first visit (drinking water is allowed). Participants will then be provided with a standardised breakfast on arrival (after consent has been obtained). This meal will be prescribed according to body mass and will provide approximately 8 kcals per kg of bodyweight made up of 13-14% protein, 51-52% carbohydrate and 35% fat. Typical ingredients used here will be; Plain bagels with margarine and a protein powder mixed with whole milk. Alternative ingredients matching the nutritional content of this meal will be explored in light of special dietary preferences (i.e. vegan) or food allergies (i.e. lactose intolerance).

It is also important that participants avoid general exercise and drinking alcohol or coffee in the 48 hours (2 days) leading up to this first visit (and the remaining two visits), this will be made clear in the participant information sheet**.** Very strenuous exercise must be avoided 72 hours **(3 days)** prior.

Participants will be asked to record all food and drink consumed the day before this first visit. They will then be asked to replicate this diet the day before the remaining visits using their dietary record to guide them. Therefore, all meals leading up to each visit will be standardised and will not confound the results.

The measurements taken during this visit include; basic anthropometry (body mass, waist circumference, body fat %); blood pressure; biochemical variables (HbA1c and blood lipids and CRP); Energy expenditure (at rest, during slow steady state treadmill walking and at numerous intensities on an arm ergometer machine); and measures of objectively determined free-living physical activity, sedentary behaviour and posture.

Anthropometry measurement at visit 1

Arterial blood pressure will be measured in the sitting position. Three measurements will be obtained and the average of the last two measurements will be used.

Basic anthropometric measures such as body mass and body fat percentage (measured through bio impedance analysis) will be taken alongside height and waist circumference (midpoint between the lower costal margin and iliac crest). These will be recorded to the nearest 0.1kg, 0.5%, 0.5cm and 0.5cm respectively.

Biochemical variables collected at visit 1

During visit one a blood sample will be taken whereby HbA1c (Glycated Haemoglobin), CRP (C-Reactive Protein) and lipid profile will be measured. All venepuncture will be undertaken by trained health care professionals and all biochemical analyses will be conducted blinded to treatment condition. All participants will be sent an individual results letter highlighting their main clinical results after the familiarisation visit. With the participants consent, all results will be copied to their GP. Any participant whose Hba1c is 6.5% or above will be sent an individual results letter strongly advising them to make an appointment with their GP as soon as possible to discuss their results in more detail, as this is indicative of Diabetes. Similarly, any participant whose HbA1c is between 6 and 6.4% will also be sent an individual results letter advising them to make an appointment with their GP if they are concerned, as this is indicative of pre-diabetes. With the participants consent, the GP will also be notified of the situation and sent a results letter

Energy Expenditure testing during visit 1

During visit one, the energy expenditure at rest, while walking at 3km/hr and while performing arm ergometry at different intensities will be measured via respiratory gas collected from the participants using the Cortex or GEM breath-by-breath automated gas-analysis system.

In order to assess resting energy expenditure, each participant will be asked to sit quietly (refraining from movement) for 30 minutes while wearing a gas mask/hood. Expired gas data will then be collected over the latter half of this 30 minute period once values have stabilised.

Following this, walking will be performed at 3km.hr on a treadmill for 10 minutes (also while wearing a gas mask), from which expired gas data will be collected in the latter 5 minutes.

Light arm ergometry while seated will then be performed at 5 minute intervals (across numerous intensities) from which expired gas data will be collected in the 2nd 3rd and 4th minutes, discarding both the first and last minute. Participants will also remove the face-mask for 5minutes in between each 5 minute bout in order to allow participants to recover and for energy expenditure outputs to return to their resting level prior to the next activity bout.

This data will consequently dictate the intensity of arm ergometry to be performed when breaking up sitting time, as we would like the energy expenditure of these seated breaks to closely resemble that of light walking. This allows investigators to further elucidate the role of posture alone as energy expenditures will be kept relatively consistent with that of previous research, allowing direct comparisons to be made.

Objective measures of physical activity at visit 1

Participants will be issued with an Actigraph accelerometer to measure the time spent sedentary and in moderate to vigorous physical activity under free-living conditions. Participants will be asked to wear this accelerometer (placed on right anterior axillary line) for 7 days after the familiarisation visit, up to 7 days leading up to the treatment conditions and throughout the experimental conditions in order to monitor any potential changes in physical activity levels and make sure participants have avoided exercise in the days leading up to the treatment conditions. Outputs will include steps per day, total body movement (counts per day), and time in sedentary, light, moderate and vigorous-intensity physical activity as determined by counts per minute cut points proposed by Freedson et al. [21]

Posture and sedentary time data collection at visit 1

Postural allocation (sitting and standing) and walking will be quantified using an activPAL physical activity monitor (PAL Technologies, Glasgow, Scotland). The activPAL is a single-unit monitor based on a triaxial accelerometer that is worn midline on the anterior aspect of the thigh and attached directly onto the skin using medical dressing. The monitor produces a signal related to thigh inclination and has been shown to be a valid and reliable measurement tool for determining posture during activities of daily living in a healthy population [22]. Outputs from this device include; time spent sitting, standing, stepping and sit-to-stand transitions. The device will be worn for 7 days after the familiarisation visit, (alongside the Actigraph) and throughout experimental conditions.

Experimental treatment conditions (Visits 2 and 3)

In the weeks following this initial familiarization visit, participants will be assigned to receive the following two treatment conditions (A: Prolonged sitting and B: Light arm ergometry breaks) in a random order using an online randomisation tool. Each treatment condition will be carried out on one single day. In total, the study requires three separate visits to the Leicester Diabetes Centre, Leicester General Hospital, University Hospitals of Leicester; familiarisation visit, first treatment condition and second treatment condition.

Where participants have limited access to motorised transport, we will provide taxis to and from their home address to reduce ambulatory activity involved in the commute to and from each visit.

For male participants, there will be a minimum of 7 days between each treatment condition. For females, there will be a gap of one month (or near as possible) due to the potential that different phases of a women's menstrual cycle may affect their blood glucose response to a meal [23]. By testing at similar times of the month, we avoid this potential variability. However, if a female participant reports being post-menopausal on their pre-screening questionnaire then a minimum of 7 days between treatment conditions will be used.

Treatment Condition A: Prolonged sitting

During the prolonged sitting condition, participants will be restricted from walking and standing. They will be in a designated room equipped with a chair/desk and have access to a computer with internet services, books and magazines or movies throughout the day. Lavatory breaks will be permitted and a wheelchair will be used to wheel the participants to the toilet to reduce upright activity.

The day before each treatment condition, participants will be asked to consume the same type and quantity of food and drink that they recorded prior to their familiarisation visit. Participants will also be asked to avoid alcohol, caffeine and moderate intensity exercise for 2 days prior to each treatment condition (as done prior to visit one) as reproducibility data has shown that insulin and triglyceride responses to meal ingestion are good under these conditions [24]. Furthermore, there is evidence that the effect of a single exercise session on insulin sensitivity and glucose tolerance may last up to 48 hours [25]. Strenuous exercise should be avoided for 3 days prior to each treatment condition, as this has been found to increase insulin sensitivity for up to 72 hours [26].

Participants will be asked to fast the night before both treatment conditions (and prior to familiarisation as discussed previously) from 10pm onwards. On the morning of the test, participants

will have a cannula inserted into an accessible vein by a trained health care professional and the first of the blood samples will be taken (this represents time point: -1hr according to figure 1). Blood pressure measurements will be taken immediately before each blood sample alongside self-reported measures of positive affect, mood and sleepiness (see description under secondary outcomes section 3.0). Once the cannula has been inserted and the first blood sample taken, participants will then be asked to sit quietly for 60 minutes in order to achieve a steady state. During this time, participants will be expected to complete a battery of cognitive function tests lasting approximately 25 minutes (see description under secondary outcomes section 4.0).

Following this 60 minute period, participants will have another blood pressure measurement, positive affect, mood and sleepiness assessment and blood sample taken, at which point they will then be provided with a standardised mixed meal breakfast (09:00am) (time point: 0h). The meal will be prescribed according to body mass and will provide approximately 8kcals per kg of bodyweight made up of 13-14% protein, 51-52% carbohydrate and 35% fat. A mixed-meal of this nature will be used to ensure ecological validity as fat and carbohydrates are usually co-ingested in real-life situations. Blood sampling alongside positive affect, mood, sleepiness and blood pressure measurements will continue at 30, 60, 120 and 180 minutes following breakfast. A second, lunch meal (12:00pm) (with identical nutrient composition to breakfast), will then be consumed over 15 minutes. Blood pressure, positive affect, mood, sleepiness and blood sampling will continue at 30, 60, 120 and 180 minutes following lunch (Figure 1). Participants will then repeat the battery of cognitive function tests after their final blood sample is taken. All participants will remain sitting throughout the test period whilst undertaking typical sedentary pursuits such as watching TV/DVDs/reading.

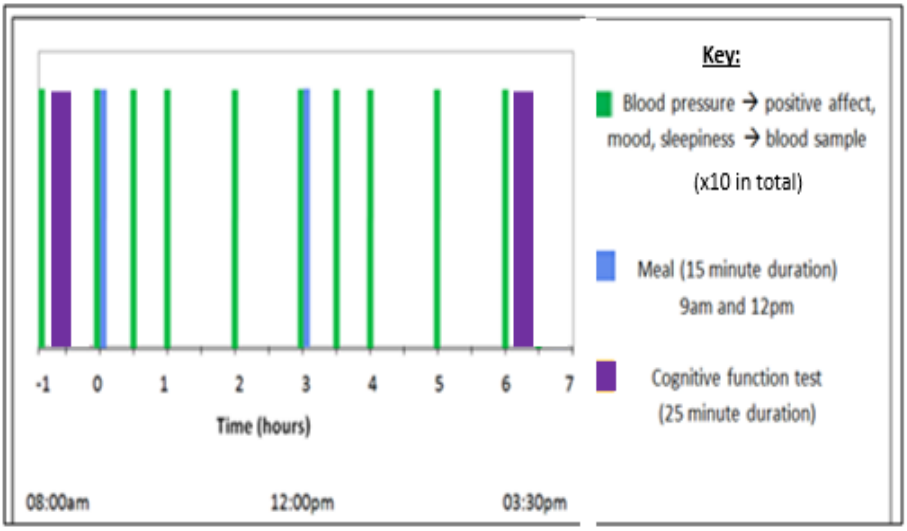

Figure 1 - Timeline for treatment condition A

Treatment Condition B: Light arm ergometry breaks

This will be identical to the prolonged sitting condition (Treatment A), but participants will be required to break up their restful sitting time with seated upper body contractile activity. This will be implemented in the form of arm ergometry breaks for 5 minutes every 30 minutes following both breakfast and lunch. In total, individuals will accumulate 12 bouts (60 minutes) of light intensity arm ergometry.

The main reason for utilising 5 minute bouts every 30 minutes was to achieve a balance between frequency and utility, as having to interrupt sitting time too regularly has less real world application, especially in an office based environment where prolonged sitting is most prevalent. Previous research has also found positive findings while interrupting sitting time in this manner [38].

Figure 2 shows a timeline for this treatment condition. The intensity (speed and resistance) of these arm ergometry breaks will have been derived from the energy expenditure tests conducted in visit 1, and we will be aiming to match the intensity of arm ergometry to that of light walking at 3km/hr.

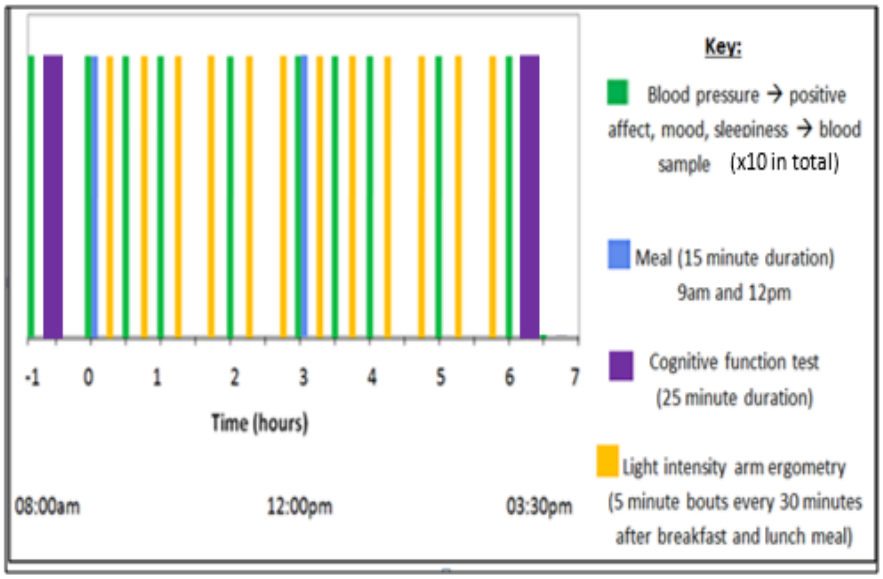

Figure 2 - Timeline for Treatment Condition B

Defining the end of the trial

The end of the trial is marked by the date at which the participants complete the last of their two treatment conditions (3 visits in total).

Primary Outcome

Glucose area under the curve (AUC); Plasma glucose will be measured using a glucose oxidase method undertaken in University Hospitals of Leicester laboratory.

Secondary Outcomes

1. **Biochemical:**

This study will also measure Insulin AUC and triglyceride AUC, as they are directly involved in the underlying physiological mechanism of sedentary behaviour [12]. Serum triglycerides will be analysed using the glycerol phosphate oxidase (GPO) assay. The Mercodia Insulin ELISA will be used to provide a quantitative determination of insulin. Triglycerides may be measured in the University Hospitals of Leicester or at another certified laboratory. In addition to traditional markers of metabolic health, we will also store samples for analysis of inflammatory proteins (including interlukin-6). Samples will be stored in secure -80oC freezers located within the Leicester Diabetes Centre and analysed at the end of the study.

2. **Blood pressure:**

Blood pressure will also be measured immediately before each blood sample as replacing sedentary behaviour with light activity breaks has been suggested to lower blood pressure (especially in diastolic state)[27]

3. **Positive affect, mood and sleepiness:**

Psychological impacts of both treatment conditions will be monitored and compared. The Felt Arousal scale [28] is a six-point scale that will be used to assess arousal level throughout the day (low arousal= 0 and high arousal= 6). The Feeling Scale [29] will be used to quantify participants feelings on an 11-point scale (very good= +5 and very bad= –5). These two single item scales will be used to plot participants' affective states throughout the experimental conditions. The modified Karolinska Sleepiness Scale [30] is on a 9-point scale (1 = extremely alert and 9 = extremely sleepy, fighting sleep) and will be used to plot participants' daytime sleepiness states throughout the experimental conditions.

4. **Cognitive function**

Cognitive function in areas such as memory, reasoning and planning has been shown to improve with acute bouts of physical activity [31 - 32]. Prolonged sitting is highly prevalent in the workplace, and cognitive function in this environment is important. By monitoring the impact of each intervention on cognitive function we can begin to quantify any implications that interrupting sitting time may cause in a workplace environment.

The cognitive function tests will consist of the semantic verbal fluency test (SVFT) [33], Hopkins verbal learning test (HVLT) [34], trail making tests A and B (TMT) [35], and rapid visual information processing test (RVIP) [36] (see Figure 3). The SVFT assesses semantic memory and language, and participants will be asked to name as many items as they can that belong to a particular category. The categories selected for each of the experimental days will be clothing (first test) and animals (second test). The HVLT assesses verbal learning and working memory, requiring immediate and delayed recall of a series of 12 words over three learning trials. Participants will be requested to undertake the delayed recall component at the end of the first set of cognitive function tests and during the second set of cognitive function tests. There will be no immediate recall component required during the second test (see Figure 3). The TMT assesses cognitive flexibility and requires connecting randomly located numbers in numerical order (e.g. 1,2,3,4) (TMT A) or numbers and letters in numerical and alphabetical order alternately (e.g. 1,A,2,B,3,C) (TMT B). The RVIP assesses sustained visual attention using numbers and requires both selective attention and working memory. This test displays a

number on screen that changes between odd and even digits and individuals must detect target sequences of three odd or three even consecutive digits. The tests will be delivered via the Sensitive Cognitive Assessment Inventory (SCAI) [37] software package and administered with standardised instructions. The SCAI computerised battery will allow accurate response timing with millisecond resolution to maximise sensitivity for small effects.

