Sitting, Standing and Light Activity: Measurement and Postprandial Metabolic Response

By

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Abstract

A high level of sedentary behaviour has recently emerged as a distinct risk factor for a number of diseases. On the other hand, a large body of evidence has shown that physical activity (PA) can prevent several illnesses. However, there are important issues regarding the accurate measurement of SB behaviour and physical activity in observational studies which are currently unresolved. Research is particularly needed to investigate the impact of characteristics of sedentary behaviour such as type/context, sedentary bout length, breaks in sedentary time on metabolic responses and accurate quantification of PA and SB is needed to evaluate current and changing physical activity and sedentary behaviour levels on health outcomes.

A number of studies have demonstrated that replacing sedentary time with light-intensity physical activity such as standing can induce a measurable metabolic benefit. However, it is unclear whether these benefits could be stimulated by simply breaking up time spent sitting down by standing up, or whether the number of transitions from sitting to standing influences metabolic changes over and above the effects of total time spent standing. The first experimental study in this thesis demonstrated, in ten overweight/obese men, that prolonged standing – where participants alternated 15 minutes of sitting with 15 minutes of standing – energy expenditure was 10.7% higher than continuous sitting (p<0.001) over an 8-hour observation period. Intermittent standing – where participants undertook 10, 1.5-minute bouts of standing in every half-hour – led to a further increase in energy expenditure of 9.0% (p<0.001). Participants oxidised 7.1 g more fat and 7.7 g more carbohydrate with intermittent standing compared with prolonged standing, but there was no significant effects of either prolonged or intermittent standing breaks on postprandial incremental glucose, insulin or triglyceride (TG) responses.

However, the intermittent protocol used in that study was clearly not feasible to implement as a practical intervention. Building on these data, the second experimental chapter involved breaking up prolonged sedentary time by undertaking sit-to-stand transitions over a short period (sitting and standing 10 times over 30 seconds, every 20 minutes) was compared to prolonged sitting in fourteen overweight/obese men. The main finding in chapter 4 was that

sit/stand trial 'chair squats' significantly increased energy expenditure by 16.6% over a 6.5-hour observation period ($p<0.0001$). Total carbohydrate oxidation was 33.9% higher in the 'sit/stand' trial than the sitting trial ($p = 0.0005$). The difference in total fat oxidation between trials over the 6.5-hour observation period was not statistically significant, but tended to be 9.7% higher in the 'sit/stand' trial, ($p = 0.11$). Postprandial insulin concentrations over the post-breakfast period were 10.9% lower in the 'sit/stand' trial than the sitting trial ($p = 0.047$), but no difference in the post-lunch period. Postprandial TG and glucose responses were not significantly different between the two trials.

Comprehensive and accurate methods of assessing sedentary time and physical activity are essential to further our understanding the links between activity behaviours and disease: misclassification due to poor measurement can attenuate the apparent association between these behavouirs and health outcomes. The aims of the third experiment chapter were therefore to compare thigh and hip positions for accelerometer placement of an ActivPAL accelerometer for the measurement of step-based physical activity and to develop an algorithm for the estimation of walking or running speed and energy expenditure from acceleration outputs from a thigh-based accelerometer. The main finding was that the thigh-based ActivPAL were capable of determining stepping activity well at speeds from 2 $km.h^{-1}$ upward, whereas the hip-based ActivPAL accelerometer and the hip-based Actigraph underestimated steps count at speed below ~ 3-4 $km.h^{-1}$. There was a strong linear relationship between vector magnitude acceleration and speed for both hip and thigh positions. The relationship between ActivPAL accelarations and oxygen uptake was very strong for both the thigh and hip positions ($R^2 \approx 0.90$), ($R^2 \approx 0.88$) respectively. Half of the participants (n = 20) were used to derive regression equations for the relationship between accelerations and oxygen uptake and these equations were tested in the other half of the cross – validation group (n = 20). The result indicated that the linear regression equation to obtain oxygen uptake from accelerometer was valid in all ActivPAL positions with standard errors of the estimate (SEE) between 3.2 to 3.7 $ml.kg^{-1}.min^{-1}$. For the hip-based, ActiGraph, the regression equations had lower accuratcy with SEE = 4.8 $ml.kg.min^{-1}$. To establish whether data generated from treadmill-based walking was applicable to free-living walking, the relationship between walking or running speed and vector magnitude accelerations was compared between the treadmill and overground walking or running on a track. The relationships were virtually identical, which suggests that estimates of oxygen uptake and

