
Title : Effects of interrupting prolonged sitting with high-intensity physical activity on postprandial metabolism

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# Effects of interrupting prolonged sitting with high-intensity physical activity on postprandial metabolism

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A thesis submitted to the University of Bedfordshire, in fulfilment of the requirements for the degree of

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**Authors Declaration**

I CHARLIE ORTON declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Effects of interrupting prolonged sitting with high-intensity physical activity on postprandial metabolism

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

3. Where I have cited the published work of others, this is always clearly attributed;

4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

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6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

7. Either none of this work has been published before submission, or parts of this work have been published as indicated on **References (p.62).**

**Signed ……..** **Date …….**

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**Abstract**

**Aims** The aim of this thesis was to explore the effects of interrupting prolonged sitting with high-intensity activity on cardiometabolic risk markers. **Methods** In study 1, participants completed 3, 8 hour trials: 1) uninterrupted sitting (SIT) 2) continuous moderate-intensity activity followed by sitting, and 3) sitting interrupted with hourly high-intensity activity (SIT-ACT). In study 2, participants completed 3, 6.5 hour trials: 1) SIT, 2) a continuous high-intensity interval exercise session followed by sitting (CON-HIE), and 3) sitting interrupted with high-intensity activity bouts (SIT-HIE). Postprandial incremental area under the curve (iAUC) was calculated for cardiometabolic risk markers and compared between conditions. Data are mean (95% confidence intervals). **Results** In study 1, glucose iAUC was not different between conditions ($p$= 0.606). Triglyceride (TG) iAUC was lower and high-density lipoprotein was higher in SIT-ACT than SIT ($p$=<0.05). In study 2, glucose iAUC was significantly lower in SIT-HIE than SIT (p=0.026), while TG iAUC was significantly lower in CON-HIE than SIT (p=0.014). **Conclusion** Study 1 observed beneficial TG and HDL responses to interrupting sitting with high-intensity activity. Study 2 observed suppressed glucose in response to interrupting sitting with high-intensity activity, but postprandial TG was reduced only in response to a high-intensity interval exercise session.

**Keywords:** Prolonged sitting; Postprandial glycaemia; postprandial insulinaemia; postprandial lipaemia; glucose; insulin; Triglycerides; High-Density Lipoproteins; High-intensity activity; Continuous moderate-intensity activity.

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## Glossary

### Trial conditions

**Study 1**

SIT – Uninterrupted sitting

MOD-CON – Sitting with a single continuous session of moderate-intensity activity

SIT-ACT – Sitting with hourly interruptions of high-intensity activity

**Study 2**

SIT – Uninterrupted sitting

CON-HIE – Sitting with a single session of high-intensity activity

SIT-HIE – Sitting +high-intensity activity breaks every 30 minutes

TG – Triglycerides

HDL – High-density lipoprotein

T2DM – Type 2 diabetes mellitus

GLUT4 – Glucose transporter 4

iAUC – Incremental area under the curve

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## 1 Introduction

A lack of regular exercise and prolonged sedentary time are significant factors contributing to the increased risk of coronary heart disease, type 2 diabetes mellitus (T2DM) and all-cause mortality (Dunstan et al., 2011). More specifically, cardiovascular mortality and cardiovascular event relative risks may be increased by 90 % and 147 %, respectively, as a result of increased total daily sedentary time (Wilmot et al., 2012b). Technological advancements have caused a global rise of prolonged sitting in the workplace, during transportation and in leisure time (Bailey, 2015). These behaviours are characterised by low energy expenditures and are associated with increased levels of obesity (Leon-Munoz et al., 2013). Furthermore, sitting for long periods also results in a prolonged absence of muscular contractions, particularly in the lower limbs (Hamilton et al., 2007). This may have a strong negative impact on metabolic processes, such as the metabolism of glucose and lipids, which are involved in the regulation of cardiometabolic disease risk (Hamilton et al., 2007).

Recent research has examined the cardiometabolic effects of frequent small amounts of physical activity for intervening prolonged periods of sitting (Dunstan et al., 2012). Dunstan et al. (2012) initiated this area of research by finding significant reductions to 7 hour glucose and insulin concentrations when interrupting prolonged sitting with 2 minutes of light or moderate intensity walking every 20 minutes over a 5 hour postprandial period. Miyashita et al., (2006) examined the effects of accumulating multiple bouts of exercise throughout the day on postprandial lipaemia and compared this with a time and intensity matched single 30 minute bout. Significant reductions to postprandial lipaemia were observed in both the intermittent and continuous activity bouts in comparison to uninterrupted sitting (Miyashita et al., 2006). It is also important to point out that the reduction observed in the intermittent condition was similar to the continuous activity group. This suggests that intermittently performing activity may induce benefits to postprandial lipaemia similar to traditional physical activity methods (Miyashita et al., 2006).

To date, studies investigating interrupting prolonged sitting on cardiometabolic risk markers have focussed on low-intensity to moderate-intensity activity. The use of high-intensity activity to interrupt prolonged sitting has not been researched. High-intensity activity could serve as a more time efficient method for individuals to reach the government physical activity recommendations when compared with lower intensities. The higher energy expenditure inherent to high-intensity exercise may also produce cardiometabolic risk benefits of a greater magnitude than lower-intensity activity (Tremblay et al., 1994). Therefore, the primary aim of this thesis is to explore the effect of high-intensity activity use to interrupt prolonged sitting on cardiometabolic risk markers.

## 2 Literature review

This chapter reviews and critically examines the literature surrounding the effect of breaking up prolonged sitting and acute high-intensity physical activity on postprandial glycaemia, lipaemia and insulinaemia. The first section explores the prevalence and negative health effects of prolonged sitting. This is followed by a comprehensive discussion surrounding the new concept of interrupting prolonged sitting with physical activity on glycaemia, insulinaemia and lipaemia. High-intensity physical activity and its strong health benefits are also discussed in comparison to traditional moderate intensity physical activity approaches. The final sections provide a summary and rationalises a novel approach to completing high-intensity physical activity as a method to break up prolonged sitting.

### *2.1 Postprandial glycaemia and insulineamia*

Blood glucose concentration (glycaemia) reflects the balance of influx of carbohydrates from the diet to the cells and muscles for immediate oxidation or storage (Blaak et al., 2012). Insulineamia is the resultant rise in insulin release from the pancreas, due to an increase in blood glucose after consumption of a meal (Blaak et al., 2012, de Vegt et al., 2001). With regards to insulin-sensitive tissues (muscle and adipose tissue), glucose transporter-4 (GLUT4) is the protein mediating glucose uptake through the process of facilitated diffusion (Ryder et al., 2001). Insulin and muscle contractions translocate GLUT4 to the sarcolemma and T-tubular system of muscle (Derave et al., 2000, Derave et al., 1999). Muscle contractions in particular are believed to increase the translocation of GLUT4 seven fold (Jensen et al., 2014), which is likely mediated by AMP-activated protein kinase (Jørgensen et al., 2006). The aetiology of chronic metabolic diseases such as T2DM and cardiovascular disease are more strongly associated with postprandial (post-meal consumption) glycaemia compared with fasting glucose levels (Blaak et al., 2012). Interventions into reducing post-meal hyperglycaemia are now an important preventative target.

*2.2 Postprandial lipaemia*

Postprandial lipaemia refers to the rise in plasma triglyceride-rich lipoproteins after the consumption of a meal (Hyson et al., 2003). Triglycerides (TG) are a form of dietary fat, which can be taken up by cells and used for energy or alternatively stored as fat. TG cannot pass freely through cell membranes. Enzymes named lipoprotein lipases break down TG into free fatty acids and glycerol, by the process lipolysis. Fatty acids are then taken up by cells through the fatty acid transporter. TG is transported around the body in blood plasma when combined with lipoproteins. The resultant increase in lipaemia as a result of meal consumption may be a better predictor of cardiometabolic disease risk in comparison to fasting levels (Bansal et al., 2007, Trombold et al., 2013). With people in modern society typically spending a larger proportion of the day in the postprandial state due to snacking and regular meal intake, postprandial metabolism is now considered a leading phenomenon in the development of cardiometabolic disease (Hyson et al., 2003).

Recent evidence has determined that elevated postprandial plasma triglyceride (TG) levels are a risk factor in the development of cardiovascular disease, independent of other lipid levels and markers of insulin resistance (Bansal et al., 2007, McBride, 2008, Nordestgaard et al., 2007). Additionally, impaired ability to clear TG rich lipoproteins and an increased hepatic secretion of very-low density lipoproteins are factors which increase cardiometabolic risk (Harchaoui et al., 2009). Measurement of TG in the fasted state does not provide the best indicator of TG metabolism, particularly as the major catabolic pathway for TG typically occurs in the postprandial state (Weintraub et al., 1996). Therefore, lowering postprandial hypertriglyceridemia may an important factor in reducing cardiometabolic disease risk.

*2.3 Prevalence of sedentary behaviour*

Sedentary behaviour is defined as 'any waking behaviour characterised by an energy expenditure ≤1.5 metabolic equivalents while in a seated or reclining posture' (Sedentary Behaviour Research NETWORK, 2012). Modern occupations and technological advancements have resulted in a global rise in sedentary behaviour, such as prolonged sitting and a reduction in physical activity (Sedentary Behaviour Research NETWORK, 2012). This is typically due to individuals spending a greater amount of time sitting in desk-based office occupations, during personal transportation and leisure-time entertainment.

Epidemiological evidence has revealed that sitting time is considerably varied amongst several European countries(Bennie et al., 2013). This epidemiological study examined data from adults aged 15-98 years (n = 27,637) across 32 European countries. It appeared that the lowest amounts of daily sitting were reported in southern (means 194–236 minutes/day) and eastern (means 191–276 minutes/day) European countries; and some of the highest amounts of daily sitting were reported in northern European countries (means 407–335 min/day) (Bennie et al., 2013). Further analyses revealed that factors such as low physical activity levels, poor general health and higher education significantly increased the likelihood of individuals being in the highest quartile of sitting time (Bennie et al., 2013). Loyen et al. (2016) also found that northern European countries reportedly had a greater time spent sitting than countries in the south of Europe. The strongest correlates with increased sitting time were current occupation (white collar occupations being the highest) and age when participants left education (Loyen et al., 2016). The odds ratio of sitting more than 7.5 hours per day was 5.00 for people with office based jobs, when compared to manual labour occupations (Loyen et al., 2016).

Overall, this research indicates that sitting is clearly prevalent amongst a large proportion of countries, in particular across north-western countries where occupations consist largely of office based work (Bennie et al., 2013, Loyen et al., 2016).

*2.4 Observational research of sitting and cardiometabolic markers*

Aside from low energy metabolism, sitting induces unfavourable changes in the regulation of cardiometabolic risk markers such as glucose and TG that may lead to increased risk of cardiometabolic disease (Hamilton et al., 2007). This is believed to be due to a lack of muscular contractions which mediate several cellular pathways in the metabolism of these markers (Hamilton et al., 2007). Much of Hamilton's work is based around studies in rat models and although the genetic, biological and behavioural make up is similar to that of humans, any responses observed in these animal models as a result of a specific treatment can only be speculated to have the same effect in humans.

A meta-analysis of 47 studies observed that prolonged sedentary time was deleteriously associated with cardiovascular disease, T2DM, cancer and all-cause, independent of physical activity levels (Biswas et al., 2015). Ekelund et al., (2016) explored whether physical activity could attenuate the detrimental effects of sedentary behaviour. Data from 16 prospective cohort studies were used which included an individual level exposure and outcome data for daily sitting time, TV viewing time and physical activity (Ekelund et al., 2016). It was observed that high levels of moderate-to-vigorous intensity physical activity (roughly 60-75 minutes per day) eliminated the increased risk of mortality associated with high amounts of sitting time (Ekelund et al., 2016). However, this level of physical activity attenuated, but did not eliminate the increased risk of mortality due to high TV viewing time (Ekelund et al., 2016).

Wilmot et al. (2012) examined 18 studies in a meta-analysis to determine how sedentary time is associated with cardiovascular disease, T2DM and cardiovascular and all-cause mortality. The study found that higher levels of sedentary time increased the relative risk of diabetes by 112 %, cardiovascular disease by 147 %, cardiovascular mortality by 90 % and all-cause mortality by 49 % (Wilmot et al., 2012a). Edwardson et al. (2012) investigated the association between sedentary time and the metabolic syndrome, which is a clustering of cardiometabolic risk markers including elevated fasting glucose levels and TG and low high-density lipoproteins. The meta-analysis used 10 prospective studies and found that people who spent a higher amount of time sedentary were 73 % more likely to have the metabolic syndrome. A cross-sectional investigation of 4757 participants (≥20 years) from the 2003/04 and 2005/06 US National Health and Nutrition Examination Survey (NHANES) explored the association between objectively assessed sedentary time and cardiometabolic risk markers (Healy et al., 2011). Significant detrimental linear associations were observed between total sedentary time and waist circumference, high-density lipoprotein (HDL), TG and insulin, independent of moderate-to-vigorous physical activity (Healy et al., 2011).

Observational research has identified an association between objectively measured sedentary time and cardiometabolic disease risk markers, independent of time in moderate-to-vigorous physical activity (Henson et al., 2013, Healy et al., 2008, Healy et al., 2011). Cross-sectional studies have also found that an increased number of breaks in sedentary time was associated with a lower body mass index and more favourable cardiometabolic risk marker levels, such as lower waist circumference, TG and 2 hour glucose (Healy et al., 2008, Healy et al., 2011, Carson et al., 2014, Henson et al., 2013). This evidence highlights the potential importance of reducing prolonged bouts of uninterrupted sitting. However, experimental research examining the effects of breaking up prolonged sitting on postprandial glycaemia and lipaemia is required to identify a causal relationship.

*2.5 Breaking up prolonged sitting and postprandial glycaemia and insulinaemia*

Previous observational data has informed a number of subsequent experimental studies investigating the effects of interrupting prolonged sitting on cardiometabolic risk markers, which have largely focused on postprandial glycaemia. A review by Benatti and Ried-Larsen (2015) examined the available evidence from prospective experimental studies regarding the effects of breaking up prolonged sitting time on cardiometabolic risk factors. The review considered the effect of intensity of breaks from sitting time, frequency of breaks and total volume of physical activity performed within the breaks across the experimental period (Benatti and Ried-Larsen, 2015). Analysis from experimental studies revealed that performing light-intensity physical activity moderate-intensity (see below for definition) is sufficient to induce beneficial cardiometabolic outcomes in individuals who are physically inactive or those with T2DM (Benatti and Ried-Larsen, 2015). According to data from Benatti and Ried-Larsen (2015), younger, healthy participants require higher intensity or higher volume of physical activity in order to render positive cardiometabolic effects, similar to that of light-intensity physical activity in physically inactive participants (Kim et al., 2014). Light-intensity physical activity does not appear to have any significant benefits in younger, healthier populations (Benatti and Ried-Larsen, 2015).

