



Title: Interrupting prolonged sitting in overweight, and obese adults and glycaemic responses: A randomised crossover study in free-living conditions

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Interrupting prolonged sitting in overweight, and obese adults and glycaemic responses: A randomised crossover study in free-living conditions

Charlotte Anne Stringer

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Abstract

Aims: The aim of the present study was to investigate 24 h interstitial glycaemia responses to interrupting prolonged sitting in free-living conditions in inactive and sedentary overweight and obese adults.

Methods: Twelve overweight and obese individuals (mean \pm SD age 47.5 \pm 9.9 y) completed two, four-day conditions in a randomised crossover design; Uninterrupted sitting (SIT): 10 h/day sitting, 7 h/day uninterrupted bouts sitting (7 x 60 min bouts), standing and walking restricted to 1.5 h/day, or interrupting sitting (INT SIT): 3 – 6 min of standing, walking, simple body-weight resistance; half squats, lunges, calf raises, knee lifts, and repeated sit-to-stand transitions every 30 min for 10 h/day. Incremental area under the curve (iAUC) was calculated using the trapezoid method.

Results: There were no significant differences observed for iAUC glucose measures between SIT and INT SIT conditions. There was no difference in sedentary behaviour between conditions, but daily stepping time and total steps increased significantly in INT SIT compared with SIT.

Conclusion: In overweight and obese participants, it may not be possible to manipulate increases or decreases in sedentary behaviour in free-living conditions. Therefore, it was not possible to compare effects of interrupted sitting versus uninterrupted sitting on glycaemia.

Key words:

Sedentary behaviour; Interrupting pro-longed sitting; Obese adults; Glycaemia; Free-living conditions

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Glossary

Cardiovascular disease – CVD

Type 2 diabetes – T2D

Light- intensity physical activity - LIPA

Moderate to vigorous physical activity – MVPA

Body mass index – BMI

1. Introduction

Sedentary behaviours, such as prolonged sitting, have been associated with adverse metabolic health markers, including abnormal glycaemia (Dunstan *et al.*, 2004), obesity (Healy *et al.*, 2008), and insulin resistance (Helmerhorst *et al.*, 2009). High levels of sedentary behaviour is a risk factor for insulin resistance, metabolic syndrome, and type 2 diabetes (T2D), independent of light and moderate-physical activity (Venables and Jeukendrup, 2009; Edwardson *et al.*, 2012; Proper *et al.*, 2011; Van Uffelen *et al.*, 2010). However, high levels of moderate-intensity physical activity (60 – 75 minutes per day) may protect against the increased mortality risk associated with high levels of sitting (Ekelund *et al.*, 2016). Sedentary behaviour is any waking behaviour characterized by an energy expenditure ≤ 1.5 metabolic equivalents (METs), while in a sitting, reclining or lying posture (Tremblay *et al.*, 2017). Multiple definitions have been used within the literature, however, the above terminology has been selected to standardise the vocabulary used (Barnes *et al.*, 2012; Tremblay *et al.*, 2017). It has been proposed that engaging in a non-sedentary bout, between two sedentary bouts throughout the day, may be beneficial for metabolic health in individuals at risk of T2D, regardless of the physical activity intensity of these non-sedentary bouts (Henson *et al.*, 2013).

It is suggested from accelerometer derived data that adults spend up to 68 % of their waking day sedentary (Dunstan *et al.*, 2012a), with archetypical waking hours of 16 h/day (Chastin *et al.*, 2015b; Hamilton *et al.*, 2007). These individuals spend approximately half of their waking day with relatively idle muscle activity (Hamilton *et al.*, 2007). Large skeletal muscles such as the quadriceps, hamstrings, rectus abdominus, oblique's, erector spinae, and gluteus muscles, that are required for posture changes such as sit-stand-transitions, subsequently become inactive. This muscular inactivity is associated with negative effects on cellular processes in skeletal muscle and tissue regulating risk factors including plasma triglycerides and high-density lipoprotein cholesterol (Hamilton *et al.*, 2007).

Frequent interruptions to sedentary time are suggested to be metabolically beneficial (Dunstan *et al.*, 2012b). Due to oxidative stress, caused by transient exaggerated

postprandial spikes in glycaemia and lipids in response to muscular inactivity, a biochemical inflammatory cascade, endothelial dysfunction, and sympathetic postprandial hyperactivity may occur (Dunstan *et al.*, 2012b). Thus, reducing the magnitude of postprandial hyperglycaemia is of public health interest.

Interruptions to prolonged sitting involving light to moderate-intensity physical activity in bouts of 2 min every 20 – 30 min have been shown to result in suppressed postprandial glycaemia (Dunstan *et al.*, 2012b; Bailey and Locke, 2015). However, most of the studies assessing glycaemic responses to interrupting prolonged sitting have been conducted in a laboratory environment (Dempsey *et al.*, 2014; Pulsford *et al.*, 2017; Thorp *et al.*, 2014). Studies of this design may not reflect free-living transitions between sitting, standing, and ambulation that would occur sporadically. Thus, the application of these laboratory interventions to free-living conditions is limited. There is limited literature that investigates the effects of interrupting sitting on glycaemic responses in free-living conditions (Duvivier *et al.*, 2013; Duvivier *et al.*, 2016; Duvivier *et al.*, 2017; Duvivier *et al.*, 2017). Participants in these studies were either of normal weight, overweight and obese, or had T2D. However, the 24 h glycaemic responses to interrupting sitting in free-living conditions has not been studied in overweight and obese individuals and this therefore requires investigation. This is important, as adiposity is a major risk factor for insulin resistance and T2D (Field *et al.*, 2001).

1.1 Aim

The aim of this study was to examine 24 h interstitial glycaemic responses to interrupting prolonged sitting in free-living conditions in overweight and obese inactive and sedentary adults.

1.2 Hypothesis

It was hypothesised that 24 h interstitial glycaemia concentrations would be attenuated during a 4-day period of interrupted sitting compared with a 4-day period of uninterrupted sitting.

2. Literature Review

2.1 Sedentary behaviour and physical inactivity

Sedentary behaviour and physical inactivity are distinct from one another. Physical inactivity has been defined as a level of physical activity insufficient to meet current recommendations of physical activity (World Health Organization, 2010). Whereas sedentary behaviour refers to any behaviour characterized as any waking behaviour which expends energy at ≤ 1.5 metabolic equivalent of task (METs) whilst in a sitting, reclining, or in a supine position (Tremblay et al., 2017).

2.1.1 Physical inactivity

An individual can be sedentary and physically active and therefore they need to be considered as two separate entities. Interventions designed to increase physical activity may be limited in reducing sedentary behaviour as individuals may feel that they have been sufficiently active and may rest for the remainder of the day, feeling satisfied that they have met physical activity guidelines (Prince *et al.*, 2014). Furthermore, increasing physical activity without decreasing time spent in sedentary behaviours has shown to have no protective effects on health (Ekelund *et al.*, 2016). It is proposed that a multitude of genes and molecular processes are inhibited by physical inactivity, which are activated by exercise (Owen *et al.*, 2010a). In contrast to the above definition of sedentary behaviour, physical inactivity refers to not meeting specific physical activity guidelines i.e. 150 min of moderate aerobic physical activity, 75 min of vigorous physical activity, or a sufficient combination of the two intensities (World Health Organisation, 2010.) Therefore sedentary behaviour and physical activity can coincide during a 24 h period, for example, an office worker who sits for 70 % of the day may also engage in sufficient levels of MVPA (Dempsey *et al.*, 2014). Furthermore, the opposite can also occur, for example an individual may not engage in sedentary pursuits and engage in light intensity physical activity such walking, standing and light ambulation but not meet the recommended physical activity levels (Van der Ploeg & Hillsdon, 2017). Independent of meeting public health physical activity recommendations (150 min/week of MVPA), adverse cardiometabolic outcomes are still present when

prolonged bouts of sedentary behaviour, such as uninterrupted sitting occurs (Dempsey *et al.*, 2014). Furthermore, it has been proposed that engaging in high levels of moderate physical activity (60 – 75 min/day) may negate the increased risk of mortality associated with high levels of un interrupted sitting (Ekelund *et al.*, 2016). From these definitions it is clear that sedentary behaviour is treated as a separate and unique paradigm from physical inactivity (Hamilton *et al.*, 2004; Hamilton *et al.*, 2007; Owen *et al.*, 2010b).

2.1.2 Sedentary behaviour

Sedentary behaviour refers to any waking behaviour which expends energy at ≤ 1.5 metabolic equivalent of task (METs) whilst in a sitting, reclining, or in a supine position (Tremblay *et al.*, 2017). However, multiple definitions have been used to define sedentary behaviour within existing literature (Barnes *et al.*, 2012; Tremblay *et al.*, 2017). Sedentary pursuits include sitting at a desk, sitting in a vehicle and sitting watching television. Modern lifestyles may augment the engagement in sedentary behaviours, with technological and computer advancements such as automation of chores at home, and transportation trends potentially playing a role in the increased prevalence of sedentary behaviour (Hamilton *et al.*, 2007). Compared to predecessors, current environments limit physical activity and are conducive to prolonged sitting (Owen *et al.*, 2010a). With these advancements in technologies of modern life, such as transportation, communication, and work place productivity, the need for physical activity has decreased since the 1960's (Church *et al.*, 2011).

It is important to highlight that sedentary behaviour is distinct from lack of physical activity as it has independent effects from inactivity including metabolism, physical function and certain health outcomes (Venables and Jeukendrup, 2009; Edwardson *et al.*, 2012; Proper *et al.*, 2011; Van Uffelen *et al.*, 2010).

2.1.3 Prolonged sitting bout

There is much discrepancy in the literature regarding the definition of a “prolonged bout”. Prolonged bouts of sitting have typically been reported as being > 30 min of uninterrupted sitting (Dempsey *et al.*, 2016a; Healy *et al.*, 2013; Henson *et al.*, 2016; Larsen *et al.*, 2015; Peddie *et al.*, 2013; Thorp *et al.*, 2014). However, several studies have reported prolonged sitting bouts as > 20 min of uninterrupted sitting (Bailey and Locke, 2015; Brocklebank *et al.*, 2017; Crespo *et al.*, 2016; Dunstan *et al.*, 2012b). Interrupting bouts of sitting every 20 min has shown to be advantageous to metabolic health and is associated with lower post-prandial glucose and insulin levels (Dunstan *et al.*, 2012b). In line with the current literature, the mention of a prolonged bout of sedentary time will refer to > 30 min of uninterrupted sitting.

2.1.4 Measuring physical activity, sedentary behaviour and physical inactivity

Sedentary behaviour can be measured either subjectively or objectively. Subjective measures include self-report questionnaires, proxy report questionnaires, and diaries. Objective measures include accelerometer, posture monitors, heart rate/ combined sensing, and multi-unit monitors. Subjective measures such as diaries and questionnaires are a cost effective, readily available and are able to capture the type of behaviour in the context of which it occurs (Atkin *et al.*, 2012). Despite the strengths identified of subjective measures, self-report measures consistently demonstrate poor validity, and are vulnerable to social influences and cultural norms (Atkin *et al.*, 2012). The use of objective measures such as accelerometry and posture monitors is increasing. Accelerometry has been widely used to measure sedentary behaviour and feasibility has been well established. Posture monitors are widely used in sedentary behaviour research and feasibility has been indicated. Furthermore, the participant burden of these devices have been classed as low to moderate. Accelerometers can detect total sedentary time including bouts of sedentary time and breaks taken. Posture monitors such as activPAL, can record time spent sitting, standing, and posture transitions. Posture devices differ from accelerometers, as they are able to distinguish between sitting and standing, whereas accelerometers may miss these breaks from sitting if no ambulation occurs. However, for posture monitors, validation in free-living

conditions is lacking (Atkin *et al.*, 2012). The activPAL is a small electronic device affixed to an individual's skin on the midline of the anterior aspect of the thigh. The small device determines posture on the basis of thigh acceleration, including gravitational component and uses proprietary algorithm (Harrington *et al.*, 2011).

2.1.5 Prevalence of sedentary behaviour

Sedentary behaviours are highly prevalent and data suggests the majority of time awake is spent in sedentary pursuits in adults (Hansen *et al.*, 2012; Matthews *et al.*, 2008). It has been reported that adults spend between 55 - 70 % of their waking day in a sedentary behaviour, which equates to 9 – 11 h/day (Matthews *et al.*, 2008; Colley *et al.*, 2011). Sitting in itself is not dangerous to health, but problematic in high doses. An increase in risk of all-cause mortality between all-cause mortality and sitting for more than 7-8 hours a day. Findings from prospective cohort studies report an association between self-reported sitting of > 8 h/day compared to < 3 h/day, and all-cause mortality in 83,034 with a follow-up period of 8.7 y (Inoue *et al.*, 2008). Additionally, another prospective cohort study, by Patel (2010) reported an association with cardiovascular mortality and self-reported sitting time < 3 vs ≥ 6 h/day.

However, an observational study of 6,329 participants suggested that adults ≥ 20 y spent between an average of 7.48 – 9.28 h/day sedentary (Matthews *et al.*, 2008). Matthews and colleagues (2008) measured physical activity objectively via an Actigraph accelerometer, which measured physical activity in 1 minute epochs, with the cut-point for sedentary behaviour as < 100 counts/min. However, this study by Matthews *et al.*, (2008) did not investigate time spent in physical activity levels; light, moderate and vigorous-intensity, therefore high levels of sitting and low levels of physical activity cannot be postulated from these data (Ekelund *et al.*, 2016). It is important to highlight that time spent wearing the waist-worn accelerometer in this study was less than previously reported for waking hours in other national surveys (Matthews *et al.*, 2008).

Conversely, an average of 8.4 h/day spent sedentary has been reported accelerometer derived data from 173 adults (53.3 ± 11.9 y) over a 7-day period (Healy *et al.*, 2007).

Participants were asked to wear an Actigraph and were provided with a diary to record non-wear and sleep times. A cut-off point of < 100 counts/minute was chosen to categorise sedentary (Healy *et al.*, 2007) Observations by Healy *et al.*, (2007) did not report patterns of sedentary time. Data from an observational office based study, measuring total sitting time in 170 adults (40.1 ± 12.7 y, 24.5 ± 3.8 kg/m²) found on workdays participants spent a significantly greater proportion of time in sedentary pursuits and significantly less time in light-intensity physical activity compared to non-working days (Clemes *et al.*, 2014). Participants wore a waist-worn accelerometer, Actigraph, for 7 days with the sedentary cut-point being <100 counts/min. On workdays, sedentary behaviours during working hours accounted for 68 % of total daily sitting (Clemes *et al.*, 2014). Furthermore, from 17:00 (on working days) participants were observed to steadily increase their sitting time in a linear fashion until participants went sleep, to reach 71 % of the waking hours sedentary (Clemes *et al.*, 2014). Individuals in predominantly desk bound occupations have a high prevalence of sedentary behaviour throughout the waking day and it can be postulated that those individuals may be at an increased risk of adverse health outcomes.

A limitation of with the above mentioned studies (Matthews *et al.*, 2008; Healy *et al.*, 2007; Clemes *et al.*, 2014) that used accelerometer data from waist worn devices such as Actigraph, are unable to determine changes in posture from sedentary to standing. Moreover, high cut-off points such as < 100 counts/min for sedentary time may miss periods of minimal ambulation that can occur throughout the day, such as filling paperwork. Light ambulation activities such as this has been reported to register at 60 counts/ min (Crouter SE, Clowers KG, Bassett DR Jr., 2006). Furthermore, it has been suggested that different cut-point used for Actigraph devices may influence estimates of physical activity intensity and the prevalence of meeting physical activity guideline (Loprinzi *et al.*, 2012). As ambulatory physical activity is an important component of total energy expenditure, capturing this activity is vital to better understand and measure the prevalence of sedentary behaviours (Crouter SE, Clowers KG, Bassett DR Jr., 2006). Finally, participants may fail to comply with study protocols as the Actigraph is not worn during sleep and requires the participants to remove the device just before sleeping and replace upon waking up. Time spent sedentary in the evening or just before sleep could be missed if the device is removed too early or replaced too late after waking up. Therefore, more direct measures should be used to estimate physical activity and sedentary levels with devices such as ActiPAL.

2.1.6 Associations of total sedentary time with glycaemia

It is thought that physical inactivity and sedentary behaviour are main factors in the onset of many chronic diseases (Bergouignan *et al.*, 2011). However, these findings were derived from bed-rest studies, which may not reflect the level of physical inactivity of the general population. Nevertheless, total physical inactivity through bedrest has shown to induce the following: ectopic fat storage, decreased fat oxidation, lipid signalling between adipose tissues and muscle, and insulin resistance (Bergouignan *et al.*, 2011). It is hypothesised that the influence of sedentary behaviour on metabolic and cardiovascular risk may be mediated through the cation of insulin, the upregulation of lipoprotein lipase activity and triglyceride uptake (Peddie *et al.*, 2013). Additionally, the aforementioned metabolic alterations are

more prevalent within obese populations resulting in a potential higher risk for these populations (Bhupathiraju, 2016).