therefore energy expenditure based on treadmill exercise are likely to be applicable to free-living conditions.

The combined findings of this thesis suggest that small increments in activity beyond sitting, especially standing, could be efficient and feasible behaviours to replace sedentary behaviour. Targeting such facets of individuals' behaviour, particularly obese adults who are likely to be the most susceptible to the health risks associated with prolonged sitting. This thesis also achieved the initial and crucial steps towards the developing novel algorithms to predict additional physiological measurements using accelerometer devices, which with future work will allow accelerometers to produce accurate and informative physiological measurements to assess physical activity behaviour in free-living conditions.

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Author's Declaration

Unless otherwise indicated by acknowledgment or reference to published literature, the presented work in this thesis is the author's own and has not been submitted for a degree at another institution.

Nabeha Hawari_____ Date_____

The findings of some of the studies have been published as follows:

Published Papers

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Jason Gill, **Nabeha Hawari**, Douglas Maxwell. Validation of a novel pocket-based device to measure and provide feedback on physical activity and sedentary behaviour. Presented by the author as a poster at the ISBNPA, Edinburgh. 3 - 6 June 2015.

Nabeha Hawari, Iqbal Alshayji, John Wilson & Jason Gill. Metabolic responses to breaking up sitting time. Journal of Sports Sciences. Presented orally by author at the BASES Conference, St. George's Park. 1-2 December, 2015

List of Abbreviations

AG	actigraph
AP	activPAL
AUC	area under curve
BMI	body mass index
CHD	coronary heart disease
CVD	cardiovascular disease
EE	energy expenditure
ELISA	enzyme-linked immunosorbent assay
HDL	high density lipoprotein
$HOMA_{IR}$	homeostasis model of assessment-insulin resistance
HR	heart rate
IAUC	incremental area under curve
INT-Stand	intermittent standing trial
IPAQ	international physical activity questionnaire
LDL	low density lipoprotein
LTPA	light-intensity- physical activity
MET	metabolic Equivalent
MVPA	moderate-to vigorous-intensity physical activity
NP VO_2	non-protein oxygen consumption
NP VCO_2	non-protein carbon dioxide production
NP RQ	non-protein respiratory quotient
PA	physical activity
PRO-Stand	prolonged standing
RER	respiratory exchange ratio
RMR	respiratory exchange ratio
SB	sedentary behaviour
SD	standard deviation
SEM	standard error of mean
SIT	sitting
STPD	standard error temperature and pressure for a dry gas
TG	triglyceride

T_2D	type 2 diabetes
$\dot{V}O_2$	rate of oxygen consumption
$\dot{V}CO_2$	rate of carbon dioxide production
$\dot{V}O_2max$	maximal oxygen consumption
VM	vector magnitude
WC	Waist circumference
WHO	World health organisation