The intensity of physical activity used to break up prolonged sitting varies across studies and is an important consideration when reviewing the research findings. Moderate-intensity physical activity is defined as 40% to <60% of an individuals' oxygen uptake reserve ($\dot{V}O_2R$)Reserve or HHR, which is equivalent of 3 to <6 metabolic equivalent tasks (MET's) (Riebe et al., 2015, Ferguson, 2014). Practitioners also use the Borg scale of rating of perceived exertion (RPE) to define intensity, with a rating of 12-14 suggesting a moderate-intensity (Muyor, 2013, Borg, 1982) Examples of moderate-intensity physical activity include brisk walking and general gardening. Vigorous intensity physical activity is defined as achieving ≥60% oxygen uptake reserve or heart rate reserve , or ≥6 metabolic equivalent

tasks (METs) (Ferguson, 2014). This can include jogging/running or heavy gardening.

Performing short bouts of light- or moderate-intensity activity to break up prolonged sitting significantly reduces postprandial glucose and insulin concentrations in apparently healthy and overweight and obese adults (Dunstan et al., 2012, Peddie et al., 2013).

The pioneering study by Dunstan et al. (2012) was the first to highlight the importance of breaking up prolonged sitting on postprandial glycaemia and insulinaemia. The three-condition randomized-crossover design study investigated the effects of breaking up prolonged sitting every 20 minutes with 2 minutes of light- or moderate- intensity activity (walking) compared with an uninterrupted prolonged sitting condition in overweight and obese individuals. Light-intensity walking was at 3.2 km/h and moderate-intensity walking was between 5.8 and 6.4 km/h on a motorised treadmill at a level surface. A total of 14 bouts were completed for both intensities, which equated to 28 minutes of activity in each condition. The postprandial period lasted for 5 h, starting after consumption of a standardised test drink (75 g carbohydrate, 50 g fat 12.8 g protein). Postprandial glucose incremental area under the curve (iAUC) was 24.1 % and 29.6 % lower in the light-intensity and moderate-intensity walking conditions, respectively, compared with the uninterrupted sitting condition (Dunstan et al., 2012). Both light- and moderate- intensity activity lowered postprandial insulin iAUC responses by 23 % relative to uninterrupted sitting. However, the study by Dunstan et al. (2012) does have some potential limitations. Firstly, breaking up prolonged sitting every 20 minutes may be impractical for some individuals and could be perceived as interruptive to work tasks in desk-based employees. Secondly, a 5 hour postprandial period was examined and only one meal was provided; longer postprandial periods with provision of a breakfast and lunch meal could better simulate a normal day. Furthermore, the meal provided was a standardized test drink, which is likely not to be generally consumed in free-living settings.

A subsequent study examined the effects of breaking up prolonged sitting with 2 minutes of light-intensity walking or 2 minutes of standing still every 20 minutes with uninterrupted prolonged sitting in young adults (Bailey and Locke, 2015). Total area under the curve (AUC) for postprandial glucose was ~16 % lower in the light-intensity walking condition compared with the standing breaks and uninterrupted sitting conditions. Standing breaks were not significantly different to levels observed in the uninterrupted sitting condition. However, this study does have some limitations. Firstly, as stated above, 2 minutes of walking every 20 minutes may be impractical for some individuals. Second, insulin was not measured making it difficult to infer whether postprandial glycaemia was reduced as a result of improved insulin sensitivity. Indeed, an acute bout of exercise may result in improved insulin sensitivity for up to 48 hours afterwards (Mikines et al., 1988).

In a different design to the above studies, Peddie et al. (2013) examined the effect of interrupting prolonged sitting with shorter and less frequent bouts, i.e., 1 minute 40 seconds of moderate intensity walking every 30 minutes, throughout a 9 hour postprandial period. This was compared to uninterrupted sitting and prolonged sitting with a 30 minute continuous bout of time and energy matched moderate intensity walking 15 minutes into the postprandial period followed by uninterrupted sitting (Peddie et al., 2013). The regular-activity-break condition lowered plasma glucose iAUC by 18.9 mmol/L compared with uninterrupted sitting and by 17.4 mmol/L compared with the single-continuous physical activity bout condition. The regular-activity-break condition also lowered plasma insulin iAUC significantly when compared with uninterrupted sitting and the continuous physical activity intervention. The effects of uninterrupted sitting and continuous physical activity on plasma insulin iAUC did not differ significantly. It was speculated that the intensity of the exercise in the single-continuous activity condition may not have been high enough to cause any changes in postprandial glucose and insulin, particularly as higher intensity physical activity has been shown to reduce these parameters compared to prolonged sitting (Magkos et al.,

2008). This study provided a liquid meal replacement to standardise macronutrient consumption across the conditions, but this may not reflect a typical meal in free-living conditions. Postprandial responses to interrupting prolonged sitting should be measured after consumption of foods that may be more regularly consumed by the target population in order to better replicate their habitual lifestyle.

An important limitation of previous studies in this area is the use of only low to moderate-intensity activity to interrupt prolonged sitting. Lower volume activity breaks of a higher intensity may be an alternative solution to offset the negative effects of prolonged sitting on postprandial glycaemia that is less time consuming to the individual.

*2.6 Breaking up prolonged sitting and Postprandial Lipaemia*

Several experimental studies have examined the effects of breaking up sitting on postprandial lipaemia. Bailey and Locke (2015) measured pre- and post-condition TG and HDL concentrations but found no significant difference between any of the conditions. A major limitation of this study is the measurement of these markers only pre- and post-condition so responses shortly after meal consumption are unknown. With hourly measures being taken, it would have been possible to calculate AUC for these variables, which may have revealed different outcomes.

Continuous bouts of moderate-intensity physical activity has been identified as an effective method for acutely reducing postprandial plasma TG concentrations (Katsanos, 2006, Malkova and Gill, 2006). A number of studies have also reported on the use of physical activity to break up periods of prolonged sitting on postprandial lipaemia. Altenburg et al. (2013) examined the effects of interrupting prolonged sitting with hourly bouts of 8 minute moderate-intensity cycling (40-60 % heart-rate max) throughout an 8 hour postprandial period. No significant effects were observed on postprandial lipaemia, possibly due to a

combination of the hourly interruptions not being frequent enough and the activity being of a low- to moderate-intensity. Therefore, more frequent interruptions or a higher intensity may be needed to achieve postprandial lipaemic benefits when interrupting prolonged sitting.

Miyashita et al. (2009) completed an experimental study involving two 1-day conditions. The conditions were uninterrupted sitting for 9 hours and prolonged sitting with intermittent activity bouts (running at 70 % of maximum oxygen uptake in six 5 minute bouts every 85 minutes). The study found that breaking up sitting with intermittent running significantly reduced postprandial TG concentrations in comparison to uninterrupted sitting (Miyashita et al., 2009). Accumulating exercise throughout a day instead of performing a traditional moderate-continuous exercise bout is an important observation as this approach could be more time efficient and an attractive addition to the traditional structured exercise approach that many individuals have difficulty adhering to. No previous studies have examined short regular bouts of high-intensity activity to interrupt prolonged sitting. Lower volume activity breaks may be an alternative solution to offset the negative effects of prolonged sitting on postprandial lipaemia. It also may be important to compare high-intensity intermittent activity to interrupt sitting time to moderate-continuous activity in order to examine whether this approach could be an alternative for improving postprandial lipaemia.

### *2.7 Mechanisms for postprandial responses to breaking up prolonged sitting*

The mechanisms explaining the beneficial effects to postprandial glycaemia from interrupting sitting are still unclear. It has been proposed that the significant changes to glucose and insulin parameters observed by Dunstan et al. (2012) could suggest improved insulin sensitivity and reduced insulin secretion. This may result in the preservation of pancreatic β-cell function if performed on a long-term basis (Dunstan et al., 2012). Peddie et al. (2013) proposed that the increases in carbohydrate oxidation with regular activity breaks, as

indicated by the observed higher respiratory exchange ratio, reflected an increased clearance of glucose from the bloodstream. The frequent short bouts of activity may also maintain an increased permeability of muscle cells to glucose (Wallberg-Henriksson et al., 1988). Another mechanism proposed is that frequently performing light activity maintains GLUT4 in a position in the cell where it can be readily recruited to the cell surface in response to minimal amounts of activity (Holloszy, 2005).The increased permeability of muscle cells to glucose and increased translocation of GLUT4 may result in an increased oxidation of glucose (Derave et al., 2000, Derave et al., 1999).

Mechanisms proposed for the attenuation of postprandial lipaemia may include increased lipoprotein lipase activity (Seip et al., 1997) and increased energy expenditure as result of physical activity (Tsetsonis and Hardman, 1996). Lipoprotein lipase is a water-based soluble enzyme that is responsible for hydrolysing TG that are found in lipoproteins into free fatty acids and glycerol (Goldberg, 1996). Miyashita et al. (2009) proposed that increased activity of lipoprotein lipase could have resulted in the increased uptake and a greater oxidation of TG in skeletal muscle, which thus resulted in an increased clearance of TG.

*2.8 High-intensity interval exercise and postprandial glycaemia*

ACSM state that high-intensity exercise is intense ranging from 5 seconds to 8 minutes in duration, and are performed at 80% to 95% of an individuals' estimated maximal heart rate. High-intensity interval training (HIIT) involves alternating low-volume intense exercise with less intense recovery periods over a number of weeks (Gibala and McGee, 2008). This type of exercise training is receiving a high amount of attention from researchers as it provides a

potentially time efficient strategy to elicit physiological adaptations similar to or greater than longer duration, higher-energy expending endurance-based exercise training (Gibala et al., 2006). Physiological adaptations include increased aerobic capacity, mitochondrial content and muscle buffering capacity (Gibala et al., 2006). A single session of HIIT will be termed high-intensity interval exercise (HIIE) in this thesis. HIIE is characterised by various combinations of short, vigorous exercise bursts interspersed with low-intensity exercise bouts or rest and is usually maintained for about 10 to 30 minutes at 70 % - 100 % of maximal oxygen consumption. A number of chronic HIIT studies have been conducted to determined cardiometabolic responses, however, the acute effects of a single HIIE session on postprandial glycaemia are relatively unknown.

Gillen et al. (2012) examined the effects of an acute, low-volume HIIE session on 24 hour glucose response in adults with T2DM. The exercise consisted of 10 x 60 second cycling bouts at 90 % heart-rate max with 60 seconds rest between bouts, compared with a no exercise condition (Gillen et al., 2012). Continuous glucose monitoring was used to determine glucose values over a 24 hour period post-exercise. The HIIE condition, which only required 10 minutes of intense exercise, resulted in reduced postprandial glycaemia throughout the 24 hour period.

One study has demonstrated that a HIIE session can cause a rapid acute ~20 % reduction in skeletal muscle glycogen levels (Metcalfe et al., 2015). This session consisted of 9 minutes 20 seconds of cycling at 60 W with two 20 second all-out sprints during a 10 minute exercise protocol with participants in a fasted state. The subjects remained fasted for 90 minutes afterwards with blood samples collected at 0, 15, 30 and 90 minutes post-exercise. No change in fasting plasma glucose levels were observed in the subjects. Regular glycogen turnover as a result of glycogenolysis is suggested to be a contributor to increased insulin

sensitivity due to the body attempting to replete glycogen stores (Jensen et al., 2011). In this instance it could be suggested that the increased rate of glycogenolysis due to the high-intensity exercise could have resulted in increased insulin sensitivity. However, no postprandial glycaemia or insulinaemia period was analysed and therefore this notion would not be possible to quantify.

Sprint-interval exercise (SIE) is another form of high-intensity exercise whereby the participant completes a number of low-volume maximal efforts (usually on a bike) against a resistance (typically around 7.5 % bodyweight). These sprints are separated with a short rest period ranging from 1-4 minutes. Ortega et al. (2015) examined insulin sensitivity after 4 x 30 second maximal sprints (SIE) using an oral glucose tolerance test 30 minutes post-exercise. Insulin sensitivity was significantly improved in the SIE protocol compared with a no-exercise control low-intensity continuous (60 minutes of 45 % $\dot{V}O_{2max}$) and continuous exercise (77 % $\dot{V}O_{2\ peak}$ for 30 minutes) (Ortega et al., 2015).Energy expenditure was significantly lower in the SIE condition in comparison to both continuous exercise conditions, which could suggest that mechanisms other than energy expenditure are responsible for the improved insulin sensitivity. In contrast, another study found that a 45 minute acute bout of endurance exercise (~ 75 % $\dot{V}O_{2\ peak}$) improved insulin sensitivity when measured the day after exercise using an oral glucose tolerance test, whereas 5 x 30 second sprints (~125 % $\dot{V}$O2max) did not (Brestoff et al., 2009).. However, Brestoff et al. (2009) used 45 minutes of cycling for the continuous exercise condition compared to 32.5 minutes for the SIE session, which may have contributed to the greater improvement in insulin sensitivity. Secondly, Brestoff et al. (2009) stated that energy expenditure between conditions was ‘different’, without stating the value; if the exercise energy expenditure was higher in the continuous exercise condition, this could have contributed to the increased insulin sensitivity. Although high-intensity interval based exercise sessions appear to result in improved postprandial glycaemia, it is

unknown whether performing the short intense bouts of activity across the day to interrupt prolonged sitting is also beneficial.