Total sedentary time, both objectively and subjectively measured, may be adversely associated with markers of cardiometabolic risk including abnormal glucose metabolism (Dunstan *et al.*, 2004; Healy *et al.*, 2008). Objectively measured free-living physical activity from 173 individuals (53.3 y, 27.2 kg/m²) found a significant association between higher sedentary time and higher 2 h glycaemic response compared to individuals with lower levels of sedentary behaviour (Healy *et al.*, 2007). However, it is not known from data available, how sedentary behaviour is accumulated throughout the waking day, which has been suggested to be pertinent in glucose metabolism (Dunstan *et al.*, 2004; Healy *et al.*, 2008). Observational data shows that after adjusting for potential confounders, high levels of sedentary behaviour were associated with significantly higher postprandial plasma glycaemia (Healy *et al.*, 2008). Data from 168 participants demonstrated that individuals with the least interruptions to sitting had significantly higher 2 h postprandial glycaemic response compared to individuals with the highest frequency of interrupted sitting (Healy *et al.*, 2008). Moreover, adverse glycaemic response may be associated with the duration of sedentary bouts, and not only the total time spent sedentary (Healy *et al.*, 2008). Therefore, sedentary behaviour accumulated in uninterrupted bouts may be detrimental to glycaemic metabolism and potentially increase the risk of developing CVD and T2D.

2.1.7 Associations of interruptions in sedentary behaviour with glycaemia

It has been suggested that replacing sedentary time with standing or light intensity activity may assist in the prevention of major non-communicable disease (Smith *et al.*, 2015). Regular ingestion of high-calorie meals, in which carbohydrate accounts for a high percentage of macronutrient composition, can lead to transient exaggerated postprandial spikes in glycaemia (Dunstan *et al.*, 2012b). These postprandial excursions, when repeated throughout the day, may generate an internal environment for the pathogenesis of CVD and T2D (O'keefe and Bell, 2007). Elevated postprandial glycaemic response may exhibit greater degrees of adverse pathogens, namely oxidative stress, compared to sustained

hyperglycaemia (Monnier *et al.*, 2006). Interrupting sedentary behaviour with light-intensity physical activity (LIPA) may be an effective mode for acutely reducing postprandial glycaemic spikes (Dunstan *et al.*, 2012b).

Individuals with higher levels of MVPA and increased LIPA time had significantly lower postprandial plasma glycaemia (Healy *et al.*, 2008). This association was observed following adjustment for potential confounding factors including WC, age, sex, and wear time of accelerometer (Healy *et al.*, 2008). Fewer interruptions in sedentary time are significantly associated with higher WC and higher post-prandial glycaemic response (Healy *et al.*, 2008). However, observations in glycaemic response to sedentary behaviour may be confounded by the influence of wearing activity monitors on participants behaviour, which thus may not reflect true habitual activities. It has previously been suggested that activity monitors may influence activity patterns of participants due to their awareness that they are being measured (Busmann *et al.*, 2001). Although beneficial glycaemic responses were observed in response to interrupting sitting with LIPA and MVPA (Healy *et al.*, 2007), it cannot be determined that this is a chronic response to interrupting sitting, as participants may have engaged in higher volumes of MVPA and LIPA to respond to demand characteristics (Fahrenberg, 1996; Busmann *et al.*, 2001).

Hyperglycaemia is one component of the metabolic syndrome (Alberti *et al.*, 2005). The likelihood of having the metabolic syndrome increased as sedentary time duration, percentage of sedentary time, and average length of sedentary bout increased (Bankoski *et al.*, 2011). Furthermore, the risk of insulin resistance, and dysglycaemia also decreases as the number of breaks from sedentary behaviour increases (Bankoski *et al.*, 2011). Another study which evaluated the associations of sedentary behaviour and glycaemic control detected a relationship between sedentary time and the metabolic syndrome (Bankoski *et al.*, 2011). Observational data from 1,367 men and women, aged ≥ 60 y was used to compare fasted glycaemic response to habitual sedentary behaviour in individuals with metabolic syndrome and those without metabolic syndrome (Bankoski *et al.*, 2011). Authors observed that individuals without metabolic syndrome had greater interruptions to sitting and

accumulated greater physical activity intensity during time spent sedentary compared to those with metabolic syndrome (Bankoski *et al.*, 2011).

Conversely, data pooled from the two prevention studies Project STAND (Sedentary Time And Diabetes; Yates *et al.*, 2012) and the Walking Away from Type 2 Diabetes Study (Wilmot *et al.*, 2011) demonstrated breaks in sedentary time, total physical activity, and MVPA were significantly inversely associated with adiposity measures, but not glycaemic measures (Henson *et al.*, 2013). The study by Henson *et al.*, (2013) was unable to further evidence previously noted beneficial glycaemic responses to interrupting sedentary behaviour. From observational studies, it is not possible to determine the minimum amount of time or intensity for interrupting sedentary behaviour that may be essential to result in glycaemic attenuation (Healy *et al.*, 2008). For Healy *et al.*, (2008) and Bankoski *et al.*, (2011) the threshold that were included as interruptions to sitting were measured from ≥ 100 counts per min for 1 min, using an accelerometer algorithm . Therefore, it may be postulated that if interruptions to sitting are accumulated at the lower end of the activity count threshold, this intensity may be insufficient to result in glycaemic improvement to interrupting sitting in observational studies.

From the epidemiological studies conducted, disparities in the effectiveness of reducing postprandial glycaemia via interrupting sitting have been observed. Furthermore, the acute effects of interrupting sedentary behaviour with LIPA are short term and may not translate to metabolic benefit over longer time scales (Van Dijk *et al.*, 2013). Therefore, investigation of glycaemic response to interrupting sitting over multiple days in those at an increased risk of insulin resistance and T2D is required.

2.2. Cardiometabolic risk factors

Cardiometabolic risk factors include abdominal adiposity, hypertension, dyslipidaemia, hyperinsulinemia and glucose intolerance. When clustering of these risk factors occurs this may lead to an increased risk of cardiovascular disease (CVD) and T2D (Fisher, 2006; Pescatello and Vanheest, 2000).

2.2.1 Type 2 Diabetes

The incidence of all diabetes has reached approximately 415 million people worldwide and is expected to rise in subsequent years (Cho, 2016). Adults with T2D account for around 90 % of all diabetes cases (Health and Social Care Information Centre: National Diabetes Audit 2012/13). The prevalence in the United Kingdom (UK) has increased by 1.74% in the years from 2004 to 2014, with men having higher incidences of T2D compared to women (Zghebi *et al.*, 2017). Almost 3.5 million people in the UK have been diagnosed with diabetes (HSCIC: National Diabetes Audit 2012/13). Furthermore, the financial burden upon the National Health Service (NHS) is estimated to be £10 billion a year, which equates to 10 % of the NHS budget. Primary care is just a fraction of the cost involved, direct and indirect care costs combined are estimated to be £23.7 billion a year (HSCIC: National Diabetes Audit 2012/13), further augmenting the need for prevention of T2D.

T2D is the outcome of interactions between ecological factors and genetic components that affect beta-cell function and tissue insulin sensitivity (Scheen, 2003; Kahn, 2001). Insulin is a polypeptide hormone produced by beta-cells within the pancreas, in response to circulating carbohydrates postprandially. Insulin resistance and insulin secretion defect are both important factors in the pathogenesis of T2D, although the mechanisms regulating the interaction between these two deficiencies and the sequence in which they occur remains unclear (Scheen, 2003). T2D differs from the autoimmune disease, Type 1 diabetes, in which a complete loss of insulin-producing cells from the pancreas occurs. Type 2 diabetes is the dysfunction of beta-cells (Kahn, 2001) and the failure of insulin to produce its homeostasis effect on target organs (skeletal and cardiac muscles, liver and adipose tissue), leading to continuous endogenous glycaemia production, despite hyperglycaemia (Ali, 2013). T2D is clinically diagnosed when a 2 h oral glucose tolerance test (OGTT) or fasting plasma glucose test provides results of ≥ 11 mmol/L and ≥ 7 mmol/L, respectively (American Diabetes Association, 2014).

Sustained hyperglycaemia impairs-stimulated glycaemia utilisation in the skeletal muscle, also referred to as glycaemia toxicity (Kurowski *et al.*, 1999) Hyperglycaemia from uncontrolled glycaemia regulation is known to cause oxidative stress and is recognised as a

causal role in the development of diabetes. Disproportional proton gradient caused by oxygen consumption leaks, due to high glycaemia states, producing reactive oxygen species (Rolo and Palmeira, 2006). Furthermore, reactive oxygen species may also be associated with the pathologic process of T2D (Rolo and Palmeira, 2006). Hyperglycaemia-induced increases in the electron transfer donors (NADH and FADH₂) increases electron flux through the mitochondrial electron transport chain, increasing Adenosine-triphosphate and Adenosine-diphosphate ratio and mitochondrial membrane potential. This leads to partial reduction of O₂ to generate reactive oxygen species (Rolo and Palmeira, 2006). Oxidative stress markers have been observed in non-diabetic obese individual which further supports the hypothesis that oxidative stress plays a role in the pathogenesis of T2D (Houstis *et al.*, 2006) Therefore, insulin-mediated glycaemia disposal is inhibited in individuals with T2D (Scheen, 2003). Oxidative stress increases during postprandial periods, when glycaemia directions fluctuate rapidly, compared to sustained elevation of hyperglycaemia (Monnier *et al.*, 2006). Therefore, oxidative stress is associated with the pathogenesis of diabetes via dysregulation of cell metabolism and homeostasis, resulting in insulin resistance in diabetics (Pitocco *et al.*, 2013).

Beta-cell dysfunction exists in individuals who are at high risk of developing the disease even when their glycaemia levels are still within normal range and beta-cell function decreases proportionally to increasing fasting glycaemia levels (Kahn, 2001; Kahn *et al.*, 2006).

2.2.2. Cardiovascular disease

The term cardiovascular (CVD) refers to disease of the heart and blood vessels, and is an umbrella term that includes coronary heart disease, stroke, cardiomyopathy, and heart failure (Wallace *et al.*, 2010). CVD, and their consequential outcomes are the most significant health issue of the western hemisphere regarding cost and prevalence (Lloyd-Jones *et al.*, 2009). Moreover, in 2014 the second main cause of death in the UK was CVD (Bhatnagar *et al.*, 2016). Although mortality rates in the UK are declining, the prevalence of

CVD for the UK is estimated to be ≥ 7 million (Bhatnagar *et al.*, 2016). The economic burden to the NHS is estimated to be £9 billion per year, and indirect costs incur a further £10 billion a year.

One regulatory pathophysiological aspect of the cardiovascular system includes reactive oxygen species which function as signals to regulate a range of processes within the cardiovascular system, including control of vascular muscle (Dröge, 2002). Oxidative stress, the disproportionate or continual imbalance between the production of reactive oxygen species and anti-oxidant defences, attributes to the pathogenesis of CVD (Touyz and Briones, 2011). Furthermore, oxidant stress mediates postprandial glycaemia and the response in reactive oxygen species is proportional to the increase in glycaemia postprandially (O'keefe and Bell, 2007). Postprandial oxidative stress may generate an increases in inflammation, and vasoconstriction of blood vessels, and increases oxidation of low-density lipoproteins, which may lead to atherosclerosis in T2D patients and non-diabetic individuals (Ceriello and Motz, 2004; Ferreira *et al.*, 2004; Weissman *et al.*, 2006).

2.2.3 Risk Factors for cardiometabolic disease and type 2 diabetes

Risk factors for CVD and T2D include visceral obesity, dyslipidaemia, hyperglycaemia, and hypertension (Alberti *et al.*, 2005). The thresholds for risk factors are as follows: visceral obesity as measured by waist circumference (WC) ≥ 94 cm and ≥ 80 cm for men and women respectively; raised triglycerides > 1.7 mmol/L; reduced HDL < 1.03 mmol/L and < 1.29 mmol/L for men and women respectively; raised blood pressure systolic ≥ 130 mm Hg and diastolic ≥ 85 mmHg; raised fasting plasma glycaemia ≥ 7 mmol/L (Alberti *et al.*, 2005); and 2 h postprandial glycemia 7.8 -11.0 mmol/L (World Health Organisation, 1999).

2.2.4 Overweight and obesity as a risk factor for cardiovascular disease and type 2 diabetes

Obesity is a major risk factor for T2D with trends and prevalence and incidences of T2D closely emulating those of obesity. Analysis of epidemiological found that the majority of the increase in T2D is due to the increased prevalence of obesity, with 85 % of those with T2D

being overweight or obese (Bhupathiraju, 2016). Individuals who are overweight (BMI ≥ 25.0 to ≤ 29.9 kg.m²) may be at an increased risk of developing T2D and CVD than their healthy weight counterparts (Field *et al.*, 2001). Obese adults with a BMI ≥ 30.0 kg.m² may have further increased risk of developing T2D and CVD (Field *et al.*, 2001). Individuals with a BMI ≥ 35 kg.m² are 20 times more likely to develop T2D over a 10 y period compared to those with a BMI ≤ 25.0 kg.m² (Field *et al.*, 2001).

Obesity is a chronic metabolic disease that has a substantial influence on the CVD system (Poirier & Eckel., 2002). Obesity contributes to an array of structural and functional adaptations to the cardiovascular system. These include; lower cardiac output, augmented peripheral resistance, left ventricular mass and left ventricular wall thickness and a deterioration in left ventricular systolic function (Bastien *et al.*, 2014). Furthermore, obesity is known to affect coronary risk indirectly through its effect on related comorbidities such as dyslipidaemia, hypertension, endothelial dysfunction and inflammation, and glucose intolerance (Wilson *et al.*, 2002).

In most obese patients, obesity is associated with a low-grade inflammation of white adipose tissue resulting from chronic activation of the innate immune system and which can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes (Bastard *et al.*, 2006). For obesity and insulin resistance to be associated with T2D, beta-cells must be unable to compensate wholly for attenuated insulin sensitivity (Kahn *et al.*, 2006; Kahn, 2001). However, obesity alone can cause some level of insulin resistance (American Diabetes Association, 2014). For instance, in obese individuals adipose tissue releases increased amounts of free fatty acids, glycerol, hormone, and pro-inflammatory cytokines that are factors involved in the development of insulin resistance (Kahn *et al.*, 2006). One theory suggests that limited ability of subcutaneous fat depots to store excess energy intake results in an excess of electron flux to intra-subcutaneous abdominal adipose tissue and abnormal sites, including liver and skeletal muscle (Seppälä-Lindroos *et al.*, 2002). An accretion of ectopic fat (desposition of lipids within cells of non-adipose tissues, which typically only store small amounts of fat) distribution in turn leads to metabolic dysfunction in the above mentioned organs (Seppälä-Lindroos *et al.*, 2002). Insulin

resistance is correlated with intrahepatic fat or “fatty liver”, and increased intramyocellular fat in the liver and skeletal muscle, respectively (Sinha *et al.*, 2002; Shulman, 2014). Non-diabetic individuals with impaired fasting glycaemia and/or impaired glycaemia tolerance postprandially have increased risk of T2D and CVD (American Diabetes Association, 2014).

Sedentary behaviour or prolonged sitting may be conspicuous in the development of these detritus metabolic and cardiometabolic risk profiles. It is proposed that the cumulative effect of too much sitting over time may contribute to shifting energy balance towards weight gain and obesity (Swartz *et al.*, 2011). Observational research suggests higher total sitting time and sitting time accumulated in prolonged bouts (> 30 min) are associated adversely with cardiometabolic risk profiles, and with premature death from cardiovascular disease and all causes. It is proposed a mechanistic contender underlying these associations is lack of muscle contractile activity. A lack of skeletal muscle activity may lead to a reduction in skeletal muscle glucose uptake via reduced translocation of GLUT4 to the cell surface and lowered efficiency of insulin (Homer, Owen, & Dunstan, 2018). Although results from meta-analysis and systematic review by Campbell *et al.*, (2017) indicated no association with sedentary behaviour and obesity. Furthermore, Campbell and colleagues postulated that the harmful effects of sitting on cardiometabolic risk are likely mediated through mechanisms other than a direct effect on obesity (Campbell *et al.*, 2017).

Waist circumference is suggested to be a more valid indicator of cardiometabolic disease risk compared with BMI (Janssen *et al.*, 2004). The recommended thresholds, highlighted above, are derivative from WC that are correlated with a BMI ≥ 30 kg.m² in relation to cardiometabolic risk (Health, 1998). Moreover, WC is associated with an increased risk of cardiometabolic disease and T2D (American Diabetes Association, 2014). Additionally, WC, as a measure of fat distribution around the abdominal region, is effective and economical at ascertaining increased adiposity measurements compared to BMI (American Diabetes Association, 2014). It is proposed that WC can identify individuals at a greater cardiometabolic risk than using BMI alone (American Diabetes Association, 2014) and the relationship between health outcomes and WC has less variability compared to BMI (Visscher *et al.*, 2001). Epidemiological studies have found WC to be a strong correlate of

T2D, and independent of BMI (Janssen *et al.*, 2004). Moreover, abdominal obesity is associated with postprandial glycaemia and impaired fasting glycaemia (American Diabetes Association, 2014). Therefore, it can be postulated that WC is a greater predictor of adverse health outcomes compared to BMI alone.