1. Introduction and Literature Review

1.1 The prevalence of cardiovascular disease

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. Coronary heart disease and stroke have emerged as main areas of concern for researchers. These conditions arise from a build-up of fatty deposits inside the arteries precipating an increased risk of blood clots on the inner walls of the blood vessels which supply the brain and heart (WHO 2011). Several studies have documented that cardiovascular disease (CVD) is one of the major causes of death worldwide, accounting for more than 17.3 million deaths per year in 2012 and 2013; this, is expected to rise to more than 23.6 million by 2030 (Mozaffarian *et al.* 2016;WHO 2011). In 2014, cardiovascular disease was the cause for a second highest cause of all deaths in the UK with 26% of all female deaths and 28% of male deaths **Table 1-1**. There are around 7 million people living with cardiovascular disease in the UK: 3.5 million men and 3.5 million women (BHF Heart Statistics 2016). The cost of CVD to the UK economy was £29.1 billion in 2004 (Luengo-Fernandez et al. 2006) and in England, more than £6.8 billion was spent on treating CVD in 2012/2013 (Bhatnagar *et al.* 2015). Each year around £11 billion is spent on healthcare costs relating to cardiovascular disease. Data from several studies suggest that the risk of cardiovascular disease can prevented by addressing behavioral risk factors.

Table 1-1: Deaths by cause and sex, UK 2014 (men, and women) (Bhatnagar et al. 2015).

Deaths by cause (%)	Men	Women
Hypertensive Disease	2,743 (1%)	3,975 (1%)
Coronary heart disease	41,364 (16%)	27,799 (10%)
Other heart disease	11,090 (4%)	14,737 (5%)
Stroke	16,222 (6%)	23,060 (8%)
All cardiovascular disease	78,240 (28%)	76,399 (26%)
Cancer	8,666 (32%)	78,916 (27%)
Respiratory disease	36,344 (14%)	38,938 (14%)
Diabetes	3,018 (1%)	3,295 (1%)
Dementia and Alzheimer	19,187 (6%)	38,724 (13%)
All other causes	53,000 (18%)	55,614 (19%)

1.2 Risk factors for cardiovascular disease

Anumber of factors influence risk of CVD which can be divided into non-modifiable and modifiable risk factors. A subset of modifiable risk factors are behavioral risk factors. These are described below and in **Table 1-2**.

1.2.1 Non-modifiable risk factors

Family history, sex, ethnicity and age are factors which cannot be modified. The risk of CVD increases approximately by 3-fold with each decade of life (Finegold *et al.* 2013). Patients with a family history of coronary artery disease have a higher prevalence of CVD (45% higher odds with sibling history) and stroke (50% higher odds with history in a first-degree relative) (Mozaffarian *et al.* 2016). Men have higher risk of CVD than premenopausal women, but after menopause the protection associated with being female is attenuated (Edmunds and Lip 2000) . Individual of South Asians or Afro-Caribbean or African-American ethnicity are at increased risk of CVD mortality than White Europeans (Cappuccio 1997). In addition, the risk of stroke is higher than White Europeans in Blacks, some Hispanic Americans, Chinese, and Japanese populations (Cappuccio 1997).

1.2.2 Major modifiable risk factors

Obesity is an independent risk factor in the incidence and development of cardiovascular disease. In Scotland in 2014, 69% of men 61% of women, aged ≥ 16 were overweight (BMI ≥ 25 kg/m2) and 26% of men and 29% of women were obese (BMI ≥ 30 kg/m^2). In the UK 26% of men and 24% of women are obese (Bhatnagar et al. 2015). A recent individual-participant-data meta-analysis of data from the Global BMI Mortality Collaboration, incorporating over 10 million participants, reported a hazard ratios of 1.42 per 5 $kg.m^{-2}$ increase in BMI above 25 $kg.m^{-2}$ for coronary heart disease and stroke (Global BMI *et al.* 2016).

Diabetes Mellitus (DM) is defined as a metabolic condition in which the pancreas does not produce sufficient insulin to regulate blood glucose levels or where the insulin produced is unable to work efficiently (WHO. 2015). DM is one of the key factors driving increasing rates of CVD, such as CHD and stroke having the condition approximately doubles the risk of developing CVD disease (BHF Heart Statistics 2016). According to British Heart Foundation 3.5 million adults in the UK have been diagnosed with diabetes, with 10% of those diagnosed are living with Type 1 diabetes and 90% with Type 2. In the United States,

data from NHANES 2009 to 2012 was reported that 21.1 million adults have diagnosed DM, 8.1 million adults have undiagnosed DM, and 80.8 million adults (35.3%) have prediabetes (eg, fasting blood glucose of 100 to < 126 mg/dL) (Mozaffarian *et al.* 2016). Obesity is a key risk factors for diabetes. Individuals with a BMI of 25kg/m^2 have a 5 times greater risk for developing diabetes than those with a BMI <20kg/m^2 with increments rising up to 93 times for those with a BMI >35kg/m^2 (Astrup and Finer 2000). The two conditions share causative factors, but do not necessarily lead to one another. However the development of diabetes/insulin resistance in the obese leads to an exponential rise in CV mortality (Astrup and Finer 2000).