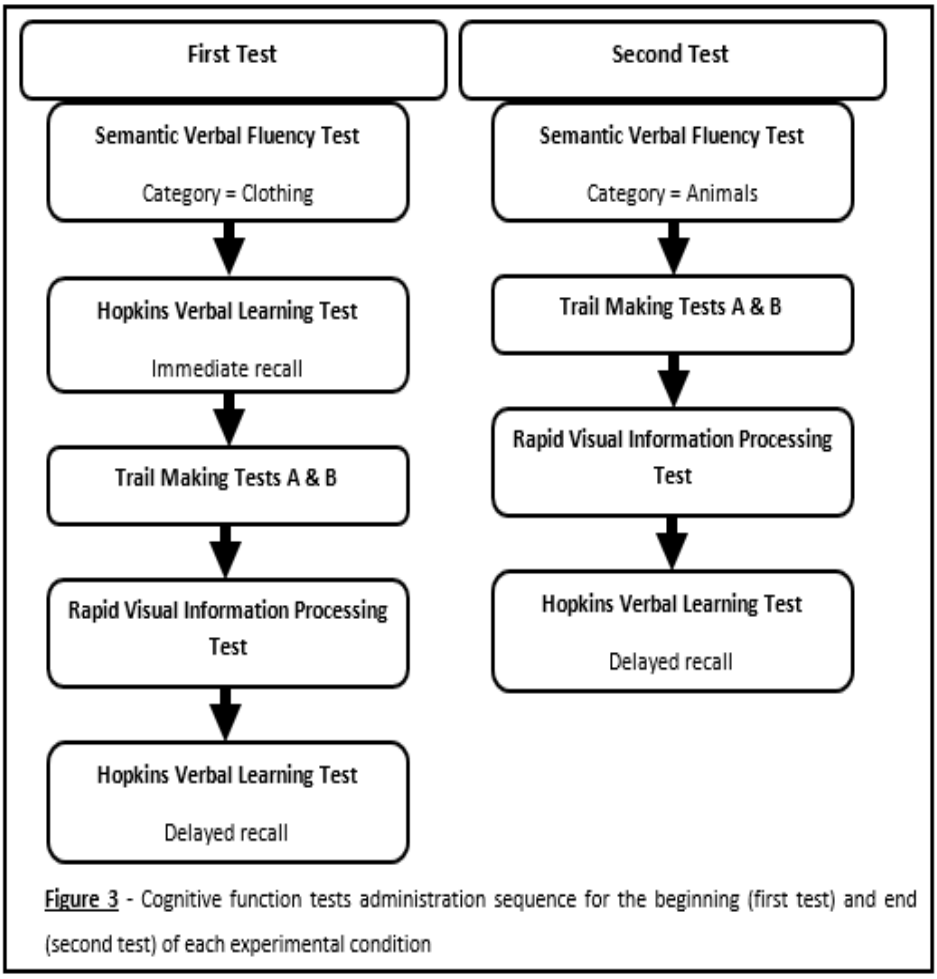

Figure 3 - Cognitive function tests administration sequence for the beginning (first test) and end (second test) of each experimental condition

5. Sleep Quality

Altering physical activity levels may affect sleep quality and therefore participants will be requested to complete a sleep diary for the night before and after each experimental condition. In addition, sleep quality will also be captured via an activity monitor located on the wrist that will be worn during these nights. Participants will be asked to post the sleep diary and accelerometers the day after the final experimental condition using prepaid envelopes provided.

Sample Size

Assuming a population standard deviation of 2.5mmol/L.hr in glucose AUC and a within-person correlation of 0.5, we estimated that we would require 13 participants in order to detect a difference of 1.8 mmol/L.hr in glucose AUC with 90% power assuming alpha = 0.05 to allow for one primary comparison (Condition A vs Condition B). These values are based on a previous Leicester-Loughborough Biomedical Research Unit study. In order to allow for a 20% drop out rate, we will need to recruit 17 participants.

Data Analysis

Each treatment condition will assess the primary (glucose) and secondary (triglyceride and insulin) AUC (Area Under the Curve) outcomes, both incremental AUC and total AUC. A direct comparison of this will be made between Treatment A and B using a paired samples t-test. Descriptive statistics (mean values and frequencies) will be calculated. Histograms will be used to identify any outliers and to assess normality. If data is found to be non-normally distributed, an appropriate transformation will be carried out or a non parametric alternative will be used. Carryover effects will not be formally tested, given the 7-day washout between treatment groups.

The calculation of the AUC (incremental and total) is critical for analysing the response to a standardised meal. Analysis of AUC will involve calculating the entire area under the curve using the trapezium rule. Incremental AUC will then be calculated by subtracting the fasting AUC from the total AUC.

We will also conduct a sensitivity analysis removing any individuals with substantial MVPA (≥15 minutes/day) in the 72 hours before each treatment visit, as this is a deviation from the pre-assessment requirements and may confound the results of this investigation. However, experience with previous studies would suggest that compliance to this aspect of the protocol is high.

Withdrawal and discontinuation from the study

Participants will be fully informed of their right to withdraw from the study at any point without needing to give a reason. If a participant withdraws their consent during the study and requests for their data not to be used, all samples will be destroyed and data will be deleted. The participant will be withdrawn from the study. If a participant withdraws from the study, but not their consent, because they are no longer able to take part in future visits, data already obtained will be used for the study.

If a participant loses capacity during the study, they will be withdrawn and identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant in question. The investigator may discontinue a participant from the study at any time if they consider it necessary to do so. Reasons for this might include, but is not restricted to:

Significant non-compliance with treatment regime or study requirements

Ineligibility (may arise during the study).

Safety Issues

We do not foresee any adverse events over and above those associated with everyday life and routine health care that could be attributable to the study. However, all participants will undergo venepuncture and cannulation, which occasionally results in bruising, swelling and temporary discomfort. Baseline safety checks will also include blood pressure (to detect underlying hypertension), reporting of drugs which may increase the risk of bleeding (warfarin, aspirin) and a measurement of triglycerides (to detect hypertriglyceridaemia).

Regarding routine measurements collected in the Arming your Health study, in the case of urgent circumstances (e.g. blood pressure 170/95, raised potassium) the individual will be strongly advised to visit their General Practitioner. In Emergencies (e.g. BP> 220/ 120 or K+ >7.0, blood sugar > 20.0mmol/l with diabetes symptoms) the individual will be referred to A+E / MAU / UHL services as considered appropriate by a doctor depending on the nature and history of the condition.

Reporting of SAE and timelines

All SAEs will be reported internally to the the sponsor (University of Leicester) using appropriate reporting forms, within 24 hours of the study team becoming aware of the event. A follow up/final SAE report will be submitted if necessary to the sponsor with 28days of the initial report.

Additional information can be provided if requested to the sponsor, University of Leicester. The principal investigator is responsible for the review and sign off the SAE, or in their absence, another appropriately qualified member of the research team.

The sponsor will ensure that all relevant information about a SUSAR which occurs during the course of the Arming your Health study that is fatal or life-threatening is reported as soon as possible to the sponsor, not later than seven calendar days after they were first aware of the reaction. Any

additional relevant information will be sent within eight days of the report. The sponsor will ensure that a SUSAR which is not fatal or life-threatening is reported to the main REC no later than 15 calendar days after they were first aware of the reaction.

The investigator site file will contain documentation for:

- SAE, SAR and SUSAR reports
- Evidence of submission of SAEs to the sponsor within 24 hours of the team becoming aware of an event
- Evidence of timely SUSAR submission to the main REC.

Access to documents and Source Data

Direct access to information gathered in this study will only be available to individuals who have been granted access. The sponsor, host institution and regulatory authorities can permit trial related monitoring, audits and inspections.

Participant Confidentiality

All staff involved in this study will make sure that the participant's anonymity is maintained. Participants will only be identified by participant ID number. Documents will be stored securely and be assessed by trial staff and authorized personnel. This procedure abides by the Data Protection Act which requires data to be anonymized as soon as possible.

Ethical Issues

Approval from the University of Leicester (sponsor), NHS REC and University Hospitals of Leicester NHS Trust R&D will be sought prior to the commencement of the research. This will ensure that all ethical and indemnity issues are dealt with. The research protocol, participant recruitment flyer, informed consent form, participant information sheet, pre-screening questionnaire and any other supporting documents will be submitted to the sponsor, NHS REC and R & D for approval. The Study Coordinator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents. The Chief Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the International Conference on Harmonisation Guidelines for Good Clinical Practice.

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Participants Information Sheet

NHS
National Institute for Health Research

ARMING your HEALTH

Leicester Diabetes Centre
Leicester General Hospital,
Gwendolen Road,
Leicester,
LE5 4PW

Mob: 07740049606
Email: mm636@le.ac.uk

Chief Investigator: Dr. Thomas Yates

The '**Arming your Health**' study: Investigating whether breaking up sedentary behaviour with seated upper body contractile activity can regulate an individual's metabolic health.

PARTICIPANT INFORMATION SHEET

You are being invited to take part in a research study but before you make a decision, it is important that you understand why the research is being carried out and what it involves. The study is being conducted by a student as part of their research degree (PhD) and this information sheet is designed to help you decide whether you would like to take part. You can talk it over with your family or friends, and if anything is not clear, or you would like to know more, we have put a contact number at the end of this leaflet allowing you to talk to us directly.

What is the purpose of the study?

Research shows that sitting for long periods of time on a regular basis is bad for our health and can leave us more susceptible to Obesity, Cardiovascular Disease, Type 2 Diabetes and premature death regardless of how much we exercise outside of these seated hours.

As sitting is so common in modern society it is vital that we explore ways to protect individuals from this worsening issue. We want to see if breaking up long periods of sitting time with short, frequent bouts of light physical activity, while remaining seated, is enough to alleviate these risk factors.

Why have you chosen to invite me?

You may have previously attended our diabetes department and provided consent to be contacted about future research or you have registered your interest by responding to one of our research advertisements and feel you meet the inclusion criteria to take part in this study.

What if I do not meet the inclusion criteria?

We will see whether you meet our inclusion criteria on your first visit to the Leicester Diabetes Centre. If it is found that you do not meet the criteria for this study we will give you general feedback on any measurements taken from your first visit, reimburse any travel costs and thank you for your time. Unfortunately you would no longer be allowed to continue in this study.

Do I have to take part?

It is your right to decide whether or not you would like to take part in this study. If you decide to take part, you can withdraw your consent from the study at any time by contacting the study team. If you do this, we will ensure that all personal details held are carefully destroyed. You can also withdraw from this research at any point without having to give any reason for doing so. Any future medical care will not be affected in any way.

If I decide to take part, what happens then?

If you would like to take part, please fill in the pre-screening questionnaire that is attached alongside this participant information sheet and send it back to us. We will then contact you to arrange your first appointment. Taking part in the study will involve three separate visits to the Leicester Diabetes Centre, at the Leicester General Hospital. In your first visit we will describe the study and go through this information sheet step by step, allowing you to ask any questions you may have. We will then ask you to sign a consent form to show that you have agreed to take part (this is not a contract and does not mean you definitely have to take part, you can still stop taking part at any point during the study). This is called 'informed consent'. This first visit will take around 2 hours and you will have the chance to meet the friendly members of our team who will fully familiarise you with what is expected on your remaining visits to the centre.

How can I prepare for my first visit?

You will be required to fast from 10pm onwards the evening prior to this first visit. (drinking water is allowed). You will then be provided with a standardised breakfast on arrival. It is also important to avoid exercise and drinking alcohol or coffee in the 48 hours (2 days) leading up to this first visit. Vigorous/ very strenuous exercise should be avoided 3 days prior. You should also record all food and drink consumed the day before your first visit, this will then be replicated the day before visits two and three.

Will you be measuring anything at the first visit?

During this visit we will check your blood pressure, height, weight, waist measurement and body fat percentage. Blood tests will also be taken to measure the levels of sugar and cholesterol in your blood.

We will also measure the amount of energy you use while at rest (for 30minutes), during a slow walking task (10 minutes) and during 5 minute bouts of light seated arm activities. During these tasks/activities, you will be expected to breathe into a tube through a special mask (see picture below), this will allow us to capture the air that you breathe in and out. We can then use this information to work out how much energy, in the form of calories, that you are using during each of the above states.

There will be plenty of time spent at rest to become accustomed to the mask prior to the analysis and participants will be reminded that they can remove the mask at any point if they feel uncomfortable. Fresh water will be readily available to reduce stuffiness while wearing the mask.

You will also be introduced to two types of activity monitors known as the 'Actigraph' and 'ActivPAL'. These are small devices worn on the waist and thigh that record movement patterns, allowing researchers to collect information on your activity levels and how much time you spend sitting. After your first study visit we would like you to wear these devices for 7 days (1 week). On the evening of your second and third visit, we would like you to wear a wrist based monitor to

observe the impact that these treatment visits have on your sleeping pattern. We would also like you to complete a sleep diary. This diary consists of 9 simple questions and will allow researchers to gain more information regarding the quality of your sleep during the nights before and after your second and third visit. We will demonstrate how to use all the devices mentioned above, and provide you with written instructions. These devices, alongside the sleep diary, will be returned at your next visit or via a pre-paid envelope addressed to the Leicester Diabetes Centre. Also, 1 week prior to your second and third visit, we would like you to wear the 'Actigraph' activity monitor to give us further information on your activity levels leading up to the treatment conditions.

What happens next?

Approximately seven days after you have been for your first visit (this may be longer depending on staff, laboratory and participant availability), you will be asked to return to the Leicester Diabetes Centre for your first of the two treatment conditions, (condition A and B are detailed on the next page). Each condition lasts 7 ½ hours (8am until 3:30pm). The order of your treatment conditions will be chosen at random. (It may be condition A followed by condition B or vice versa).

Females will require a 1 month gap between Treatment 1 and Treatment 2 to minimise the impact of menstrual cycle that could potentially skew our findings. (Unless reported to be post-menopausal in pre-screening questionnaire). Males however will require a minimum of 7 days washout period between treatments.

What are the two treatment conditions?

Condition A is referred to as the '**sitting'** condition. You will be expected to remain seated throughout this entire 7 ½ hour test period (8am – 3:30pm) whilst watching TV/DVD's, reading, using the internet, doing paperwork etc. at your will.

On the morning of the test you will come into the Leicester Diabetes Centre and have a cannula (a small tube that allows us to take blood) inserted, this will stay in throughout the day allowing our trained and experienced nurses to take regular blood samples without the need for multiple needles. After the first blood sample has been taken, you will be expected to sit quietly for an hour, following which you will be provided with a standardised breakfast.

After breakfast, we will continue to measure blood pressure and take blood samples four times over the next three hours while you are seated (at 30, 60, 120 and 180 minutes from the end of breakfast). You will then be provided with a standardised lunch and we will continue to measure blood pressure and take blood samples four times over the next three hours whilst you continue to sit (at 30, 60, 120 and 180 minutes from the end of lunch).

In total, we will take 10 blood samples over the 7 ½ hour testing period. Although this may sound like a lot, it is equivalent to approximately 8-10 tablespoons over the course of the day, which is less than a quarter of a blood donation.

At each time point, alongside blood pressure and blood samples we will also be recording your arousal, mood state and sleepiness, all of which will be self reported using a numbering scale (i.e. 1 = extremely alert and 9 = extremely sleepy, fighting sleep).

During both condition A and condition B, before breakfast and after the last blood sample of the day, participants will undergo 20 minutes of cognitive function testing. These will assess aspects of your cognitive function such as immediate recall, delayed recall and word association. More information on what these short tests consist of will be provided at the familiarisation visit.

Condition B is the **'light arm ergometry breaks'** condition. This will mimic condition A but you will be required to interrupt your sitting time with 5 minute bouts of light intensity seated activity every 30 minutes following breakfast and lunch. In total you will do 12 bouts of seated activity throughout the 7 ½ hour testing period (accumulating to 60 minutes of activity in total).

Breakdown of the study

Visit One: Familiarisation visit

- Full overview of everything involved in the study, a chance to show any concerns or ask any questions, invited to give informed consent so that we can commence with the study.
- Receive a standardised breakfast.
- Measurement of height, weight, body fat percentage, waist circumference, blood pressure, blood tests (e.g. sugar and cholesterol) and energy requirements for sitting, light walking and arm ergometry. (All of which will be conducted using the gas mask technique).
- Medical History check.
- Provided with activity monitors.