2.2.5 Postprandial glycaemia as a risk factor cardiovascular disease and type 2 diabetes

Seemingly healthy populations with impaired glycaemia metabolism, or dysglycaemia, are at an increased risk of a cardiac event and diabetes onset (Bergman *et al.*, 2013). Dysglycaemia has been defined previously as fasting glycaemic concentrations between 5.6 and 6.1 mmol/L (Gerstein, 2010). However, it has also been suggested that dysglycaemia is a fasting glycaemic concentration of ≥ 6.1 mmol/L (Takeno *et al.*, 2008). Elevated fasted and postprandial glycaemia concentrations in non-diabetic, healthy populations are risk markers for CVD (Levitan *et al.*, 2004). Impaired fasting and postprandial glycaemic response have heterogeneous pathogenesis and therefore may contribute to different rates of progression to T2D (Nathan *et al.*, 2007). Additionally, with non-diabetic populations, fasting blood glycaemia concentrations are modestly and non-linearly associated with risk of vascular disease (Emerging Risk Factors Collaboration, 2010). Conversely, an epidemiological study observed impaired fasting glycaemia to not be a risk factor for CVD mortality (Tominaga *et al.*, 1999).

However, epidemiological studies have revealed postprandial hyperglycaemia to better predict future CVD mortality compared to fasting hyperglycaemia in diabetics and normoglycaemic individuals (Mah and Bruno, 2012). Furthermore, elevated postprandial glycaemic response may be a stronger risk factor for CVD and greater indicator of CVD and T2D risk, compared to fasting hyperglycaemia (Bonora and Muggeo, 2001). Acute hyperglycaemia exacerbates vascular endothelial dysfunction in individuals with chronic hyperglycaemia and transiently impairs vascular function in healthy individuals (Mah and Bruno, 2012). Non-diabetic individuals with moderately higher glycaemic responses (< 7 mmol/l) to an OGTT have an increased cardiovascular risk than their normoglycaemic

(fasting blood glycaemia 3.9 – 5.6 mmol/L) counterparts (World Health Organisation, 1999; Hermans *et al.*, 1999).

Sustained postprandial hyperglycaemic bouts (≥ 7 mmol/L) are associated with micro and macrovascular damage, and damage to internal organs. However, adverse health outcomes may not develop until postprandial glycaemic values exceed ≥ 11 mmol/L (Stratton *et al.*, 2000). Therefore, identifying and reducing consistently elevated postprandial glycaemic concentrations in non-diabetic populations is of public health interest to attenuate the risk of T2D.

2.2.6 Sedentary behaviour as a risk factor for cardiovascular disease and type 2 diabetes

Physical inactivity and large volumes of sedentary time are independently associated with increased cardiometabolic morbidity and mortality in a dose-dependent manner (Bassuk and Manson, 2005). Adults at a high risk of T2D (BMI ≥ 25 kg/m², high waist circumference, low physical activity levels, and being ≥ 40 y, or ≥ 25 y, and some black and minority ethnic groups), the time spent sedentary is strongly and adversely associated with cardiometabolic health and may be a more important indicator of poor health compared to moderate–vigorous physical activity (MVPA) levels (Henson *et al.*, 2013; Harding *et al.*, 2006).

Active individuals that engage in the recommended intensity and duration (150 min/week MVPA) of physical activity are not protected against the adverse health outcomes associated with sedentary behaviour (Ekelund *et al.*, 2016). Although meeting the recommended physical activity guidelines does not eliminate mortality risk, a smaller increase was observed in those that did meet these recommendations compared to those who engaged in ~ 35 min of moderate intensity physical activity per week (Ekelund *et al.*, 2016). However, those engaging in high volumes (60-75 min/day) eliminate the increased mortality risk associated with high sitting time (Ekelund *et al.*, 2016). Therefore, interventions in inactive individuals need further investigation to attenuate the increased mortality risk associated with sedentary behaviour. High volumes of sitting, typically assessed as total daily sitting or time spent viewing television, have been associated with increased risk of all-cause mortality.

Data pooled from 16 studies, analysing over 1 million individuals suggests that individuals who spend ≤ 4 h/day and engaged in low levels of physical activity (≤ 2.5 MET-hour/week) had higher all-cause mortality rates compared to individuals who sat ≤ 4 h/day and engaged in high levels of physical activity (≥ 35.5 MET-hour/week) (Ekelund *et al.*, 2016). However, data suggests that individuals who sat 8 h/day and engaged in high levels of physical activity had increased risk of all-cause mortality compared to individuals who sat ≤ 4 h/day and engaged in high levels of physical activity (≥ 35.5 MET-hour/week) (Ekelund *et al.*, 2016). Therefore, high levels of physical activity may protect against adverse health outcomes of sedentary behaviour.

Associations of CVD mortality risk with daily sitting are estimated to be 2.7 times greater in those with high levels of sitting compared to those with low levels of sitting (Weller and Corey, 1998). Furthermore, uninterrupted sitting, an accumulation of sedentary time in an extended continuous bout (Tremblay *et al.*, 2017), predicted increased CVD risk in 73,743 women independent of age and recreational energy expenditure (Manson *et al.*, 2002). However, when the association between biomarkers of CVD risk and weekly time spent TV viewing was investigated there were no significant relationships with low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, insulin, C-peptide, and glycated haemoglobin over an 8 y period (Fung *et al.*, 2000).

Commuting to work via a car is classified as a sedentary activity. Owning a car and/or TV is associated with increased risk of acute myocardial infarction (Van Craenenbroeck and Conraads, 2012). A longitudinal cohort study followed participants for 21 y and measured the associations between sedentary behaviours and cardiovascular disease mortality rates. Out of 7,700 men, those who reported sitting in a car for > 10 h/week had a 50 % increased risk of dying from CVD compared to those who reported < 4 h/week (Warren *et al.*, 2010). When reported hours for sedentary behaviours were combined (viewing TV and sitting in a car), those that reported > 23 h/week of sedentary behaviour had a 37 % increased risk of CVD mortality compared to those that had reported < 11 h/week (Warren *et al.*, 2010). Additionally, a cross sectional study showed time spent in cars to be associated with higher BMI, WC, and cardiometabolic risk score (Hoehner *et al.*, 2012).

Furthermore, sedentary behaviour may contribute to weight gain and obesity which increases the risk of developing T2D and CVD (Field *et al.*, 2001). A recent systematic review reported that only WC and sedentary behaviour are significantly associated with one another whereas body weight, BMI, and % of body fat were not associated with sedentary behaviour (Campbell *et al.*, 2017). It was reported that each 1 h/day increase in sedentary behaviour resulted in 0.02 cm WC increase over five years (Campbell *et al.*, 2017).

2.3 Experimental research on glycaemic response to interrupting sitting

Presently, experimental evidence suggests that both LIPA and MVPA interruptions to sitting have acute beneficial effects on glycaemic control (Chastin *et al.*, 2015a). The evidence for postprandial glycaemic attenuation from interrupting prolonged sedentary behaviour has largely been observed over an acute period typically lasting up to one day (Bailey *et al.*, 2015; Dempsey *et al.*, 2014; Dunstan *et al.*, 2012b; Pulsford *et al.*, 2017). However, some studies have investigated postprandial glycaemic response over longer durations of 3 - 8 days (Duvivier *et al.*, 2016; Duvivier *et al.*, 2013; Thorp *et al.*, 2014; Larsen *et al.*, 2015; Altenburg *et al.*, 2016). The details of such studies are outlined in Table 1 and are discussed in the following sections in more detail.

A recent study examined the response of postprandial glycaemia in response to interrupting sitting over one and three days, and found that there may be different pathways in which postprandial glycaemic concentration is reduced (Bergouignan *et al.*, 2016). Interrupting prolonged sitting with light and moderate-intensity physical activity bouts over one day have been suggested to stimulate contraction-mediated glycaemia uptake pathways (Bergouignan *et al.*, 2016). Furthermore, moderate-intensity physically activity bouts over one day may increase GLUT4 translocation, glycogen synthesis, and oxidative phosphorylation (Bergouignan *et al.*, 2016). Additionally, LIPA interruptions over three days have been suggested to stimulate contraction-mediated and insulin-mediated glycaemia uptake pathway and GLUT4 translocation (Bergouignan *et al.*, 2016).

2.3.1 Laboratory studies on glycaemic response to interrupting sitting

2.3.2 Interrupting prolonged sitting with walking

Research has demonstrated that interrupting sitting with 2 min bouts of light intensity walking every 20 min for 5 – 7 h results in a decrease in glucose total area under the curve (AUC) and incremental area under the curve (iAUC) by 9 % to 55 % compared with uninterrupted sitting in laboratory environments (Brocklebank *et al.*, 2017; Pulsford *et al.*, 2017; Dunstan *et al.*, 2012b; Larsen *et al.*, 2015; Bailey and Locke, 2015). However, the greatest reduction seen was when

interstitial glycaemia concentrations were measured over 5 h in response to a test meal drink in a mixture of healthy weight, overweight, and obese adults (Brocklebank *et al.*, 2017). In comparison, lower glycaemia attenuation has been observed in obese and overweight individuals when glycaemia was measured via capillary and venous blood samples in response to an OGTT or standardised mixed meals (Dunstan *et al.*, 2012b; Pulsford *et al.*, 2017; Larsen *et al.*, 2015; Bailey and Locke, 2015). This suggests that analysing singular capillary or venous blood samples over multiple periods (e.g. every 30 min) over a 2 h postprandial period may miss potential important glycaemic responses in overweight and obese individuals and underestimate attenuation in response to interrupting sitting time. Therefore, measuring glycaemic response with samples at a higher frequency (e.g. every 15 min) over a longer duration, may provide deeper insight into postprandial glycaemic response to interrupting sitting.

Overweight and obese adults and those diagnosed with type 2 diabetes may have the greatest magnitude in attenuation of postprandial glycaemic response to interrupting sitting with light intensity walking (Dempsey *et al.*, 2016a; Dunstan *et al.*, 2012b; Henson *et al.*, 2013; Larsen *et al.*, 2015). One such study instructed overweight and obese participants to rise from a seated position every 30 min and walk on a treadmill for 3 min at 3.2 km.h⁻¹ and then return to the seated position (Dempsey *et al.*, 2016a). Participants performed this posture change 12 times during 7 h, totalling 36 min of light-intensity walking (Dempsey *et al.*, 2016a). This physical activity intensity and frequency attenuated postprandial plasma iAUC glycaemic response by a statistically significant 38.8 % compared to 7 h of uninterrupted sitting (Dempsey *et al.*, 2016a). Furthermore, walking at 3.2 km.h⁻¹ for 2 min every 20 min over 5 h resulted in a significant reduction in positive iAUC postprandial glycaemia in overweight and obese participants by 24.1 % (Dunstan *et al.*, 2012b). Furthermore, a 9 % reduction in glycaemia AUC in response to light intensity walking for 2 min every 20 min was observed in inactive middle-aged men (Pulsford *et al.*, 2017). However, these studies only investigated the acute effects of interrupting sitting on glycaemic responses over a period of one day (Dempsey *et al.*, 2016a; Dunstan *et al.*, 2012b; Pulsford *et al.*, 2017), therefore, the persistent or carryover effects of light intensity walking in overweight and obese individuals cannot be postulated.

The persistent effects of interrupting sitting with light intensity walking has been examined over a 24 h period (Crespo *et al.*, 2016). Nine overweight and obese individuals interrupted sitting with four bouts of 10 min walking, two bouts of 20 min walking, and two bouts of 30 min walking at 1.0 mph over an 8 h interval, totalling 2.5 h of light intensity walking (Crespo *et al.*, 2016). Twenty-four-hour interstitial glycaemia was measured to observe three, 2 h postprandial episodes; breakfast between 0815-0845, lunch between 1200-1230 and dinner at participants elected time but standardised between conditions, and cumulative 6 h postprandial glycaemic response and glycaemic responses during sleep (Crespo *et al.*, 2016). A 24 % decrease in 6 h postprandial glycaemia iAUC was found for light intensity walking compared to uninterrupted sitting and these glycaemic benefits persisted overnight (Crespo *et al.*, 2016). Furthermore, a recent study investigated the 24 h carryover effects of interrupting sitting with light intensity walking over 7.5 h on postprandial glycaemic responses (Henson *et al.*, 2016). On day one, participants were asked to interrupt sitting with 5 min of walking at a self-perceived light intensity on a treadmill (rate of perceived exertion between 10 – 12) every 30 min. At 0800 h the next day, participants returned and remained seated for 7.5 h. Postprandial iAUC glycaemic response was significantly reduced by 28 and 17 % on day one and two, respectively (Henson *et al.*, 2016). Another study explored the cumulative effects of three consecutive days of interrupting sitting with light intensity walking on postprandial glycaemic response compared to uninterrupted sitting (Larsen *et al.*, 2015). Nineteen overweight and obese individuals were instructed to interrupt their sitting with treadmill walking at 3.2 km.h⁻¹ for 2 min every 20 min over a 6 h period for three days. Postprandial iAUC for glycaemia reduced by 32 and 31 % on day one and three, respectively for light-intensity walking (Larsen *et al.*, 2015). However, no effect of time was found across the three-day protocol. Therefore, three accumulative days of interrupting sitting does not enhance, but sustains, the magnitude of the reduction in plasma glycaemia concentrations, compared to one day of interrupting sitting.

Data indicates that interrupting prolonged sitting has the highest impact on metabolic health in individuals with strong risk factors for T2D, such as obesity, physical inactivity, and dysglycaemia (Liu, 2015). Interrupting sitting for 3 - 5 min every 30 min with moderate-intensity walking in overweight and obese individuals, and overweight and obese individuals with T2D, have resulted in

a higher attenuation in glycaemic response compared to when interrupting sitting occurred for 2 min every 20 min (Peddie *et al.*, 2013; Dunstan *et al.*, 2012b; Dempsey *et al.*, 2016a). Therefore, the frequency and time spent in light-intensity activity may be an important factor for glycaemic reduction in overweight and obese individuals. From the current literature, it appears that both light and moderate walking intensities attenuate postprandial glycaemia when interrupting sedentary behaviour, and thus both should be used in protocols aimed at reducing glycaemic responses.

2.3.3 Breaking up sitting with standing

Another method of interrupting prolonged sitting for postprandial glycaemic benefits is with standing.

However, a study examining postprandial glycaemic response to interrupting sitting with standing found no significant difference in glycaemia AUC compared to uninterrupted sitting (Pulsford *et al.*, 2017) Participants were instructed to interrupt their sitting for 2 min bouts of standing every 20 min over a duration of 7 h (Pulsford *et al.*, 2017). Furthermore, interrupting sitting with standing for 2 min bouts every 20 min over a 5 h period resulted in no significant for difference for glycaemia iAUC compared to uninterrupted sitting and light walking compared to uninterrupted sitting (Bailey and Locke, 2015). For both these studies participants were of a normal healthy weight (Pulsford *et al.*, 2017; Bailey and Locke, 2015) suggesting that interrupting sitting with standing for healthy weight individuals may not a great enough metabolic stimulus to reduce postprandial glycaemic response.

However, In overweight and obese individuals standing results in significant glycaemic attenuations (Henson *et al.*, 2016; Thorp *et al.*, 2014). Twenty-two overweight and obese dysglycaemic postmenopausal women interrupted sitting with standing for 5 min every 30 min over a 7.5 h period Henson, 2016 #40}. Glycaemia iAUC reduced by 34 %, compared to uninterrupted sitting during this period and was also reduced by 19 %the following day (Henson *et al.*, 2016). Furthermore, a significant decrease of 11.1 % was found for glycaemic iAUC response in overweight and obese adults when sitting was alternated with standing every 30 min, totalling 4 hours over 8 h (Thorp *et al.*, 2014). Glycaemic response to interrupting sitting was similarly

effective on day 1 and day 5 of this protocol, suggesting that there is no cumulative effect of interrupting sitting with standing (Thorp *et al.*, 2014).

Conversely, no significant difference for postprandial glycaemia was observed in overweight and obese individuals when prolonged or intermittent standing was performed over 8 h (Hawari *et al.*, 2016). Participants completed three protocols in a randomised cross over design: uninterrupted sitting for 8 h, sitting and prolonged standing for 15 min every 30 min totalling 4 h/day, and sitting with intermittent standing 10 times every 30 min totalling 4 h/day (Hawari *et al.*, 2016). This suggests that standing alone as a mode of interrupting sitting, irrelevant of the frequency of sit to stand transitions, may be unable to attenuate postprandial glycaemic responses in overweight obese individuals.

There is conflicting research regarding the benefits of interrupting sitting with standing on postprandial glycaemia. One explanation for the attenuation of postprandial glycaemia may be standing duration. Standing for 28 and 36 min, accrued in bouts of 2 min every 20-min, resulted in no significant difference in postprandial glycaemia over a 5 and 7 h period, respectively (Bailey and Locke, 2015; Pulsford *et al.*, 2017). However, standing for an accumulated 60 min and 240 min, resulted in significant glycaemic reduction over a 7.5 and 8 h period, respectively (Henson *et al.*, 2016;Thorp *et al.*, 2014). It is important to note that significant attenuation was observed in overweight and obese individuals who are at an increased risk of dysglycaemia and T2D (Liu, 2015)., compared to no benefit being observed in healthy weight individuals. Therefore, in dysglycaemic individuals, it could be postulated that significant beneficial impacts of standing on postprandial glycaemia may be dependent on standing duration.

2.3.4 Interrupting sitting with resistance exercise

There a limited number of studies (see Table 1) that investigate the effects of interrupting sitting with resistance exercise on glycaemic response (Dempsey *et al.*, 2016a; Fletcher *et al.*, 2017). It has been proposed that performing body weight resistance exercises utilises larger muscle groups compared to walking and standing and therefore may promote an increased energy expenditure and higher glucose uptake(Fletcher *et al.*, 2017).