*2.9 High-intensity exercise and postprandial lipaemia*

A number of experimental studies have examined the effect of SIE on postprandial TG concentrations (Freese et al., 2011, Gabriel et al., 2012). Freese et al., (2011) examined the effect of SIE on postprandial lipaemia using three, 2-day conditions. This involved two-exercise conditions and a no-exercise control (performed on day one), with one exercise session replenishing expended energy post-workout (exercise-energy balanced) and the other not (exercise-energy-deficit). Both exercise sessions required the participant to perform a sprint interval protocol using four, 30 second all-out sprints against a resistance of 0.088 kp/kg fat-free mass. Following a 5 minute warm-up, the first sprint began. Four minutes of active recovery was undertaken between each sprint. The exercise session was completed at ~19:00 hours. The exercise sessions were compared with a control of uninterrupted sitting where the participant stayed at home and refrained from exercise. On day two of each condition, participants underwent a three-hour postprandial observation commencing at 10:00 hours. The energy-deficit exercise condition significantly reduced postprandial TG iAUC compared to both the energy-balanced exercise condition and control (Freese et al., 2011). Furthermore, the energy-balanced exercise condition did not differ significantly from uninterrupted sitting (Freese et al., 2011). It was concluded that SIE is an effective way to attenuate rises in postprandial lipaemia when an exercise-energy-deficit is maintained. Gabriel et al. (2012) compared SIE (5 x 30 seconds maximal sprints against 7.5 % bodyweight with 4 minutes of active rest between) with a single 30 minute moderate continuous brisk walking bout (7 km/h) and uninterrupted. The trials were performed over two days. Exercise conditions were performed on day 1 at ~14:00 hours. Participants then rested throughout the remainder of the day. A postprandial period was then observed on day two starting at ~8:45am with the meal providing approximately 56 % of fat, 33 %

carbohydrate and 11 % protein. Findings of the study were comparable to that of Freese et al. (2011) with SIE attenuating the postprandial rise in TG, compared with uninterrupted sitting (Gabriel et al., 2012). It was also noted that the SIE significantly reduced postprandial TG compared with brisk walking. This difference would not have been as a result of activity energy expenditure as this was lower in the SIE condition (Gabriel et al., 2012). Other mechanisms such as increased lipoprotein lipase activity may be an important mechanism determining the greater postprandial TG response following SIE (Mann et al., 2014). However, it may not be safe, well-tolerated, or appealing to some individuals in the general population to perform SIE, particularly those who are at moderate or high risk of medical complications or those who are sedentary. HIIE, rather than SIE may be a more appropriate alternative. Furthermore, the effects of HIIE across a day is still unknown and may be a better tolerated form of physical activity.

Another study examined moderate continuous exercise (45 minutes at 60 % $VO_{2max}$ on cycle ergometer) versus high-intensity interval cycling (10 x 60 seconds at 90-100 % $VO_{2max}$ with 60 seconds active rest between at 30W) (Gross, 2015). A postprandial period was observed immediately after the exercise through the use of an oral-glucose tolerance test. A largely noticeable, albeit non-significant reduction in the rise of glucose was observed in the high-intensity interval exercise session compared with moderate-continuous-intensity exercise and the control condition (uninterrupted sitting) (Gross, 2015). The lack of statistical significance was possibly due to the low sample size used (n = 5), which would most likely result in insufficient statistical power to observe any difference between conditions. With a strong general trend and large effect sizes in the data across conditions (particularly high-intensity compared with other conditions), it is of interest to test the effects of this high-intensity protocol on a larger sample size. Furthermore, the sample included four males and one female, with the female having a significantly lower cardiorespiratory fitness compared with the males. Thus, between sex differences could have contributed to the variability in results and thus the lack of significance. Condition order was also not randomised, which

could increase potential bias i.e. learning effects of study. Gross (2015) performed an oral glucose tolerance test (OGTT) to identify the effects the exercise conditions had on postprandial glycaemia. Although, a useful way to examine insulin sensitivity, this does not represent a normal meal consumption, whereas the use of standardized meals to commence a postprandial period could be used to replicate a normal lifestyle.

Currently there is a lack of research into the effects of HIIE on postprandial lipaemia, particularly when postprandial lipaemia is observed on the same day as the exercise is performed. This is possibly due to the effects of lipoprotein lipase potentially not being realised until later following physical activity, which its peak activity potentially occurring 6 hours post-activity (Seip et al., 1997).

### *2.10 Breaking up prolonged sitting versus continuous physical activity*

Breaking up prolonged sitting can provide sedentary populations with an alternative to completing single continuous bouts of activity. These small bouts of activity may be a good alternative to allow sedentary populations to lower their cardiometabolic risk when barriers such as low physical fitness and lack of time are present. A number of studies suggest that completing accumulated intermittent activity across the day is at least as effective as a single continuous bout of physical activity (Murphy et al., 2000, Mestek et al., 2008, Gill et al., 1998, Miyashita, 2008, Altena et al., 2004). Holmstrupp et al. (2014) investigated the effects of a 1 hour bout of morning physical activity versus intermittent walking activity bouts and uninterrupted sitting on glucose excursions and insulin secretion over 12 hours in young, obese individuals. Both activity conditions required participants to walk at an intensity of 60-

65 % $\dot{V}O_{2max}$ (Holmstrup et al., 2014). For all conditions, six small meals (15 % protein , 65 % carbohydrate, 20 % fat) were consumed at 2 hour intervals (Holmstrup et al., 2014). It was found that the short, frequent activity bouts attenuated glucose excursions and insulin concentrations in comparison to uninterrupted sitting and the moderate continuous activity bout condition (Holmstrup et al., 2014). However, the liquid-based meal used in this study may not represent typical meal consumption in free-living conditions. Despite this limitation, the provision of 6 meals throughout the day was a notable strength of the study as it could be considered to better simulate free-living conditions as participants were in a continuous postprandial state.

Miyashita et al. (2006) examined the effects of accumulating multiple bouts of physical activity throughout the day on postprandial lipaemia and compared this with a time and intensity matched single continuous 30 minute physical activity bout. Physical activity was completed on the same day as the postprandial period and significant reductions in postprandial lipaemia were observed in both conditions compared with uninterrupted sitting (Miyashita et al., 2006). The same group also conducted a later study where they compared the effect of continuous moderate physical activity with prolonged uninterrupted sitting vs. breaking up prolonged sitting with intermittent activity on postprandial plasma TG concentrations (Miyashita et al., 2008). Three two-day conditions were completed. On day 1, participants attended the laboratories at 09:00 hours to perform either 1) uninterrupted sitting until 16:00 hours 2) a moderate-continuous bout of brisk walking (self-selected pace) performed between 15:00 hours and 15:30 hours and 3) ten 3 minute bouts of brisk walking (at the same self-selected pace condition every 30 minutes across the 7 hour period (Miyashita et al., 2008). On day 2, participants underwent a 7 hour observational postprandial period after consumption of a standardised high-fat test meal consisting of white bread, cheddar cheese, butter, mayonnaise, potato crisps, whole milk, and milkshake powder. The macronutrient content of each test meal was 56 % fat, 33 % carbohydrate, and

11 % protein (Miyashita et al., 2008). Two test meals were provided; the first in the morning for breakfast to commence the postprandial period and the second 3 hours into the postprandial period (Miyashita et al., 2008). The study found that accumulating activity was as effective at reducing postprandial TG compared with a time and intensity matched moderate continuous bout of physical activity performed the afternoon before. Both activity conditions significantly reduced postprandial TG compared to uninterrupted sitting (Miyashita et al., 2008). Limitations of the study include the sample population who were healthy, young males and the effects observed may not represent other populations such as those who are sedentary. Furthermore, it is unknown whether interrupting sitting with high-intensity activity is effective in reducing postprandial lipaemia compared with an energy-matched continuous moderate-intensity physical activity bout.

### *2.11 Summary and rationale*

Prolonged sitting is associated with a number of cardiometabolic diseases and risk markers for cardiometabolic disease, such as postprandial glycaemia and lipaemia. A number of studies have reported acute reductions in postprandial glycaemia and lipaemia when prolonged sitting is interrupted with light or moderate-intensity activity. However, the effects of high-intensity activity used to break up prolonged sitting has not been investigated and this could be an effective method that may be less time consuming and potentially more effective at negating the effects of prolonged sitting compared with lower intensity activity breaks. There is also a lack of research comparing the effects of breaking up prolonged sitting with high-intensity activity to moderate-continuous activity on postprandial glycaemia and lipaemia, so it is unknown whether interrupting sitting with high-intensity activity could be an effective alternative or addition to traditionally recommended structured exercise.

Furthermore, it is unknown whether the postprandial benefits reported following a single continuous HIIE session are observable if this exercise is spread across the day in order to break up prolonged sitting.

*2.12 Aims*

Study 1

The aim of study 1 was to compare the effects of 1) breaking up prolonged sitting with high-intensity physical activity over a single day to 2) performing a single, energy matched moderate-intensity continuous physical activity bout in the morning followed by uninterrupted sitting and 3) uninterrupted sitting on postprandial glycaemia and lipaemia in sedentary, inactive adults.

Study 2

The aim of study 2 was to compare the effects of 1) breaking up prolonged sitting with high-intensity physical activity to 2) a volume, intensity and duration matched single high-intensity intermittent exercise session performed in the morning followed by uninterrupted sitting and 3) uninterrupted sitting on postprandial glycaemia and lipaemia.

## 3 General methods

The purpose of this general methods section is to describe all methods that apply to both study 1 and 2. Any methods which differ between the two studies are stated in their individual sections.

### *3.1 Ethical approval*

Ethical approval was granted by the University of Bedfordshire Institute for Sport and Physical Activity Research Ethics Committee for study 1 and study 2 prior to recruitment or any testing procedures (ethical approval numbers 2015ISPAR004 and 2016ISPAR006 for studies 1 and 2, respectively). Both studies conformed to the standards set by the Declaration of Helsinki. All study conditions took place within the Sport and Exercise Science Laboratories at the University of Bedfordshire. For both studies, participants were required to provide written informed consent following a written and verbal explanation of the nature of the research and the associated risks. All participants were fully informed of the experimental protocols and the associated risks, both verbally and in writing before providing written consent. Opportunity was given to participants to ask any further questions before giving consent to participate.

### *3.2 Preliminary testing*

Preliminary measures took place at least one week prior to commencement of first main trial. Anthropometric measures were taken from all participants during the respective preliminary testing visits. Standing height was measured without shoes to the nearest 0.1 cm with a stadiometer (Horltain Ltd., Crymych, UK). During standing height measurements, participants were asked to take a deep breath prior to measurement and look straight ahead with their head upright. Body weight was measured to the nearest 0.1 kg using electronic

weighing scales (BC41MA Segmental Body Composition Analyser, Tanita Corp., Tokyo, Japan). Participants were asked to wear light clothing and to remove their socks before body weight measurement. Body composition was measured after an overnight fast on the morning of the first main trial in each study using bioelectrical impedance analysis using the Tanita BC-418 Segmental Body Composition Analyzer (BC41MA Segmental Body Composition Analyser, Tanita Corp., Tokyo, Japan).

*3.3 Inclusion and exclusion criteria*

Inclusion and exclusion criteria were the same for studies 1 and 2. Sedentary males and females aged 18-55 years were invited to participate in both studies. Inclusion criteria were self-reported sitting of ≥7 h on an average day. In addition, individuals who were highly active engaging in more than 150 minutes of moderate-to-vigorous-intensity activity a week were excluded. Exclusion criteria included any known blood borne disease, pregnancy, clinically diagnosed diabetes, taking glucose-lowering and/or lipid-lowering medication, self-reported average daily sitting <7 hours, any known physical activity contraindications, major illness/injury, allergies to the test meals being provided, or other health issues that may limit the ability to perform the necessary activity bouts in the relevant studies. Due to the transient changes that occur in glucose metabolism during the female menstrual cycle (Valdes and Elkind-Hirsch, 1991), females were tested in the follicular phase only (days 1-10) for their main trial visits. This was determined via self-report at the time of scheduling laboratory visit dates and was verbally confirmed on the morning of each condition. Females using birth control that prevented menstruation were permitted to attend their main trials at any time. Participants were required to provide answers to a food preferences questionnaire (Appendix 1, see appendices), a health questionnaire (Appendix 2, see appendices), short-form IPAQ questionnaire (Appendix 3, see appendices) and domain-specific sitting form

(Appendix 4, see appendices) prior to participation in any testing procedures to screen for the above criteria.

### *3.4 Standardisation of pre-trial physical activity and dietary intake*

For both studies, a minimum of six days washout between trials was used to eliminate any carryover effects. Participants were required to refrain from any exercise 48 hours prior to each main trial to reduce the effects that physical activity may have on sensitivity and responsiveness to insulin (Mikines et al. 1988). On the day of each condition, participants were asked to attend the laboratories after an overnight fast for ≥9 hours. Participants were provided with a food diary and a set of digital scales (Salter Disc Electronic Kitchen Scale,, Homedics Group Ltd., Kent, UK) to be used the day prior to their first main trial to record the volume and timings of all food and liquids consumed. Participants were asked to replicate this food intake the day prior to each subsequent main trial day (Bailey et al., 2016). Participants were asked to minimise the amount of activity performed before attending the laboratories for each condition by travelling to the laboratory by car.

### *3.5 Standardisation of experimental protocol meals*

For both studies, two standardised test meals were provided during each main trial. Resting daily energy requirements were calculated using the Mifflin equation (Mifflin et al., 1990a). The Mifflin equation is based on body weight, height, age and gender. This value was then multiplied by 1.4 to represent a sedentary day:

- Males: Resting daily energy = (10 x weight) + (6.25 x height) - (5 x age) + 5
- Female:(10 x weight) + (6.25 x height) - (5 x age) – 161

Meals were consumed in their entirety within a 15 minute period in an isolated food consumption area adjacent to the test laboratory. Consumption time was recorded in the first main trial and participants were asked to replicate this as closely as possible in subsequent trials.

*3.6 Whole blood glucose analysis*

Whole blood was immediately analysed following venous (study 1) or finger prick (study 2) blood collection for determination of blood glucose concentrations using the YSI 2300 STAT plus glucose and lactate analyzer (YSI Inc., Yellow Springs, OH, USA). The YSI uses a steady state measurement methodology, where membrane based glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide. The difference between the sample generated plateau current and the initial baseline current is proportional to the glucose concentration. The YSI was calibrated at the start of each testing day and every 45 minutes thereafter.

*3.7 Plasma Insulin analysis*

Insulin was analysed using commercially available enzyme-linked immunosorbent assay kits (Mercodia, Uppsala, Sweden) using 25 μl of plasma. To eliminate intraassay variation, samples from each participant were analysed in the same run.

*3.8 Statistical analysis*

Incremental area under the curve (iAUC) was calculated for all metabolite data using the trapezoidal method IAUC represents concentrations of a given metabolite over a certain period. It is considered the best representation of TG and glucose over a number of time points as it more accurately describes responses to food (Carstensen et al., 2003, Le Floch et al., 1990). It also permits comparisons with previous research (Dunstan et al., 2012, Henson et al., 2016).