Visit Two: Condition A (or Condition B depending on randomisation order)

- Remain seated for 7 ½ hours.
- Have breakfast at 9am and lunch at 12pm.
- Frequent blood pressure and blood samples taken (alongside self report of your mood state, arousal and sleepiness).
- Short 20 minute cognitive function tests at the beginning and end of the day.

Visit Three: Condition B (or condition A depending on randomisation order).

- This will replicate visit two but will interrupt sitting time with 5 minutes of light intensity arm ergometry every 30 minutes after breakfast and lunch.
- Frequent blood pressure and blood samples taken (alongside self report of your mood state, arousal and sleepiness).
- Short 20 minute cognitive function tests at the beginning and end of the day.

Study complete

Additional Reminders

Please do not consume any food from 10pm onwards the evening prior to each study visit. (Water is allowed).

Wear both activity monitors provided (ActiGraph and ActivPAL) for 7 days after 'visit one' and return them to the researchers at 'visit two'.

Please avoid very strenuous exercise for 3 days prior to visits 2 and 3. Do not drink alcohol or coffee in the 48 hours (2 days) leading up to each visit.

Make a note of all food eaten on the evening prior to visit one and replicate this the evening prior to visits two and three.

What is a cannula and are there any risks?

A cannula is a small flexible tube that is inserted into a vein to allow blood samples to be taken. As with any object that punctures the skin there is a risk of infection but using a clean technique when putting it in will substantially reduce this risk.

Where will the cannula be put and how is it inserted?

Your cannula will usually be placed in a vein in the lower arm/hand. The doctor/nurse will try to avoid the hand you use for writing; however this may not be possible. The healthcare worker will clean their hands using soap and water or alcohol gel/hand sanitizer and wear disposable gloves. The area around a suitable vein will be cleaned using a recommended product. A tight strap called a tourniquet will be placed around your arm to help identify the best vein to use. The cannula will be inserted through the skin into a vein, using a needle and when it is correctly in position the needle will be removed leaving only the cannula in the vein. The cannula will then be flushed through with sterile salty water (saline) to ensure it is working.

Is it painful and can it fall out?

There may be a small amount of pain or discomfort as the cannula is inserted and should pass very quickly once the cannula is in place. The cannula will be secured with a see-through dressing and there is usually no need for the cannula to be bandaged. A cannula may fall out if the dressing becomes loose. Please inform staff if the dressing becomes loose.

When will the cannula be taken out?

The cannula will be removed at the end of each day, or earlier if a problem occurs.

What happens after removal of the cannula?

When the cannula has been taken out, the place where it has been may feel slightly bruised. This sensation can last for up to one week and is quite normal. The dressing which is put over the site after removal can usually be taken off within a couple of hours.

Will I get any refreshment whilst I attend the study visits?

Yes, we will provide breakfast and lunch for your second and third visit. At your first visit we will provide breakfast on arrival.

What are the possible benefits of taking part?

We cannot guarantee any direct benefits of taking part in this study, however in addition to helping advance medical and scientific knowledge, we believe you may benefit by having information made available to you, should you wish. For instance, we will be able to inform you of; the levels of fat in your bloodstream; your body fat percentage; the number of calories you expend each day and how much you could increase this amount if you were to reduce your sitting habits. Wearing gold standard physical activity monitors throughout this study will also allow researchers to quantify your sitting habits. This information might be of personal interest and help you to make decisions about your health. This study will also contribute to the ongoing work aimed at the prevention and management of Type 2 diabetes.

What are the risks of taking part?

Taking part involves minimal risk for you, just the inconvenience of taking the time to participate in the study. A small amount of bruising from having bloods taken is possible but will be minimal.

The tests in the study are not designed for clinical diagnosis, but in the unlikely event that we find an abnormality with the blood results, for instance a HbA1c (blood sugar) level above 6.5% (indicative of diabetes), this will be discussed directly with you and you will be advised to see your GP as soon as possible. Providing you have given consent for us to do so, we shall also send a copy of results to your GP.

What if something goes wrong?

It is very unlikely that you would be harmed by taking part in this type of research study. However, if you wish to complain or have any concerns about the way you have been approached or treated in connection with the study, you should ask to speak to Dr. Thomas Yates on **0116 258 7453** who will do his best to answer your questions. If you remain unhappy and wish to address your concerns or complaints on a formal basis, you should contact Patient Information & Liaison Service at pils.complaints.compliments@uhl-tr.nhs.uk. The Firs, c/o Glenfield Hospital, Groby Road, Leicester. LE3 9QP Freephone: 0808 1788337. Your legal rights to claim compensation for injury where you can prove negligence are not affected.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Data will be stored either in locked filing cabinets or in password protected databases which are only accessible by members of the research team. Any information that is shared will have your name and address removed so that you cannot be recognised from it. Information collected will not be used for any other purpose than that explained here. Study data and procedures may also be looked at by authorized people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant.

What will happen to the results of the research study?

The results of the study may be published in a professional journal, but you will not be identified by name in any publications. You will be informed about the overall results of this study when it has finished.

Who is organising and funding the research?

This study is being organised and co-ordinated by the Diabetes Research Centre, University of Leicester. It is predominantly funded by the Leicester-Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee (REC) to protect your safety, rights, wellbeing and dignity. This study has been given a favourable opinion by the Research Ethics Committee East Midlands – Leicester South. This study has also been reviewed by the University of Leicester who is sponsoring this study.

Will I get study and travelling expenses?

Travelling expenses (in the region of £10 per visit) can be reimbursed. The reimbursement application is simple and can be filled out during study visits. We are also able to issue a ticket so that you do not have to pay to park your car at the Leicester General Hospital.

What do I do if I decide to volunteer?

We are pleased that you are considering taking part in our research study. For the next step please fill in the pre-screening questionnaire attached to this e-mail and simply send it back to us on the e-mail address below. If it has already been established that you do not have access to e-mail and you have received this participant information sheet by post, please fill in the questionnaire by hand and return them to us in the pre-paid envelope attached to this paper copy. A member of the study team will then contact you. We look forward to welcoming you for your first visit.

What do I do if I want to withdraw from the study?

All participants are free to withdraw from the study at any time without needing to give a reason. If the participant loses capacity to participate during the study, they will be withdrawn and only data collected up to that point will be retained and used. The study investigator may also withdraw a participant if it is deemed necessary to do so, for example for non-compliance with study procedures.

Contact for further information

In the meantime, the researchers involved in this study will be pleased to discuss any questions or concerns that you may have. If you have any further questions about this research please e-mail the team at mm636@le.ac.uk or call us on 07740049606, (if unavailable, leave a message and we will get back to you as soon as possible).

Thank you for taking the time to read this participant information sheet

Pre-screening Questionnaire

ARMING your HEALTH

Pre-screening Questionnaire

(Please cross (☒) the options that apply to you by clicking the relevant boxes).

Participant ID No:
Address:

I have read the information sheet provided and would like to be considered to take part in the 'Arming your Health' study. I understand that I will have to sign a consent form at visit one if I am eligible to take part.

Date:

Date of Birth:

Please complete the section below if you would like to take part in the 'Arming your Health' study.

Do you have diabetes? ☐ Yes ☐ No ☐ Not sure

Have you had a stroke? ☐ Yes ☐ No

Are you a smoker? ☐ Yes ☐ No

Have you ever had Cardio-vascular disease?
(Disease of the heart or blood vessels) ☐ Yes ☐ No ☐ Not sure

Do you have Chronic Obstructive Pulmonary Disease?
(For example chronic bronchitis or emphysema). ☐ Yes ☐ No ☐ Not sure

Do you suffer from claustrophobia to an extent that it may prevent you from wearing the mask outlined in the participant information sheet? ☐ Yes ☐ No

Do you have any food allergies or requirements? ☐ Yes ☐ No
If so, please state here:

Has your doctor ever told you that you have heart trouble? ☐ Yes ☐ No

Do you have any injuries that may prevent you being able to walk? ☐ Yes ☐ No
If YES, give details below:

List any medication you are currently taking?

If you are female, what is your menstrual status?
Post-menopausal ☐ Or Pre-menopausal ☐ N/A ☐

Your approximate height: and weight:

Please include your telephone number and e-mail address so we can contact you
Tel: ☎ **E-mail:**
Please call me: ☐ Morning (9am-12pm) ☐ Afternoon (12pm-5pm) ☐ Evening (5pm-7pm)

Informed Consent Form

NHS
National Institute for Health Research

ARMING your HEALTH

The 'Arming your Health' study

Study ID ☐

CONSENT FORM: Version 3 - 19/01/2016

Title of project: ***The 'Arming your Health' study*: Investigating whether breaking up sedentary behaviour with seated upper body contractile activity can regulate an individual's metabolic health.**

Chief Investigator: Dr Thomas Yates

Please Initial Every Box

1) I confirm that I have read and understand the 'Arming your Health' participant information sheet, version 3.0, dated 19/01/2016. I have had the opportunity to ask questions and have had them answered satisfactorily. ☐

2) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without any future care or legal rights being affected. ☐

3) I understand that relevant sections of my medical notes and/or study data may be looked at by responsible individuals from the study team, the sponsor, the NHS trust or from regulatory authorities where it is relevant to my taking part in the research. I give permission for these individuals to access my records. ☐

4) I agree to being contacted with details of future research and for my details to be stored on the University of Leicester database. ☐

☐ Yes ☐ No

5) I agree to my GP being informed of my participation in the study ☐

☐ Yes ☐ No

6) I agree to take part in the above study. ☐

Name of participant	Date	Signature
Researcher taking consent	Date	Signature

University Hospitals of Leicester NHS

Individual Results Letter Template

Leicester-Loughborough
Diet, Lifestyle and Physical Activity
Biomedical Research Unit

NHS
National Institute for
Health Research

Leicester Diabetes Centre
Leicester General Hospital,
Gwendolen Road,
Leicester,
LE5 4PW

Tel : 0116 258 XXXX
Email: your.name@uhl-tr.nhs.uk
Web: www.ll.dlpa.bru.nihr.ac.uk

Name
Address

XX/XX/XXXX

Dear ***Forename Surname***,

Thank you for attending the 'Arming your Health' study on XX/XX/XXXX. Your results alongside the normal ranges are listed below:

Results from the 'Arming your Health' study
Appointment Date: XX/XX/XXXX
Height: ***X.XX***m
Weight: ***XX.XX***kg
Body Mass Index: ***XX.X***kg/m^2
Blood pressure: **XXX/XXmmHg**
Cholesterol: **X.Xmmol/L**
HbA1c: **X.X %**

Desirable Values	
Body Mass Index:	**Below 25kg/m^2** **Below 23kg/m^2 if South Asian**
Blood Pressure:	**Below 150/90mm/Hg**
Cholesterol:	**Below 5mmol/L**
HbA1c:	**Below 6.0%**

Diabetes diagnosis: HbA1c of 6.5% or above

Pre-diabetes diagnosis: HbA1c between 6.0 and 6.4%

The results from your HbA1c test indicate that you do not have Diabetes and fall within the desired range of below 6.0% (X.X %).

If we have not done so already, a member of our research team will be in contact with you to explain what happens next. Alternatively, please call us on ***07740049606*** if you would like to speak to someone about your results.

Yours sincerely

Dr/Professor .....
(This will be a named medic on the 'Arming your Health' delegation log).

GP Results Letter Template

Leicester-Loughborough
Diet, Lifestyle and Physical Activity
Biomedical Research Unit

NHS
National Institute for
Health Research

GP Name
GP Address

Dear Dr

The following patient has enrolled in a research study called 'Arming your Health' at the Leicester Diabetes Centre (based in the Leicester General Hospital). This study administered a voluntary HbA1c test on XX/XX/XXXX. The results of this HbA1c test alongside general health screen outcomes are listed below:

Patient ID: **XX**
Patient: **XX**
Date of Birth: **XX/XX/XXXX**

Height: **X.XXm** Weight: **XX.XXkg** BMI: **XX.Xkg/m2**

Waist Circumference: **XXXcm** Blood Pressure: **XXX/XXmmHg**

Blood Results:

HbA1c Result	**X.X %**
Total Cholesterol Result	**X.Xmmol/L**
LDL Cholesterol Result	**X.Xmmol/L**
HDL Cholesterol Result	**X.Xmmol/L**
Triglycerides Result	**X.XXmmol/L**
White Cell Count	**X.X/L**
Red Blood Count	**X.X/L**
Haemoglobin	**X.Xg/L**
Haemocrit	**X.XL/L**
Platelet Count	**X.X/L**
Neutrophil Count	**X.X/L**

The results from this HbA1c test indicate that this patient does not currently have Diabetes and falls within the desired range of less than 6.0%. If you feel you need any further information from us please do not hesitate to contact us on **07740049606**.

Yours sincerely

Dr/Professor.....
[This will be a named medic on the 'Arming your Health' delegation log).

Appendix Five - Publications related to work in this thesis

Peer reviewed publication stemming from Chapter Two: (*full text article shown below*).

- McCarthy, M., Edwardson, C. L., Davies, M. J., Henson, J., Bodicoat, D. H., Khunti, K., Dunstan, DW., James, JA. and Yates, T., 2017. Fitness Moderates Glycemic Responses to Sitting and Light Activity Breaks. ***Medicine and Science in Sports and Exercise***. DOI: 10.1249/MSS.0000000000001338.

Peer reviewed publication stemming from Chapter Three: (*full text article shown below*).

- McCarthy, M., Edwardson, C.L., Davies, M.J., Henson, J., Rowlands, A., King, J., Bodicoat, D.H., Khunti, K. and Yates, T., 2017. Breaking up sedentary time with seated upper body activity can regulate metabolic health in obese high risk adults: A randomised crossover trial. ***Diabetes, Obesity and Metabolism***. DOI: 10.1111/DOM.13016.

Peer reviewed publication stemming from Chapter Four: (*full text article shown below*).

McCarthy, M., Edwardson, C.L., Davies, M.J., Henson, J., Gray, L., Khunti, K. and Yates, T., 2017. Change in Sedentary Time, Physical Activity, Body weight, and HbA1c in High-Risk Adults. ***Medicine and Science in Sports and Exercise***, *49*, 1120-1125.

Fitness Moderates Glycemic Responses to Sitting and Light Activity Breaks

MATTHEW MCCARTHY[1,2,3], CHARLOTTE L. EDWARDSON[1,2], MELANIE J. DAVIES[1,2], JOESPH HENSON[1,2], DANIELLE H. BODICOAT[1,2,4], KAMLESH KHUNTI[1,4], DAVID W. DUNSTAN[5,6], JAMES A. KING[2], and THOMAS YATES[1,2]

[1]Diabetes Research Centre, University of Leicester, Leicester Diabetes Centre, Leicester General Hospital, Leicester, Leicestershire, UNITED KINGDOM; [2]National Institute for Health Research (NIHR) Leicester Biomedical Research Centre (BRC), Leicester Diabetes Centre, UNITED KINGDOM; [3]Health Sciences, University of Leicester, Leicester, UNITED KINGDOM; [4]National Institute for Health Research (NIHR) Collaboration for Leadership in Applied Health Research and Care-East Midlands (CLAHRC-EM) Leicester Diabetes Centre, Leicester, UNITED KINGDOM; [5]Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, AUSTRALIA; and [6]Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Victoria, AUSTRALIA

ABSTRACT

MCCARTHY, M., C. L. EDWARDSON, M. J. DAVIES, J. HENSON, D. H. BODICOAT, K. KHUNTI, D. W. DUNSTAN, J. A. KING, and T. YATES. Fitness Moderates Glycemic Responses to Sitting and Light Activity Breaks. *Med. Sci. Sports Exerc.*, Vol. 49, No. 11, pp. 00–00, 2017. **Purpose**: This study aimed to experimentally determine whether cardiorespiratory fitness (CRF) modifies postprandial glycemia during prolonged sitting and investigated the potentially blunting influence this may have on the benefits of interrupting postprandial sitting time with light activity breaks. **Methods**: Thirty-four adults (18 women; 16 men; mean ± SD age, 40 ± 9 yr, body mass index, 24.5 ± 3 $kg \cdot m^{-2}$) undertook two 7.5-h experimental conditions in a randomized order: 1) Prolonged sitting; 2) Sitting interspersed with 5 min light walking bouts every 30 min. Blood samples were obtained while fasting and postprandially after ingestion of two identical meals. Incremental area under the curve (iAUC) was calculated for glucose and insulin throughout experimental conditions. Maximal exercise testing quantified peak oxygen consumption ($\dot{V}O_2$ peak) as a measure of CRF. A repeated-measures ANOVA investigated whether $\dot{V}O_2$ peak modified glucose and insulin iAUC between conditions. **Results**: Breaking sedentary time with light walking breaks reduced blood glucose iAUC from 3.89 ± 0.7 to 2.51 ± 0.7 $mmol \cdot L^{-1} \cdot h^{-1}$ ($P = 0.015$) and insulin iAUC from 241 ± 46 to 156 ± 24 $mU \cdot L^{-1} \cdot h^{-1}$ ($P = 0.013$) after adjustment for $\dot{V}O_2$ peak and sex. A significant interaction between treatment response and $\dot{V}O_2$ peak was observed for glucose ($P = 0.035$), but not insulin ($P = 0.062$), whereby the treatment effect reduced with higher CRF. Average blood glucose iAUC responses for a man at the 25th centile of CRF within our cohort (42.5 $mL \cdot kg^{-1} \cdot min^{-1}$) went from 5.80 to 2.98 $mmol \cdot L^{-1} \cdot h^{-1}$ during the prolonged sitting and light walking break conditions respectively, whereas average responses for a man at the 75th centile of CRF (60.5 $mL \cdot kg^{-1} \cdot min^{-1}$) went from 1.99 to 1.78 $mmol \cdot L^{-1} \cdot h^{-1}$. Similar trends were observed for women. **Conclusions**: Individuals with low CRF gained the most metabolic benefit from breaking prolonged sitting with regular bouts of light walking. **Key Words:** SEDENTARY BEHAVIOR, TYPE 2 DIABETES, PHYSICAL ACTIVITY, CARDIORESPIRATORY FITNESS, POSTPRANDIAL METABOLISM

Adults in developed western countries typically spend 50% to 70% of their waking hours sat down (30), making sedentary behavior the new reference of modern living. Greater time spent in sedentary behaviors (defined as sitting or reclining with low energy expenditure) has been associated with an increased likelihood of metabolic syndrome (10), diabetes, cardiovascular disease (CVD), and all-cause mortality (4,27). The evidence of which appears to be the strongest and most consistent for the risk of type 2 diabetes mellitus (T2DM) (4).