Thirteen adolescents (16.4 ± 1.3 y, BMI 20.6 ± 2.5 kg.m⁻²) completed four experimental conditions with a minimum of 24 h wash out period between conditions (Fletcher *et al.*, 2017). Participants were instructed to perform 2 min of body weight resistance exercises (half squats, calf raises, knee lifts, and step ups) each for 30 s (Fletcher *et al.*, 2017). Four experimental conditions were as follows: two high energy diet conditions; uninterrupted sitting for 6 h, interrupting sitting 2 min of body weight resistance exercises (half squats, calf raises, knee lifts, and step ups) each for 30 s completed every 18 min, and two standard energy diet conditions with either uninterrupted sitting or interrupting sitting with bodyweight resistance exercises. Postprandial glycaemic response was measured over three-time periods; 2 h post the first meal, 2 h post the second meal, and over the entire 6 h trial duration (Fletcher *et al.*, 2017).

Glycaemic response was measured via an interstitial continuous glucose monitor (Fletcher *et al.*, 2017). Compared to sitting conditions, interrupting sitting conditions resulted in a significant reduction of 36.0 mmol.L.h and 35.9 mmol.L.h for iAUC for the first meal. However, this reduction was no longer significant over the 6 h period between sitting conditions and interrupting sitting conditions. However, this finding was observed in adolescents of normal weight, therefore they may have been more insulin sensitive and the effects of puberty may have increased postprandial glycaemic response variability within and between participants (Fletcher *et al.*, 2017).

In adults with T2D, simple resistance exercises (body weight half squats, calf raises, gluteal contractions and knee raises) when performed for 3 min every 30 min resulted in a 39.2 % reduction in glycaemia iAUC compared to uninterrupted sitting (Dempsey *et al.*, 2016a). Participants performed these resistance exercises in nine, 20 s exercise segments (Dempsey *et al.*, 2016a). Twenty-four inactive overweight and obese participants with T2D completed three 8 h conditions; uninterrupted sitting, sitting plus 3 min bout of light-intensity walking (3.2 km h⁻¹) interruptions to sitting every 30 min, and sitting with interruptions for 3 min consisting of simple resistance activities interruptions every 30 min. Simple resistance activities included; alternating between body weight half-squats, calf raises, gluteal contractions, and knee raises. The 3 min was divided into a total of nine 20-s movement segments. This sequence was selected to maximise continuous muscle activation over the 3-min period. Resistance type exercises have been

suggested to use larger muscle groups and increase muscle activity, compared to sitting. Therefore simple resistance activities could provide a stimulus for increased energy expenditure, resulting in increased glycaemic uptake and an postprandial glycaemic reduction (Tikkanen *et al.*, 2013). Therefore, performing simple resistance exercises that utilise large muscles and potential increased glucose uptake, may be beneficial to postprandial glycaemic response to interrupting sitting.

Table 1 Summary of studies examining glycaemic responses to prolonged sitting and interrupting sitting in laboratory conditions

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Bailey and Locke, 2015)	10 non-obese adults (7 men, 3 women)	Randomised cross-over Glycaemic outcome: Capillary glycaemia (fasting and hourly),	1. Uninterrupted sitting: 5 h uninterrupted sitting 2. Stand: interrupting sitting every 20 min for 2 min 3. Walk: interrupted sitting every 20 min for 2 min with light-intensity walking Diet: x 2 drinks totalling 80.3 g CHO	<i>LW vs SIT</i> 5 h blood glucose iAUC by 15.9 % <i>STAND vs SIT</i> No significant difference <i>LW vs STAND</i> 5 h blood glucose iAUC by 16.5 %
(Brocklebank et al., 2017)	17 (13 complete data sets) Age: 54.4 ± 5.1 y BMI: 28.0 ± 4.5 kg/m ² WC: 95.3 ± 10.5 cm Setting: participants workplace	Randomised 3-period, 3-treatment crossover 24 h washout between condition days Activity monitoring: activPAL and ActivGraph (from start of trial 1 until end of trial 3) Glycaemic outcomes: Postprandial interstitial glycaemia concentration:	1. Uninterrupted sitting: 5 h uninterrupted sitting 2. Stand: interrupted sitting every 20 m and stood still for 2 min, for 5 h. 3. Walk: interrupted sitting every 20 min with 2 min of self-perceived light intensity walking (RPE 9) for 5 h. Diet: x2 200 mL drink (600 kcal, 73.6 g CHO, 23.6 g protein, 23.2g fat)	<i>SIT vs. LW</i> ▼ 5 h Glucose iAUC by 55.5 % <i>SIT vs LW vs STAND</i> Glucose iAUC _t no significant difference

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Dempsey <i>et al.</i> , 2016a)	24 (14 men, 10 women) with type 2 diabetes Age: 62 ± 6 y BMI: 33.0 ± 3.4 kg/m ² WC Not reported Setting: Laboratory	Randomised cross-over 6 – 14 washout between condition days Activity monitoring ActiGraph – sedentary time Glycaemic outcomes: Venous glycaemia (fasting and postprandial – 30 min intervals)	1. Sitting 7 h 2. Sitting and 12 light intensity walking (3.2 km.h) bouts for 3 min, every 30 min (totalling 36 min) 3. Sitting and 12 simple resistance activities (SRA) bouts for 3 min, divided into 9 segments lasting 20 s, (totalling 36 min) Diet: Standardised meal (12 – 15 % protein, 55 – 58 % CHO, 29 – 31 % fat, 822.9 ± 124.3 kcal/meal)	<i>LW vs. SIT</i> v 7 h blood glucose iAUC by 38.8 % <i>SRA vs. SIT</i> v 7 h blood glucose iAUC by 39.2 %

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Crespo <i>et al.</i> , 2016)	9 (7 men, 2 women) Age: 30 ± 15 y BMI: 29 ± 3 kg.m ⁻² WC: Not reported Setting: Laboratory, stimulated office environment	Randomised cross over 4 consecutive weeks 7 day wash out days between conditions Activity monitoring: ActivPAL, GENEActiv, Bioharness Glycaemic outcomes: 24 h Postprandial interstitial glycaemia – 5 min intervals	1. Sitting 8 h 2. Sitting and standing for 10 min (x 4) 20 min (x 2) and 30 min (x 2) (totalling 2.5 h) 3. Sitting and cycling at 20 W for 10 min (x 4) 20 min (x 2) and 30 min (x 2) (totalling 2.5 h) 4. Sitting and walk at 1.0 mph or 10 min (x 4) 20 min (x 2) and 30 min (x 2) (totalling 2.5 h)	<i>STAND vs SIT</i> 5 % v 24 h interstitial glucose AUC 5 % v 6 h postprandial interstitial glucose iAUC <i>CYCLE vs SIT</i> No significant difference in 24 h interstitial glucose AUC 11 % v 6 h postprandial interstitial glucose iAUC <i>WALK vs SIT</i> 7 % v 24 h interstitial glucose AUC 8 % v 6 h postprandial interstitial glucose iAUC
Dunstan <i>et al.</i> , 2012	19 (11 men, 8 women) Age 54 ± 5 y BMI 31 ± 4 kg/m ² Setting: Laboratory	Randomised 3-period, 3-treatment cross-over Minimum 6 day washout Activity monitoring - ActiGraph Glycaemic outcomes: Venous plasma glycaemia (fasted and postprandial)	1. Sitting 5 h 2. Sitting and 14 light-intensity walking (3.2 km/h) bouts of 2 min, every 20 min, totalling 28 min 3. Sitting and moderate-intensity walking (5.8 – 6.4 km/h) bouts of 2 min, every 20 min, totalling 28 min	<i>LW vs SIT</i> v 5 h positive glucose iAUC by 24.1 % <i>MW vs SIT</i> v 5 h positive glucose iAUC by 29.6 %

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Fletcher <i>et al.</i> , 2017)	13 adolescences Age: 16.4 ± 1.3 y BMI: 20.6 ± 2.5 kg/m ² WC: Setting: Laboratory	Cross-over factorial randomised trial Minimum 1 day washout Glycaemic outcomes: Fasted and postprandial interstitial glycaemic response	<ol style="list-style-type: none"> 1. Sitting 6h (high energy diet) 2. Sitting and body weight resistance exercises: body-weight resistance exercises and included 30-s half squats, 30-s calf raises, 30-s knee lifts and 30-s step-ups, totalling 18 min (high energy diet) 3. Sitting 6 h (standard energy diet) 4. Sitting and body weight resistance exercises: body-weight resistance exercises and included 30-s half squats, 30-s calf raises, 30-s knee lifts and 30-s step-ups totalling 18 min (standard energy diet) 	<p><i>INT SIT vs SIT</i></p> <p>1st meal – v 36 mmol/L/h glucose iAUC</p> <p>2nd meal - v 35.9 mmol/L/h glucose iAUC</p> <p>Total 6 h – No significant difference for glucose AUC</p> <p><i>HE vs SE</i></p> <p>1st meal – v 55 mmol/L/h glucose iAUC</p> <p>Total 6 h – v 75.7 mmol/L/h glucose iAUC</p>
(Hawari <i>et al.</i> , 2016)	10 (men) normoglycemic overweight/ obese Age: 33.0 ± 13.0 y BMI: 28.3 ± 2.8 kg/m ² WC: 100.2 ± 9.5 cm Setting:	Randomised cross-over Minimum 7 day washout Glycaemic outcomes: Venous glycaemia concentration (fasted and postprandial)	<ol style="list-style-type: none"> 1. Sitting (SIT): 8 h 2. Sitting and prolonged standing: 8 h (16 x 15 min bouts of standing, every 30 min, totalling 4 h) 3. Sitting and intermittent standing: 8 h (160 x 90 sec standing followed by 30 sec sitting) <p>Diet: Standardised meal</p>	No significant difference for blood glucose between 3 trials

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Henson <i>et al.</i> , 2016)	22 (women) postmenopausal Age: 68.6 ± 4.7 y BMI: 32.9 ± 4.7 kg/m ² WC: 102.0 ± 9.0 cm Setting:	Balanced incomplete block design (participants completed 2 of the 3 conditions) Day 1. 22 participants completed Day 2. 17 participants completed Washout between conditions 7 and 22 days Glycaemic outcome: Plasma glycaemia, (fasting and postprandial)	Day 1. 1. Sitting – 7.5 h 2. Sitting 6.5 h and 12 standing bouts of 5 min every 30 min, totalling 60 min 3. Sitting 6.5 h and 12 bouts of light intensity walking (1.5 – 4.0 km/h) for 5 min every 30 min, totalling 60 min - Day 2. 1. Sitting, uninterrupted 7.5 h Diet: standardised breakfast and lunch (both 58 % fat, 26 % CHO, and 16 % protein. 15 min to consume)	Day 1. <i>STAND vs SIT</i> ▼ glucose iAUC by 34 % <i>LW vs SIT</i> ▼ glucose iAUC by 28 % No significant differences between conditions Day 2. <i>STAND vs SIT</i> ▼ glucose iAUC by 19 % <i>LW vs SIT</i> ▼ glucose iAUC by 17 % No significant difference between conditions
(Larsen <i>et al.</i> , 2015)	19 (11 men, 8 women) overweight/ obese Age: 57 ± 2 y BMI: 33 ± 1 kg/m ² WC: Setting: laboratory	Randomised cross-over 3 consecutive days per condition Minimum 12 day washout Glycaemic outcomes: Plasma glycaemia, venous blood plasma glucose (fasted and postprandial)	1. Sitting 6 h (limit movements to activities necessary to daily living) 2. Sitting and 17 light intensity walking (3.2 km/h) bouts of 2 min every 20 min Diet: meal tolerance test 75 g carbohydrate, 50 g fat,	<i>LW vs SIT</i> ▼ glucose iAUC by 32 % and 31 % on day 1 and 3 respectively No effect of time, across 3 days protocol for any outcomes

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Peddie <i>et al.</i> , 2013)	70 (10 men, 60 women) Age 25.9 ± 5.3 y BMI 23.6 ± 4.0 kg/m ² WC 76.0 ± 10.7 cm Setting: laboratory	Randomised cross-over design Minimum 6 day washout Glycaemic outcomes: Venous blood glucose (fasted and postprandial)	1. Sitting 9 h (SIT) 2. Sitting and walking 30 min continuous (CON LW) 3. Sitting and walking bouts of 1 min 40 s every 30 min (INT SIT)	<i>INT SIT vs SIT</i> v plasma glucose iAUC by 39 % <i>CON LW vs SIT</i> No significant difference <i>INT vs CON LW</i> v plasma glucose iAUC by 37 %
(Pulsford <i>et al.</i> , 2017)	25 (men) inactive Age: 40.2 ± 12.2 y BMI: 26.1 ± 4.1 kg/m ² WC: 87.3 ± 9.4 cm Setting: laboratory	Cross-over design Minimum 6 day washout Glycaemic outcomes: Oral glycaemia tolerance test, venous blood glucose (fasted and postprandial) Matsuda index	1. Sitting 2. Sitting and (?) standing bouts of 2 min every 20 min, totalling (?) 3. Sitting and (?) light intensity (2 mph) walking bouts of 2 min every 20 min, totalling (?) Diet: 75 g glycaemia drink (consumed within 2 min)	<i>LW vs SIT</i> v glucose AUC by 9 % <i>STAND vs SIT</i> No significant difference for glucose AUC

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Thorp <i>et al.</i> , 2014)	23 (17 men, 6 women) Age: 48.2 ± 8.0 y BMI: 29.6 ± 4.1 kg/m ² WC: male: 99.9 ± 10.5 cm, female: 100.4 ± 11.3 cm Setting: laboratory	Randomised cross-over 5 day experimental conditions Minimum 7 day washout Mixed test drink – consumed 10 min Activity outcomes: ActiGraph (run-in phase till end of each experimental condition) activPAL (at all times) Glycaemic outcomes: Venous plasma glycaemia (fasting and postprandial – 0, 60, 120, 180, and 240 min post meal)	1. Sitting 8 h 2. Sitting 4 h and 4 standing bouts for 30 min alternating posture every 30 min, (totalling 4 h of standing) Diet: standardised test drink (75 g CHO and 50 g fat), standardised meals providing 70 % EE intake (53-55 % CHO, 12-15 % protein, 30-33 % fat)	<i>STAND</i> vs <i>SIT</i> v iAUC glucose by 11.1 % No significant difference for fasted glucose

2. Free-living studies

The majority of research into the glycaemic response to interrupting sitting has taken place in laboratory settings (Pulsford *et al.*, 2017; Thorp, 2014; Bailey, 2015; Henson, 2016; Dempsey, 2016; Larsen, 2015; Crespo, 2016; Hawari, 2016; Dunstan, 2012; Peddie, 2013). It can be postulated that compliance to protocols for interrupting sitting maybe easier to achieve within a controlled laboratory setting compared to free-living conditions (Hillsdon *et al.*, 1995). Within laboratory based studies compliance does not appear to be an issue due to the controlled environment as participants are closely observed and instructed when to complete their physical activity breaks. It is imperative to explore the effects of interrupting prolonged sitting in real-life settings to inform public health guidelines based on interventions that are effective and achievable in day-to-day life. There has been limited research that examines postprandial glycaemia responses in free-living conditions (Duvivier *et al.*, 2016; Duvivier, 2017; Duvivier *et al.*, 2013; Brocklebank, 2017; Altenburg, 2016). The details of such studies are outlined in Table 2 and are discussed here in more detail.

The first study to assess the cumulative effects of interrupting sitting in free-living conditions compared three, 4-day activity regimes on glycaemia concentrations in eighteen young healthy weight (21 ± 2 y, 22.6 ± 2.6 kg²) individuals (see Table 1) (Duvivier *et al.*, 2013). The activity regimes consisted of: (a) Sitting for 14 h, (b) Exercise: 13 h sitting with 1 h of vigorous exercise, and (c) Minimal intensity physical activity: 8 h sitting with 4 h walking and 2 h of standing (Duvivier *et al.*, 2013). No significant difference was found between each condition for fasted and postprandial AUC for glycaemia after an OGTT (Duvivier *et al.*, 2013). Although novel in its methodology, the authors were unable to find a reduction in AUC for glycaemia. However, the authors did not measure 24 h glycaemic response over the four condition days. Postprandial glycaemic attenuation has previously been observed to not increase in magnitude over a period of 3 days (Larsen *et al.*, 2015). Therefore, by only measuring glycaemic responses on the final day of multiple days of interrupting sitting may miss important postprandial changes that may have occurred at an earlier time point. Furthermore, dietary intake was not controlled before the start and during each condition. Therefore, glycaemic responses may have been affected by potential

differences in dietary intake between conditions. In healthy young adults, no significant difference was reported for glycaemia after participants were instructed to remain sedentary for 8 h/day for six days (Altenburg *et al.*, 2016). However, participants were unable to significantly increase their sedentary behaviour time during this period (Altenburg *et al.*, 2016). Participants were requested to sit for 8 h/day, four of which were required to be uninterrupted, and the remaining four hours participants were permitted to interrupt their sitting for a maximum of 15 min/h to allow for activity necessary for daily living (Altenburg *et al.*, 2016). When normal habitual days were measured by accelerometry, uninterrupted sedentary time was 3.6 h/day. During the increased sedentary days, participants were unable to significantly increase their uninterrupted sedentary time. Participants were also unable to significantly increase their prolonged sedentary time and glycaemic control was unaffected by the experimental period (Altenburg *et al.*, 2016).