AUC = (Timepoint 1 + Timepoint 2) / 2

iAUC = AUC – (Baseline value x Time between timepoints)

iAUC was added for all timepoints to calculate iAUC for entire trial

Analyses were completed using statistical software package IBM SPSS statistics version 22.0 (SPSS INC., Armonk, N.Y., USA). Figures were created using Microsoft excel 2015. Prior to any inferential statistics, descriptive statistics were created to check central tendency (mean, median), and dispersion (standard deviation, minimum, maximum) of the data. Normality of results obtained for each of the conditions were assessed through the use of Quantile-Quantile (Q-Q) plots. These standard graphical methods were preferred over null hypothesis significance testing to check statistical assumptions (Grafen and Hails, 2002). Linear mixed models were used to determine any differences in dependant variables between conditions. This type of analysis was preferred as it can accurately model different covariate structures for repeated-measures data and model between-subject variability (Vandenbogaerde and Hopkins, 2010, West et al., 2006). Fixed and random factors for the linear mixed model were fit for each variable. The fixed factors were study condition and the

covariates age, gender, body fat percentage and baseline concentrations. Subjects were entered as random factors. The most appropriate covariance structure model was chosen using the smallest Hurvich and Tsai's criterion (AICC) in accordance with the principal of parsimony. Normality and homogeneity of variance of the residuals for all variables were checked using Q-Q plots and scatter plots, respectively. The main effect for condition was analysed by plotting the mean values. Sidak adjusted post-hoc comparisons were used to examine the differential effects of pairs of conditions if a significant main effect was present. 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, 1977). Data are presented as mean (95 % confidence interval [CI]) unless stated otherwise. P≤0.05 was considered significant.

*3.9 Sample size calculations*

Sample size was calculated using the software package G*Power (Faul, 2009). The primary outcome variables for this study were postprandial glucose and triglyceride iAUC. For postprandial glucose iAUC, sample size calculations were based on previous research which observed a significant 16% reduction in postprandial glucose AUC over 5 hours (effect size, *F*=0.61) when prolonged sitting was interrupted with light-intensity walking 2 minutes every 20 minutes versus uninterrupted sitting (Bailey and Locke, 2015). It was estimated that nine participants would be required for both of the three-condition crossover design studies to detect a minimum intervention effect of 16 %, a within-person correlation of 0.6, power of 95 % and an α of 0.05.

For postprandial TG iAUC, sample size calculations were based on previous research that observed a 16 % reduction (effect size, *F*=0.45) in postprandial triglycerides iAUC when prolonged sitting was interrupted with brisk walking 3 minutes every 30 minutes over 7 hours or a continuous 30 minute bout of moderate-intensity physical activity performed in the morning followed by uninterrupted sitting compared with an uninterrupted sitting condition

(Miyashita et al., 2008). It was estimated that 12 participants would be required for both three-condition crossover design studies to detect a minimum intervention effect of 16 %, a within-person correlation of 0.6, power of 95 % and an α of 0.05. To allow for potential dropout, 14 participants were recruited for each study.

## 4 Study 1 Methods

### *4.1 Study overview and participants*

Fourteen (seven female) sedentary and inactive adults participated in this study (mean age 29 ± 9 years, height 172.8 ± 5.9 cm, weight 78.5 ± 20.4 kg, $\dot{V}O_{2max}$ 38.6 ± 4.2 ml/kg/min, BMI 26.1 ± 5.8 kg/m$^2$). This was a three-condition randomised crossover design study. Participants completed three 8-hour conditions. Condition order was pre-determined using an incomplete counterbalance randomised order using the Latin square method.

The 8 hour experimental conditions were as follows 1) *uninterrupted sitting*, 2) *Continuous moderate-intensity physical activity*, and 3) *Sitting +high-intensity activity breaks*.
All physical activity for this study (maximal oxygen uptake test and activity during experimental conditions) consisted of brisk walking/jogging on a motorised treadmill (Woodway PPS55Med-I, GmbH, Germany).

### *4.2 Preliminary testing visit*

Participants attended a preliminary testing session prior to the experimental protocols where they became accustomed to the study environment and use of the Borg rating of perceived exertion (RPE) scale (Borg, 1982). Participants then completed a maximal oxygen uptake ($\dot{V}O_{2max}$) test on a motorised treadmill (Woodway PPS55Med-I, GmbH, Germany). Expired air was measured continuously during exercise using an online gas analysis system (Cortex Metalyzer 3B, GmbH, Germany). Participants began the test with a 3 minute stage at a speed they felt they could maintain for 30 minutes. The speed then increased by 1 km/h every 3 minutes until volitional exhaustion. $\dot{V}O_{2max}$ was taken as the highest $\dot{V}O_2$ value averaged over a 10-second period and was accepted as having been achieved when meeting ≥2 of the following end-point criteria: 1) heart rate within 10 bpm of age predicted maximum (220 – age), 2) respiratory exchange ratio >1.15, 3) plateau of $VO_2$ despite increasing workload, and 4) RPE ≥18 (Mier et al., 2012). Expired air data was averaged for

the last 60 seconds of each stage and used to individually calibrate the ambulatory heart rate and activity monitor (Actiheart, CamNtech Ltd., Cambridge, UK), which was worn during the subsequent experimental condition conditions. Data from the $\dot{V}O_{2max}$ test was used to calculate the speed eliciting 60 % and 85 % $\dot{V}O_2$ Reserve ($\dot{V}O_2$ R) for the main conditions. $\dot{V}O_2$ R was selected instead of $\dot{V}O_{2max}$ to determine exercise intensity as it is suggested that this method provides an equivalent relative intensity for individuals of different fitness levels (Dalleck and Dalleck, 2008). $\dot{V}O_2$ reserve was calculated by subtracting 3.5 from each participant's $\dot{V}O_{2max}$. A linear regression was used to determine the corresponding treadmill speed for each participant that elicited 60 % and 85 % $\dot{V}O_2$ R, respectively, which was subsequently used in the respective trial conditions. 60 % $\dot{V}O_2$ R was used in the continuous moderate-intensity physical activity condition as this is considered moderate intensity physical activity (Mann et al., 2013). 85 % $\dot{V}O_2$ R was used for the sitting + high-intensity activity breaks condition as this is considered a high-intensity (Mann et al., 2013).

*4.3 Experimental protocol*

Upon arrival, participants were weighed and fitted with an Actiheart to be worn throughout the experimental condition. Participants had a cannula inserted into an antecubital vein and a fasting blood sample was collected. The 8 hour postprandial period then commenced after the participant had returned to their seat following consumption of a standardised breakfast meal. The trial conditions were as follows:

1) *Prolonged, uninterrupted sitting (SIT)*

For the prolonged sitting condition, participants were seated uninterrupted in an upright position at a desk for 8 hours. When participants were in the seated position during their conditions they were advised to avoid excessive movement.

*2) Sitting with continuous moderate-intensity activity (MOD-CON)*

Similar to prolonged sitting; however a 30 minute moderate-intensity activity bout was completed 30 minutes into the postprandial period at an intensity of 60 % $\dot{V}O_2$ reserve, on a motorised treadmill followed by uninterrupted sitting for 7 hours 30 minutes.

3) *Sitting with high-intensity activity breaks (SIT-ACT)*

Similar to prolonged sitting; but hourly 2 minute 32 second bouts of high-intensity activity at 85 % $\dot{V}O_2$ Reserve were completed. The first bout of activity was initiated at the same time in the postprandial period as the start of activity bout in MOD-CON. Conditions MOD-CON and SIT-ACT were matched for activity energy expenditure.

Participants were supervised throughout the experimental conditions by a member of the research team to ensure adherence to the protocols. Water was provided *ad libitum* during the first main condition and the total volume consumed was recorded and replicated in subsequent conditions through provision of equal volumes every 2 hours. During sitting time for each trial, participants only rose from the chair to void (toilets were located approximately 30 metres from the test laboratory) and walk to the research kitchen to consume their standardised breakfast and lunch meals (located approximately 10 metres from the laboratory). Participants worked on a laptop computer, read books, watched DVDs, or talked. For study protocol schematic, see Figure 1.

*4.4 Measurement of activity energy expenditure during conditions*

An ambulatory heart rate and activity monitor (Actiheart, CamNtech Ltd., Cambridge, UK) was worn during each experimental condition in order to quantify activity energy expenditure during the conditions. The Actiheart is a compact, chest-worn monitoring device that records heart rate, inter-beat-interval (IBI), and physical activity in one combined light-weight waterproof unit. It is designed for capturing HRV data and for calculating and measuring activity energy expenditure. The Actiheart has been deemed as technically reliable and valid, where energy expenditure and walking and running intensity can be estimated accurately (Brage et al., 2005).

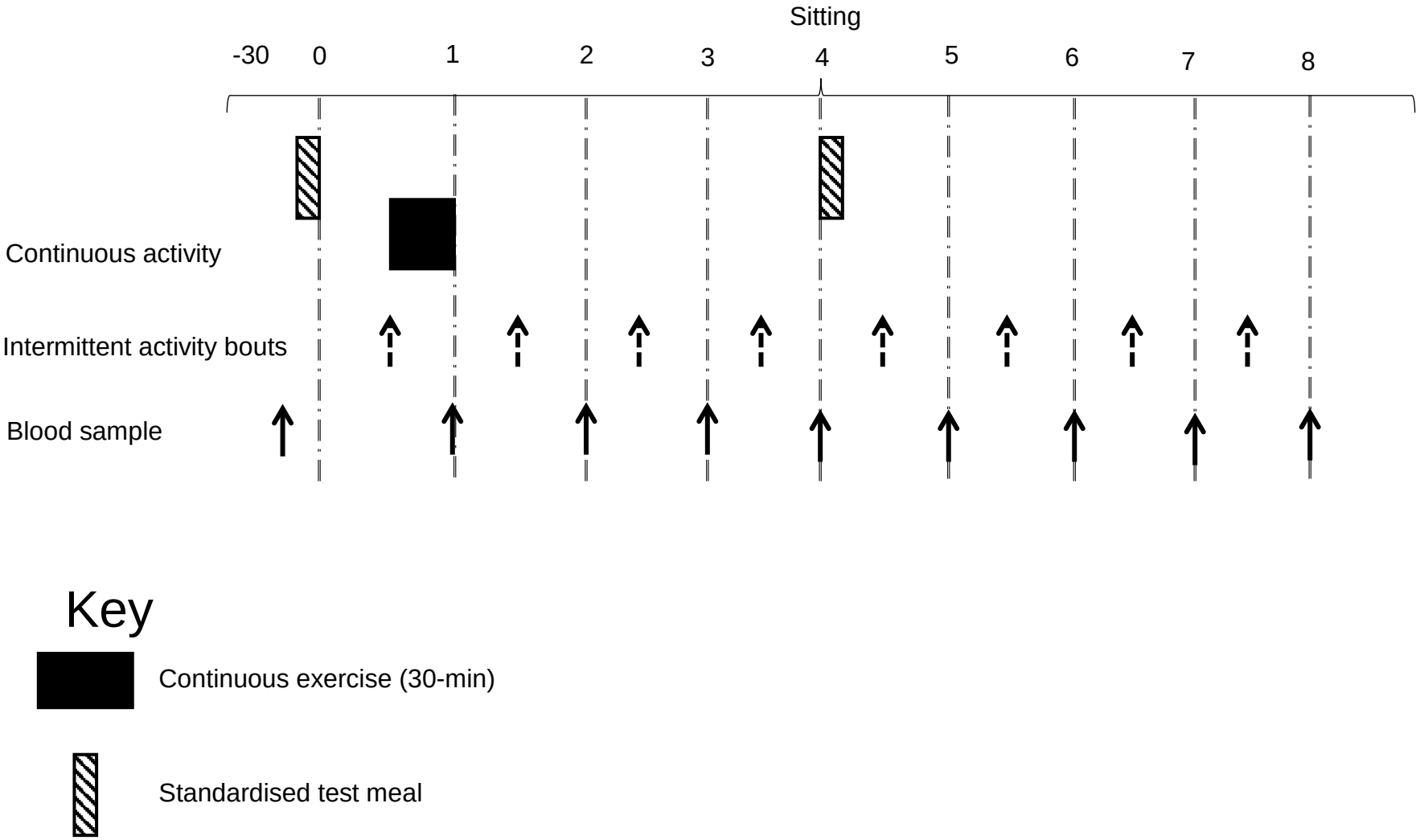


**Figure 1.** Study 1 protocol schematic

*4.5 Standardised test meals*

A standardised mixed-meal breakfast was consumed at -15 min in an isolated food consumption room adjacent to the test laboratory. The meal provided 15 % of estimated daily energy requirements for each participant, which was calculated using the Mifflin equation (Mifflin et al., 1990) (see general methods). Consumption time for the meal (≤15 minutes) was recorded and replicated in subsequent conditions. A standardised lunch meal was also provided at 4 hours, which also consisted of cornflakes and milk, but provided 30 % of estimated daily energy requirements (Mifflin et al., 1990). Both test meals consisted of a macronutrient composition of 30 % fat, 55 % carbohydrate and 15 % protein.

*4.6 Blood sampling and biochemistry*

Ten blood samples were collected in total during each trial. The first was a fasted sample followed by samples at the following postprandial time points: 60, 120, 180, 240, 300, 360, 420 and 480 minutes. Venous blood was collected via a cannula (Vasofix, B. Braun medical Ltd, Sheffield, UK) inserted into an antecubital vein during experimental conditions. Two samples were collected into 4.9 ml EDTA-containing vacuettes (Vacuette, Greiner Bio-One, Austria). From the samples, 50 μl of whole blood was pipetted into a microcuvette and analysed immediately to determine glucose concentrations using the YSI 2300 STAT plus glucose and lactate analyser (see general methods). 80 μl of whole blood was then pipetted onto two separate Reflotron test strips (Roche Diagnostics, Burgess Hill, UK) for determination of TG and HDL concentrations using the Reflotron Plus system (Roche Diagnostics, Burgess Hill, UK). The Reflotron plus is a compact reflectance photometer for fully automatic evaluation of Reflotron test strips. The instrument completes all functions such as heating, automatic calibration, test execution, evaluation and calculation of results.

Following removal of blood for glucose and lipid determination, the vacuettes were then spun using a refrigerated centrifuge (Heraeus, Heraeus Multifuge X3R,Thermo Scientific) immediately at 1500 x g for 10 min at a temperature of 4$^{o}$C. The plasma supernatant was then dispensed into 2 ml cryovials and stored at -80 $^{o}$C for later batch analysis of insulin. The intraassay coefficient of variation for standards in insulin analysis were 14%.