However, recent epidemiological evidence has suggested that physical activity levels and cardiorespiratory fitness (CRF) may moderate these associations, such that the association between sedentary time and markers or outcomes of health may be weaker in those with higher fitness levels (7,23,25), or those undertaking greater physical activity (11). This suggests that sedentary behavior may be a less important determinant of health in those with adequate CRF or those that are physically active. Although experimental evidence largely confirms that breaking prolonged bouts of sitting with light-intensity walking can significantly reduce postprandial blood glucose and insulin in healthy non-obese individuals (2,24), in those who are overweight and obese (9,26), and in those with

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dysglycemia (15), no previous experimental trials have investigated whether these responses are modified by CRF or habitual physical activity levels.

CRF in particular is an important candidate for further investigation, because it is one of the strongest predictors of morbidity and mortality (19). Cardiorespiratory fitness has been shown to moderate the deleterious impacts of other exposures, such as body mass index (BMI), whereby obese individuals with moderate to high CRF levels have a lower risk of morbidity and mortality outcomes compared with normal weighted individuals with low CRF levels (12). It is therefore plausible that high levels of CRF may also protect against the deleterious impacts of prolonged sedentary behavior. Therefore, we hypothesized that CRF would modify the postprandial glucose response to breaking prolonged sitting with light walking breaks with lower CRF levels being associated with greater reductions to postprandial plasma glucose.

METHODS

Study design. All participants attended the Leicester Diabetes Centre on three separate occasions between September 2014 and September 2015. The first visit involved consent, familiarization and a fitness assessment which was followed by two experimental condition visits that were at least 7 d apart. This was a randomized crossover trial, whereby each participant took part in two experimental treatment conditions in a random order, thereby acting as their own controls. Order randomization was conducted by a statistician using an online tool. Due to the nature of the trial, participants were not blinded to their randomized order; however, all outcomes including blood assays were analyzed blinded to the experimental condition that they derived from. Before commencing, this study received ethical approval from the University of Leicester-Health Sciences Department and from the local NHS Research and Development Committee.

This trial was also registered with ClinicalTrials.gov (NCT0493309).

Participants. Thirty-six nonobese adults (BMI, $<30\ kg \cdot m^{-2}$) age between 25 and 55 yr who worked in a predominantly seated environment were recruited from the general public via study-specific information distributed in the community, around the University of Leicester campus and University Hospitals of Leicester NHS Trust. Two individuals were withdrawn following enrolment in the study due to a change in personal circumstances ($n = 2$). This left 34 participants who went on to complete the remaining experimental conditions. This is detailed in Figure 1.

Exclusion from taking part in this study came under the following circumstances: an inability to communicate in spoken English, a BMI $\geq 30\ kg \cdot m^{-2}$, pregnancy, steroid usage, regular smoking habits, diagnosed T2DM, CVD, or psychotic illness. As our study was predicated on having a broad range of fitness levels, and considering that most of the variance in CRF is explained by habitual physical activity levels (6), we stratified recruitment by self-reported leisure time physical activity. Consequently, we enrolled 12 inactive (0 min of MVPA per week), 12 moderately active (≥75 min to <150 min of MVPA per week), and 12 highly active (≥150 min of MVPA per week) individuals (see Table S-1, Supplemental Digital Content 1, Scope of CRF levels captured, http://links.lww.com/MSS/A960).

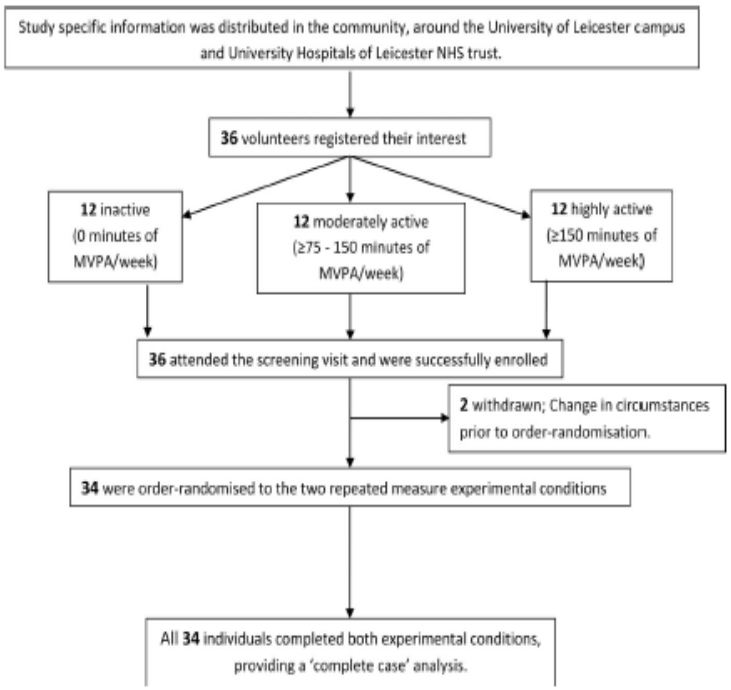

FIGURE 1—Trial CONSORT Profile.

Consent, familiarization, and fitness assessment visit. On arrival, a researcher described in detail all study procedures and written informed consent was obtained. Participants were then shown the designated experimental area for the study.

A venous blood sample was taken to assess HbA1c and confirm absence of T2DM (<6.5% [<47.5 mmol·mol^{-1}]) (29). Body weight (Tanita TBE 611; Tanita, West Drayton, UK), waist circumference (midpoint between lower costal margin and iliac crest), and height were measured to the nearest 0.1 kg, 0.5 cm, and 0.5 cm, respectively.

To assess CRF, participants undertook a maximal incremental exercise test on a motor driven treadmill (Technogym Excite® 700). Following a 3-min warm-up at 4 km·h^{-1} (0% incline), participants would walk or jog at a constant speed that they felt comfortable with (6, 8, 10, or 12 km·h^{-1}) while elevations in treadmill gradient occurred at a rate of 0.5% every 30 s. All participants received encouragement to continue this exercise for as long as possible. The test was terminated upon volitional exhaustion. Throughout the test, gas was sampled continuously and analyzed using a Metalyser 3B gas analyser (Cortex 3B; Cortex Biophysik, Leipzig, Germany). Peak oxygen consumption ($\dot{V}O_2$ peak) was calculated using the highest 10-s average throughout the testing period. Before each test, the gas analyser was calibrated according to the manufacturer's recommendations. As a safety precaution, a 12-lead electrocardiogram was performed by a cardiac nurse for each participant at rest and during the exercise test.

Finally, participants were issued with two activity monitors; an ActiGraph GT3X+ accelerometer (Pensacola, FL) worn on the right anterior axillary line, and an activPAL3 physical activity monitor (PAL Technologies, Glasgow, UK) worn on the midline anterior portion of the right thigh. Participants were required to wear these for seven consecutive days, allowing insight into their habitual sitting and physical activity levels.

Experimental procedure. Participants were asked to avoid alcohol and caffeine for the 48 h preceding experimental treatment conditions. Because the influence of an acute bout of physical activity on insulin sensitivity can persist for 48 h (17), avoidance of moderate and vigorous physical activity for this timeframe was also instructed. Continuation in this study was subject to participants being able to confirm their compliance with these restrictions. After an ethical amendment to the protocol during this study, a subset of participants was asked to wear an accelerometer in the 2 d leading up to each experimental condition to confirm adherence to the exercise restriction (see Table S-2, Supplemental Digital Content 2, Activity data leading up to experimental conditions, http://links.lww.com/MSS/A961).

Participants fasted from 10:00 PM, the evening before each visit, and were asked to keep a record of all food eaten during the day leading up to their first experimental condition. This could then be replicated before their second experimental condition in an attempt to eliminate the potentially confounding influence of preexperimental food intake.

Participants underwent two separate 7.5-h experimental treatment conditions:

1. Prolonged sitting—participants sat in a designated room (occupied with a desk, books, and laptop with internet services) while minimizing excessive movement. Lavatory breaks were permitted using a wheelchair to and from the lavatory to further reduce unnecessary movements that could otherwise confound the study.
2. Light walking breaks—participants emulated the above, but interrupted sitting time with 5-min bouts of walking at a light intensity of 3 km·h^{-1} on the treadmill (Technogym Excite® 700) every 30 min. These bouts were performed 12 times, totalling 1 h of activity and 6.5 h of sitting throughout the course of the experimental day.

On arrival, participants had a cannula fitted into an accessible vein from which 10-mL samples were obtained throughout the day. Immediately following the two fasting samples (depicted at timepoints −1 and 0 in Fig. 2), participants were given a standardized meal consisting of 8 kcal·kg^{-1} of body weight, with a macronutrient composition reflective of coingestion in modern western diets (14% protein, 51% carbohydrate, and 35% fat). Once consumed (within ≤15 min), blood sampling commenced at 30, 60, 120, and 180 min thereafter, enabling us to capture the postprandial period. An identical meal was

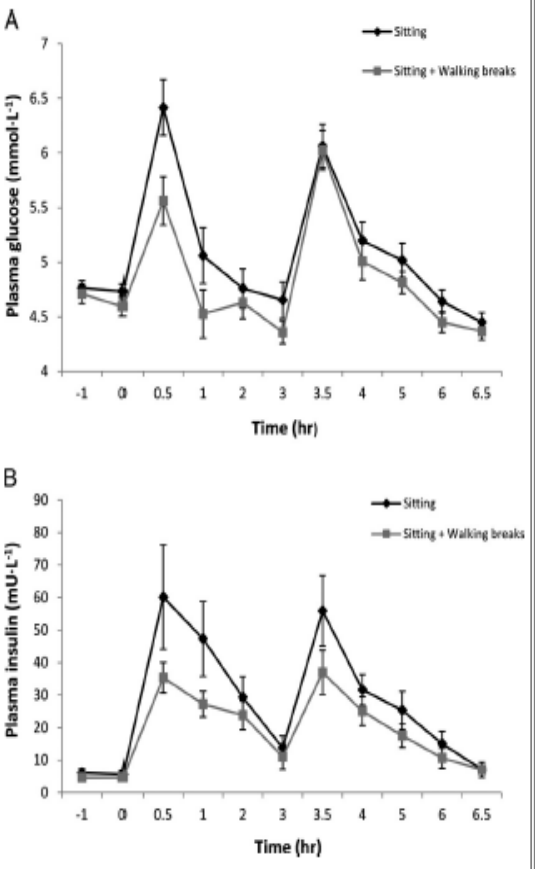

FIGURE 2—Effect of treatment condition on average blood glucose (A) and Insulin (B).

then issued (time point 3 in Fig. 2) and sampling continued in a similar fashion at 30, 60, 120, 180, and 210 min after this. Participants were supervised by study staff to ensure compliance with the protocol and were asked to wear an activPAL monitor to objectively confirm sitting and walking times during each experimental condition (see Table S-3, Supplemental Digital Content 3, Sitting and walking data during experimental conditions, http://links.lww.com/MSS/A962). *Ad libitum* water consumption was also noted and made consistent between conditions.

Biochemical analysis. Glucose was analysed on the day of collection by the University Hospitals of Leicester Pathology Department, using standard enzymatic techniques with commercially available kits (Beckman, High Wycombe, UK).

Centrifuged (4°C) plasma samples were stored in −80°C freezers and insulin was analysed from these collectively at the end of the trial using an electrochemiluminescence assay (Meso Scale Discovery, Maryland, USA). Each sample was ran in duplicate to ensure reliability of readings. Duplicate sample values with ≥20% variability were reanalyzed. Ambient conditions of the laboratory were kept consistent to reduce variability between assays.

Free-living activity monitor processing. ActivPAL data were downloaded using the manufacturers software (activPAL Professional Research Edition, PAL technologies, Glasgow, UK) and "Event" csv files were processed using a validated automated algorithm in STATA (StataCorp LP, College Station, TX) described in detail elsewhere (28).

Actigraph data (100 Hz) were downloaded using the manufacturer's software (ActiLife version 6.10.4, Lite Edition), reintegrated into 60-s epoch files and processed using a bespoke tool (KineSoft, version 3.3.76; KineSoft, New Brunswick, Canada [www.kinesoft.org]). Freedson cut points were used to categorize activity intensities (13). Nonwear time was defined as a minimum of 60 min of continuous zero counts, and when assessing habitual activity levels, days with at least 10 h of wear time were required to be considered valid.

The minimum amount of valid days utilised for both ActivPAL and ActiGraph data was 3 d.

Statistical analysis. Descriptive characteristics of those who completed this study are summarized overall (n = 34) and stratified by sex (Table 1) for descriptive purposes.

Missing glucose and insulin data during the experimental conditions accounted for roughly 2% of overall required samples (34 of 1496) (see Table S-4, Supplemental Digital Content 4, Summary of missing glucose and insulin data, http://links.lww.com/MSS/A963). These 34 missing data points were imputed using a regression model previously developed for an acute trial investigating breaking sedentary behavior (15). This approach uses key predictors (BMI, ethnicity, age, fasting values, and treatment condition) to derive a regression equation for the glucose and insulin values at each individual time point, this regression equation is then used to impute missing values.

The incremental area under the curve (iAUC) of glucose and insulin was calculated for each experimental condition. Total AUC was calculated by applying the trapezium rule and further subtraction of fasting levels gave a single value of iAUC for each participant. Using iAUC as opposed to total AUC is common practice in acute interventions where fasting levels should be unaffected by the intervention (20). Glucose iAUC was defined *a priori* as the primary outcome.

The effect of light walking breaks compared with continuous sitting on outcomes (glucose and insulin iAUC) and whether CRF modified this response was assessed using a repeated-measures ANOVA. Treatment was entered as a within-person variable, with CRF (as a continuous variable) entered as a between-subjects covariate. Sex was also entered as a between-subjects factor. "Treatment by CRF" and "treatment by sex" interaction terms were investigated to assess the modifying effect of fitness and sex respectively. Sex was included in the model given that it is a strong determinant of fitness and an important potential confounder. Treatment by CRF interactions were further explored by calculating the linear regression coefficients within each treatment condition. To highlight the direction of significant interactions, derived average glucose iAUC values for men and women at the 25th, 50th, and 75th centiles of the CRF distribution are shown in Figure 3.