High blood pressure is defined as constant systolic and diastolic blood pressure (BP) above 140/90mmHg (Mackay and Mensah 2004). 30% of adults in the UK have high blood pressure and up to half are not receiving treatment (BHF Heart Statistics 2016). Data from NHANES 2011 to 2012 observed that 17.2% of US adults are not aware they have hypertension. The prevalence of hypertension in men and women ≥ 18 years of age was 29.7% and 28.5%, respectively (Mozaffarian *et al.* 2016). Moreover, higher risk has been indicated in those aged between 40 and 89 years, as for every 20mmHg systolic or 10mmHg diastolic increase in blood pressure is a doubling of stroke ischaemic heart disease mortality (Lewington *et al.* 2002). Unhealthy diet is estimated to be accountable for half of hypertension whereas physical inactivity and obesity are both accountable for about 2% each (Bhatnagar *et al.* 2015)

Cholesterol is a fatty substance transported by in the circulation in particles called lipoproteins. While many lipoprotein subclasses exist, broadly speaking low-density lipoproteins (LDL) are the most atherogenic lipoprotein species which carry cholesterol from the liver to the cells, and high-density lipoprotein (HDL) is responsible for reverse cholesterol transport, carrying cholesterol away from the cells and back to the liver to be broken down, and are associated with lowering of atherogenic risk (Mackay and Mensah 2004). High level of cholesterol can led to atherosclerosis, limiting blood flow through the arteries and increasing the possibility of heart attack and stroke. High total cholesterol TC, low-density lipoprotein LDL-cholesterol and triglyceride levels TG, and low levels of high-density lipoprotein HDL-cholesterol can increase the risk of coronary heart disease and ischaemic stroke. Beyond this, there is a substantial body of evidence that high levels of triglyceride, particularly in the postprandial state, is associated with increased CVD risk and are mechanistically implicated in the atherosclerotic disease process (Bansal *et al.*

2007;Chapman *et al.* 2011; Goldberg *et al.* 2011; Mora *et al.* 2008; Nordestgaard *et al.* 2007).

1.2.3 Behavioural modifiable risk factors

Daily smoking is one of the top three leading risk factors for CVD and contributed to an estimated 6.2 million deaths in 2010 (Mozaffarian *et al.* 2015). Nearly one in five adults in the UK smoke cigarettes which close to 10 million adults, and 20.000 UK deaths from CVD disease can be attributes to smoking each year (BHF Heart Statistics 2016). In England in 2013, an estimated 78,200 deaths among adults aged 35 and around 17% among older were attributed to smoking (Bhatnagar *et al.* 2015).

A number of dietary factors as associated with CVD risk. There is evidence that diets high in saturated fat intakes are associated with higher CVD risk and replacement of saturated with unsaturated fats leads to lower risk (Sacks *et al.* 2017). Data on the association between carbohydrate intake and CVD risk is less clear. Replacing saturated fat with refined carbohydrates and sugars does not appear to reduce CVD risk, (Sacks *et al.* 2017), but diets high in complex carbohydrates, particularly low glycaemic index carbohydrates may lead to lower CVD risk (Fleming and Godwin 2013). Increasingly, dietary studies are moving beyond considering single nutrients and are instead investigating dietary patterns. There is increasing evidence that adopting a Mediterranean diet, characterised by high intakes of vegetables, legumes, fruit, nuts, grains and fish is associated with lower CVD risk (Sacks *et al.* 2017). A meta-analysis of prospective cohort studies showed that each 2% of calories from unsaturated fat was associated with a 23% higher risk of CHD (RR, 1.23; 95% CI, 1.11–1.37) (Mozaffarian *et al.* 2016).