Further exploration of glycaemic response to interrupting sitting in free-living conditions has recently been examined in individuals with T2D (Duvivier *et al.*, 2016). Nineteen healthy, overweight and obese individuals, diagnosed with T2D completed three, four day experimental conditions; Sit: 14 h/day sitting, Exercise: 1 h/day cycling (5 x 20 min, with 5 min rest between) and 13 h/day sitting, and Sit less: 2 h/day walking, 3 h/day standing, and 9 h/day sitting (Duvivier *et al.*, 2016). The iAUC for 24 h glycaemic response was significantly lower during Sit less compared to Sit. Furthermore, 24 h glycaemic responses were not significantly different between Sit less and Exercise conditions (Duvivier *et al.*, 2016). However, traditional bouts of prolonged exercise, such as in those in the Exercise condition may result in hypoglycaemia in individuals with T2D. The study by (Duvivier *et al.*, 2016) postulated that implementing interventions targeting frequent interruptions to may promote attenuated hypoglycaemic fluctuations. However, these findings were observed in individuals with T2D and thus the findings cannot be extrapolated to the wider population, or those at an increased risk of developing T2D. Furthermore, Duvivier *et al.*, (2016) investigating the effect of replacing SEDENTARY BEHAVIOUR time as opposed to increasing frequency of interruptions.

On such study to investigate the glycaemic response to reducing sitting time on those at an increased risk of developing T2D found no significant difference in fasted glycaemia or postprandial glycaemic response following an OGTT (Duvivier *et al.*, 2017). Twenty-four sedentary

overweight and obese (64 ± 7 y, 29 ± 2 kg/m²) participants competed two, four-day randomised conditions; Sit: restrict walking and standing to 1 h/day each, and sit for the remainder of waking hours, and Sit less: replace at least 7 h/day of sitting with ≥ 4 h/day of self-perceived light-intensity walking and ≥ 3 h/day standing. Participants were also requested to interrupt their sitting every 30 min (Duvivier *et al.*, 2017). Fasted and postprandial glycaemic samples were only collected and analysed after the last day of each condition (day 5) and not during each condition day. Similar assumptions to (Duvivier *et al.*, 2013) study can be made regarding potentially missed glycaemic responses as the effects of interrupting sitting on glycaemic responses may only be acute (< 1 day) in nature. Therefore, investigation into the effect of interrupting sitting on glycaemic responses in overweight and obese individuals over multiple interrupting sitting days is required.

Table 2. Summary of studies examining glycaemic responses to prolonged sitting and interrupting sitting in free living conditions

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Altenburg <i>et al.</i> , 2016)	7 (men) healthy Age: 21.4 ± 2.3 y BMI: 21.8 ± 1.4 kg/m ² Setting: Two days laboratory, with 6 consecutive free-living days in the middle	Pilot study – Randomised order Glycaemic outcomes: Postprandial venous glycaemia measured hourly. C-peptide and triglycerides	Day - 6 - -1: Habitual activity Day 1: Sat reclined for 7 h Day 2-7: Sedentary days Remain seated for at least 8 h between 7:00 – 20:00, of which 4 h uninterrupted Day 8: As day 1 Diet: x 2 Standardised high fat drink (hour 1, and hour 6) Standardised pre-visit meal and snack (15 ± 5 g fat, 70 ± 10 g CHO, 25.8 ± 5.2 g protein, 526.7 ± 40.4 kcal and 0.6 g fat, 48.6 g CHO, 2 g protein, 213 kcal respectively)	No significant difference for glucose and triglycerides ^ postprandial C-peptide

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Brocklebank <i>et al.</i> , 2017)	17 (13 complete data sets) Age: 54.4 ± 5.1 y BMI: 28.0 ± 4.5 kg/m ² WC: 95.3 ± 10.5 cm Setting: participants workplace	Randomised 3-period, 3-treatment crossover 24 h washout between condition days Activity monitoring: activPAL and ActivGraph (from start of trial 1 until end of trial 3) Glycaemic outcomes: Postprandial interstitial glycaemia concentration:	Uninterrupted sitting: 5 h uninterrupted sitting Stand: interrupted sitting every 20 min and stood still for 2 min, for 5 h. Walk: interrupted sitting every 20 min with 2 min of self-perceived light intensity walking (RPE 9) for 5 h. Diet: x2 200 mL drink (600 kcal, 73.6 g CHO, 23.6 g protein, 23.2g fat)	<i>SIT vs. LW</i> v 5 h Glucose iAUC by 55.5 % <i>SIT vs LW vs STAND</i> Glucose iAUC _t no significant difference

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Duvivier <i>et al.</i> , 2013)	18 (2 men, 16 women) healthy Age: 21 ± 2 y BMI: 22.6 ± 2.6 kg ² Setting: free-living conditions	Randomised cross-over 10 day wash out Activity outcomes: activPAL (continuously) Glycaemic outcomes: Oral glycaemia tolerance test (75 g glycaemia, 250 ml water) Venous glycaemia concentrations (fasting and postprandial, 15, 30, 45, 60, 90, and 120 min), C-peptide and insulin	1. Sitting: 14 h/day, 4 days 2. Sitting and exercise: 13 h, 1 h vigorous cycling, 4 days 3. Sitting and minimal intensity physical activity: 8 h, 4 h walking, 2 h standing, 4 days Diet: 250 ml water and 75 g glycaemia	No significant difference for baseline or postprandial glucose
Duvivier., <i>et al.</i> 2016	19 (14 men, 6 women) with type 2 diabetes Age 63 ± 9 y BMI 30.5 ± 3.3 kg/m ² Setting: Free living conditions	Randomised cross-over 10 day wash out Glycaemic outcomes: 24 h interstitial glycaemia concentrations	Sitting: 14 h sitting/ day, 4 days Exercise: 1 h cycling (20 min bout, 5 min rest) 13 h sit/day, 4 days Sit less: accumulate 2 h walking, 3 h standing (sitting interrupted every 30 min) /day. 4 days	<i>SITLESS vs SITTING</i> v 24 h glycaemia <i>SITLESS vs EXERCISE</i> No significant difference for 24 h glucose

Reference	Populations	Study design/ Outcomes	Methods	Findings
(Duvivier <i>et al.</i> , 2017a)	24 (13 men, 11 women) sedentary overweight and obese individuals Age: 64 ± 7 y BMI: 29 ± 2 kg/m ² Setting: Free living conditions	Randomised cross-over 10 day wash out Activity outcomes: activPAL Glycaemic outcomes: 190 min oral glucose tolerance test	Sitting: 13.5 h/day sitting, self-perceived light-intensity walking 0.7 h, standing 1.4 h/day, 4 days Sit less: accumulate 4.3 h/day self-perceived light-intensity walking, 4 h/day standing (sitting interrupted every 30 min) /day. 4 days	<i>SIT</i> vs <i>SITLESS</i> No significant difference for postprandial and fasted AUC

3. Research design and methods

3.1 Participants

Thirteen overweight or obese, sedentary and inactive adults ($n = 5$ men, and $n = 8$ women) aged 18 – 65 y, with a BMI of ≥ 25 kg/m² to 45 kg/m² (Organization, 1995) , and a waist circumference of ≥ 102 cm for men and ≥ 88 cm for women (Wang *et al.*, 2003) were recruited via advertisement and word of mouth at the University of Bedfordshire and in the local community. Participants were screened prior to the preliminary visit to determine sedentary behaviour and physical inactivity via the International Physical Activity Questionnaire (IPAQ) (see appendix A), and domain specific sitting time questionnaire (Marshall *et al.*, 2010) (see appendix B) retrospectively. Exclusion criteria were: sitting < 7 h/day, MVPA > 150 min/week, working night shifts, current smokers or smoked within the past year, had an artificial pacemaker, known contraindications to physical activity (see appendix C), self-reported diagnosis of diabetes, experimental drug use, alcohol abuse, any known blood borne disease, pregnancy, and taking glycaemia-lowering and/or lipid-lowering medication (see appendix D). Informed consent (see appendix E) obtained from all eligible participants prior to testing procedures. Ethical approval was obtained from the University of Bedfordshire Institute for Sport and Physical Activity Research Ethics Committee.

3.2 Sample size calculations

Previous research reported a 44% reduction in free-living 24 h glucose iAUC in a sit less condition compared with a sitting condition (Duvivier *et al.*, 2016). As this study is in non-diabetic participants, a conservative 20% variance was assumed as the intervention is expected to have a smaller effect. Based on this, it was estimated that a minimum of six participants would be required to provide 95% power to detect a significant change in 24 h glucose iAUC with an α of 0.05, a within-person correlation of 0.5, and an estimated within-group variance of 20% in this two-treatment crossover design.

3.3 Preliminary testing

Participants were met by a researcher at their workplace, a communal meeting space, or the University of Bedfordshire laboratory for preliminary measures. Participant height was measured to the nearest 0.1 cm using a portable stadiometer (Marsden HM-250, Invicta plastics, Rotherham). Waist circumference was measured at the midpoint between the lowest rib and the iliac crest to the nearest 0.1 cm (Wang *et al.*, 2003). Body mass (kg) and body fat % were measured using the Tanita BC-418 Segmental Body Composition Analyzer (Tanita Corp., Tokyo, Japan). This measure was used to assess the eligibility of participants in regards to BMI. The bio-impedance analysis device uses eight polar electrodes, a single-point load cell weighing system in the scale platform and an algorithm to estimate body composition (Völggi *et al.*, 2008). Bio-impedance is a valid and reliable measure for overweight and obese adults (Franssen *et al.*, 2014).

3.4 Study design

A within-subjects randomised crossover study was conducted and comprised of two, 4-day free-living conditions, separated by a 72 h washout period (see Figure 1). Participants were randomised using an online tool that allocated trial order (www.randomizer.org). A washout period of three days was included to avoid carryover effects as a single session of exercise may improve insulin sensitivity for up to 48 h (Mikines *et al.*, 1988). During the washout period, participants were instructed to refrain from engaging in MVPA and from consuming alcohol. Participants were instructed to replicate their dietary intake (see meal standardisation) and refrain from taking part in any structured exercise during the two, 4-day conditions (Duvuvier *et al.*, 2013; Duvuvier *et al.*, 2016). Abstinence from alcohol and structured exercise was also required 48 h prior to commencement and during both conditions. Participants were provided with a diary for each day of both conditions to record their activity in 60 min blocks from 06:00 – 23:00 hours to encourage adherence to the protocols (see appendix F). Within seven days following the preliminary visit participants were met at their workplace, a communal space, or the University of Bedfordshire laboratory for initial setup of the glucose monitoring device and postural monitor. This setup occurred twenty-four hours before study commencement (on a Monday). A FreeStyle Libre (Abbott Diabetes Care, Abbott Laboratories Ltd, Berkshire, UK) flash glucose monitor (FGM) was fitted to each participant at the midline of the upper arm (see Flash Glucose Monitoring section). Subsequently, participants were fitted with an activPAL (PAL Technologies, Glasgow, Scotland) activity monitor (see Sedentary Behaviour and Physical Activity Assessment section). Participants were instructed to adhere to the following two, 4-day conditions: 1) Sitting, and 2) Interrupted Sitting.

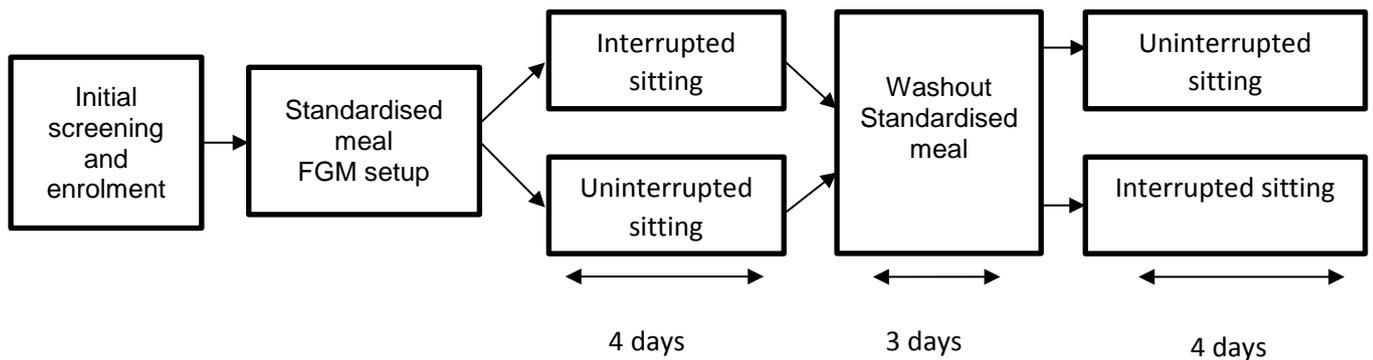


Figure 1. Schematic of study design FGM; Flash glucose monitoring

3.5 Experimental Conditions

Following preliminary measures and device set up participants were provided with verbal and written protocol instructions. These are outlined in the sections below.

3.6 Uninterrupted sitting experimental condition

Similar to previous research, Altenburg *et al.* (2016), during the Uninterrupted Sitting (SIT) condition, participants were instructed to increase their sitting time as much as possible and to remain seated for at least 10 h/day during each day of the four condition days (see appendix G). Participants were asked to spend 7 of these 10 sedentary hours/day uninterrupted, except for visiting the toilet. Participants were not required to remain sedentary for 7 unbroken hours, but rather accumulate 7 bouts of a 1 h uninterrupted sitting bout. During the remaining 3 hours, participants were permitted to interrupt their sitting time once per hour, up to a maximum of 15 min to allow them to carry out activities necessary for daily living, such as cooking and cleaning. Participants were instructed to restrict standing and stepping to a maximum of 1.5 h/day during the sitting condition. Participants were given an activity log to note each activity per hour. This was provided for participants to enable them to visualise their behaviour and to promote condition compliance.

3.7 Interrupted sitting experimental condition

During the Interrupted Sitting (INT SIT) condition, participants were asked to interrupt their sitting time at least once every 30 min with bouts of activity lasting 3 to 5 min during each day of the four condition days (see appendix H). Participants were instructed to accumulate a total volume of 6 to 10 min of interruptions in sitting time per hour, per day (84 to 140 min/day). The frequency and duration of interruptions to sitting was selected as current literature has indicated beneficial postprandial glycaemic responses (Henson *et al.*, 2016; Dempsey *et al.*, 2016a; Duvivier *et al.*, 2016; Duvivier *et al.*, 2017). Participants were provided with a list of suggested mobile device, and personal computer applications and tools to alert/remind them to interrupt their sitting at least every 30 min. Participants received suggestions on different activities that they could use to interrupt their sitting including walking, standing, stair climbing, and simple resistance activities (e.g. body weight half squats, lunges, calf raises, knee lifts, and repeated sit-to-stand transitions). Interrupting sitting with these types of activities has elicited acute beneficial glycaemic responses in laboratory conditions (Bailey and Locke, 2015; Dempsey *et al.*, 2016a; Thorp *et al.*, 2014). Participants were informed they could select an activity that best suited the situation or environment they were in to reduce any potential embarrassment from breaking “cultural norms”. For example; if a participant was in a meeting, they could simply rise from a seated position to standing rather than perform body weight squats. However, participants were informed that a range of activities were required to be performed, and they could not just pick one activity. As above, Participants were given an activity log to note each activity per hour. This was provided for participants to enable them to visualise their behaviour and to promote condition compliance.

3.8 Meal standardisation

Participants were provided with two standardised instant pasta meals (464.0 ± 2.0 kcal, carbohydrate 80.7 ± 2.8 g, protein 18.2 ± 1.2 g, and 7.0 ± 1.1 g fat) to consume on the evening, at the same time, before the start of each four-day condition (see Figure 1). Participants were also provided with electronic weighing scales (Salter Disc Electronic Kitchen Scale, HoMedics Group Ltd, UK) and asked to weigh and record volume and timings of all food and beverage intake in a

food diary during the first 4-day condition and were asked to replicate the exact volume and timings of consumption during their second four-day condition. Participants were provided with a second meal log to record replicated food to ensure protocol adherence. The participants were asked to consume a minimum of three meals during each experimental condition day that contained at least 50 % carbohydrate, in addition to any snacks that the participants wished to consume. A list of example meals containing a minimum of 50 % carbohydrate was provided to each participant (see appendix J).

3.9 Flash Glucose Monitoring

A FreeStyle Libre FGM sensor was inserted subcutaneously into the back of the upper arm during the preliminary visit. Interstitial glucose monitoring, using the Freestyle Libre, is valid when compared to capillary blood glucose reference values using a YSI analyser (Yellow Springs Instrument, Yellow Springs, OH) (Bailey *et al.*, 2015). Furthermore, the Freestyle Libre FGM provides accurate measurements of interstitial glucose corresponding with capillary plasma glucose over 14 days, and is unaffected by participant characteristics (Sekido *et al.*, 2017). An interstitial glucose monitoring device positioned on the arm was selected to measure changes in glycaemic concentrations rather than the abdomen due to potential deterioration of biometric values. Biomechanical factors such as movement and pressure can distort the tissues physiology around the subcutaneously inserted wire sensor and ultimately impact upon sensor performance (Charleer *et al.*, 2018). Furthermore, abdominal glycaemic concentration have been reported to be 10 – 18 % lower and report elongated hypoglycaemic episodes compared to than finger capillary measures (van der Valk *et al.*, 2002). This may be due to site specific characteristics such as excessive subcutaneous fat (van der Valk *et al.*, 2002). Finally, the Freestyle Libre is a factory calibrated device which does not require any further calibration from the user, compared to other interstitial device which required multiple daily capillary sample calibrations. Thus, this device was selected to reduce participant burden and comfort. The accuracy of the factory calibrated flash glucose monitoring device has been reported to be similar across the 14 day wear time, apart from the first day of wear. It has been reported that the first day of wear has the lowest accuracy. This

may be due to the body's inflammatory response to the sensor being inserted, which has been shown to affect glycaemic concentrations in interstitial fluid (Bailey *et al.*, 2015). To attenuate any confounding effects, a 24 h run in period was selected.