Two-tailed $P \leq 0.05$ was considered significant. Analyses were performed with SPSS (version 24). Results are presented as

TABLE 1. Metabolic, demographic, and anthropometric characteristics taken at baseline.

Baseline Characteristics	Overall (n = 34)	Male (n = 16)	Female (n = 18)
Age (yr)	41 (15)	35 (17)	43 (13)
BMI (kg·m^{-2})	23.8 (6.1)	25.9 (5.1)	22.7 (4.6)
Body weight (kg)	66.5 (23.5)	82.2 (19.6)	59.9 (9.0)
Waist circumference (cm)	78.5 (13)	83 (12.8)	75 (10.0)
HbA1c (%)	5.3 (0.3)	5.3 (0.3)	5.3 (0.4)
HbA1c (mmol·mol^{-1})	34 (3)	34 (3)	34 (3)
Total cholesterol (mmol·L^{-1})	5 (1.2)	5.1 (1.2)	5.0 (1.2)
Ethnicity			
White European	26 (76.5)	13 (81)	13 (72)
Black and minority ethnic	8 (23.5)	3 (19)	5 (28)
Accelerometer variables			
Habitual sedentary time (average minutes per day)	564 (92)	547 (164)	595 (126)
Habitual MVPA time (average minutes per day)	35 (28)	43 (47)	32 (21)
Accelerometer wear time (average minutes per day)	892 (117)	899 (118)	890 (97)
Habitual step counts (average per day)	8048 (4551)	9048 (7030)	7451 (3941)
Fitness test			
$\dot{V}O_2$ max (mL·kg^{-1}·min^{-1})	41.3 (18.9)	50.3 (19.6)	34.0 (7.9)

Data are presented as median (interquartile range) or n [%].

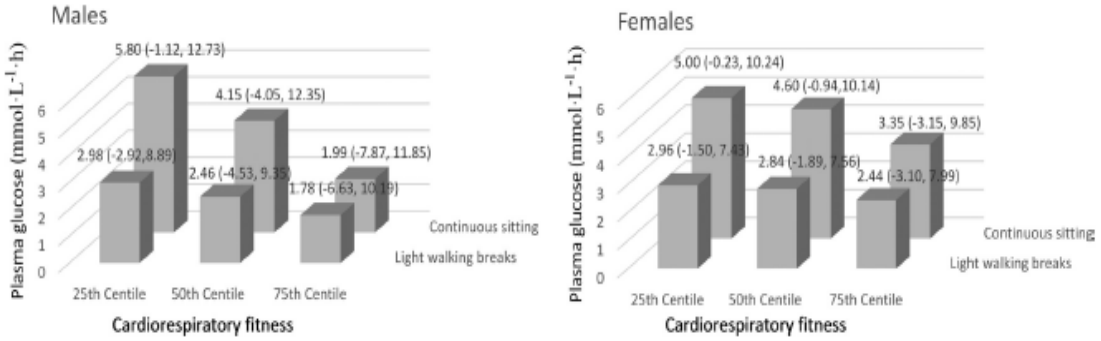

FIGURE 3—Predicted glucose values (with 95% CI) at sex-specific centiles of CRF 25th centile of CRF corresponds to 42.5 mL·kg^{-1}·min^{-1} for men, and 32.1 mL·kg^{-1}·min^{-1} for women. 50th centile of CRF corresponds to 50.3 mL·kg^{-1}·min^{-1} for men, and 34.0 mL·kg^{-1}·min^{-1} for women. 75th centile of CRF corresponds to 60.5 mL·kg^{-1}·min^{-1} for men, and 39.9 mL·kg^{-1}·min^{-1} for women. Predicted glucose iAUC values were derived from the below equations gained from linear regression models entering glucose iAUC within each condition as the dependant variable with CRF and sex entered as independent variables. 95% CI values show the variability around the derived estimates; negative values represent postprandial glucose concentrations that are suppressed below fasting levels. The derived glucose iAUC values and 95% CI are within the range observed in this study (minimum observed glucose iAUC = −9.73 mmol·L^{-1}·h^{-1}, maximum observed glucose iAUC = 16.50 mmol·L^{-1}·h^{-1}) Glucose iAUC during prolonged sitting condition = 11.81 − (0.21; 95% CI, 0.05–0.38) × CRF + 3.00 if men. Glucose iAUC during walking breaks condition = 5.12 + (−0.07; 95% CI −0.21 to 0.08) × CRF + 0.72 if men.

mean ± SE or regression coefficient (95% confidence interval [CI]) unless stated otherwise.

RESULTS

The key characteristics of those who successfully completed all three study visits are displayed in Table 1 (n = 34). Stratification of these characteristics for both men and women is also presented here.

Overall treatment condition effect. The average postprandial concentrations of glucose (A) and insulin (B) witnessed throughout the 7.5-h testing periods for both experimental conditions ("prolonged sitting" and "light walking breaks") are depicted in Figure 2. There was a significant main effect of treatment for both glucose (F (1, 31) = 6.67, P = 0.015) and insulin (F (1, 31) = 7.00, P = 0.013) iAUC after adjustment for fitness and sex. Interrupting prolonged sitting time with light walking breaks reduced blood glucose iAUC by 35% (from 3.89 ± 0.7 mmol·L^{-1}·h^{-1} to 2.51 ± 0.7 mmol·L^{-1}·h^{-1}) and insulin iAUC by 35% (from 241 ± 46 mU·L^{-1}·h^{-1} to 156 ± 24 mU·L^{-1}·h^{-1}).

Impact of CRF and sex. There was a significant treatment by CRF interaction for glucose iAUC (F (1, 31) = 4.89, P = 0.035). The treatment by CRF interaction for insulin iAUC failed to reach significance (F (1, 31) = 3.76, P = 0.062). There was no treatment by sex interaction for glucose (F (1, 31) = 1.77, P = 0.194) or insulin (F (1, 31) = 1.54, P = 0.223) iAUC.

Stratified analysis revealed that each unit increment in CRF (per mL·kg^{-1}·min^{-1}) was associated with a lower glucose iAUC (−0.21 mmol·L^{-1}·h^{-1}; 95% CI −0.38 to −0.05) (P = 0.013) in the prolonged sitting condition, whereas there was no association between CRF and glucose iAUC during the light walking breaks condition (−0.07 mmol·L^{-1}·h^{-1}; 95% CI, −0.21 to 0.07) (P = 0.335). In contrast, each unit increment in CRF was associated with a lower insulin iAUC (−10.93 mU·L^{-1}·h^{-1}; 95% CI, −19.48 to −2.37) (P = 0.014) in the prolonged sitting condition and a lower insulin iAUC (−6.35 mU·L^{-1}·h^{-1}; 95% CI, −10.90 to −1.83) (P = 0.007) in the light walking breaks condition.

Figure 3 uses the derived regression coefficients to show how the predicted average difference between conditions for glucose iAUC changes as CRF increases for men and women. This demonstrates that average blood glucose iAUC response for a man at the 25th centile of CRF within our cohort went from 5.80 to 2.98 mmol·L^{-1}·h^{-1} (from prolonged sitting to light walking breaks, respectively), whereas average responses for a man at the 75th centile went from 1.99 to 1.78 mmol·L^{-1}·h^{-1}. Similar trends were observed for women.

DISCUSSION

This study found that interrupting prolonged sitting with regular light walking breaks reduced postprandial glucose and insulin levels in a healthy cohort. However, CRF modified the response for glucose such that individuals with lower levels of fitness received incrementally greater reductions in postprandial glucose. For example, the average response for a man at the 25th centile of CRF within our population ($\dot{V}O_2$ peak of 42.5 mL·kg^{-1}·min^{-1}) demonstrated relatively high postprandial glucose levels during prolonged sitting (5.80 mmol·L^{-1}·h^{-1}) but was able to almost half this level through using regular light walking breaks. In contrast, the average response for a man at the 75th centile of fitness ($\dot{V}O_2$ peak of 60.5 mL·kg^{-1}·min^{-1}) demonstrated relatively low levels of postprandial glucose during prolonged sitting (1.99 mmol·L^{-1}·h^{-1}) but only reduced this by a further 11% through using regular light walking breaks. The same pattern was demonstrated for women. These results were supported by further analysis which demonstrated that CRF was inversely associated with postprandial

glucose during prolonged sitting, whereby every unit increment in $\dot{V}O_2$ peak (per $mL \cdot kg^{-1} \cdot min^{-1}$) was associated with an average reduction of 0.21 $mmol \cdot L^{-1} \cdot h^{-1}$ in glucose iAUC values. Taken together, our results suggest that having high CRF or using regular light walking breaks in those with low CRF can both reduce postprandial levels of glucose during periods of prolonged sitting activity. Elevated postprandial glucose levels are implicated with the development of T2DM and CVD (5) and therefore strategies to promote healthy glycemic responses when sedentary are of high importance.

Our observation that those with higher CRF demonstrate less metabolic benefit from light activity breaks is consistent with previous experimental research that has tended to show relatively lower metabolic benefits of light activity breaks in healthy cohorts (1,22) compared with both those with high risk of chronic disease (9,15). Our findings also correspond to cross sectional research that has shown the influence of sedentary time on a cluster of cardiometabolic issues to be significantly less pertinent in those with higher fitness levels (7,23,25). The concept that fitter individuals may gain less pronounced health benefits from lower levels of sitting time is supported by cross-sectional research that have stratified data by habitual MVPA level, finding that individuals with higher MVPA levels display significantly weaker associations between sedentary time with HbA1c (3), inflammation markers (16), and all-cause mortality (11).

In contrast, a recent meta-analysis found that the association between sedentary time and health outcomes persisted in sufficiently active individuals (4). However, this pooled analysis was predominantly derived from self-reported measures of sedentary time and MVPA which are prone to bias and consequently may have been insensitive to detecting true interactions. It should also be noted that although observational research linking sedentary behavior to health is plentiful, the vast majority have investigated the confounding rather than the modifying influence of physical activity (4,27) or fitness (25).

The growing observational and experimental data has supported new guidance and recommendation calling for reductions in sitting time (18). However, if the findings of the current study continue to be supported by further research, there may be reasonable grounds to embark on a more personalized/tailored approach to T2DM prevention. Precision medicine is important given that a one size fits all recommendation is rarely effective. For example, interventions to reduce sitting time may be optimized by targeting those with poor CRF, whereas those with high CRF may be better served by interventions aimed at maintaining CRF and physical activity levels across the lifespan. However, it should be noted that median levels of CRF within our population for men and women were 50.3 and 34.0 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively, and that the average reductions in postprandial glucose at this level of CRF was 41%. As the majority of the general population within the age range included in this study are estimated to fall below the median levels of fitness within our population (8), the importance of interrupting sitting time with light activity breaks is likely to remain generalizable to the majority of the population.

This research also suggests that increasing CRF levels may be a viable way to protect against the potential harms of prolonged sitting. Although there are genetic contributions to fitness, the largest contributor to an individual's fitness is their time spent in MVPA (6). Participation in regular MVPA outside of seated hours may therefore offer some protection, particularly in seated occupations such as driving.

Our observation that fitter individuals experienced less pronounced postprandial glycemic excursions during prolonged sitting may result from favorable physiological adaptations stemming from regular engagement in MVPA (one of the main determinants of fitness), such as increased skeletal muscle GLUT 4 protein expression (14). This would also leave less scope for further improvement, potentially explaining why the benefits of interrupting sitting time with light activity breaks appear to be blunted in those with higher fitness. However, given that CRF is determined by a mixture of both MVPA engagement and genetics (6), we cannot distinguish between behavioral and genetic mechanisms driving the results of the current study.

This study has some important limitations. Although this study provides an initial proof-of-concept from which future research can tailor to alternative study cohorts, findings should not be generalized outside the population investigated. In particular, given that the population utilised in this study were healthy, the extent to which CRF modifies responses in high risk or clinical population remains to be investigated. Our second limitation is that despite instructions to standardize food intake, and refrain from caffeine and alcohol consumption leading up to treatment conditions, we did not objectively test participant compliance and relied on self-reported adherence. In addition, fitness assessments were only conducted at one timepoint, thus direct causality cannot be inferred. Future interventions that actively set out to manipulate fitness levels and assess prospective change in experimental data are required to elucidate direct causality. Another concern was that those with higher fitness in this study were predominantly men and conversely, those with lower fitness were predominantly women. However, our results were adjusted for sex, and it was not found to modify the treatment effect for glucose which was in contrast to CRF. Therefore, the correlation between sex and CRF is unlikely to be confounding the results of this study.

In conclusion, participants with lower fitness had worse postprandial glucose and insulin responses during prolonged sitting, and were able to gain greater metabolic benefit through breaking their sitting time with light activities compared with individuals with higher fitness. Future interventions aimed at alleviating the deleterious metabolic impacts of sedentary behavior may therefore be optimized by tailoring to CRF levels of the general population.

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Aside from the information disclosed above, authors declare no conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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WILEY

ORIGINAL ARTICLE

Breaking up sedentary time with seated upper body activity can regulate metabolic health in obese high-risk adults: A randomized crossover trial

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Aims: To investigate the impact of performing short bouts of seated upper body activity on postprandial blood glucose and insulin levels during prolonged sitting.

Methods: Participants undertook two 7.5-hour experimental conditions in randomized order: (1) prolonged sitting only and (2) sitting, interspersed with 5 minutes of seated arm ergometry every 30 minutes. Blood samples were obtained while fasting and throughout the postprandial period after ingestion of two standardized meals. The incremental area under the curve (iAUC) was calculated for glucose and insulin throughout each experimental condition. A paired samples *t*-test was used to assess the difference in iAUC data between conditions for glucose (primary outcome) and insulin (secondary outcome).

Results: Thirteen obese adults (7 women, 6 men; mean ± standard deviation [s.d.] age: 66 ± 6 years; body mass index 33.8 ± 3.8 kg/m^2) completed this investigation. Compared with the prolonged sitting-only condition, the implementation of seated arm ergometry every 30 minutes significantly reduced mean blood glucose iAUC (from 7.4 mmol/L/h [95% confidence interval (CI) 5.2, 9.5] to 3.1 mmol/L/h [95% CI 1.3, 5.0]; P = .001). Significant reductions in mean insulin iAUC (from 696 mU/L/h [95% CI 359, 1032] to 554 mU/L/h [95% CI 298, 811]; P = .047) were also observed.

Conclusion: Performing short bouts of arm ergometry during prolonged sitting attenuated postprandial glycaemia despite maintaining a seated posture. This may have clinical significance for those with weight-bearing difficulty who may struggle with postural change.

KEYWORDS

exercise, glucose metabolism, glycaemic control, insulin resistance, randomised trial, type 2 diabetes

1 | INTRODUCTION

Greater time spent sedentary (defined as sitting or reclining with low energy expenditure [EE]),[1] is increasingly being recognized as an independent risk factor for morbidity (especially type 2 diabetes)[2-5] and mortality,[2,4-6] associations that persist after controlling for moderate-to-vigorous physical activity (MVPA) levels.[2-5] Associations between sedentary behaviour and health may be attenuated, however, when engaging in very high levels of physical activity (typically in the region of ≥60 min/d).[6]

Epidemiological findings have been strengthened by recent experimental evidence showing beneficial effects of interrupting prolonged sitting on markers of metabolic health, particularly postprandial glycaemia. For example, interrupting sitting time with regular bouts of light-intensity[7-10] and moderate-intensity[8,11,12] walking have been shown to be effective at reducing postprandial blood glucose levels in overweight and obese adults,[8] those with dysglycaemia,[7,12] those with diagnosed type 2 diabetes,[10] and healthy, normal-weight populations.[9,11] Breaking up prolonged

sitting time with standing[7,13,14] or light resistance activities[10] (while in a standing posture) have also proven to be effective.

Interrupting sitting time with upright (non-seated) activities therefore appears to be a viable way of attenuating postprandial glucose. Whether these improvements can be replicated by introducing upper body muscle activity while maintaining a seated posture is currently unknown. Addressing this question will help clarify whether it is the posture of sitting that is driving the association with poor health or whether it is the resulting generalized muscular inactivity. Importantly, investigating non-weight-bearing strategies for reducing sedentary behaviour will also have important clinical implications for individuals who have restricted mobility or find standing difficult. In addition, strategies for breaking sedentary behaviour that have been investigated to date not only overlook those with weight-bearing difficulty, but have also been criticized for being disruptive and non-conducive to the working day.[15] Given that seated strategies would not require vacating the desk area, this could present a more appealing option for sedentary workers.