*4.7 Statistical analysis*

Glucose, insulin, TG and HDL iAUC was calculated for the 8 hour trial condition period. For full statistical analysis procedures see general methods. Activity energy expenditure was compared between conditions using a dependant *t* – test.

## 5 Study 1 Results

Q-Q plots revealed that all dependant variables were normally distributed. Residuals for data for all dependant variables for all conditions were deemed to be homogenous and normally distributed. Mean iAUC values for each dependant variable across each condition can be found in Table 1.

### *5.1 Activity energy expenditure*

The activity energy expenditure for the 30 minute continuous moderate-intensity activity bout in the MOD-CON condition was 661 kJ (476, 828). In the SIT-ACT condition, energy expenditure for all of the high-intensity activity bouts combined was 732 kJ (539, 891). Activity energy expenditure did not differ significantly between the MOD-CON and SIT-ACT conditions ($p$ = 0.236).

### *5.2 Postprandial glucose and insulin concentrations*

Fasting glucose levels did not differ significantly between conditions ($p$ = 0.709). For glucose concentrations over time see figure 2. There was no significant main effect of condition on 8-hour postprandial glucose iAUC ($p$ = 0.606; see Table 1). Cohen's d effect sizes for differences between conditions were as follows: SIT and MOD-CON (0.20, small effect size); SIT and SIT-ACT (0.15, small effect size); MOD-CON and SIT-ACT (0.34, small effect size).

**Table 1.** Incremental area under the curve values for glucose, insulin, triglycerides and high-density lipoprotein.

| | SIT | MOD-CON | SIT-ACT |
|---|---|---|---|
| Glucose iAUC (mmol/L·8 h) | 0.36 (-1.17, 1.90) | -0.27 (-1.93, 1.38) | 0.82 (-0.81, 2.45) |
| Insulin iAUC (mmol/L·8 h) | 172.90 (128.75, 217.06) | 163.76 (119.14, 208.37) | 151.27 (106.20, 196.34) |
| TG iAUC (mmol/L·8 h) | 1.355 (-0.458, 3.168) | 0.113 (-1.71, 1.94) | -0.876 (-2.725, 0.973)* |
| HDL iAUC (mmol/L·8 h) | -0.128 (-0.911, 0.655) | 0.176 (-0.607, 0.959) | 0.861 (0.076, 1.646)* |

**Note.** Values are means + 95 % confidence intervals. iAUC incremental area under the curve. TG: Triglycerides, HDL: High-density lipoprotein, SIT: Uninterrupted sitting, MOD-CON: continuous moderate-intensity activity, SIT-ACT: High-intensity intermittent activity.

*significant difference for TG iAUC between SIT-ACT and SIT ($p$ = 0.035). Significant difference for HDL iAUC between SIT and SIT-ACT ($p$ = 0.037).

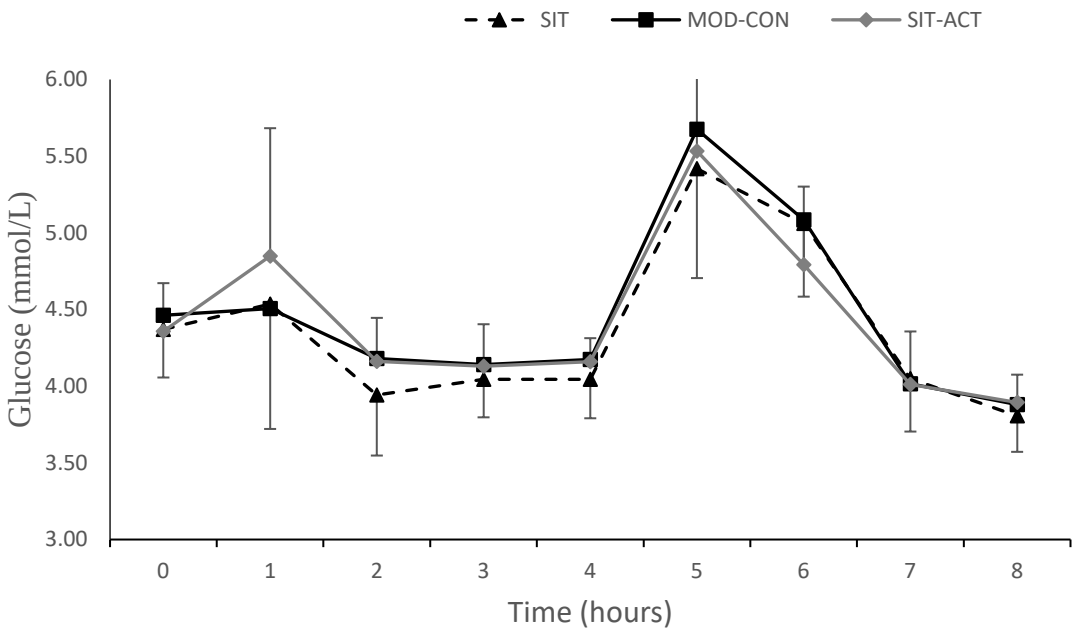


**Figure 2** Whole blood glucose concentrations (mmol/L) over time for the uninterrupted sitting (SIT), Continuous moderate-intensity physical activity (MOD-CON) and Sitting +high-intensity activity breaks (SIT-ACT) conditions. Values are mean and 95 % confidence interval. Some error bars have been omitted for clarity.

Fasting plasma insulin did not differ significantly between conditions ($p$ = 0.889). For plasma insulin concentrations over time see figure 3. No significant main effect of condition on postprandial insulin was observed ($p$ = 0.653; see Table 1). Cohen's d effect sizes for differences between conditions were as follows: SIT and MOD-CON (0.10, small effect size); SIT and SIT-ACT (0.24, small effect size); MOD-CON and SIT-ACT (0.13, small effect size).

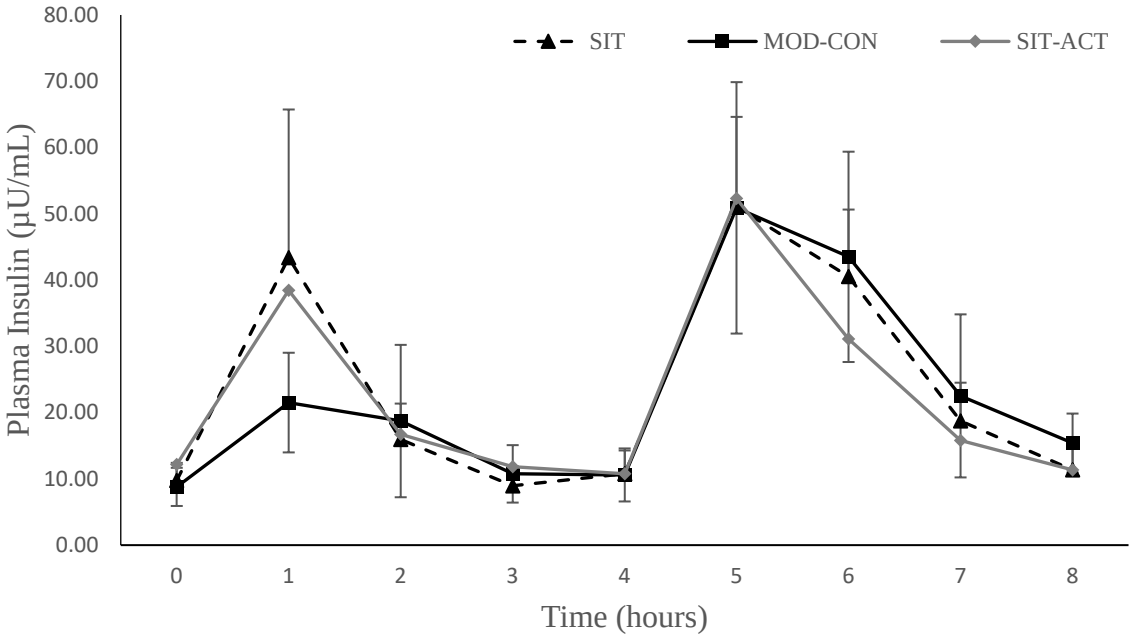


**Figure 3** Plasma Insulin concentrations (µU/mL) over time for the uninterrupted sitting (SIT), Continuous moderate-intensity physical activity (MOD-CON) and Sitting +high-intensity activity breaks (SIT-ACT) conditions. Values are mean and 95 % confidence interval. Some error bars have been omitted for clarity.

*5.3 Postprandial lipid concentrations*

Fasting TG concentrations did not differ significantly ($p$ = 0.534). For TG concentrations over time, see figure 4. There was a significant main effect of condition ($p$ = 0.038) for TG iAUC. Mean postprandial TG iAUC for SIT-ACT was significantly lower than SIT ($p$ = 0.035, d = 0.62). The Cohen's d effect size was medium for this difference. No significant differences were observed between SIT and MOD-CON (d = 0.35) or between MOD-CON and SIT-ACT (d = 0.27) (both $p \geq 0.361$). Cohen's d effect sizes were small for these differences.

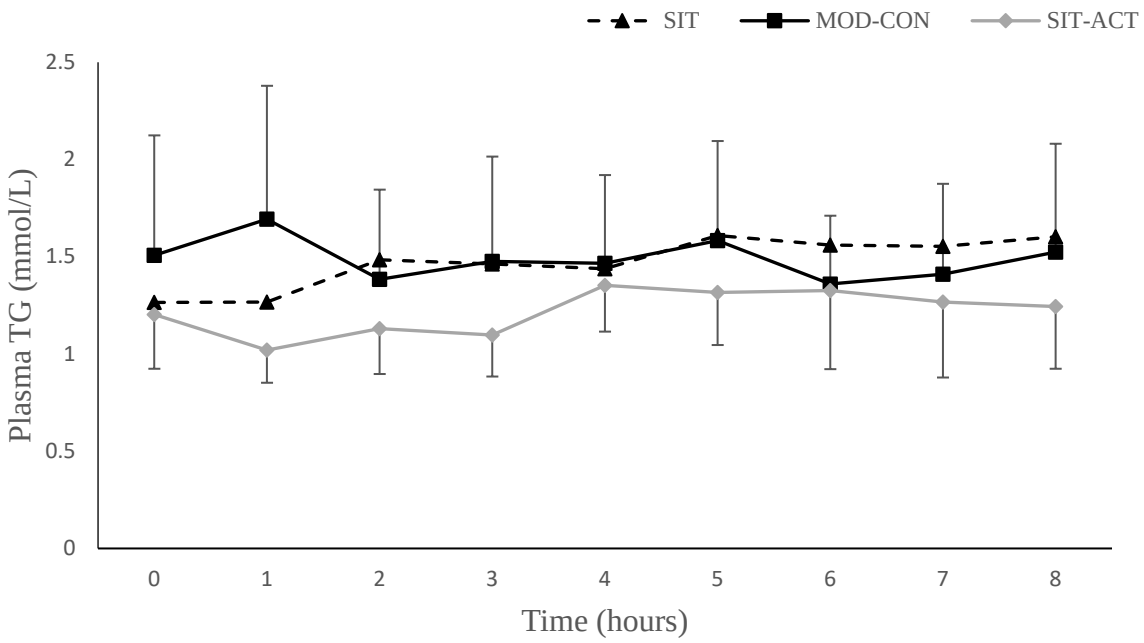


**Figure 4** Plasma triglyceride concentrations (mmol/L) over time for the uninterrupted sitting (SIT), Continuous moderate-intensity physical activity (MOD-CON) and Sitting +high-intensity activity breaks (SIT-ACT) conditions. Values are mean and 95 % confidence interval. Some error bars have been omitted for clarity.

Fasting HDL concentration did not differ significantly between conditions ($p$ = 0.317). For HDL concentrations over time, see figure 5. There was a significant main effect of condition ($p$ =0.034) for postprandial HDL iAUC. The mean postprandial HDL iAUC for SIT-ACT was significantly higher than SIT ($p$ = 0.037, d = 0.64). Cohen's d effect size was medium for this difference. No significant differences were observed between SIT and MOD-CON (d = 0.20) or between MOD-CON and SIT-ACT (d = 0.44) (both $p$'s ≥ 0.211). Cohen's d effect sizes for these conditions were small.

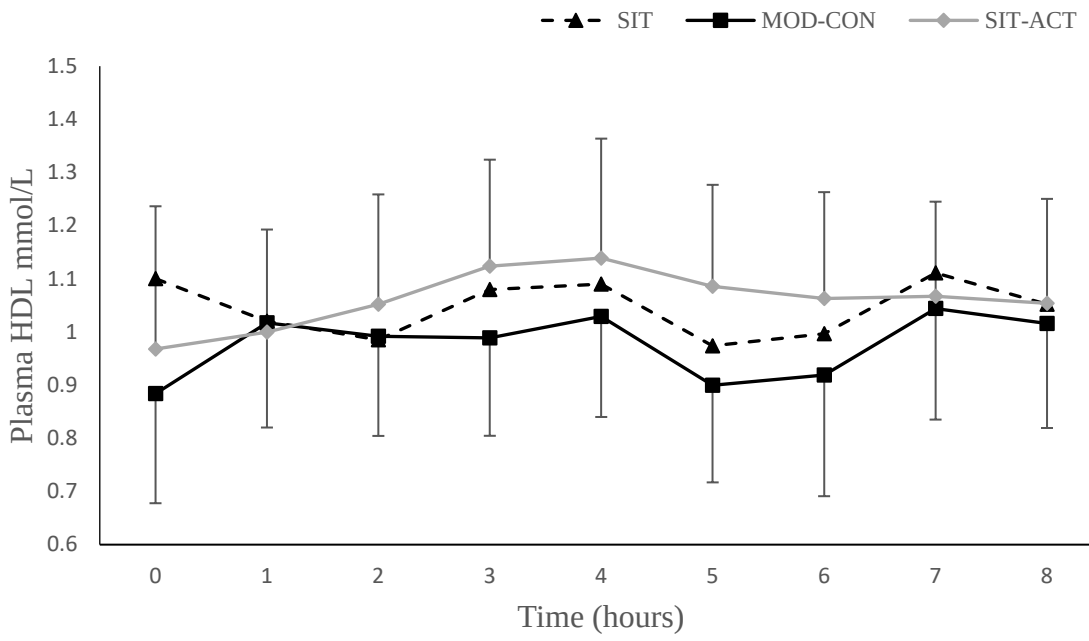


**Figure 5** Plasma HDL concentrations (mmol/L) over time for the uninterrupted sitting (SIT), Continuous moderate-intensity physical activity (MOD-CON) and Sitting +high-intensity activity breaks (SIT-ACT) conditions. Values are mean and 95 % confidence interval. Some error bars have been omitted for clarity.