Each participant was asked to wear the monitor for a total of 11 full days. An initial 1 h calibration was required to set up the monitor. To reduce user error, clear written and verbal instruction were provided for the FGM receiver (See Appendix K). Participants were asked to scan the FGM sensor at least once every 8 h via the FreeStyle Libre receiver to ensure that the sensor data continues to sync with FGM receiver, although they could do this more frequently if desired. An in-built alarm system, on the FGM receiver, was set in accordance with each participant's waking and sleeping times, with an alarm every 4 h during waking hours to ensure no data transfer was missed. Additionally, participants were advised to scan the sensor when they woke up, at lunch, and when they went to bed.

Glycaemia concentrations were measured in the interstitial fluid of the subcutaneous tissue every minute and data averaged for each 15-min period was stored and transmitted to a reader. The reader was downloaded by the research team at the end of the last condition day. Interstitial glycaemia iAUC, total area under the curve (tAUC) and positive area under the curve (pAUC) responses were calculated using the trapezoidal method for day 4 of each condition, to measure cumulative glycaemic effects of interrupting sitting. Positive iAUC was calculated as AUC above the baseline value only (Brouns *et al.*, 2005).

3.10 Sitting time and physical activity assessment

Sitting time and physical activity was monitored during the 11-day period in which each participant was involved with the study via an activPAL activity monitor. The monitor was attached to the skin on the anterior aspect of the right thigh using a medical grade adhesive dressing. Continuous wear was achieved by waterproofing the device with a small flexible sleeve further covered by a medical grade adhesive dressing (Hypafix Hypoallergenic Tape, BSN Medical Limited, Hull, UK), which allowed participants to shower and bathe. ActivPAL uses algorithms to discriminate the commencement and conclusion of each period spent sitting/lying, standing, and

stepping, as well as stepping speed, step counts, and postural transitions by thigh positions (Harrington *et al.*, 2011). ActivPAL uses an inbuilt equation: $\text{MET} \cdot \text{h}^{-1} = (1.4 \cdot d) + (4 - 1.4) \times (c / 120) \times d$ (where c = cadence (steps per minute) and d = activity duration (in hours)) to estimate an indirect metabolic equivalent (Harrington *et al.*, 2011). Participants were provided with written (see appendix L) and verbal instructions on how to affix the activPAL dressing and consumables to enable participants to change dressings to clean activPAL placement site or in case of malpractice (Edwardson *et al.*, 2016).

Participants were required to keep a sleep and work log to differentiate between sleep and wake time (see Appendix K). Data for postural allocation and energy expenditure was averaged over days 1-4 of the Uninterrupted Sitting condition, days 1-4 of the Interrupted Sitting condition, and the wash out period. Energy expenditure was quantified as metabolic equivalent of task (MET) by the activPAL device to determine sedentary bouts. ActivPAL is a valid measure of METS when compared to indirect calorimetry, especially when sedentary and LIPA is performed (Harrington *et al.*, 2011).

3.11 Statistical Analysis

All statistical analysis was performed using IBM SPSS statistics version 21 (SPSS Inc, Armonk, N.Y., USA). Data are presented as mean (95% confidence interval) unless otherwise specified. Quantile quantile plots were used to assess normal distributed of data preceding analysis. Statistical significance was set at $p \leq 0.05$. The differences in outcome variables between conditions was analysed using linear mixed model with activity condition as a fixed factor and participants as a random factor with adjustment for potential covariates (FGM analysis: WC, baseline glucose values on day 4, and activPAL analysis: activPAL wear-time, and WC) explaining residual outcome variances. Dietary carbohydrate consumption, age and sex were considered as covariates, but were not used because they were not significantly associated with any of the outcome variables (iAUC, tAUC, pAUC). Outcome variables included; time spent sedentary, accumulation of sedentary bouts, sit-to-stand transitions, time spent in physical activity (stepping and standing) and interstitial glycaemia response (iAUC, tAUC, pAUC) on day 4 of each condition.

Cohens' d effect sizes were calculated to describe the magnitude of differences between conditions; 0.2, 0.5 and 0.8 indicated a small, medium or large effect, respectively (Cohen, 1988).

4. Results

Forty-seven participants were assessed for eligibility, however 34 participants were excluded prior to randomisation (see Figure 2.). After screening, 13 participants were included in the study. The anthropometrical, glycaemia, and carbohydrate intake data were reported in Table 3. One participant withdrew midway through the study and was excluded from the analysis.

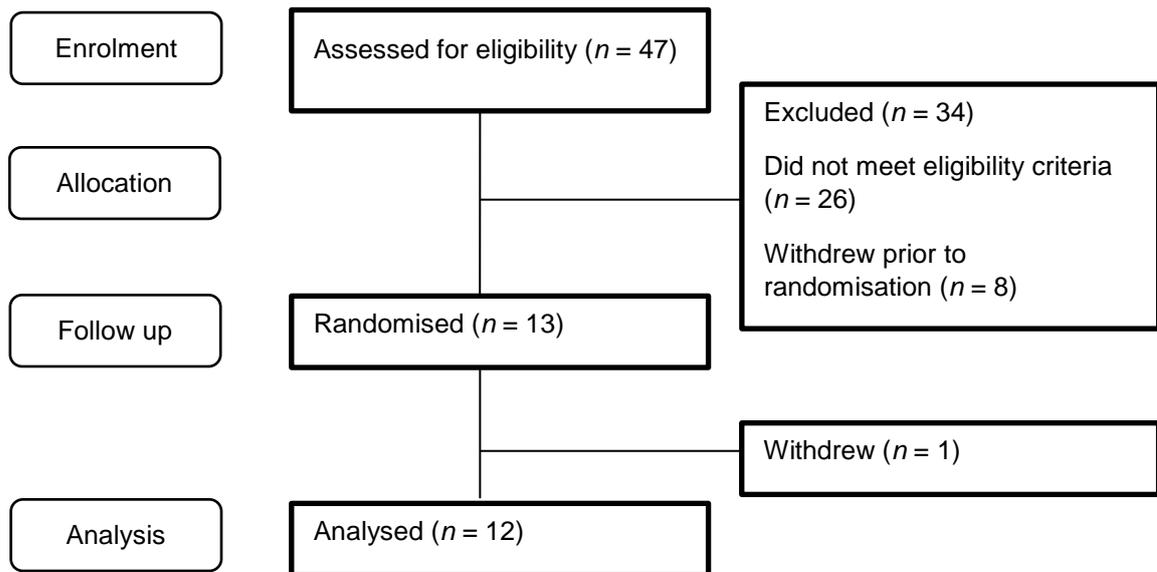


Figure 2. CONSORT diagram showing participant flow through study (crossover design).

Table 3. Participant characteristics

Variables	Mean \pm SD
<i>N</i>	12
Men (n)	4
Age (y)	47.5 \pm 9.9
Height (cm)	167.5 \pm 8.5
Weight (kg)	93.9 \pm 13.3
BMI (kg/m ²)	33.3 \pm 5.3
WC (cm)	107.8 \pm 8.9
BF (%)	39.4 \pm 7.9
Baseline glycaemia (mmol/L)	5.5 \pm 0.8

Data presented as mean \pm SD. BMI; body mass index, WC; waist circumference, BF; body fat percentage

4.1 Sitting time and physical activity

Table 4 shows a significant difference for mean total steps ($p = .001$, $d = 1.18$). Furthermore, time spent stepping increased significantly by 51.8% ($p = < 0.001$, $d = 1.04$).

Compared to SIT (3265.75 [2210.41, 4321.09]) average total steps increased in INT-SIT (5507.27 [4418.36, 6596.17]) on day 4 by 2241.52 steps ($p = .001$, $d = 1.18$). Time spent in light-intensity physical activity for INT SIT (0.67 [0.52, 0.82]) compared to SIT (0.48 [0.34, 0.63]) increased significantly by 40.8 % ($p = .007$, $d = 0.73$). During INT SIT (1.47 [1.18, 1.76]), total time spent in MVPA significantly increased by 57.3 % ($p = < 0.001$, $d = 1.25$) compared to SIT. Compared to SIT, the number of total sedentary bouts were not significantly different in INT SIT ($p = 0.872$, $d = 0.05$). SIT conditions ($p = 0.872$, $d = 0.05$).

Table 4. *Sitting time and physical activity during the experimental conditions*

Variables	SIT	INT SIT	P value	d
Sedentary bouts				
Number of sedentary bouts lasting 0-30 min	46.68 (38.93, 54.42)	46.57 (38.61, 54.52)	0.980	.01
Time spent in sedentary bouts lasting 0-30 min	5.09 (3.98, 6.2)	5.79 (4.65, 6.93)	0.238	0.35
Number of sedentary bouts lasting 30-60 min	3.17 (1.56,4.78)	4.85 (3.17, 6.51)	0.118	0.59
Time spent sedentary for durations 30-60 min	2.22 (1.25, 3.18)	2.99 (1.99, 3.99)	0.210	0.44
Number of sedentary bouts lasting 60-120 min	2.09 (1.21, 2.98)	1.42 (0.49, 2.35)	0.269	0.42
Time spent sedentary for durations 60-120 min	2.64 (1.49, 3.79)	1.68 (0.48, 2.88)	0.216	0.46
Number of sedentary bouts lasting >120 min	.334 (-.02, 0.69)	0.11(-0.26, 0.48)	0.320	0.35
Time spent sedentary for durations >120 min	.81 (-.84, 1.71)	.24 (-0.69, 1.17)	0.329	0.29
Total number of sedentary bouts	52.27 (45.05, 59.49)	52.96 (45.52, 60.40)	0.872	.05
Total sedentary bouts (h/day)	10.75 (9.07, 12.44)	10.75 (9.05, 12.46)	0.996	0.28
Number of sedentary to upright transitions	51.94 (44.74, 59.14)	52.76 (45.35, 60.18)	0.845	.09

Variables	SIT	INT SIT	P value	<i>d</i>
Physical activity				
Light stepping time (h/day)	0.48 (0.34, 0.63)	0.67 (0.52, 0.82)	.007*	0.73
Moderate to vigorous stepping time (h/day)	0.84 (0.56, 1.12)	1.47 (1.18, 1.76)	.000*	1.25
Total stepping time (h/day)	1.33 (.96, 1.69)	2.14 (1.77, 2.51)	.000*	1.04
Total steps per day	3265.75 (2210.41, 4321.09)	5507.27 (4418.36, 6596.17)	.001*	1.18
Standing time (h/day)	4.32 (3.11, 5.53)	4.39 (3.18, 5.62)	0.864	.03
Data presented as	mean (95 %	confidence intervals)	<i>p</i> =	0.05

4.2 Twenty-four-hour interstitial glycaemic

Table 5 illustrates that there were no significant differences for 24 h iAUC between SIT and INT-SIT. Furthermore, there were no significant differences for 24 h pAUC or 24 h tAUC between conditions.

Table 5. Mean interstitial glycaemia response on day 4

Variables	SIT	INT SIT	<i>P</i> value	<i>d</i>
24 h interstitial glycaemia				
iAUC (mmol/L·24 h ⁻¹)	4.17 (-2.25,10.87)	7.32(.62,14.01)	.472	.27
pAUC(mmol/L·24 h ⁻¹)	147.45 (135.11, 159.79)	142.88 (130.54, 155.22)	.189	.21
tAUC (mmol/L·24 h ⁻¹)	138.74 (126.69, 150.78)	135.57 (123.54,147.61)	.683	.15
Interstitial glycaemia waking h				
iAUC (mmol/L·16 h ⁻¹)	5.13 (.05, 10.21)	5.81 (.71, 10.91)	.840	.07
pAUC (mmol/L·16 h ⁻¹)	107.851 (98.88, 116.82)	103.87 (94.88, 112.86)	.201	.25
tAUC (mmol/L·16 h ⁻¹)	101.93 (93.81, 110.04)	98.56 (90.43, 106.69)	.272	.23

Data presented as mean (95 % confidence interval) *p* = 0.05. iAUC incremental area under the curve, pAUC positive area under the curve, tAUC total area under the curve.

5. Discussion

In this randomised cross-over study in inactive overweight and obese adults, no significant difference in 24 h glycaemia was found in response to increased steps, LIPA, and MVPA for INT SIT condition. These findings address important gaps in the existing literature by examining the potential postprandial glycaemic response to interrupting sitting in normoglycemic overweight and obese individuals in free-living conditions.

Although participants accumulated an average of 10.75 h/day total sitting time for SIT condition in the current study, this was not accrued in uninterrupted bouts as instructed. The quantity of waking hours spent sedentary in durations of 60-120 min and > 120 min indicates that on average, 3.45 h of uninterrupted sitting lasting at least 60 min was accrued during the SIT condition. This was less than half the instructed volume of sitting in that condition (≥ 7 h). Although participants reduced their total uninterrupted sitting lasting at least 60 min by 44 % in INT SIT compared to SIT, this was not significant, which may indicate that this study lacked power for this variable. One explanation for participants not accumulating ≥ 7 h of uninterrupted sitting may include participants' inability to increase their total sitting time during SIT compared to their normal habitual behaviour. The inability to increase total sitting time has also been observed in previous free-living studies (Altenburg *et al.*, 2016). Participants in the study by Altenburg *et al.*, (2016) were healthy active adults and were unable to increase sitting time over a 6 day period. The authors postulated that adverse associations between sitting and postprandial glycaemia may only arise when sedentary behaviour is sustained for an increased number of consecutive days. Furthermore, without a sizable amount of total sitting time, attenuation of postprandial glycaemic response may not occur when interrupting sitting time.

Observational data has suggested that sedentary individuals sit for approximately of 7.48 – 9.28 h/day sedentary (Matthews *et al.*, 2008). Additionally, in office workers, total sitting time was observed to be between 8.46 – 10.15 h/day (Clemes *et al.*, 2014). The total time spent sitting for both conditions in this current study is in line with previous findings (Clemes *et al.*, 2014; Matthews *et al.*, 2008). As this study was carried out in sedentary office workers in free-living conditions,

which included participants' place of work, the effect of occupational requirements on uninterrupted sitting should be determined in future research. A 5 h difference in sitting time between uninterrupted sitting and interrupting sitting regimes has previously been reported in response to protocols investigated interruptions to sitting time in free-living conditions (Duvivier *et al.*, 2017; Duvivier *et al.*, 2016; Duvivier *et al.*, 2013). It can be postulated that individuals who have high levels of sitting (≥ 10 h/day) may accrue multiple interruptions to sitting throughout the day regardless of total sitting time. Independent of total sitting time, individuals with higher frequency of breaks to sitting time are beneficially associated with reduced WC, triglycerides, and 2 h plasma glucose compared to those that do not interrupt their sitting frequently (Healy *et al.*, 2008). Although no significant reduction in 24 h glycaemic response to interrupting sitting was found in this study, it may be postulated that shorter duration sitting time with frequent interruptions to sitting may still result in health benefits.

The data from the current study extend the conclusions of Duvivier *et al.*, (2013) who did not find a significant effect of interrupting sitting on postprandial glucose in healthy weight normoglycemic individuals in free-living conditions. Furthermore, the present study is also in agreement with Duvivier *et al.*, (2017) who did not find a significant reduction in fasting or postprandial glucose in overweight and obese individuals in response to interrupting sitting with stand and self-perceived light-intensity walking in free-living conditions. Participants completed two activity regimes in the study by Duvivier *et al.*, (2017) in a randomised cross-over design, which consisted of Sit (standing and stepping restricted to 1 h/ day with the rest of the day spent sitting), and Sit Less (7 h/day of sitting replaced with 4 h/day of self-perceived light intensity walking and 3 h/day of standing). Participants were requested to interrupt sitting with standing and walking in a preferable frequency of 30 min (Duvivier *et al.*, 2017). However, participants in the study by Duvivier *et al.*, (2017) significantly decreased their sitting time concurrently with an increase in standing and stepping in their Sit Less condition compared to the Sit condition. In the current study, participants were unable to achieve a reduction in total sitting time.

Previous research investigating the effects of interrupting sitting in free living conditions in T2D have found reductions in 24 h glucose iAUC on day 4 of the interrupting sitting condition compared

to day 4 of the control condition Duvivier, 2016. Participants performed three activity regimes in the study by XXX: Sitting (14 h/day sitting with walking, and stepping restricted to 1 h/day), Exercise (13 h/day sitting, 1 h/day supervised cycling), and Sit Less (9 h/day sitting, with 2 h/day walking, and 3 h/day standing). Participants were requested to interrupt sitting with standing and walking in a preferable frequency of 30 min the during Sit Less condition (Duvivier *et al.*, 2016). When participants reduced their sitting time by 37 % (from 13.7 h to 8.9 h for Sitting compared to Sit Less), this resulted in a significant reduction of 24 h iAUC glycaemic response (Duvivier *et al.*, 2016). Nevertheless, when sitting time was reduced by 8.7 % from Sit to the Exercise condition no significant difference in 24 h iAUC glycaemic response was observed (Duvivier *et al.*, 2016).