The aim of the present study was to investigate whether performing short, frequent bouts of seated upper body activity (using similar EEs to light-intensity walking) can attenuate postprandial glycaemia.

2 | MATERIALS AND METHODS

2.1 | Trial design

Each participant attended the research centre on three separate occasions between May and August 2016. The first visit involved consent, familiarization and EE measurement. This was followed by two experimental condition visits that were at least 7 days apart. A randomized crossover design was used, whereby each participant took part in 2 experimental treatment conditions in a random order, thereby acting as their own controls. Order randomization was conducted by a statistician using an online tool. Because of the nature of the trial, participants were not blinded to their randomized order, but all outcomes, including blood assays, were analysed blinded to the experimental condition from which they derived. Before commencing the present study, we received ethical approval from the National Health Service (NHS) East Midlands - Leicester South Research Ethics Committee. This trial was registered with ClinicalTrials.gov (NCT02909894).

2.2 | Participants

Fourteen obese adults (body mass index [BMI] ≥30 kg/m^2) deemed to be inactive (failing to meet physical activity guidelines for MVPA, defined as 150 minutes per week of self-reported moderate-intensity physical activity or 75 minutes per week of self-reported vigorous-intensity physical activity)[16] and at high risk of type 2 diabetes according to the Leicester Practice Risk Score[17] were identified and recruited from a database.[18] The Leicester Practice Risk Score calculates risk of type 2 diabetes based on 6 variables (age, sex, ethnicity, BMI, family history of the disease and antihypertensive drug usage); all individuals eligible for the present study scored within the top 10% for risk within their family practice.

Exclusion criteria were as follows: an inability to communicate in spoken English; diagnosed type 2 diabetes; cardiovascular disease; psychotic illness; pregnancy; steroid usage; regular smoking habit; or an inability to walk without an assistive device.

One individual was withdrawn as a result of having a glycated haemoglobin (HbA1c) concentration indicative of type 2 diabetes. This left a total of 13 participants who went on to complete the trial. This process is shown in Figure 1.

2.3 | Consent, familiarization and EE assessment visit

On arrival, a researcher described in detail all study procedures and written informed consent was obtained.

As a part of the screening process, a venous blood sample was taken to assess HbA1c levels, and confirm absence of type 2 diabetes (HbA1c <6.5% [<47.5 mmol/mol]).[19] Body weight (Tanita TBE 611: Tanita, West Drayton, UK), waist circumference (midpoint between lower costal margin and iliac crest) and height were measured to the nearest 0.1 kg, 0.5 and 0.5 cm, respectively.

During this first visit, we also undertook arm ergometry EE testing. Specifically, we sought to identify the power output (watts) necessary to elicit the desired EE during the main experimental condition. To allow comparison of metabolic responses to arm ergometry with previous findings that have examined the impact of light walking (3 km/h),[7-10] we aimed to match participants' arm ergometry EE to their 3 km/h walking EE. To achieve this, EE was captured: (a) while at rest; (b) while walking at 3 km/h and (c) while performing arm ergometry at various power outputs. In order for EE to be derived throughout each of these three domains, participants wore a face mask that was directly attached to a breath-by-breath gas-analysis system (Metalyser 3B; Cortex Biophysik, Leipzig, Germany). Oxygen uptake and carbon dioxide production were used to calculate EE via indirect calorimetry.[20] Before undertaking each testing occasion (detailed below), the gas analyser was calibrated according to the manufacturer's recommendations.

To assess EE while at rest (phase a), each participant sat quietly (refraining from movement) for 30 minutes. Expired gas data were collected over the final 15 minutes of this 30-minute period, once values had stabilized.

To assess EE while walking at 3 km/h (phase b), participants wore the face mask while walking on a motor driven treadmill (Technogym Excite 700) for 10 minutes. Expired gas data were collected in the latter 5 minutes.

To assess EE during seated arm ergometry (phase c), participants wore the face mask while pedalling at various wattages on an arm ergometer (Monark Rehab Trainer 881 E; HaB International Ltd, Southam, UK). Participants performed three 5-minute bouts of arm ergometry, with the first bout standardized to a wattage of 15 W for 5 minutes. For the remaining bouts, investigators manipulated the resistance of the arm ergometer and/or the speed at which the participants pedalled until the wattage of arm ergometry initiated an EE that matched that of light walking (this ranged from 15 to 35 W). Expired gas data were collected in the second, third and fourth minute, discarding both the first and last minute from each bout. The face-mask was removed for 5 minutes in between each bout in order for EE outputs to return to their resting level prior to the next measurement. From these 3 bouts, the wattage of arm ergometry that

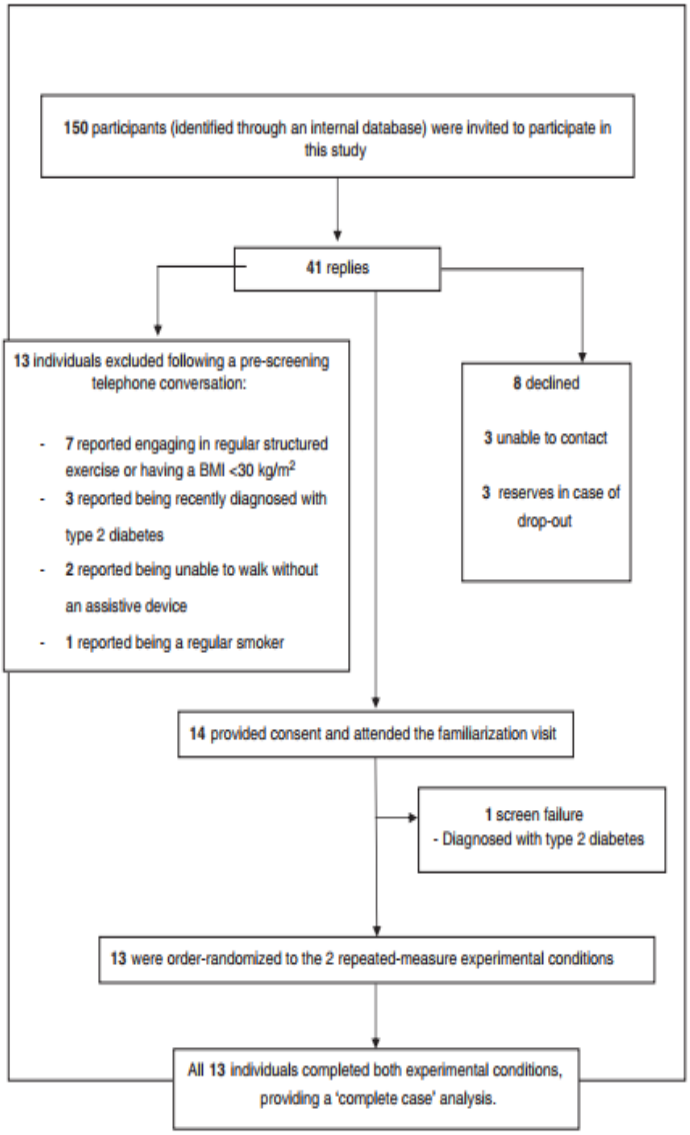

FIGURE 1 CONSORT diagram showing participant flow

most closely resembled the average EE of light walking was prescribed in the subsequent experimental condition.

Finally, participants were issued a GENEActiv accelerometer (ActivInsights Ltd, Huntingdon, UK) to wear on their non-dominant wrist for 24 h/d for 7 consecutive days, allowing quantification of habitual physical activity and sedentary behaviour levels.

2.4 | Experimental procedure

Participants were asked to avoid alcohol and caffeine for 48 hours preceding experimental conditions and to replicate their diet in the 24 hours before main trials. Given that the influence of an acute bout of physical activity on insulin sensitivity can persist for 48 hours,[21] avoidance of MVPA for this timeframe was also instructed. GENEActiv accelerometers were worn in the 2 days leading up to each experimental condition to confirm compliance with the exercise restriction. Participants fasted from 10.00 PM on the evening before main trials, with only water permitted to drink.

The 2 experimental treatment conditions that formed this repeated measures crossover trial were as follows. (1) Prolonged sitting only. Participants sat in a designated room for 7.5 hours (occupied with a desk, books and laptop with internet services) while minimizing excessive movements. Lavatory breaks were permitted using a wheelchair to and from the lavatory to further reduce unnecessary movements that could confound the study. (2) Arm ergometry breaks. Participants emulated the 7.5-hour prolonged sitting condition, but every 30 minutes they performed 5 minutes of arm ergometry. These bouts were performed 12 times, totalling 1 hour of seated upper body activity and 6.5 hours of sedentary time throughout the course of the experimental day. As mentioned previously, the intensity of arm ergometry performed was dictated by phase (b) and (c) of the EE testing performed during visit 1. The selected arm ergometry intensities closely resembled the EE achieved during the 3 km/h light-intensity walk for each participant.

On arrival at the research centre, participants had a cannula fitted into an accessible vein from which 10-mL samples were obtained

throughout the day. Immediately after the 2 fasting samples (time points − 1 and 0 in Figure 2), participants were given a standardized breakfast meal consisting of 8 kcal per kg body weight, with a macronutrient composition reflective of co-ingestion in modern western diets (14% protein, 51% carbohydrate and 35% fat). Once breakfast had been consumed (within ≤15 minutes), blood sampling commenced at 30, 60, 120 and 180 minutes thereafter, enabling us to capture the postprandial period. An identical lunch meal was then issued (time point 3 in Figure 2) and sampling continued in the same fashion at 30, 60, 120 and 180 minutes afterward. Participants were supervised by study staff to ensure compliance with the protocol and were asked to wear an activPAL monitor to objectively confirm sitting time during both experimental conditions. *Ad libitum* water consumption was made consistent between conditions.

2.5 | Measuring mood during experimental conditions

The Feeling Scale[22] was used to quantify mood/effect prior to each blood sample (10 times in total) for both experimental conditions. Participants were asked to estimate their current mood state on an 11-point scale (+5 = very good; 0 = neutral; −5 = very bad) throughout the day.

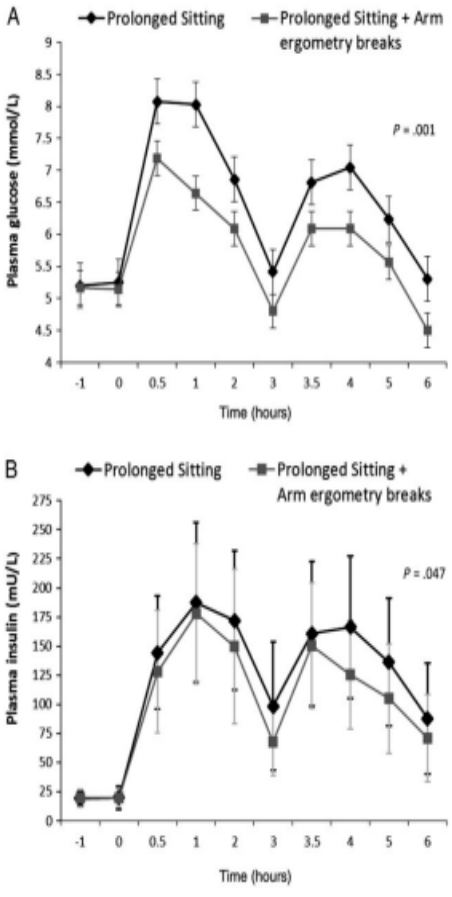

FIGURE 2 Analyte AUC data between experimental conditions

2.6 | Safety

Incidences of hypoglycaemia (defined as glucose levels <4 mmol/L) during the final measurement period before lunch (3 hours after breakfast) and in the final measurement period of the day (3 hours after lunch) were also investigated during each experimental condition.

2.7 | Free-living activity monitor processing

ActivPAL proprietary software (activPAL Professional V5.9.1.1) was used to create processed csv event files in order to quantify postural data collected during the 7.5-hour experimental conditions. GENEActiv .bin files were analysed using R package GGIR version 1.2-11 (http://cran.r-project.org).[23,24] Habitual data were included if participants had >16 hours of wear-time recorded during the 24-hour day of interest, and if they had >3 valid days of data collected. MVPA was calculated using an acceleration threshold of 100 mg.[25] MVPA bouts were identified as ≥10 minutes of consecutive 5-second epochs, where 80% of epochs were equal to, or higher than, the 100-mg threshold. Time spent in the ranges 0 to 50 mg and 50 to 100 mg was used to establish sedentary (minus sleep time) and light activity, respectively.

A summary of all GENEActiv data collected at each phase of the study is detailed in Table S1. ActivPAL data collected during experimental conditions is detailed in Table S2.

2.8 | Biochemical analysis

Glucose (primary outcome measure) was analysed on the day of collection by the University Hospitals of Leicester pathology department using standard quality controlled enzymatic assays with commercially available kits (Beckman, High Wycombe, UK).

Centrifugated plasma samples (spun at 3000 *g* for 10 minutes immediately after extraction) were stored in −80°C freezers. Insulin (secondary outcome measure) was analysed from these collectively at the end of the trial using an electrochemiluminescence assay (Meso Scale Discovery). Each sample was run in duplicate to ensure reliability of readings. Duplicate sample values with ≥20% variability were reanalysed. Ambient conditions of the laboratory were kept consistent.

2.9 | Sample size

The primary aim of the present study was to assess the difference in postprandial glucose levels between the two experimental treatment conditions. Assuming a population standard deviation (s.d.) of 2.5 mmol/L/h in glucose incremental area under the curve (iAUC) and a within-person correlation of 0.5, 13 participants were required to complete the study in order to detect a difference of 1.8 mmol/L/h in blood glucose iAUC between the experimental conditions with 90% power ($\alpha = 0.05$).

2.10 | Statistical analysis

Missing glucose and insulin data during the experimental conditions (highlighted in Table S3) resulted from an inability to draw enough

blood from the cannula at given time points and accounted for ~3.7% of required samples (19 out of 520). These 19 missing data points were imputed via a regression model used previously.[7] The iAUCs of glucose and insulin were calculated for each experimental condition. Total area under the curve (AUC) was calculated by applying the trapezium rule. Subtraction of the fasting area from this total then gave a single value representing iAUC for each participant. Using iAUC as opposed to total AUC is common practice in acute interventions where fasting levels should be unaffected by the intervention.[26] Each outcome (glucose and insulin iAUC) was compared between treatments using a paired samples *t*-test. Data from the Feeling Scale were averaged across each condition and analysed using a paired *t*-test. All statistical analyses were performed using IBM SPSS Statistics (Version 22.0) and statistical significance was set to $P < .05$ throughout. Data distribution was interpreted by visual inspection and through the Shapiro–Wilk test. Normally distributed descriptive data and experimental data are presented as mean ± s.d. and mean (95% confidence interval [CI]), respectively, while all non-parametric data are reported as median (interquartile range) unless specified otherwise. For the experimental data, the unstandardized residuals were checked for normality.

3 | RESULTS

The descriptive characteristics of those who completed the present study are summarized in Table 1 ($n = 13$). The study characteristics show that the EE of arm ergometry breaks conducted in the experimental condition was similar to that achieved through a light-intensity walk at 3 km/h (4.5 vs 4.6 kcal/min, respectively); however, the average respiratory exchange ratio (RER) was higher during arm ergometry compared with light-intensity walking (1.00 vs 0.84; $P < 0.001$).

3.1 | Experimental data

Biochemical results collected during each experimental condition are shown in Figure 2.

The mean glucose iAUC response during the arm ergometry breaks condition (3.1 [95% CI 1.3, 5.0] mmol/L/h) was significantly lower than the mean glucose iAUC response to the prolonged sitting-only condition (7.4 [95% CI 5.2, 9.5] mmol/L/h; $P = .001$). This was also the case for mean insulin iAUC (554 [95% CI 298, 811] mU/L/h vs 696 [95% CI 359, 1032] mU/L/h; $P = .047$).

3.2 | Physical activity and sedentary time data

Physical activity and sedentary behaviour data are shown in Table S1. Free-living accelerometer data collected after the familiarization visit ($n = 13$), showed that participants spent, on average, 644 ± 106 min/day sedentary and only engaged in a median (IQR) of 2 (0, 13) min/d of purposeful MVPA, thus confirming the inactive nature of the present study cohort.