## 6 Study 1 discussion

The effect of breaking up prolonged sitting with frequent bouts of high-intensity physical activity had not been investigated before this study. The main finding of this study was that breaking up prolonged sitting with short bouts of high-intensity activity (2 minutes 32 seconds) every hour lowered postprandial TG and increased postprandial HDL in sedentary adults. This study is comparable to the findings of previous research, where significant reductions to plasma TG were observed from breaking up sitting with moderate-intensity physical activity (Murphy et al., 2000, Miyashita et al., 2009, Gill et al., 1998). The findings from the present study provide further evidence that completing intermittent physical activity to break up prolonged sitting may be an effective intervention to reduce cardiometabolic disease risk.

Small bouts of activity performed on an hourly basis could be considered as a time-efficient method for sedentary individuals to incorporate more physical activity into their lifestyle. Kim et al. (2014) found that breaking up 9 hours of prolonged sitting with hourly bouts of light intensity walking (25% $\dot{V}O_{2max}$) induced lower TG responses in nonobese healthy recreationally active young subjects. These reductions were observed the day after performing the activity (Kim et al., 2014). Similar to the present study, treadmill activity (walking / running) was used which may have been the reason for the comparable results. However, Kim et al. (2014) also performed a continuous moderate-intensity (1 hour at 65% $\dot{V}O_{2max}$) which resulted in significant reductions to TG compared with uninterrupted sitting and hourly interruptions to sitting. This may have been due to a higher-intensity activity being performed.

Accordingly, Altenburg et al. (2013) investigated breaking up 8 hours of prolonged sitting with 8 minute hourly bouts of cycling (50 – 60% heart rate reserve). No differences were observed to postprandial TG between uninterrupted sitting and the hourly bouts of cycling in healthy, recreationally active individuals (Altenburg et al., 2013). Results from Altenburg et al. (2013) are inconsistent with the present study. Firstly, this may be due to cycling being

used rather than treadmill activity (walking/running). It may also be due to a lower intensity of activity being used. Although, Kim et al. (2014) found differences between prolonged sitting and hourly bouts of low-intensity walking the next day. Higher-intensity activity may be needed in order to observe reductions to TG on the day of activity. Or the use of running/walking may increase the possibility of reductions to TG.

There are some potential mechanisms which may have resulted in the reduction of TG in the SIT-ACT condition compared to uninterrupted sitting in the current study. There may have been an increased lipoprotein lipase activity, which may have increased TG uptake into skeletal muscle (Seip et al., 1997). However, an increase in lipoprotein lipase activity may not be the sole mechanism involved in the reduction of TG. In the present study, physical activity was performed on the same day as postprandial testing. This study design differs to some previous research in which the postprandial monitoring period occurred the day after experimental physical activity was performed (Miyashita et al., 2008). Increased TG clearance the day after activity is hypothesized to be a secondary effect of a reduction in VLDL-apolipoprotein-B100 secretion (Magkos et al., 2006). It is proposed that activity of lipoprotein lipase in skeletal muscle is at its highest at ~4 hours after activity (Katsanos, 2006). In the present study, it could be presumed that the reduction to TG in SIT-ACT may be as a result of an increase in the metabolism of TG in the latter parts of the day. Another mechanism for the reduction of TG in SIT-ACT is the reduced rate of hepatic very low-density lipoprotein secretion (VLDL) (Gill et al., 2001). Continuous moderate-intensity activity has previously increased 3-hydroxybutyrate (3-OHB) which is thought to have increased fatty acid oxidation rather than re-esterification and VLDL synthesis (Gill et al., 2001). This could therefore suggest a reduced rate of hepatic VLDL secretion, which has previously been observed in studies on rats (Fukuda et al., 1991). Increased activity energy expenditure from activity is also a possible factor in the reduction of postprandial TG in SIT-ACT compared with SIT (Tsetsonis and Hardman, 1996). However, in the present study, energy expenditure was not significantly different between activity trials. It is therefore

difficult to attribute reductions in TG solely to increased energy expenditure, particularly as MOD-CON did not differ significantly in postprandial TG compared with SIT. In the present study, it is difficult to pin the effects on TG in SIT-ACT to a singular mechanism, particularly as cardiometabolic markers such as non-esterified fatty acids (NEFA) and 3-OHB were not examined. An increased postprandial serum 3-OHB concentration would suggest that NEFA is being oxidised at a greater rate, which would reduce the availability of TG to bind to very-low density lipoproteins (Seip et al., 1997, Fukuda et al., 1991). This may result in lower levels of plasma TG.

This is the first study to show that acutely breaking up prolonged sitting significantly increases postprandial HDL. Bailey and Locke, (2015) observed pre- and post-trial HDL concentrations and found no difference between uninterrupted sitting and activity conditions. A chronic study examined the effects of introducing sit-stand workstations in adult nonobese healthy office workers, which resulted in a reduced sitting time of more than 2 hours a day after 3 months (Alkhajah et al., 2012). The reduced sitting time resulted in an increase to HDL (Alkhajah et al., 2012). The potential mechanisms for changes to HDL due to SIT-ACT in the present study are thought to be as a result of an increase in lipoprotein lipase activity. Previous studies have suggested that a decrease in lipoprotein lipase activity results in a decrease to HDL (Bey and Hamilton, 2003, Goldberg et al., 1988). However, it is still fairly unclear why physical activity in SIT-ACT in the present study reduced HDL, and this warrants further investigation.

Postprandial glucose iAUC did not differ between any of the conditions in this study. Previous studies examining interruptions to sitting time with light- and moderate-intensity walking for 2 minutes every 20 minutes have revealed significant reductions in postprandial glucose over a 5 hour postprandial period (Bailey and Locke, 2015, Dunstan et al., 2012). The reason for the discrepancy in findings are unclear, particularly as high-intensity activity was used which would provide a greater activity stimulus. The relatively low glucose levels in the uninterrupted sitting condition could have meant a reduced scope for improvement in this

postprandial marker. It may also be due to a sufficient level of insulin sensitivity in the participants in order to maintain postprandial glycaemia. Further investigation into the effects of interrupting sitting with high-intensity activity on postprandial glucose is required.

This study does have some limitations. Firstly, it is difficult to attribute the effects of interrupting sitting with high-intensity activity on TG and HDL to a specific mechanism. If 3-OHB and NEFA were analysed, it could provide a better mechanistic understanding explaining the responses observed. Secondly, the meals provided in the present study consisted of the same foods. This may not be an accurate representation of a typical diet consumed in free-living conditions and therefore may limit the generalisability of the results. It is unclear whether the reductions in TG in SIT-ACT are due to the high-intensity activity or due to breaking up sitting with intermittent activity. However, the beneficial responses to interrupting sitting in the current study is consistent with previous light and moderate intensity studies. High-intensity activity may be too intense for sedentary populations and therefore could have lower adherence compared with lower intensity activity. Although, studies have previously stated that high-intensity activity is more enjoyable than low intensity activity which could improve exercise adherence (Bartlett et al., 2011). Chronic responses to interrupting sitting with high-intensity activity are unknown and require further investigation.

In conclusion, postprandial TG and HDL concentrations are beneficially altered in response to interrupting sitting with high-intensity activity on an hourly basis. No benefit was observed from continuous moderate-intensity activity so a reduction in cardiometabolic risk may be greater with interrupting sitting.

## 7 Study 2 Methods

### *7.1 Study overview and participants*

Twelve (seven female) sedentary and inactive adults participated in this study (mean age 23 ± 4 years, height 170.2 ± 8.2 cm, weight 66.7 ± 14.5 kg, waist circumference 79.7 ± 10.3, $\dot{V}O_{2max}$ 36.2 ± 7.5 ml/kg/min). This was a three-condition randomised crossover design study. Participants completed three 6.5-hour conditions. Condition order was pre-determined using an incomplete counterbalance randomised order using the Latin square method.

The 6.5 hour experimental conditions were: *Prolonged, uninterrupted sitting (SIT), Single continuous high-intensity interval exercise session (CON-HIE) and Sitting + high-intensity activity breaks (SIT-HIE).*

### *7.2 Preliminary testing*

Participants attended a preliminary testing session prior to the experimental protocols where they became accustomed to the study environment and the use of the Borg RPE scale (Borg, 1982). Waist circumference was measured at the level of the umbilicus at the end of gentle expiration to the nearest 0.1cm using an adjustable tape measure. Three measures were taken and the average used. Height, weight and body fat were also measured (refer to general methods)

Participants then competed a cycling graded exercise test (GXT) on a Lode bike (Excalibur sport, Cranlea, Birmingham, UK) in order to determine workload that would be required to cycle at 90 % $\dot{V}O_{2max}$ during main conditions. The test begun at 100 W and increased by 25 W every 3 minutes until volitional exhaustion. Pulmonary gas exchange was measured using an online, breath-by-breath gas analysis system (Cortex Metalyzer 3B, GmbH, Germany), calibrated daily for barometric pressure, temperature and humidity. Heart rate was measured

beat-by-beat and averaged over each minute via a telemetric heart rate (HR) monitor (Polar, FS1, Cranlea, Birmingham, UK). $\dot{V}O_{2max}$was recorded as the highest $\dot{V}O_2$ value in ml/min/kg averaged over a 10-sec period and was accepted as having been achieved when meeting ≥2 of the following end-point criteria: 1) heart rate within 10 bpm of age predicted maximum, 2) respiratory exchange ratio > 1.1, 3) plateau of $\dot{V}O_2$ despite increasing workload and 4) RPE ≥ 18. The 90 % $\dot{V}O_{2max}$ workload was calculated through the individual linear relationship between workload and $\dot{V}O_2$ from the GXT.

*7.3 Experimental protocol*

Upon arrival, participants were weighed and rested for 30 minutes before a fasting capillary blood sample was collected. The 6.5 hour postprandial period commenced immediately after the participant had consumed a standardised breakfast meal.

1) *Prolonged, uninterrupted sitting (SIT)*

For the prolonged sitting condition, participants were seated uninterrupted in an upright position at a desk for 6.5 hours.

2) *Sitting with a single continuous high-intensity interval exercise session (CON-HIE)*

Similar to prolonged sitting, but a high-intensity interval exercise session was completed 45 minutes into the postprandial period. The exercise session consisted of a warm up for 1 minute at 60 W before completing 10 x 60 second cycling bouts at 90 % $\dot{V}O_{2max}$ with 1 minute of active rest cycling at 60 W between bouts ((total 20 minutes of cycling). The final bout did not include any active rest afterwards. The exercise session finished 1 hour 5 minutes into postprandial period and was followed by uninterrupted sitting for the remainder of the condition.

3) *Sitting with intermittent high-intensity activity bouts (SIT-HIE)*

Similar to prolonged sitting, but half-hourly bouts of high-intensity cycling at 90 % $\dot{V}O_{2max}$ for 1 minute were completed. Each bout included 30 seconds of cycling (60 w) before and after the 60 seconds of high-intensity cycling (total 20 minutes of cycling). The first bout of activity was initiated at the same time in the postprandial period as the start of activity in the continuous high-intensity cycling condition (45 minutes into the postprandial period). Conditions CON-HIE and SIT-HIE were time and duration matched.

Participants were supervised throughout the experimental conditions by a member of the research team to ensure adherence to the protocols. When participants were in the seated position during their conditions they were advised to avoid excessive movement. Participants were transported in a wheelchair to the research kitchen (to consume their standardised test meals) and to the lavatory when required. Participants worked on a laptop computer, read books, watched DVDs, or talked. Water was provided *ad libitum* during the first main condition and the total volume consumed was recorded and replicated in subsequent conditions through provision of equal volumes every 2 hours. For full study protocol schematic, see Figure 6.

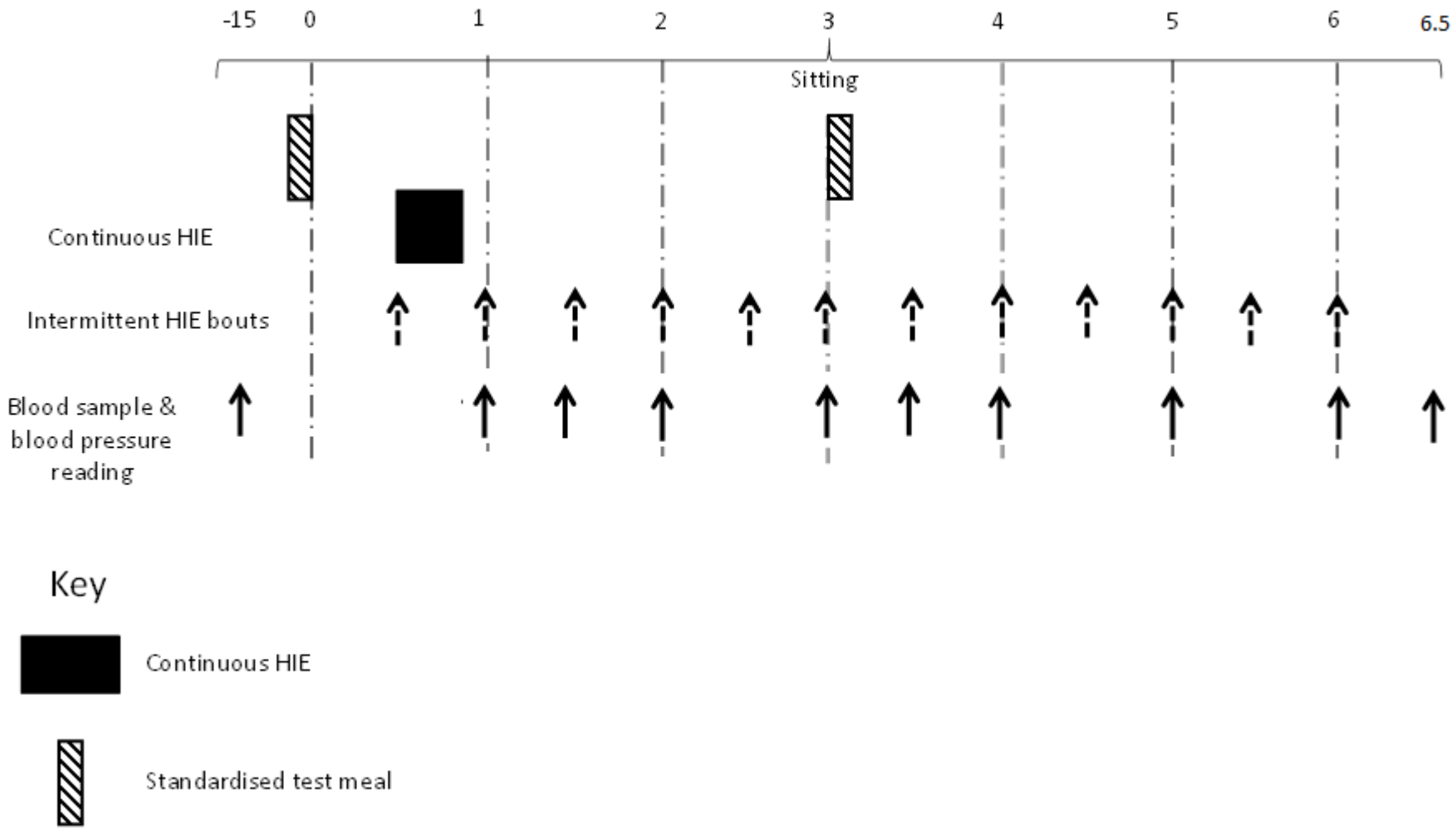


**Figure 6** Study 2 protocol schematic

*7.4 Standardised test meals*

A standardised mixed-meal breakfast was consumed at -15 minutes (cornflakes, whole milk and croissant) providing 30 % of estimated daily individual energy requirements, calculated using the Mifflin equation (Mifflin et al., 1990b) (see General Methods). This meal contained 57 % carbohydrate, 29 % fat and 14 % protein. A standardised lunch meal was provided at 3 hours, which consisted of white bread, chicken, butter, chocolate and crisps, providing 30 % of estimated daily energy requirements (Mifflin et al., 1990) (see General Methods). This meal contained 47 % carbohydrate, 39 % fat and 14 % protein