A requirement for participant inclusion in the present study included self-reported sitting for ≥ 7 h/day. As participants reported high levels of sedentary behaviour during recruitment, it may be postulated that participants were unable to increase their total daily and uninterrupted bouts of sitting time significantly during SIT condition, compared to their habitual sitting behaviours (Altenburg *et al.*, 2016). However, no habitual accelerometry data was collected prior to study commencement. Participants were unable to increase their sedentary time during the SIT condition compared to INT SIT.

It is important to note that in the current study, participants were not required to meet a minimum total time threshold of walking, standing and body weight resistance exercises in INT SIT condition. This was to allow participants to select an activity that suited their environment, during the interruption to potentially increase adherence to the INT SIT protocol. Participants were instructed to interrupt their sitting every 30 min over a 10 h period in INT SIT condition. Therefore, the lack of change in total sitting time between the conditions is not surprising as the intervention focused on interrupting sitting not, not reducing total sedentary time.

During the free-living study by Duvivier *et al.* (2016) energy intake was standardised for all meals and snacks on day 4 and dinner on day 3 of each of their conditions. During this present study, participants were instructed to record a food diary for the first four condition days and then replicate the exact food intake and timings during the second four condition days. Participants were also provided with a pre-packaged pasta meal to consume for dinner the evening before each condition

started. Furthermore, throughout the current study participants were instructed to consume at least 50 % carbohydrates in each meal, therefore participants may not always be able to consume their normal diet. A recent study by Duvivier *et al.*, (2017) instructed that participants adhered to their normal diet. One explanation for the lack of findings within this study may be due to macronutrient compositions of meal consumed. Although instructions were given on the percentage of carbohydrates consumed during each meal, no guidance was provided on recommended composition of protein, fat, glycaemic index and the portion size of each meal. The co-ingestion of fat and carbohydrate during a meal may reduce the postprandial glycaemic response (Collier and O'dea, 1983). Obese individuals may often consume foods with a high percentage of fat in greater quantities than healthy weight individuals (Westerterp-Plantenga *et al.*, 1996). Therefore, future studies investigating postprandial glycaemic response to interrupting sitting, in obese individuals should control for macronutrient composition and portion size. In addition to the SIT and INT SIT instructions, food diary restrictions may have been too burdensome for participants which may be one explanation as to why the experimental protocols not followed.

It may be that those without existing metabolic impairment, occasionally altering positions for 6 - 12 min per hour each day in this study, may not represent a sufficient stimulus to alter postprandial glucose. Interruptions to sitting may have to involve more frequent and with and at an increased volume of physical activity stimulus to be of benefit to the attenuation of glycaemic responses (Dempsey *et al.*, 2016b). This may have implications on future study designs examining interrupting prolonged sitting in clinical populations.

The selected parameters (24 h and waking h iAUC, pAUC, and tAUC) for glycaemic response in the current study were chosen due to the continuous wear of the glucose monitoring device. However, flash glucose monitoring systems typically receive historic glucose every 15 min, for 8 h. This type of system reports glucose trends but not real time glycaemic responses, and capillary blood glycaemia may be more accurate in detecting rapidly changing directions of glycaemic change, but provides less time points to analyses samples (Bailey *et al.*, 2015). Furthermore, capillary and venous glycaemic blood sampling measures can only capture a single reading and may miss extreme variability over a certain time frame (e.g. over 30 min) (Bailey *et al.*, 2015).

Additionally, this study used flash measurements of interstitial glucose, which have been shown to differ in time and magnitude compared to blood glucose samples (Kulcu *et al.*, 2003). Interstitial glucose monitoring is a non-invasive method of monitoring glycaemic concentrations. This method uses interstitial fluid that surrounds the cells subcutaneously. Glucose travels from blood vessels and capillaries, through cell walls into the interstitial space. Samples are obtained via a transcutaneous sensor typically inserted into the arm or abdomen, and then sent to a transmitter or receiver, (Riddell and Perkins, 2009). During glucose uptake, blood glucose concentration increases quickly, and as glucose is transferred into the interstitial space from blood, interstitial glucose is a lower concentration, and lags compared to blood glucose. These differences represent physiological variation in glucose uptake and utilisation rather than diminished sensor sensitivity (Kulcu *et al.*, 2003). Therefore, sensors may have failed to capture glycaemic responses that had rapidly changing velocities and magnitudes. The lack of findings for 24 interstitial glycaemic response to interrupting sitting may have been due to measurement method of FGM. Future studies should investigate over a longer duration (> 14 days) as this would provide accurate and valid glycaemic trends, and patterns in glycaemic response to interrupting sitting.

It is important to highlight that there were occasions when participants' 24 h glucose measurements fell markedly below their baseline during the SIT condition in the present study. Therefore, due to potential postprandial glycaemic variability within this current study, sedentary participants may have abnormal glycaemic uptake (Buckley *et al.*, 2013). It is possible that interstitial glucose concentration fell below baseline due to baseline glucose being higher in individuals with poorer glucose tolerance (Young and Benton, 2014). It has been suggested that adults with poorer glucose tolerance may have developed hyperinsulinemia to compensate for possible insulin resistance. (Young and Benton, 2014). However, insulin was not measured during this study, thus, possible insulin mechanisms cannot be attributed to the data observed. This may be the circumstance for participants within in this current study. However, no OGTT was completed prior to study to act as a reference point. One possible explanation for no significant difference in 24 h glycaemia between conditions in the current study may be due to minimal differences in physical activity and sitting time observed. Total time spent sedentary was the same between

conditions (10.75 h/day). Total steps and time spent stepping significantly increased by 68 and 61 %, respectively for INT SIT condition compared to SIT, indicating that participants engaged with walking as an interruption to sitting. Furthermore, time spent in LIPA and MVPA significantly increased by 40 and 75 %. Total mean time spent in sedentary bouts up to 120 min decreased not statically, by 70 %. Total mean time spent in sedentary bouts up to 60 min decreased not statically, by 36 %. Moreover, shorter interrupted sitting increased, but not significantly by 35 and 14 % for mean sedentary bouts lasting 30 min and under 30 min, respectively. It can be suggested that as standing time did not increase in INT SIT condition compared to SIT participants did not engage in standing as an interrupting sitting method. One explanation for the lack of attenuation in glycaemic response for the INT SIT condition compared to SIT may be due to the volume and duration of standing accumulated in this present study, as interrupting sitting with standing has previously shown to reduce glycaemic iAUC over an 8 h duration in overweight and obese individuals (Thorp *et al.*, 2014). In this current study, participants were requested to complete a variety of physical activities throughout the day to interrupt their sitting. No minimum quantity thresholds were applied to each particular physical activity, only the request that an activity was performed for 3-5 min every 30 min. No minimum quantity thresholds were selected regarding the amount of each activity used in INT SIT because standing (Thorp *et al.*, 2014), walking at a self-perceived light intensity (Duvivier *et al.*, 2013; Brocklebank *et al.*, 2017), walking at a moderate intensity (Dunstan *et al.*, 2012b; Larsen *et al.*, 2017), and simple resistance exercise (Dempsey *et al.*, 2016a) have all shown significant reductions in glycaemic response compared to uninterrupted sitting. As previous free-living studies used mixed interrupting sitting methods (i.e. walking and standing), significant postprandial glycaemic reductions cannot be attributed to one singular interrupting sitting method (Duvivier *et al.*, 2017; Duvivier *et al.*, 2016; Duvivier *et al.*, 2013). However, in current study participants were not able to effectively interrupt sitting compared to SIT.

5.1 Strengths and limitations

The main strength of this current study is that flash glucose monitoring was used to record average interstitial glucose every 15 min from the start until the end of experimental procedures in addition to an objective measure of sitting, standing and stepping. This resulted in 384 glucose observations over a 96 h period. Previous studies have analysed and reported frequency of continuous interstitial samples at 5 min intervals using continuous glucose monitors (Brocklebank *et al.*, 2017; Duvivier *et al.*, 2017; Duvivier *et al.*, 2016b). However, compared to continuous glucose monitoring, flash glucose monitoring using Freestlye Libre does not require user calibration, unlike continuous glucose monitors (Bailey *et al.*, 2015). Continuous glucose monitoring requires users to perform four fingerpick capillary tests per day (Brocklebank *et al.*, 2017; Heinemann and Freckmann, 2015). Factory calibrated glucose monitoring devices, which do not require finger prick sample calibration may affect glucose stability, particularly postprandially (Castle and Ward, 2010). However, factory calibrated devices reduces the burden on participants (Bailey *et al.*, 2015). Furthermore, this study included the measurement of 24 h glycaemic response over four days and included nocturnal glycaemic responses as sleep may impact total AUC glycaemic response to interrupting sitting (Morselli *et al.*, 2010). Another strength of this study is that it was conducted in participants' workplace and home, rather than a laboratory, increasing the ecological validity of the findings.

This study has some limitations. This study was not powered to compare sexes, therefore it was not possible to compare sex difference in physical activity between conditions. Anecdotally, women informed the research team that compliance was challenging to adhere to over the study duration due to household chores and family commitments. However, men reported less challenges with adherence to the protocol, as they had more opportunity to be sedentary. It is possible that this difference corresponds with duties associated with traditional gender roles. A possible explanation for this may be attributed to domestic duties and responsibilities that women have regarding having a family (Matthews *et al.*, 2008). Future studies should investigate potential sex differences

between men and women's adherence to uninterrupted sitting and interrupting sitting protocols and explore the glycaemic response to such interventions in free-living conditions

Due to study design, female participants' menstrual cycle could not be accounted for due to the FGM sensor only being able to measure interstitial glycaemic responses reliably for 14 consecutive days. Therefore, the influence that menstrual cycle may have on glycaemic responses cannot be ruled out (Pulido and Salazar, 1999).

It was unfeasible to blind participants to glycaemic response as synchronisation of receiver and interstitial sensor was required every 8 h and the FGM displayed glycaemic readings on the inbuilt screen as participants synced their device. This may have influenced the quantity of composition of dietary intake over the duration of the first four condition days and could have influence physical activity and sedentary behaviour. Participants were instructed to consume the same caloric intake for each condition by following a food diary. Although such diaries may be unreliable in absolute terms (Duvivier *et al.*, 2013) participants did not alter their diet between conditions as no changes in dietary intake were reported during conditions. However, participants may have inaccurately reported energy intake or underreported (Goris *et al.*, 2000). However, social bias may lead overweight obese individuals to underreporting food intake which has low social desirability to confirm to social desirability (Hebert *et al.*, 1995; Lissner, 2002).

Although participants were provided with instructions and demonstrations on how to adhere to the study protocols upon recruitment, no other activity feedback was given. This may be a limitation of the study. A recent study (Duvivier *et al.*, 2013), investigating glycaemic response to interrupting sitting in free-living conditions, provided feedback throughout the conditions to participants on how to better adhere to the study protocol by assessing activity data after condition day (Duvivier *et al.*, 2013). During screening for (Duvivier *et al.*, 2017) recent study participants performed a one day try-out of the Sit Less protocol to ensure that they could achieve the requirements of this regime. Although encouragement and contact were made with participants throughout the current study to assist with adherence, it was not feasible to meet with participants daily or provide physical activity feedback. Additionally, to better explore the ecological benefits of interrupting sitting time on postprandial glycaemia, the environment in which research is conducted

should be reflective of habitual behaviour (Altenburg *et al.*, 2016). Therefore, future research should investigate the effectiveness of interrupting sitting on postprandial glycaemic response compare to habitual sitting glycaemic response, rather than larger imposed sedentary bouts as individuals may be unable to increase their prolonged and total sitting time. This will provide the scientific research discipline with more representative metabolic outcomes compared to compulsory sitting time, which may not reflect individual's characteristic sitting behaviours. As participants may have overestimated their prolonged sedentary time during screening for this study, pre-enrolment objective assessments of habitual activity may be required to identify suitable participants for such studies. Future research should also examine the chronic outcomes from interrupting sitting on 24 h glycaemic response in free-living conditions in overweight and obese individuals.

5.2 Conclusion

To conclude, in overweight and obese participants, it may not be possible to manipulate increases or decreases in sedentary behaviour in free-living conditions, therefore, it was not possible to compare effects of interrupted sitting versus uninterrupted sitting on glycaemia. However, there was an increase in physical activity (steps) in the interrupted sitting condition, but this had no effect on glycaemia. Further research to effectively reduce sedentary behaviour in free-living conditions is required to inform public health guidelines.

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Appendices

Appendix A. *International physical activity questionnaire*

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

No vigorous physical activities → **Skip to question 3**

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

No moderate physical activities → **Skip to question 5**

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

No walking → **Skip to question 7**

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

8. If your total engagement in moderate-to-vigorous physical activity is 150 min/week or more, has this been a regular weekly occurrence over the past 3 months?

Yes

No

9. How often does your job require you to sit for long periods of time during your work-shift? (please circle one option below)

All **most** **some** **little of the time** **never**

10. On average, how many hours per day do you spend watching TV? (please circle one option below)

Less than 1 hour **1-2 hours** **2-3 hours** **3-4 hours**

More than 4 hours

Appendix B. Domain-specific sitting time questionnaire

Domain-specific sitting time questionnaire (Marshall *et al* 2010)

Please estimate how many hours you spend SITTING EACH DAY in the following situations: (Please write your answer)

	<u>On a WEEK Day</u>		<u>On a WEEKEND Day</u>	
	Hours	Minutes	Hours	Minutes
While travelling to and from places				
While at work				
While watching television				
While using a computer at home				
In your leisure time, NOT including Television (e.g., visiting friends, Movies, dining out, etc.)				

Appendix C. Physical Activity Questionnaire example



PAR-Q - Please circle the correct answer.

1. Have you ever been told by your doctor that you have a heart condition and advised only to participate in physical activity approved by your doctor?
Yes/No

2. Do you experience any chest pains when you participate in physical activity?
Yes/No

3. Have you recently experienced any chest pains whilst not participating in physical activity?
Yes/No

4. Do you ever lose consciousness?
Yes/No

5. Do you ever lose your balance as a result of dizziness?
Yes/No

6. Do you have any problems with your bones and joints that could cause further problems if you participate in physical activity?
Yes/No

7. Are you aware of any reasons as to why you should not participate in physical activity?
Yes/No

Name: Signature: Date:

Appendix D. Pre-study health questionnaire



Sport and Exercise Science Laboratories
 Polhill Avenue
 Bedford MK41 9EA

PRE-STUDY HEALTH QUESTIONNAIRE

To be completed by all participants before participating in practical sessions.

Name:

Age:..... Gender: M / F

1 Are you in good health? Yes / No
 If no, please explain:

2 Are you pregnant or have you given birth in the last 6 months? Yes / No

3 How would you describe your present level of moderate activity?

<	once	per	month
once	times	per	month
2-3	times	per	week
4-5	times	per	week
> 5 times	per week		

4 Have you suffered from a serious illness or accident? Yes / No
 If yes, please give particulars:

5 Are you recovering from an illness or operation? Yes / No
 If yes, please give particulars:

6 Do you suffer, or have you ever suffered from:
 Respiratory conditions (asthma, bronchitis, tuberculosis, other)? Yes / No
 Diabetes? Yes / No
 Epilepsy? Yes / No
 High blood pressure? Yes / No

Heart conditions or circulation problems:
 (angina, high blood pressure, varicose vein, aneurysm, embolism, heart attack, other)?

Do you have chest pains at any time? Yes / No
 Do you suffer from fainting/blackouts/dizziness? Yes / No

Is there any history of heart disease in your family? Yes / No

7 Are you currently taking medication ? Yes / No
If yes, please give particulars:

8 Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? If yes, please give particulars: Yes / No

9 Have you had to consult your doctor, or had hospital treatment within the last six months? Yes / No

10 Are you currently fitted with a pacemaker? Yes / No

11 Have you, or are you presently taking part in any other laboratory experiment? Yes / No

12 Has your body weight been stable for the past 6 months (i.e. not varied by more than 2 kg/4.4 lb)? Yes / No

13 Are you currently dieting? Yes / No

14 Do you have any food allergies? Yes / No
If yes, please state what this allergy is.....

DECLARATION

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Name of participant (please print) _____

Signature of participant _____

Date: _____

Name of Experimenter (please print) _____

Signature of Experimenter _____

Date: _____

Appendix E. Participant information sheet and participant consent form example



INFORMATION SHEET

Blood sugar responses to interrupting prolonged sitting with short bouts of activity

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate.

What is the aim of the project?

The aim of this study is to evaluate the effects of interrupting prolonged sitting with short bouts of activity on blood sugar levels that will be measured continuously day and night.

What type of participant is needed?

We require adults aged 18-55 years who sit for an average of 7 hours or more per day and have a higher than average body weight for their height. Participants excluded from this study are individuals with any known blood borne disease, pregnancy, diagnosed diabetes, taking glucose-lowering and/or lipid-lowering medication, has an artificial pacemaker, any known health problems that prevent you from taking part in physical activity, or other health issues that may limit the ability to perform the necessary activity bouts.

As it is not feasible to list every medical condition, it is possible that individuals with other medical conditions, not given above, may be excluded from the study.

What will participants be asked to do?

You will be required to attend the University of Bedfordshire Sport and Exercise Science Laboratories on two separate occasions. The first visit will last for approximately 2 hours. During this visit, you will have your height, weight, and body fat levels measured. You will then be fitted with a small sensor which goes into the skin on the back of your arm that will measure your blood sugar levels continuously. You will also have an activity monitor stuck to your thigh. You will be asked to wear both the blood sugar monitor and activity monitor for 12 to 14 days continuous days. During the 11 days, you will be asked to scan the blood sugar monitor attached to your arm 3 times a day

using a small device that we will provide to you. You will be shown how to do this and have an opportunity to practice doing it with the research team, and provided with written instructions.