The median (IQR) of MVPA data collected in the 2 days leading up to the prolonged sitting-only condition (0 [0, 10] min/d) and in the 2 days leading up to the arm ergometry breaks condition (0 [0, 7] min/d) confirm adherence to the standardized exercise restriction.

TABLE 1 Metabolic, demographic and anthropometric characteristics taken at familiarization alongside important in-study characteristics

Characteristic	Overall (N = 13)
Age, years	66 ± 6
Women, n (%)	7 (54)
BMI, kg/m^2	33.8 ± 3.8
Body weight, kg	93.2 ± 13.2
Waist circumference, cm	105 ± 16
HbA1c, %	5.5 ± 0.4
HbA1c, mmol/mol	37 ± 4
Total cholesterol, mmol/L	4.6 ± 0.6
Resting heart rate, bpm	60 ± 6
Systolic blood pressure, mm Hg	140 ± 13
Diastolic blood pressure, mm Hg	79 ± 9
White European, n (%)	13 (100)
Experimental characteristics	
Energy intake per experimental meal, kcal/meal	746 ± 106
Prescribed power output of arm ergometry, average watts	20 ± 4
EE while walking at 3 km/h, average kcal/min	4.6 ± 1.0
EE at prescribed wattage of arm ergometry, average kcal/min	4.5 ± 0.9
Average RER while walking at 3 km/h, VCO_2/VO_2	0.84 ± 0.07
Average RER at prescribed power output of arm ergometry, VCO_2/VO_2	1.00 ± 0.07

Data are presented as mean ± s.d. unless otherwise indicated.

3.3 | Mood, tolerance and safety

Mean ± s.d. self-reported feeling scores throughout the day were 3.1 ± 1.1 and 2.7 ± 1.2 for the prolonged sitting-only and arm ergometry breaks conditions, respectively ($P = .101$ for difference), demonstrating positive mood states during both conditions. All participants completed the required number of arm ergometry bouts, and none reported musculoskeletal pain or discomfort.

Two participants did have asymptomatic hypoglycaemia during the final measurement of the day during the arm ergometry breaks condition, with no incidences reported during the prolonged sitting condition.

4 | DISCUSSION

The present study is the first to investigate the metabolic impact of interrupting postprandial prolonged sitting time with regular bouts of upper body activity while remaining seated. Our results show that introducing 5 minutes of arm ergometry every 30 minutes while remaining in a seated posture is well tolerated and can attenuate postprandial blood glucose and insulin levels by ~57% and ~20%, respectively, compared with prolonged sitting only. The fact that the observed reductions in glucose coincided with reductions in insulin concentration is suggestive of improved insulin sensitivity during the seated activity breaks condition using upper body muscle activation.

These findings are consistent with the majority of experimental research to date. For example, experimental studies that have interrupted prolonged sitting with 3 km/h walking breaks have led to clinically significant reductions in postprandial blood glucose by 28%[7] when implemented for 5 minutes every 30 minutes, by 39% when implemented for 3 minutes every 30 minutes[10] and by 16%[9] to 24%[8] when implemented for 2 minutes every 20 minutes post-meal. Our findings add to this evidence by showing that regular bouts of seated arm ergometry may also be a viable method of improving postprandial glycaemia. Moreover, despite closely matching the energy demand of arm ergometry breaks to that of the 3 km/h walking bouts used in previous studies, we achieved a larger reduction in postprandial glucose iAUC than that observed in those studies, even compared with those operating activity breaks at the same time intervals.[7]

Given that arm ergometry breaks were implemented while maintaining a seated posture, our findings could not have been driven by postural change, and benefits to postprandial glycaemia may be attributed to other factors. For instance, physical activity breaks are accompanied by increases in muscle activation. These increases in muscle activation not only raise EE, but also increase blood flow and upregulate GLUT-4 expression in a dose-dependent manner, which helps to restore homeostasis of postprandial glycaemia.[27,28] Greater intensity of muscle activation in the smaller muscle mass during arm ergometry may have been necessary to achieve the same EE elicited by a 3 km/h walk. In turn, this greater muscle activation may have compensated for the limited muscle mass involved, and may explain the enhanced blood glucose utilization observed in the present study. This was supported in the present study by the higher RER observed during the arm ergometry compared with the energy-matched walking, suggesting a greater relative intensity. Previous research has shown that enhanced postprandial blood glucose regulation is observed after higher-intensity physical activity bouts compared with energy-matched lower-intensity physical activity bouts.[29-31] Thus, the higher intensity of arm ergometry, compared with light walking, may have helped augment reductions in postprandial glucose. Further research is needed to assess whether reductions in postprandial glucose are also observed when using arm ergometry at a perceived light intensity.

The present study suggests an alternative strategy to help regulate postprandial glycaemia while sitting, in a population at high risk of type 2 diabetes. Not only are arm ergometry breaks an alternative strategy, but they may even act as a sole strategy for individuals with weight-bearing difficulty such as wheelchair users and those with severe peripheral neuropathy, which is thought to affect up to half of all people diagnosed with type 2 diabetes.[32] Given the disruptive nature of alternative strategies, such as frequent walking breaks, seated activity may also appeal to office workers who find it difficult to leave their desk or office space at regular intervals throughout the day. Portable lightweight desktop arm ergometers may also be of use in a hospital environment to improve postprandial glycaemia in patients who are bed-bound yet able to sit upright.

The main strength of the present study lies in the exploration of a novel strategy to alleviate the deleterious impacts of prolonged sitting bouts on postprandial glycaemia in a population at high risk of developing type 2 diabetes recruited through a primary care setting; however, it is important to acknowledge some limitations. Although comparing our findings to those observed when introducing 3 km/h walking breaks,[7-10] we did not include a third experimental walking condition which may have strengthened our conclusions. In addition, this study was not designed to elucidate the potential mechanisms underpinning the acute reductions in postprandial glucose and insulin concentrations observed when employing seated activity breaks; however, the study was specifically designed to establish proof-of-concept for the efficacy of employing seated arm ergometry breaks as a method of acutely reducing postprandial glucose concentrations during prolonged sedentary behaviour. This is clinically important given that exaggerated postprandial glucose oscillations are associated with the development of type 2 diabetes,[33] cardiovascular disease[33-35] and obesity.[33] Even small elevations in postprandial glycaemia are thought to contribute to the development of atherosclerosis and subsequent coronary heart disease events.[36]

Although a sample size of 13 provided adequate power for comparison between experimental conditions, the small sample makes it harder to generalize findings beyond the specific population recruited to this study. Given that efforts to manipulate blood glucose control are thought to be more pronounced in those with worse glycaemia,[37] the potential of such interventions in a population diagnosed with type 2 diabetes would also be intriguing and warrants further investigation. Future intervention studies observing the impacts of seated activity breaks using more ecologically valid regimes in settings outside of the laboratory (such as the home, or in a hospital environment) would also be of interest. The ability to emulate reductions in postprandial glycaemia through regular bouts of electro-stimulated muscular contractions would also be an interesting focal point for future research given recent links to improved insulin sensitivity[38] and its potential application to non-weight-bearing populations. Likewise, given that arm ergometers are not easily accessible to all, engaging in seated upper body resistance band exercises could also pose as an intriguing alternative for future research. Future research exploring the minimum time, frequency and intensity that should be used when implementing activity breaks to bring about clinically significant improvements in postprandial glycaemia is warranted to promote more attractive, feasible and sustainable strategies. In addition, given that 2 participants were found to be over the threshold for asymptomatic hypoglycaemia at the end of the arm ergometer condition, the safety of the current regime needs further investigation in those at high risk or with diagnosed type 2 diabetes, particularly in the 24 hours after the intervention. Further research applying hyperinsulinaemic-euglycaemic clamp techniques could also be used to give more detailed insight into the dynamics of glucose metabolism when employing seated upper body breaks during prolonged sedentary behaviour.

In conclusion, the present study shows that seated arm ergometry breaks are a viable way to attenuate postprandial glycaemia. This suggests that breaking up the posture of sitting may not be necessary to elicit glycaemic benefit and that interventions to reduce sedentary behaviour should not focus solely on postural change.

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Conflict of interest

All authors declare support from the NIHR Collaboration in Applied Health Research and Care for Leicestershire, Northamptonshire and Rutland alongside the Health Research Collaboration for Leadership in Applied Health Research and Care – East Midlands. M. M., T. Y., M. J. D., C. L. E., B. H. D., J. H., A. R. and J. K. declare support from the NIHR Leicester Biomedical Research Centre. K. K., M. J. D. and T. Y. were members (K. K. chair) of the NICE PH 38 (Preventing type 2 diabetes: risk identification and interventions for individuals at high risk) Program Development Group. M. J. D., K. K., and T. Y. are academic leads for a diabetes prevention programme selected to be part of Healthier You: The NHS Diabetes Prevention Programme in collaboration with Ingeus UK Ltd. All authors declare no support from any other organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work, no other relationships or activities that could appear to have influenced the submitted work. Aside from the information disclosed above, the authors declare no competing interests.

Author contributions

M. M., T. Y. and C. L. E. conceived and designed the research question. M. M. collected the data. All authors interpreted the data. C. L. E. and A. R. processed accelerometer data. M. M., T. Y. and C. L. E. analysed the data. M. M. drafted the initial version. All authors revised the paper for important intellectual content.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Change in Sedentary Time, Physical Activity, Bodyweight, and HbA1c in High-Risk Adults

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[1]Department of Health Sciences, University of Leicester, Leicester, Leicestershire, UNITED KINGDOM; [2]Leicester-Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit, National Institute for Health Research (NIHR), Loughborough, UNITED KINGDOM; [3]Diabetes Research Centre, University of Leicester, Leicester Diabetes Centre, Leicester General Hospital, Leicester, UNITED KINGDOM; and [4]NIHR Collaborations for Leadership in Applied Health Research and Care (CLAHRC), Leicester, UNITED KINGDOM

ABSTRACT

MCCARTHY, M., C. L. EDWARDSON, M. J. DAVIES, J. HENSON, L. GRAY, K. KHUNTI, and T. YATES. Change in Sedentary Time, Physical Activity, Bodyweight, and HbA1c in High-Risk Adults. *Med. Sci. Sports Exerc.*, Vol. 49, No. 6, pp. 1120–1125, 2017. **Purpose**: In recent years, there has been a migration toward the use of glycated hemoglobin (HbA1c) in determining glycemic control. This study aimed to quantify the associations between changes in body weight, sedentary time, and moderate to vigorous physical activity (MVPA) time with HbA1c levels for a 3-yr period among adults at high risk of type 2 diabetes. **Methods**: This study reports baseline and 3-yr follow-up data from the Walking Away from Type 2 Diabetes study. ActiGraph GT3X accelerometers captured sedentary time and MVPA. Linear regression examined the independent associations of changes in sedentary time, MVPA, and body weight with HbA1c between baseline and 3-yr follow-up. **Results**: The sample composed of 489 participants (mean age = 64.2 ± 7.3 yr, body mass index = 31.7 ± 5.1, 63.4% male) with valid baseline and follow-up accelerometer, body weight, and HbA1c data. After adjustment for known confounders, an increase in MVPA time (per 30 min·d^{-1}) was associated with a decrease in HbA1c percentage ($\beta = -0.11$ [−0.18 to −0.05], $P = 0.001$), and an increase in body weight (per 6 kg) was associated with an increase in HbA1c percentage ($\beta = 0.08$ [0.04–0.12], $P < 0.001$). The presence of dysglycemia at baseline (HbA1c ≥ 6.0%) strengthened these associations ($P < 0.001$ for interactions). Change in sedentary time was not significantly associated with change in HbA1c after adjustment for change in MVPA time. **Conclusion**: Increases in MVPA and body weight were associated with a reduction and increase in HbA1c, respectively, particularly in those with dysglycemia. Quantifying the effect that health behavior changes have on HbA1c can be used to inform prevention programs.
Key Words: TYPE 2 DIABETES, PRIMARY CARE, SEDENTARY BEHAVIOR, DYSGLYCEMIA

EPIDEMIOLOGY

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic diseases and accounts for between 7% and 14% of health care expenditure globally (40). Both the prevalence and the cost of T2DM in the United Kingdom are projected to rise in the future with 17% of the National Health Service (NHS) budget required for its treatment by 2035 (19). Given this current and projected increase in burden, health care policies and recommendations targeting prevention are gaining national and international traction with defined budgetary commitments (27,29).

Lifestyle interventions have consistently been shown to reduce the risk of, and slow progression to, T2DM in high-risk populations and form the cornerstone of diabetes prevention recommendations and programs (10,26). There has been a wealth of good quality interventional and epidemiological evidence quantifying the combined and individual effect of lifestyle factors in improving glucose regulation and reducing the risk of T2DM based on outcomes from an oral glucose tolerance test (fasting and 2-h postchallenge glucose levels) (11). However, such data no longer reflects clinical reality and decision making processes. Since the inclusion of HbA1c within the diagnostic framework for T2DM (36), there has been a migration toward HbA1c in the classification of diabetes risk and assessment of diabetes prevention programs run within routine care (20,26,27). This change is reflective of greater clinical utility of HbA1c compared with plasma glucose derived from an oral glucose tolerance test. For example, HbA1c does not need to be measured fasting, is a better indicator of chronic hyperglycemia, is less affected by any short-term illness-related changes in plasma glucose levels, and shows lower intertest variability (20). Given the abundant shift in focus toward HbA1c in recent years, there is a requirement to extend

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prevention research by quantifying the effect of lifestyle change on this metabolic marker.

Increased physical activity and weight loss have consistently been shown to independently reduce the risk of T2DM and are key behavioral targets for prevention programs that have been translated into real-world settings (6,21). The importance of these factors on change in HbA1c needs further elucidation, although recent research is encouraging. For example, obese individuals with currently normal HbA1c levels (5.2%–5.6%) have a greater chance of developing early onset T2DM than a lighter individual with currently higher levels (5.7%–6.4%) (25). In addition to physical activity and body weight, high levels of sedentary time, defined as any sitting or reclining behaviors undertaken with low energy expenditure, have also been associated with poor metabolic health (8), increased risk of T2DM (4,8,34), cardiovascular disease (4,34), and mortality (4,34). Recent cross-sectional links between sedentary time and insulin sensitivity have also emerged, which further support the potentially detrimental effect of sedentary time upon glycemic control (5,39).

The aim of this article is to use a prospective data set to quantify the association between changes in moderate to vigorous physical activity (MVPA), sedentary time, and body weight with changes in HbA1c using a population at high risk of T2DM recruited from primary care for a 3-yr period.

METHODS

Research design. This study performed an observational cohort analysis using baseline and 3-yr follow-up data from the Walking Away from Type 2 Diabetes trial, the design and results of which are described elsewhere (37,38). In brief, this was a randomized controlled trial that evaluated the effectiveness of a pragmatic structured education program aimed at increasing physical activity and promoting healthy lifestyles for 3 yr among those who were at high risk of T2DM.

Participants. Individuals taking part in the trial were recruited through 10 primary care practices in Leicestershire, United Kingdom, in 2010. Individuals were recruited based on having a high risk of T2DM defined using the Leicester Practice Risk Score (13). The score calculates risk based on six variables (age, sex, ethnicity, body mass index, family history of the disease, and antihypertensive drug usage). Individuals ranked within the top 10% within their family practice were invited to take part in the study. Those with T2DM diagnosed at baseline, with established T2DM or currently taking steroids, were excluded.

Informed consent was obtained from all eligible participants, and full ethical approval from the local ethics committee was granted for the trial.

Demographic data. Information regarding medication, ethnicity, smoking status, and home postcode (used to calculate index of multiple deprivation [IMD] score) was obtained after an interview administered protocol conducted by health care professionals. The IMD scores are publically available continuous measures of compound social and material deprivation, which are calculated using a variety of data including current income, employment, health, education, and housing.

Anthropometric data. Body weight, body fat percentage (Tanita TBE 611; Tanita, West Drayton, UK), and height were measured to the nearest 0.1 kg, 0.1%, and 0.1 cm, respectively.

Biochemical data. Venous blood samples were obtained after a 12-h overnight fast. All assays were measured in the same laboratory using stable methodologies and conducted by individuals blinded to the patients' identity. Glycated hemoglobin (HbA1c) was analyzed using the Bio-Rad Variant II HPLC system (Bio-Rad Clinical Diagnostics, Hemel Hempstead, UK). All venipuncture was undertaken by trained phlebotomists. Data collection procedures between baseline and follow-up were standardized.