*7.5 Blood sampling and biochemistry*

Capillary blood samples were collected via a finger prick method. Prior to finger prick blood collection, the participant's hand was first placed in a bowl of warm water for two minutes to allow a greater blood flow to the area. The selected fingertip was then wiped with an alcohol swab and allowed to dry. A single use safety lancet (Haemolance Plus Lancet, Prospect Diagnostics, Dronfield, UK) was then used to pierce the fingertip. The first drop of blood was wiped away with a tissue and the finger was then squeezed at the proximal end and blood drops were collected into two EDTA- microvettes (Microvette CB300 EDTA, Sarstedt Ltd, Leicester, UK) for preservation of insulin. Finger prick blood samples were taken from participants in volumes of 400-600 µl. Ten samples were collected in total during each trial; the first fasted and then at the following time points during the postprandial period: 75, 105, 150, 210, 240, 270, 330, 390 minutes. 50 µl (2 samples of 25µl) of whole blood was drawn immediately from the microcuvettes for the analysis of blood glucose using the YSI (see General Methods). The microcuvettes were then spun in a centrifuge (Heraeus, Pico 17, Thermo Scientific) at 2000 x g for 5 minutes. 100 ul of the plasma was then extracted and stored in a cryovial at -80 ºC for later analysis of TG and insulin. 5 µl of plasma was used for analysis of TG which was completed via the use of spectrophotometry. 25 µl of plasma was

used for analysis of insulin (see General Methods for protocol). The intraassay coefficients of variation for plasma insulin analysis were 12%.

*7.6 Statistical Analysis*

Glucose, insulin and triglycerides iAUC was calculated using the trapezoidal method for the 6.5 hour trials condition period. For full statistical analysis procedures of iAUC see General Methods.

## 8 Study 2 Results

Q-Q plots revealed data for all dependant variables was normally distributed. Residuals for all dependant variables across all conditions was homogenous and normally distributed. Mean iAUC for all dependant variables from each condition can be found it Table 2.

### *8.1 Postprandial glucose and insulin*

Fasting glucose levels not differ significantly between conditions ($p$ = 0.445). For glucose concentrations over time see figure 7. There was a significant main effect of condition for 6.5 hour postprandial blood glucose iAUC ($p$ = 0.028; see Table 2). Postprandial blood glucose iAUC for SIT-HIE was significantly lower than SIT ($p$ = 0.026, d = 0.99). The Cohen's d effect size was large for this difference. No significant differences were observed between SIT and CON-HIE (d = 0.37, small-medium effect size) or between SIT-HIE and CON-HIE (d = 0.62, medium effect size) (both $p$'s ≥ 0.235).

**Table 2.** Incremental area under the curve values for glucose, insulin and triglyceride concentrations.

| | SIT | CON-HIE | SIT-HIE |
|---|---|---|---|
| Glucose iAUC (mmol/L·6.5 h) | 5.52 (4.53-6.50) | 4.79 (3.81-5.78) | 3.59 (2.61-4.58)* |
| Insulin iAUC (μU/mL·6.5 h) | 102.26 (83.20-121.32) | 103.64 (84.59-122.69) | 94.67 (75.62-113.71) |
| TG iAUC (mmol/L·6.5 h) | 2.015 (1.300-2.730) | 0.981 (0.266-1.695)* | 1.624 (0.909-2.338) |

**Note.** Values are means + 95 % confidence intervals. iAUC incremental area under the curve. TG: Triglycerides, SIT: Uninterrupted sitting, CON-HIE: Single continuous high-intensity interval exercise session, SIT-HIE: Sitting + high-intensity activity breaks.

*significant difference for glucose iAUC between SIT-HIE and SIT ($p$ = 0.026). Significant difference for TG iAUC between SIT and CON-HIE ($p$ = 0.014).

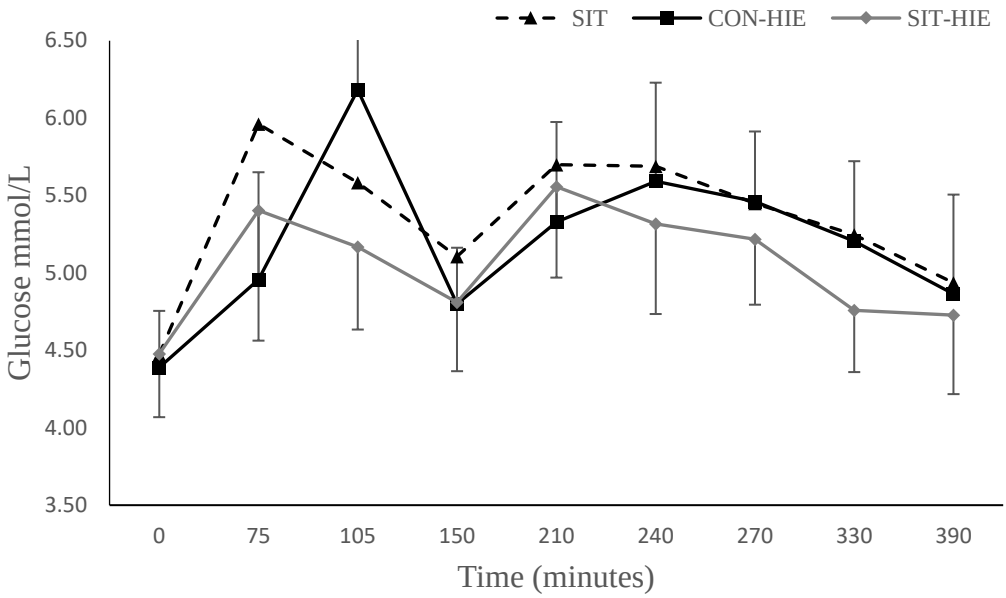


**Figure 7** Whole blood glucose concentrations (mmol/L) over time for the uninterrupted sitting (SIT), Single continuous high-intensity interval exercise session (CON-HIE) Sitting + high-intensity activity breaks (SIT-HIE) conditions. Values are mean and 95 % confidence interval. Some error bars have been omitted for clarity.

Fasting insulin did not differ significantly between conditions ($p$ = 0.312). For insulin concentrations over time, see figure 8. There was no main effect of condition on postprandial insulin iAUC responses (p = 0.743; see Table 2). The Cohen's d effect sizes for differences between conditions were as follows: SIT and CON-HIE = 0.04; SIT and SIT-HIE = 0.20; CON-HIE and SIT-HIE = 0.24. All of these effect sizes for differences between conditions were small.

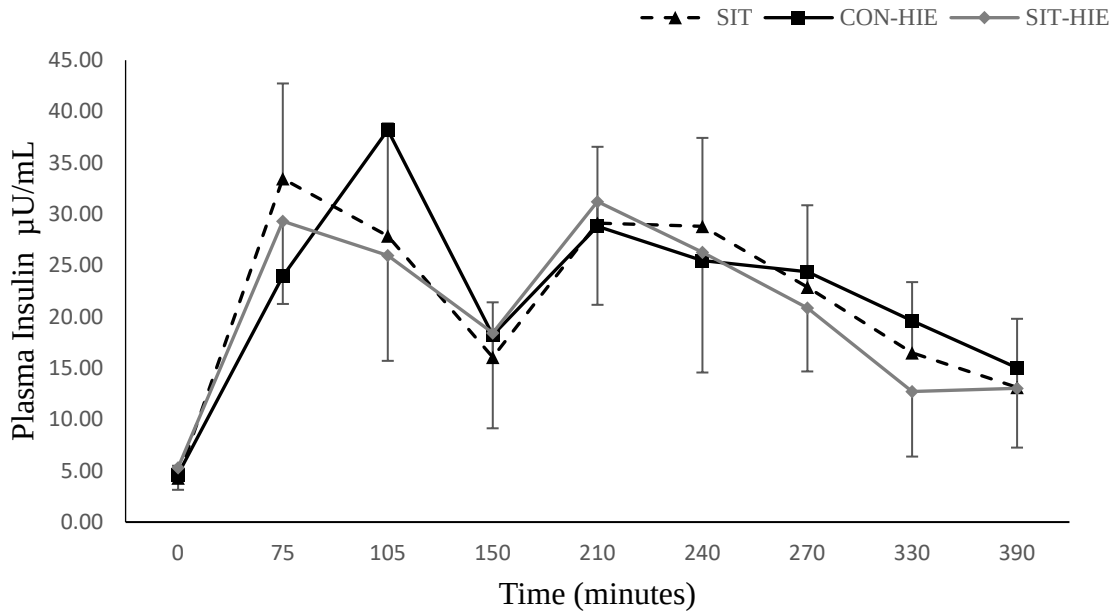


**Figure 8** Plasma Insulin concentrations (µU/mL) over time for the uninterrupted sitting (SIT), Single continuous high-intensity interval exercise session (CON-HIE) Sitting + high-intensity activity breaks (SIT-HIE) conditions. Values are mean and 95 % confidence interval. Some error bars have been omitted for clarity.

*8.2 Postprandial Triglycerides*

Fasting TG concentrations did not differ significantly between conditions ($p$ = 0.208). For TG concentrations over time, see figure 9. There was a significant main effect of condition on postprandial TG iAUC responses ($p$ = 0.027; see Table 2). Postprandial TG iAUC for CON-HIE was significantly lower than SIT ($p$ = 0.014, d = 0.73). The Cohen's d effect size for this difference was large. No significant differences (both $p \geq 0.243$) were observed between SIT and SIT-HIE (d = 0.28) or between CON-HIE and SIT-HIE (d = 0.46). The Cohen's d effect size for these differences were small and medium, respectively for these conditions.

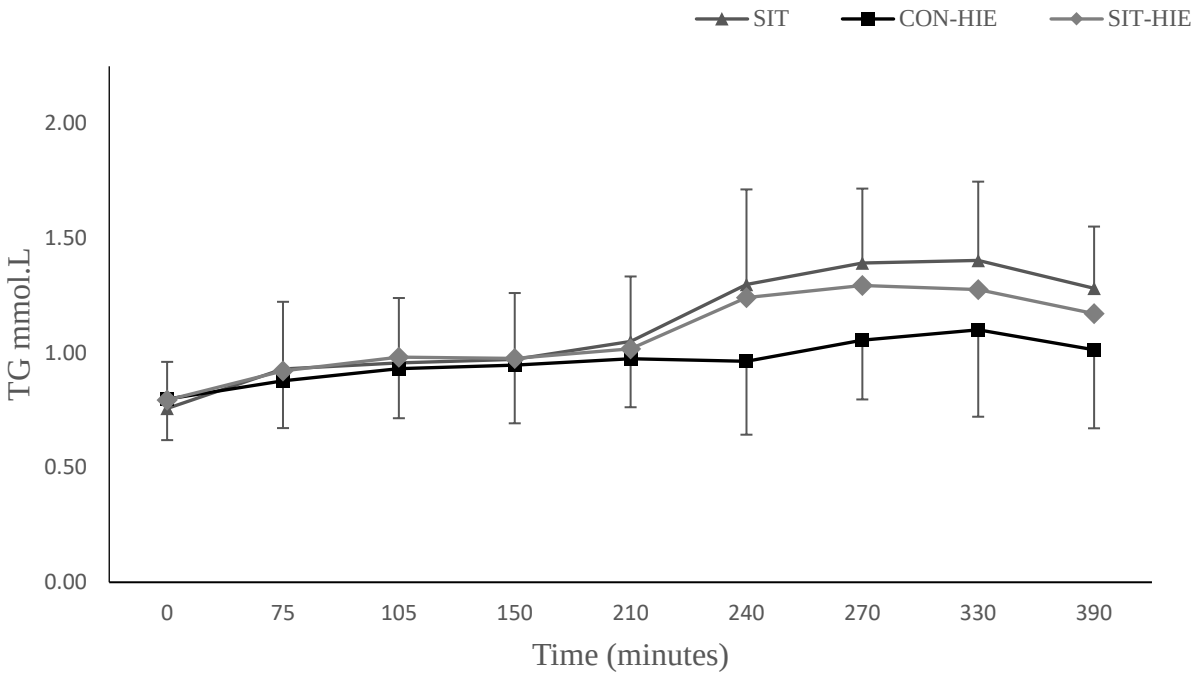


**Figure 9** Plasma Triglycerides (mmol/L) over time for the uninterrupted sitting (SIT), Single continuous high-intensity interval exercise session (CON-HIE) Sitting + high-intensity activity breaks (SIT-HIE) conditions. Values are mean and 95 % confidence interval. Some error bars have been omitted for clarity.