During the 11 days that you are fitted with the blood sugar and activity monitors, you will be asked to complete the two conditions described below. The first condition will be undertaken on days 1-4 and the second will be undertaken on days 8-11. There will be a 3 day gap between each of these conditions where you continue your normal daily routines. You will also be asked to record all food and beverages you consume during days 1-4 and replicate this consumption exactly during days 8-11.

IMPORTANT: We will ask you not to take part in any planned exercise throughout the study period.

The conditions are:

- 1) Sitting – You will be asked to sit for as much as possible during these 4 days. You will be asked to restrict the amount of walking and standing you do.
- 2) Interrupted Sitting – You will be asked to regularly interrupt your sitting time using a range of simple physical activities between 7am and 9pm on each of the 4 days. We will demonstrate some example activities to you. To remind you to interrupt your sitting regularly, we will also ask you to download a phone app or computer software to help you with this.

On the days between these two conditions, you will be asked to continue your normal daily routine, but not to engage in any exercise. A member of the research team will also contact you daily to check everything is working ok with your blood sugar and activity monitors.

The final visit to the laboratory will last approximately 30 minutes, where you will return your blood sugar and activity monitors.

What are the possible risks of taking part in the study?

There is a small risk of inflammation or infection at the site of insertion of the blood sugar sensor in your arm. This will be minimised by only using sensors that have been kept secure in their sterile packaging and ensuring the skin is appropriately cleaned before insertion of the sensor. There is a small risk that the medical grade dressing used to attach the activity monitor to the skin could cause irritation. Please remove the device and attachment if this occurs and contact the research team to discuss an alternative dressing withdrawal from the project. Please contact the research team if you have any queries regarding the blood sugar or activity monitors during the study.

What if you decide you want to withdraw from the project?

If, at any stage you wish to leave the project, then you can. There is no problem should you wish to stop taking part and it is entirely up to you. There will be no disadvantage to yourself should you wish to withdraw.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice in accordance with the Data Protection Act (1998). All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to senior members of the research team. Access to identifiable data (name, address etc.) will be limited to selected members of the research team and will be kept on secure University computers. This information and other personal details will not be included in analysis, or in publications or reports. All information collected during the study will be identified by a unique code so that you cannot be identified from it. All data will be kept on secure computer servers and in locked filing cabinets within a locked office at the University of Bedfordshire.

What will happen to the results of the study?

Everyone that takes part in the study will receive the results of the study once available. The results may be presented at academic conferences and/or published in academic journals.

What if I have any questions?

Questions are always welcome. Please contact one of the following research team members to ask questions:

Miss Charlotte Stringer: Charlotte.Stringer@study.beds.ac.uk, 07752 145017

Dr Daniel Bailey (Academic supervisor): Daniel.Bailey@beds.ac.uk, 01234 793237

Many Thanks,
Charlotte Stringer, Daniel Bailey
Institute for Sport and Physical Activity Research
University of Bedfordshire,
Bedford Campus,
Polhill Avenue,
Bedford
MK41 9EA

Participant Consent Form

UNIVERSITY of BEDFORDSHIRE

Title of study: Blood sugar responses to interrupting prolonged sitting with short bouts of activity

I confirm that I understand the nature of the practical test above and what is involved in the protocol outlined. I further confirm that my health is normal and the information given on the health/medical questionnaire is accurate and complete.

My agreement to participate in the experiment is made of my own free will, and not in response to financial or other inducements (e.g. peer pressure). I confirm that I am not currently participating in an experimental trial.

The attention of volunteers is drawn to the fact that in the case of injury to persons or damage to property no claim for damages can succeed against University of Bedfordshire or against its employees unless legal liability resulting from negligence can be proved.

Name: _____

Signed: _____

Date: _____

Email address: _____

Phone number: _____

Researcher: _____

Signed: _____

Date: _____

Appendix F. Daily activity record sheet example

Time	Condition	Activity during the hour e.g. asleep, sat, stood	Duration
Example 1	SIT	Sat	1 hour
Example 2	INT SIT	Sat Stood Sat	30 mins 5 mins 25 mins
06:00			
07:00			
08:00			
09:00			
10:00			
11:00			
12:00			
13:00			
14:00			
15:00			
16:00			
17:00			
18:00			
19:00			
20:00			
21:00			
22:00			
23:00			

Appendix G. Uninterrupted Sitting Instructions

What to do during your Sitting condition

Dear Participant,

During the Sitting condition we want you to sit as much as possible. Please follow these instructions from dates [.....] to [.....]

1. Remain seated for a minimum of **10 hours** a day while you are awake on each of the **4 days**.
2. For **7 of these 10 hours**, please sit continuously uninterrupted for the full hour, i.e. do not get up and move around for the whole hour – only get up if you need to go to the toilet. Apart from toilet breaks, please remain seated for as long as possible.
3. Please limit standing and walking to a maximum of 1.5 hours per day. Where possible please use public transport or a car to travel to and from places to limit your activity levels.

Please do not engage in any structured exercise, or consume any alcohol during the whole study (including the three washout days)

Appendix H. Interrupting Sitting Instructions

Interrupting Sitting Instructions

Dear Participant,

During the Interrupting Sitting condition, you will be asked to break up your sitting every 30 minutes for each of the 4 days. This will take place on dates [.....] to [.....]

Each day please break up your sitting time with 3 – 5 minutes of light or moderate physical activity every 30 minutes for **a minimum of 10 hours** on each day. For example, you may break up your sitting time for 6 hours while at work and 4 hours while at home. **This means engaging in a minimum of 20 breaks per day.**

If you have a long commute to work, need to sit through a long meeting, or cannot stand within a particular hour for a different reason, that is ok as long as you break up your sitting time for at least 10 hours over the day from when you wake up to when you go to bed.

Please ensure you engage in a minimum of 1.5 hours of standing or physical activity (e.g. from the activities below) on each of the 4 days.

Here are some suggestions for activities you can do during your breaks:

- Walking at a slow pace (or quicker if you wish to)
- Walking on the spot
- Taking the stairs
- Standing (you could stand when you are unable to engage in the other activities e.g. during meetings)
- Repeatedly get up and down from your chair (chair squats)
- Body weight half squats
- Calf raises
- Butt squeezes
- Knee raises

You will be given a demonstration on how to perform these exercises.

Please do not engage in any structured exercise, or consume any alcohol during the whole study (including the three washout days)

Appendix I. 4 day weighed food diary

4 day weighed food diary

Introduction

We would like you to fill out a weighed food diary of all the food and drink you consume during your first 4 condition days. You will need to take the food diary with you so you can record what you eat and drink whilst you are not at home.

Please replicate this food and drink intake exactly (amount consumed and timings) during your second 4 condition days.

Please return a copy of your food diary to us.

Each diary entry should include (See example sheet):

- Everything you eat and drink, it could be a main meal or small snack (including sweets and even water).
- Please include extras like sauces, gravies and dressings that you put on your food.
- Please give as much information as possible about the amount and type of food or drink you are consuming (weight of the food or drink, any brand names e.g. Heinz baked beans).
- If possible, please put any packaging in clear bags provided. Especially if you cannot weigh the items (i.e. crisp packet eaten out and about)
- Include the time of day, try to say if the meal or snack is for breakfast, lunch or dinner.
- Please list all of the ingredients in whatever item you eat or drink. e.g. If at lunchtime you have a ham sandwich you should list the amounts and type of foods (e.g. 2 slices of honey roast ham; 15 grams of iceberg lettuce; two slices of wholemeal bread, SEE EXAMPLE DIARY).
- There are sections on the diary to enter the different meals you eat (breakfast, lunch, dinner etc), separated by a grey bar. If you need more space to write in each section, just go over the grey shaded bar into the next section and start the next meal after the next grey shaded area (see example).

Weighing your food and drink

If you cannot weigh your food or drink, please provide a description of the amount of food or place any packaging in the plastic wallet provided and explain the quantity of the packaged food you have eaten – just so you can replicate this during your second condition.

Here is an example of how to weigh a ham sandwich with lettuce (**make sure the scales are set to weigh in grams**):

- Turn on the scales by pressing the ON button
- **Weigh the empty container.** Place the container (e.g. plate) you will be eating from, on the scales. You should use this plate to weigh the ingredients on. **Make sure you write the weight of the empty plate.** You should repeat this process if you eat out of or off an additional container (e.g. if you have a plate of toast and a bowl of cereal)
- Before putting any food on the plate press the **ZERO** button, the scales should now have the plate on but display no weight (**0.00 grams**)
- Add to the plate both pieces of bread (remember to list the type (e.g. wholemeal) in the 'Food & Drink' column. Record the weight (in grams) of the bread in the '**Weight served**' column.
- Press zero to reset the scales to **0.00 grams** and add any butter or spread to the bread. List the type of and brand of spread and record the weight of the butter in the appropriate sections.
- Press zero to reset the scales and repeat the process with the lettuce.
- Whenever a new ingredient is added, the scales should be zeroed so that the weight of each ingredient can be recorded separately as it is added to the plate.
- Use the weight on packaging to help diary entries if you consume the total contents of the packet.
- You should also record what drink you consume e.g. 1 small glass of water, 1 bottle of lucozade. Ideally weight the liquid you consume too if you can.
- Any **leftovers**, not eaten, should be re-weighed and noted in the diary. If there is food leftover, re-weigh the container or containers that you have eaten or drunk from (with any leftovers on/in) and write this in the '**weight leftover**' column on the same line as where you have written the weight of the empty container. Put a tick next to the foods or drinks that are left on/in the container.

	Food & Drink (Please describe in detail)	Weight served (g)	Weight leftover(g) (tick items left)	Leave blank (office use only)
Weight of empty container _____g - remember to weigh your empty plate or container-		_____g		
remember to include Drinks Snacks Wrappers Time eaten: _____ Type of meal: (circle) B. L. D. S.				
Weight of empty container _____g - remember to weigh your empty plate or container-		_____g		
remember to include Drinks Snacks Wrappers Time eaten: _____ Type of meal: (circle) B. L. D. S.				
Weight of empty container _____g - remember to weigh your empty plate or container-		_____g		
remember to include Drinks Snacks Wrappers Time eaten: _____ Type of meal: (circle) B. L. D. S.				

Appendix J.

Example of meals and snacks

Breakfast

- Toast with butter and jam
- Bagel with butter
- Porridge with milk – Flavoured; Golden syrup etc. or plain
- Cheerios with milk
- Shredded Wheat with milk
- Cornflakes with milk
- Granola with milk
- Toast Waffles – with or without topping; syrup, fruit etc.
- Scotch Pancakes – with or without topping; butter, syrup etc.
- Toaster Pastries e.g. Pop Tarts
- Granola bars
- Fruit; e.g. banana

Lunch

- Pre-made shop brought sandwich, crisps and chocolate bar (meal deals)
- Homemade sandwich, crisps and chocolate bar
- Bagel with butter and cream cheese
- Jacket Potato with cheese and beans
- Pasta meal e.g. pre-packed pasta salad, pasta bake
- Microwave packet or risotto, with optional extras e.g. tuna, cheese, eggs, ham
- Sausage roll
- Samosa
- Savoury Eggs – scotch eggs

Dinner

- Sausage and mash with gravy and vegetables
- Spaghetti Bolognese sauce with garlic bread
- Chilli con carne with rice
- Packaged pizza
- Curry with rice and naan bread
- Pasta Bake
- Lasagne with garlic bread
- Breaded cod with oven baked chips and vegetables
- Cottage/ Sheppard's pie and vegetables
- Cannelloni
- Oven baked pie (prepackaged or homemade) with oven baked chips and vegetables
- Toad in the hole with mash and vegetables
- Noodle dish

Drinks and Snacks

- Fizzy drinks
- Water
- Coffee and/or Tea
- Fruit
- Crumpet with topping; butter, syrup, jam, chocolate spread
- Biscuits / cookies
- Granola bar / cereal bar
- Chocolate bar
- Crisps
- Rice crackers
- Cake
- Sweets

Appendix K. Flash Glucose Monitoring instructions

Flash Glucose Monitoring

Once you have been fitted with your flash glucose sensor, you will be required to connect the receiver sensor at least 3 times a day, as shown in the picture. This is done by holding the reader within 4 cm of the sensor. The reader will beep when the reader has been successfully synced. If the sync is not successful, the reader will not beep and you will need to try again in 15



monitoring
to the
picture.
sensor.
reader will
seconds.

This will need to be done **every 8 hours**. The recommended times for this are; upon waking, midday/ at lunch, and before bed. However, there is no maximum amount of times that you can connect your device to the sensor. You are advised to do this at the recommended times **and** whenever you remember to throughout the rest of the day and night.

The reader battery should last for 7 days, and there will be a charging icon visible. To fully charge the battery, please charge the reader for at least 3 hours. This can be done over-night.

The sensor is water resistant and can be worn while bathing and showering but **MUST NOT** be submerged longer than 30 minutes in water.

Note: The reader is not water resistant and should **NEVER** be submerged in water or other liquids.

Appendix L. *Thigh Monitor Instructions (activPAL)*

Thigh Monitor Instructions (activPAL)

How do I wear the monitor?

- The Thigh Monitor is attached directly onto the skin and positioned on the front of the thigh, roughly 1/2 of the way between hip and knee with the stick man standing up (see picture).
- Please wear the monitor **every day for the 12-14 days you take part in the study**. We will remove it when you return to see us during your final laboratory visit.
- Please wear the Thigh Monitor continuously (24 hours/day)
- The Thigh Monitor **can be** worn during sleep and is water resistant (so fine to wear in the bath or shower) but please **do not wear it** when swimming or in the sea.
- The adhesive patch that sticks the Thigh Monitor to your skin may last up to 8 days but to avoid skin irritation you may want to change the adhesive patch.

Note: The Thigh Monitor will emit a green flash every 6 seconds. This is an indication that it is working and recording data.



How do I change the adhesive patch?

- You can watch this video for guidance on how re-attach your Thigh Monitor: https://www.youtube.com/watch?v=BuaRHZ_BOA4
- Remove the Thigh Monitor from your thigh and peel the adhesive patch off the Thigh Monitor. The monitor is covered in a waterproof sleeve and wrapped in one adhesive patch—please make sure that these remain on the monitor when you do this (they make the monitor waterproof).
- With an alcohol prep wipe provided, thoroughly wipe down the area of your leg where the Thigh Monitor was attached.
- Position the Thigh Monitor in the same spot as previously on your thigh (or on the other thigh if you have had a slight irritation), ensuring that the stick man on the front of the Thigh Monitor is standing up (head facing upwards).
- Peel the covering off an adhesive patch (provided in your pack) and place it over the Thigh Monitor. Press the patch onto your skin, starting from the middle out towards the edges peel back the top layer of the patch and smooth out the air bubbles and wrinkles as much as possible to ensure that the Thigh Monitor is firmly secured to your thigh.
- If you require assistance re-attaching your Thigh Monitor, or if you experience any skin irritation whilst wearing it, please call Charlotte Stringer on 07752145017.

What else do I need to do?

- It is important that you fill in the **Daily Log** on the following pages every day for the 11 days while you are wearing the monitor.
- This helps us to look specifically at the data from when you were awake.

How to fill in the daily activity monitor log

- The log is divided into 11 days. Please complete each question for all of the 11 days. Please try and be as accurate as possible—record the exact times if you can, or at least to the nearest 5 minutes of your estimated times.
- Start by writing the **date** in the top row.
- Record the time that you **woke up** and the time that you actually **got out of bed**. We ask for these two times because people sometimes spend time in bed before going to sleep or getting up and we are interested in distinguishing between actual sleeping time and time in bed before sleep or once awake, for example going to bed and watching TV for an hour before going to sleep.
- Please write **AM or PM** next to your times.
- Record the time that you **started** and **finished** work. This allows us to look at the data recorded whilst you were at work.
- Record what time you **got into bed** to go to sleep and the time that you actually **went to sleep time**. (i.e., the estimated time that you fell to sleep not the time that you got into bed). This is important as the monitor cannot tell the difference between asleep and awake times.
- Please record your sleep time first thing in the morning when you wake up along with recording your wake time and time that you got out of bed.
- If you remove either device for longer than 10 minutes during the day please note down the **time that you removed the device**, the **time length that the device is removed** and the **reason why you removed the device**. This is particularly important as we cannot tell from the data if you are lying down or whether you have removed the device and are just not wearing it (the data looks the same when we look at it).
- Being as accurate and thorough as possible when completing this log enables us to look at your data more accurately.
- If you have any questions about the log please contact Charlotte Stringer on 07745215017.

ID Number:

Day and date	Wake up	Got out of bed	Started work	Finished work	Got into bed	Went to sleep	Times during the day when I took my leg monitor off and why	Any other comments
<i>Example: Mon 17th Dec</i>	<i>0700am</i>	<i>0715am</i>	<i>0900am</i>	<i>1700pm</i>	<i>2300pm</i>	<i>2330pm</i>	 <i>1600pm for 45 minutes to go swimming</i>	
Date:								
Date:								
Date:								
Date:								
Date: (washout)								
Date: (washout)								
Date:								

(washout)								
Date:								
Date:								
Date:								
Date:								