Accelerometer data. Participants were asked to wear an accelerometer (ActiGraph GT3X, Pensacola, FL) on the right anterior axillary line above the hip for seven consecutive days during waking hours at both baseline and 3-yr follow-up. Data were collected in 60-s epochs. Freedson cut points, using counts in the vertical axis only, were used to categorize sedentary time (<100 counts per minute) and MVPA time (≥1952 counts per minute) (9). In addition, MVPA time accumulated in bouts ≥10 min (allowing for a 2-min exception in the intensity threshold) was also derived. Non–wear time was defined as a minimum of 60 min of continuous zero counts, and days with at least 600 min of wear time were considered valid (16). To be included in the analysis, a minimum of any four valid days was required (32). Accelerometer files were processed using KineSoft V3.3.76, a commercially available analytical software (KineSoft, Loughborough, UK).

Statistical analysis and data inclusion. From the 808 individuals randomized into the Walking Away from Type 2 Diabetes trial at baseline, 489 (61%) had valid measures of accelerometer data, body weight, and HbA1c at both baseline and 3 yr and were subsequently included in this analysis. Of the 319 participants who were not included in this analysis, 289 were excluded on the basis of failing to meet the minimum accelerometer wear time requirements, whereas 30 did not provide biochemical data at both time points. The results of this intervention are reported elsewhere (38) with no significant changes to MVPA, sedentary time, or body weight for the overall cohort. All analyses were conducted using IBM SPSS Statistics (version 22.0), and statistical significance was set at $P < 0.05$. Only participants with valid measures of accelerometer data, body weight, and HbA1c at both baseline and 3 yr were included in the following analyses.

Linear regression models examined the independent associations between changes in MVPA, sedentary time, and body weight with a change in HbA1c for the 3-yr period. Changes in all variables were calculated as 3-yr follow-up data minus baseline data. Beta-coefficients representing changes in "HbA1c %" reflect absolute changes in HbA1c units and not relative statistical percentage changes. Change

TABLE 1. Demographic, cardiometabolic, and anthropometric characteristics of participants.

Characteristics	Walking Away from Type 2 Diabetes Trial Participants (n = 489)
Age (yr)	64.2 ± 7.3
Male	310 (63.4)
Current smokers	30 (6.1)
Family history of diabetes (first degree)	169 (34.5)
Body mass index (kg·m^{-2})	31.7 ± 5.1
Cardiometabolic variables	
Total cholesterol (mmol·L^{-1})	5.2 (4.5–6)
HDL cholesterol (mmol·L^{-1})	1.4 (1.2–1.6)
Ethnicity	
White European	441 (90.2)
South Asian	31 (6.3)
Other	17 (3.5)
Diagnosis	
Normal glycemic function (HbA1c < 6%)	319 (65.2)
Dysglycemia (HbA1c ≥ 6%)	170 (34.8)

Continuous parametric results are displayed as mean ± SD or number (percentage), and continuous nonparametric results are displayed as median (interquartile range).

data for MVPA and sedentary time were displayed in 30-min·d^{-1} unit increments for ease of interpretation. The lifestyle intervention arm of the Diabetes Prevention Program targeted a 7% reduction in body weight (2); change data for body weight in the current study were therefore displayed in 6-kg unit increments, as this represents a 7% difference in the average body weight of our cohort. Analyses were adjusted for the following variables: age, sex, ethnicity, beta-blocker use for hypertension, IMD score, change in accelerometer wear time, and baseline measures of HbA1c, body weight, sedentary time, and MVPA. Smoking status was also added as a measure of deprivation. Additional models simultaneously added change in all variables (MVPA, sedentary time, and body weight) into the same model to establish the extent to which associations with HbA1c were independent of one another. A sensitivity analysis was conducted to see whether using MVPA time accumulated from bouts lasting ≥10 min (in line with public health physical activity guidelines [35]) influenced the findings.

In addition, we also set out to investigate whether glycemic status at baseline independently modified associations by adding interaction terms to the model. Interaction significance was set to $P < 0.01$. Glycemic status was defined as having dysglycemia (HbA1c ≥ 6.0% at baseline) or normal glycemia (<6% at baseline). Significant interactions were followed up with stratified analyses. A threshold of 6.0% was chosen to make the analysis consistent with recommendation for UK populations (26) and with international guidance (20).

Although commonly used, a cutoff value of <100 counts per minute to categorize sedentary time may be too high, particularly in older adults (1). We therefore ran a further sensitivity analysis to address whether similar results were yielded if sedentary time was categorized at a lower cutoff value of <50 counts per minute. Similarly, Freedson cut points for MVPA (≥1952 counts per minute) may underestimate time spent in MVPA (12); therefore, we conducted a sensitivity analysis to determine whether a lower cut point (≥1041 counts per minute) influenced our findings.

RESULTS

Those included in this analysis had a similar ethnic breakdown and baseline sedentary time compared with those who were excluded. There were also no significant differences in sex between those included and excluded. However, those excluded had a higher social deprivation score (22.7 vs 17.6, $P < 0.001$), were more likely to be younger (61.8 ± 9.1 vs 64.2 ± 7.3 yr, $P < 0.001$), have a higher body mass index (33.6 ± 6 vs 31.7 ± 5.1 kg·m^{-2}, $P < 0.001$), and engage in less MVPA at baseline (32.7 ± 25.1 vs 40.3 ± 27.6 min·d^{-1}, $P < 0.001$). Table 1 reports the demographic, anthropometric, and cardiometabolic characteristics of those included in the study analysis.

Table 2 reports baseline and 3-yr follow-up data for key anthropometric, cardiometabolic, and accelerometer derived measures.

Table 3 displays the adjusted associations of changes in sedentary time, MVPA, and body weight with HbA1c change.

Sedentary time. After adjustment for known confounders, greater sedentary time (per 30 min·d^{-1}) was associated with an increase in HbA1c (β = 0.02% [0.01 to 0.03], P = 0.021). This association disappeared after further adjusting for a change in MVPA (β = 0.01% [−0.01 to 0.02], P = 0.402).

MVPA time. An increase in MVPA time (per 30 min·d^{-1}) was significantly associated with a decrease in HbA1c (β = −0.14% [−0.20 to −0.08], $P < 0.001$) after adjustment for potential confounding variables. This remained significant aft er further adjustment for changes in both sedentary time and body weight (β = −0.11% [−0.18 to −0.05], P = 0.001).

Body weight. When adjusting for all covariates, including change in MVPA, an increase in body weight (per 6 kg) was associated with significantly greater HbA1c levels (β = 0.08% [0.04 to 0.12], $P < 0.001$).

Sensitivity analyses revealed that these results were largely unaffected when using MVPA accumulated in bouts ≥10 min (see Table, Supplemental Digital Content 1, Sensitivity analysis showing adjusted associations when using MVPA time accumulated in bouts ≥10 min, http://links.lww.com/MSS/A852), when using lower cut points for sedentary time (see Table, Supplemental Digital Content 2, Sensitivity analysis showing adjusted associations when using lower sedentary time cut points, http://links.lww.com/MSS/A853), or when using lower MVPA cut points (see Table, Supplemental

TABLE 2. Body weight, physical activity, and HbA1c characteristics at baseline and 3-yr follow-up.

Characteristics	Baseline	3 yr
HbA1c (%)	5.8 (5.6–6.1)	5.7 (5.4–5.9)
HbA1c (mmol·mol^{-1})	39.9 (37.7–43.2)	38.8 (35.5–41.0)
Body weight	87.6 (79.3–98.9)	87.1 (78.1–98.1)
Accelerometer variables		
Wear time (h·d^{-1})	14.4 (13.5–15.2)	14.3 (13.5–15.1)
Sedentary time (min·d^{-1})	542 (477–597)	566 (499–632)
Total MVPA (min·d^{-1})	21 (12–41)	16 (7–33)
MVPA (min·d^{-1} accumulated in bouts ≥10 min)	4 (0–10)	3 (0–10)

Results displayed as median (interquartile range).

TABLE 3. Multiple linear regression models for changes in sedentary time, MVPA, and body weight with HbA1c.

	Sedentary Time Change (per 30 min·d^{-1})[a]	MVPA Time Change (per 30 min·d^{-1})[b]	Body Weight Change (per 6 kg)[c]
Model 1			
HbA1c change (%)	0.02 (0.01 to 0.03), P = 0.021	−0.14 (−0.2 to −0.08), P < 0.001	0.09 (0.06 to 0.13), P < 0.001
~HbA1c change (mmol·mol^{-1})	0.2 (0.03 to 0.38), P = 0.021	−1.5 (−2.2 to −0.88), P < 0.001	1.0 (0.62 to 1.4), P < 0.001
Model 2			
HbA1c change (%)	0.01 (−0.01 to 0.02), P = 0.402	−0.13 (−0.2 to −0.07), P < 0.001	0.09 (0.05 to 0.12), P < 0.001
~HbA1c change (mmol·mol^{-1})	0.1 (−0.11 to 0.26), P = 0.402	−1.4 (−2.15 to −0.74), P < 0.001	1.0 (0.56 to 1.34), P < 0.001
Model 3			
HbA1c change (%)	0.004 (−0.01 to 0.02), P = 0.615	−0.11 (−0.18 to −0.05), P = 0.001	0.08 (0.04 to 0.12), P < 0.001
~HbA1c change (mmol·mol^{-1})	0.04 (−0.13 to 0.22), P = 0.615	−1.2 (−1.93 to −0.53), P = 0.001	0.9 (0.49 to 1.26), P < 0.001

Data are unstandardized regression coefficients (95% CI), P value.
Model 1: adjusted for age, sex, smoking status, ethnicity, beta-blocker use for hypertension, change in accelerometer wear time, IMD score, baseline HbA1c, baseline body weight, baseline sedentary time, and baseline MVPA time.
Model 2: adjusted for all covariates in model 1 and [a] MVPA time change, [b,c] sedentary time change.
Model 3: adjusted for the same covariates as model 2 and [a,b] body weight change, [c] MVPA time change.

Digital Content 3, Sensitivity analysis showing adjusted associations when using lower MVPA time cut points, http://links.lww.com/MSS/A854).

When interaction terms were added to the model, they revealed that glycemic status at baseline significantly modified the independent associations between a change in MVPA (P < 0.001) and a change in body weight (P < 0.001) with a change in HbA1c. Following up on this interaction, stratification by glycemic status showed that those with dysglycemia had stronger associations compared with those with normal glycemia (Table 4). For individuals with dysglycemia, each 30-min increase in MVPA per day was associated with a 0.17% (0.04–0.29) decrease in HbA1c in the fully adjusted model (including change in sedentary time and body weight, P = 0.012), and each 6-kg increase in body weight was associated with a 0.19% (0.11–0.27) increase in HbA1c (P < 0.001). Glycemic status did not significantly modify the association between sedentary time and HbA1c and, therefore, did not warrant further stratification.

DISCUSSION

Although the effect of physical activity and weight loss interventions on HbA1c has been well established in those with T2DM (3,33), the effect of individual lifestyle components on HbA1c in nondiabetic populations is not as well defined. This study helps to address this evidence gap by quantifying the relative importance of changes to MVPA, sedentary time, and body weight in the regulation of HbA1c levels in those at high risk of T2DM.

The current study demonstrated that both change in MVPA and body weight were independently associated with change in HbA1c, whereby every 30-min increase in MVPA per day was associated with a 0.11% (1.2 mmol·mol^{-1}) decrease in HbA1c and every 6-kg increase in body weight was associated with a 0.08% (0.9 mmol·mol^{-1}) increase. Of note, we found that there was a significant interaction with glycemic status whereby those with dysglycemia at baseline had stronger associations of MVPA and body weight with HbA1c, further supporting the importance of lifestyle change in those with nondiabetic hyperglycemia.

Using linear scaling from data published for the large Atherosclerosis Risk in Communities study (30), it is suggested that each 0.1 absolute percentage increase in HbA1c (1.1 mmol·mol^{-1}) is associated with a 6.1% increased risk of diabetes, a 1.8% increased risk of coronary artery disease, a 3% increased risk of stroke, and a 1.1% increased risk of all-cause mortality in nondiabetic populations. Therefore, the change in HbA1c associated with a 30-min change in MVPA or a 6-kg change in body weight is likely to be clinically meaningful in a nondiabetic population.

Our findings are consistent with that of the Finnish Diabetes Prevention Study (FDPS), which achieved around a 0.2% reduction in HbA1c for 3 yr with an intervention aimed at achieving a 5% body weight loss, at least 30 min of MVPA per day, and a healthy diet in those with impaired glucose tolerance (22).

However, results from the Look Ahead study, which focused on achieving similar parameters to the FDPS, observed a reduction in HbA1c of 0.36% (3.9 mmol·mol^{-1}) for a 4-yr period (23). This supersedes both the results of the FDPS mentioned earlier and the associations that attaining such parameters would have in the current study. However, their use of overweight and obese diabetic participants may have steepened the gradient for improvement beyond that observed in nondiabetic populations.

Results of the current analysis are also consistent with cross-sectional analyses from national surveys, which have reported

TABLE 4. Associations between change in MVPA and body weight with a change in HbA1c stratified by glycemic status.

	MVPA Time Change (per 30 min·d^{-1})[a]	Body Weight Change (per 6 kg)[b]
Impaired glycemic function (HbA1c ≥ 6%)		
HbA1c change (%)	−0.17 (−0.29 to −0.04), P = 0.012	0.19 (0.11 to 0.27), P < 0.001
~ HbA1c change (mmol·mol^{-1})	−1.8 (−3.19 to −0.4), P = 0.012	2.1 (1.17 to 2.98), P < 0.001
Normal glycemic function (HbA1c < 6%)		
HbA1c change (%)	−0.07 (−0.13 to −0.01), P = 0.031	0.04 (0.01 to 0.08), P = 0.012
HbA1c change (mmol·mol^{-1})	−0.8 (−1.47 to −0.07), P = 0.031	0.5 (0.1 to 0.82), P = 0.012

Adjusted for age, sex, smoking status, ethnicity, beta-blocker use for hypertension, baseline body weight, change in accelerometer wear time, IMD score, baseline HbA1c, baseline MVPA, baseline sedentary time, changes in sedentary time, [a] changes in body weight, and [b] changes in MVPA.

associations of MVPA, but not sedentary time, with HbA1c (14,28,31) and extend previous research that has demonstrated the effect of physical activity and body weight with risk of T2DM based on fasting or 2-h glucose values (11).

The current study supports MVPA and body weight as key targets for the prevention of T2DM when assessed by HbA1c. However, the results for change in sedentary time were more equivocal. Although sedentary time has been associated with an increased risk of T2DM (4,34) and metabolic syndrome (8), the degree to which this is independent of MVPA or total physical activity levels has remained controversial (24). This study found that although change in sedentary time was associated with change in HbA1c, the findings were attenuated when adjusted for MVPA. This finding is in contrast to studies which have found associations between sedentary time and 2-h postchallenge glucose and levels of insulin sensitivity (15,18,39), in addition to experimental interventions that have found improved postprandial glucose responses with reductions to sitting time (7,17). This discrepancy in findings could result from the properties of HbA1c, which reflect average glucose concentration and may therefore be less sensitive to the more subtle effects on postprandial responses and peripheral insulin sensitivity.

The Walking Away from Type 2 Diabetes randomized controlled trial (37), from which the data in this analysis were derived from, experienced no differences between control and lifestyle intervention groups, with a small decrease in activity levels for the entire cohort (38). A wide range of variation in both directions allowed the current analysis to be undertaken but demonstrates the challenging nature of initiating and sustaining the amount of physical activity required to elicit clinically significant results.

The main strength of this study is that it provides novel prospective evidence in a high-risk primary care population using objective measures of sedentary behavior, physical activity, and body weight. Despite the prospective nature of this study, direct causality cannot be inferred, and it is possible that unmeasured lifestyle factors were confounding relationships. In addition, although the study population is likely to be broadly representative of those referred into diabetes prevention pathways within primary care, their high-risk nature means the results are not be generalizable to the general population.

In conclusion, increasing MVPA and reducing body weight both have favorable influences on HbA1c levels in those identified as being at high risk of T2DM through a primary care setting. Through the use of regression modeling, this study is able to quantify the effect that manipulating important behavioral targets would have on HbA1c levels. This addresses an important limitation and can be used to inform future diabetes prevention interventions within primary care. Given the observational nature of this study, further research is needed to confirm these results.

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Aside from the information disclosed, authors declare no conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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