## 9 Study 2 Discussion

This is the first study to examine the effect of breaking up prolonged sitting with frequent bouts of high-intensity intermittent cycling on postprandial metabolism. The main finding of this study was that interrupting prolonged sitting with short bouts of high-intensity cycling (2 minutes every 30 minutes) significantly lowered postprandial glycaemia compared to uninterrupted sitting. Light and moderate-intensity activity has typically been used in order to break up periods of prolonged sitting in previous studies (Bailey and Locke, 2015, Dunstan et al., 2012, Healy et al., 2008, Peddie et al., 2013).The total time cycling at a high-intensity in each of the activity conditions (SIT, CON-HIE and SIT-HIE) in the present study was 10 minutes with a total activity duration of 20 minutes. Dunstan et al. (2012) and Bailey and Locke (2015) used 2 minutes of walking every 20 minutes over 5 hours to interrupt prolonged sitting in their studies. If Dunstan et al. (2012) and Bailey and Locke (2015) had used a postprandial period the same length of time as in the current study (6.5 hours) the total time performing physical activity would have been 38 minutes. It could therefore be suggested that performing shorter and less frequent bouts of high-intensity activity could cause beneficial responses in postprandial glycaemia compared with light- or moderate-intensity interruptions in sitting time.

In the present study, engaging in a single session of HIIE in the morning followed by uninterrupted sitting (CON-HIE) did not change postprandial glycaemia in comparison to uninterrupted sitting (SIT). Gillen et al. (2012) examined the effects of an acute, low-volume HIIE session on 24 hour glucose response in adults with T2DM. The exercise consisted of 10 x 60 second cycling bouts at 90 % heart-rate max with 60 seconds rest between bouts, compared with a no exercise condition (Gillen et al., 2012). The results of the present study do not support this as the activity performed in the morning thus did not appear to improve glucose metabolism throughout the rest of the day. This may have been due to the a few reasons. Firstly, Gillen et al. (2012) performed high-intensity activity in the fasted state and then proceeded with a 24 hour glucose analysing period. The present study performed high-

intensity activity in the postprandial state, therefore making it difficult to make comparisons due to differing nutritional status of the performance of activity. It may also be due to the present study examining a 6.5 hour postprandial period. If a longer duration glucose analysis was used, a difference may have been observed for the CON-HIE condition. The nature of the frequent bouts of activity in SIT-HIE will have interrupted periods of muscular inactivity thus maintaining glucose metabolism. Regular muscular contractions throughout the day, particularly in the lower limbs, are important for regulating glucose (Hamilton et al., 2007). The regular contractions in SIT-HIE could have maintained GLUT4 in a position on muscle to allow a greater uptake of glucose and thus increased oxidation (Holloszy, 2005).The frequent bouts of activity may have also increased contraction-stimulated glucose transport in skeletal muscle resulting in lower circulating concentrations (Richter and Hargreaves, 2013).

This study observed that a single high-intensity interval cycling session performed in the morning significantly lowered postprandial TG compared to uninterrupted sitting in sedentary adults. Gabriel et al. (2012) found a significant reduction to postprandial TG the day after a single session of sprint interval exercise. The use of sprint interval exercise may not be tolerable for sedentary populations. It may be of interest to perform more tolerable high-intensity in order to reduce postprandial rises in TG. Interestingly, the present study saw no reductions to postprandial TG in SIT-HIE compared with SIT. SIT-HIE was time and duration matched to CON-HIE and so it was hypothesized that similar reductions would be observed. This is the first study to investigate the use of high-intensity activity to interrupt prolonged sitting and so difficult to pin reasons for the lack of difference to TG in SIT-HIE.

The reductions in postprandial TG in CON-HIE can be attributed to a number of potential mechanisms. Continuous moderate-intensity activity can increase circulating levels 3-OHB which is suggested to increase the oxidation of fatty acids rather than re-esterification and VLDL synthesis (Gill et al., 2001). A decreased re-esterification of fatty acids and VLDL

synthesis could result in a reduced rate of hepatic VLDL secretion (Fukuda et al., 1991) Lipoprotein lipase activity may have also increased TG uptake into skeletal muscle (Seip et al., 1997). Lipoprotein lipase has been shown to be at its highest activity ~4 hours after exercise (Katsanos, 2006). In the present study, the physical activity in CON-HIE was performed 45 minutes after consumption of the first standardised meal. The total postprandial period duration was 6.5 hours which would allow lipoprotein lipase to reach its peak activity in the latter periods of the condition and contribute to reductions in circulating TG  In the present study, it is difficult to attribute the effects on TG to a singular mechanism, particularly as cardiometabolic markers such as lipoprotein lipase, NEFA and 3-OHB were not examined. An increased postprandial serum 3-OHB concentration would suggest that NEFA would be oxidised, which would reduce the availability for TG to bind into very-low density lipoproteins (Seip et al., 1997). An increase in lipoprotein lipase concentration would suggest an increase in skeletal muscle's ability to uptake more TG.

High-intensity physical activity may not be appealing or tolerable across all individuals, particularly those who are sedentary and physically inactive and thus not often exposed to such stressors. However, in the present study, both activity conditions appeared to be well tolerated by participants of a sedentary nature. Furthermore, previous research found that high-intensity activity is more enjoyable than lower intensity activity which could contribute to increased exercise adherence (Bartlett et al., 2011). It could therefore be suggested that high-intensity activity is an effective, time-efficient method for sedentary individuals to interrupt their sitting time.

This study does have some limitations. Firstly, the effects of high-intensity intermittent activity on TG are difficult to be attributed to a specific mechanism. If 3-OHB and NEFA were analysed, it could provide a clearer picture as to why effects to lipid parameters were observed. High-intensity activity may be intolerable for sedentary populations and therefore could result in a lower adherence compared with lower intensity activity. Although, studies have previously stated that high-intensity activity is more enjoyable than low intensity activity

which could improve exercise adherence (Bartlett et al., 2011). HDL was not measured in the present study and study 1 suggests high-intensity activity to interrupt prolonged sitting could be effect. HDL should therefore be explored in future studies. A significant reduction in postprandial glycaemia was observed in SIT-HIE compared to SIT, whereas postprandial lipaemia was significantly reduced in CON-HIE compared to SIT. Therefore, it is difficult to decide which is ultimately better for postprandial health. If a combination of SIT-HIE and CON-HIE was developed it could result in significant reductions to both glucose and TG, this warrants further investigation. Another limitation is the provision of a relatively large test meal 45 minutes prior to the high-intensity interval exercise session commencing. This may not replicate a typical lifestyle, as exercise at a high-intensity this length of time after a large meal could cause gastrointestinal discomfort. However, in the present study, no participants indicated the occurrence of such symptoms. This study aimed to standardise the nutritional status of the participants for when activity was performed in the CON-HIE and SIT-HIE conditions by commencing the first bout of activity 45 minutes post-meal. Future studies examining breaking up sitting time with high-intensity activity should consider the timing of test meals when designing their protocols and ensure any symptoms of gastrointestinal discomfort are reported.

In conclusion, postprandial concentrations of glucose were beneficially affected in response to high-intensity cycling used to interrupt prolonged sitting. Postprandial TG concentrations were reduced when a single high-intensity interval activity session was performed in the morning followed by uninterrupted sitting. Future research should aim to establish whether a combination of continuous moderate-intensity activity and high-intensity intermittent activity could beneficially effect glucose and TG.

## 10 Summary and Integration of findings

This thesis has examined a novel approach of utilising high-intensity physical activity to interrupt bouts of prolonged sitting. Study 1 found that interrupting prolonged sitting with high-intensity activity for 2 minutes 32 seconds every hour can significantly reduce postprandial TG concentrations and increase postprandial HDL concentrations. Study 2 found that interrupting prolonged sitting with high-intensity activity for 2 minutes every 30 minutes significantly reduces postprandial glucose and that a single continuous high-intensity activity bout performed in the morning can significantly reduce postprandial TG during subsequent uninterrupted sitting. These results indicate the beneficial effects of interrupting prolonged sitting with high-intensity physical activity.

Both studies have shown that the use of high-intensity activity to interrupt prolonged sitting could be an alternative option to reduce cardiometabolic risk in people who have limited time or the exercise capacity to complete single sessions. In some respects, both studies have shown that interruptions to sitting can be more beneficial to postprandial cardiometabolic health than a single session of physical activity. Study 2 showed that frequent interruptions to sitting reduces postprandial glucose compared to uninterrupted sitting, whereas a single session of volume and intensity matched activity did not. Study 1 has shown that a single continuous moderate-intensity activity session did not reduce postprandial TG and increase HDL compared with uninterrupted sitting, whereas high-intensity intermittent activity did. However, the results of the two studies in this thesis are not consistent with regards to TG responses to interrupting sitting with high-intensity activity bouts resulting in significant reductions in this cardiometabolic risk marker in study 1, but no change in study 2. The reason for this is not clear. It could be due to different modalities of physical activity being used. Study 1 examined treadmill activity (walking/running) and study 2 examined cycling. A comparison of these two different modalities in order to interrupt prolonged sitting needs further investigation. It could also be suggested that due to the postprandial period in study 1 being longer than study 2. If study 2 had a longer postprandial observation period, a

difference may have been observed in to TG in the sitting +high-intensity intermittent breaks condition. Another reason for this discrepancy could be the differing frequency of interruptions, with study 1 examining interruptions every hour and study 2 every 30 minutes. Furthermore, the duration of bouts in study 1 were slightly longer than study 2.

This thesis has shown beneficial cardiometabolic responses to breaking up sitting time with high-intensity activity. Benatti and Ried-Larsen, (2015) suggested that performing light-intensity activity is sufficient enough to induce significant beneficial cardiometabolic outcomes in individuals who are physically inactive or those with T2DM. This is an important point to consider in the context of the present study which examined the effects of high-intensity activity. If light-intensity activity is sufficient to elicit beneficial cardiometabolic effects in physically inactive individuals, then high-intensity activity may not be required and may not as well tolerated in an inactive population. However, higher intensity activity used to break up sitting time could produce beneficial cardiometabolic effects of a greater magnitude compared to light-intensity activity. Furthermore, the current research is the first to report beneficial postprandial HDL responses to breaking up sitting time and it is possible that higher intensity activity breaks are required to elicit improvements in this cardiometabolic risk marker.

A common stated barrier preventing people from completing regular physical activity is a lack of time or motivation (Arzu et al., 2006). Shorter and less regular bouts of activity used to break up sitting time, as shown in the present studies, can have elicit beneficial cardiometabolic effects. This may provide individuals who have a perceived lack of time or motivation an alternative physical activity strategy to reduce their risk of cardiometabolic disease. Engaging in short hourly bouts of high-intensity activity may also be more beneficial than a continuous bout of moderate-intensity physical activity.

The present studies have focussed around the potential cardiometabolic benefits of high-intensity activity used to break up sitting. For some populations, such as older adults, or individuals with mobility problems, this may not be feasible or tolerable. For these

populations, light-intensity activity breaks may be sufficient to induce beneficial responses to postprandial metabolism (Benatti and Ried-Larsen, 2015). An additional point to consider with regards to special populations is the terminology used in the description of sedentary behaviour. Much of the literature uses the phrase ‘breaking up sitting’. For populations such as those with a spinal cord injury or impaired mobility, breaking up ‘sitting’ may not a possibility as they are unable to stand unassisted. Breaking up sedentary behaviour may be a more appropriate term and research should investigate the cardiometabolic effects of breaking up sedentary time in these populations.

Based on the results from both studies, it could be suggested that high-intensity activity can be used to elicit cardiometabolic benefits from breaking up sitting time. However, the use of this approach may be impractical in some circumstances such as in office environments where individuals may find it uncomfortable or difficult to perform such activity. Participants in the present studies interrupted their sitting by performing activity on a treadmill or cycle ergometer and not having access to such equipment may prevent individuals from engaging in high-intensity activity. However, alternative modes of activity such as brisk walking along corridors or climbing up and down stairs could be performed. Further research should aim to establish sustainable strategies for breaking up sitting by leveraging the surrounding environment such as the workplace, school and home (Keadle et al., 2017)**.** .

## 10.1 Conclusion

In conclusion, this thesis has observed beneficial postprandial cardiometabolic responses to interrupting sitting with high-intensity activity. Interrupting sitting with physical activity has been shown to be more effective than continuous moderate-intensity activity at reducing postprandial TG concentrations However, further research is required to investigate responses to interrupting prolonged sitting with high-intensity activity in special populations, such as individuals with T2DM. Furthermore, long-term studies to evaluate the chronic responses to interrupting prolonged sitting with high-intensity activity requires investigation to further inform public health guidelines.

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## Appendices

### Appendix 1 Food preferences

**Food Preferences**

Please circle the number which best describes your liking of the following foods/drinks.
Focus on how much you like the foods/drinks rather than how frequently you consume them:

Cornflakes

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

□ Unsure

Whole milk

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

□ Unsure

White bread

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

□ Unsure

Butter

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

□ Unsure

Wafer thin Roast chicken slices

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

□ Unsure

Ready salted crisps

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

□ Unsure

Cadbury milk chocolate

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

□ Unsure

All butter Croissant

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

□ Unsure

**Appendix 2 PRE-EXERCISE HEALTH QUESTIONNAIRE**


Sport Science and Physical Activity

Polhill Avenue

Bedford MK41 9EA

**PRE-TEST MEDICAL QUESTIONNAIRE**

To be completed by all subjects 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 Do you currently smoke? Yes / No

4 How would you describe your present level of moderate activity?
< once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

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

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

7 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

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

9 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

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

11 Are you currently using oral contraceptives? Yes / No

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

13 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

14 Are you currently dieting? Yes / No

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

**PLEASE READ THE FOLLOWING CAREFULLY**

Persons will be considered unfit to do the experimental exercise task if they:

have a fever, suffer from fainting spells or dizziness;

have suspended training due to a joint or muscle injury;

have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;

have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;

have anaphylactic shock symptoms to needles, probes or other medical-type equipment.

have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella)

have a history of infectious diseases (e.g. HIV, Hepatitis B); and, if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum;

**DECLARATION**

I hereby volunteer to be a subject in experiments/investigations as stated in the information sheet.

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 subject (please print) _____

Signature of Subject _____ Date: _____

Name of Experimenter (please print)_____

Signature of Experimenter _____ Date: _____

**Appendix 3**

# 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.

**Name**:…….. **Date**:……..

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 4**

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)**

| | On a WEEK Day | | On a WEEKEND Day | |
|---|---|---|---|---|
| | 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.) | | | | |