Physical inactivity, defined as an activity level insufficient to meet public health guidelines (Department of Health 2011), has been identified as the fourth leading risk factor for mortality, CHD and type 2 diabetes (WHO 2011). Insufficient physical inactivity is associated with an increase the risk of all-cause mortality by 20%–30% and also is one of the key factors in prediabetes, diabetes and hypertension. 150 minutes of physical activity of moderate intensity per week can reduce the risk of Ischemic heart disease and diabetes risk by 30%, and 27% respectivly (Al-Nooh *et al.* 2014).

Recent evidence indicates that sedentary behaviour, which includes watching TV, overall daily sitting time, and time spent sitting in cars is another factor associated with increased

risk of cardiovascular disease and all-cause mortality (Owen et al. 2010b). Sedentary behaviour is one of the modifiable key factors driving increasing rates of CVD (Bauman *et al.* 2013), and sedentary behaviour in adults has shown reasonable evidence of a causal relationship with all-cause mortality (Biddle *et al.* 2016). The work in this thesis, and the remainder of this chapter will focus mostly on sedentary behaviour and physical inactivity.

Table 1-2: Risk Factors (Mackay and Mensah 2004).

Major Modifiable Risk Factors	Behavioural Modifiable Risk Factors	Non-Modifiable Risk Factors
Abnormal blood lipids	Tobacco use	Heredity or family history
High blood pressure	Unhealthy diets	Age
Diabetes mellitus	**Physical inactivity**	Gender
Obesity	**Sedentary behaviour**	Ethnicity or race

1.3 Physical activity and cardiovascular disease

Physical activity has been defined as "any bodily movement produced by skeletal muscles that results in energy expenditure above the basal metabolic level" (Vanhees *et al.* 2012). Within this overall definition, light intensity physical activity (LTPA) has been defined as those activities that increase energy expenditure at the level of 1.6–2.9 METs (where 1 MET is equivalent to resting metabolic rate) such as slow walking (less than 2.0mph) (2.0 METs), cooking (2.0 METs), and washing dishes (1.8 METs) and moderate-to-vigorous physical activity (MVPA) is defined as activities with an energy expenditure of at least 3 METs (Carr and Mahar 2012). A large body of epidemiological evidence has shown that engaging in high levels of MVPA is associated with lower risk of a number of adverse health outcomes including CVD and diabetes (Hu *et al.* 1999; Manson *et al.* 2002; Nocon *et al.* 2008; Wijndaele *et al.* 2011). According to (WHO 2017), adults (18-64 years) should accumulate at least 150 minutes of moderate-intensity aerobic activity such walking, cycling, or 75 minutes (1 h and 15 min) of vigorous-intensity aerobic activity such as running, throughout the week. Physical activity can reduce the risk of CVD events by 30% to 50% (Mozaffarian *et al.* 2008). Warburton et al (Warburton *et al.* 2010) undertook a systematic review of prospective cohort investigations including over 200 studies of male and female subjects from all over the world, between 1985 and 2007. Overall these studies found a 31% reduction in all-cause mortality in more active compared with less active individuals and a reduction in mortality risk of 45% for fit comparted with unfit individuals. The fit and physically active had a 42% lower diabetes risk than their inactive and unfit counterparts.

In recent years, there has been growing evidence that other aspects of activity behaviours beyond moderate-to-vigorous physical activity are also associated with health outcomes. In particular, sedentary behaviour has been highlighted as a behaviour associated with adverse health outcomes.

1.4 The definition of sedentary behaviour, physical inactivity and physical activity

The word 'sedentary' comes from the Latin 'sedere' (to sit) and can refer to any waking sitting or reclining posture, such as watching television, using the computer, driving a car, or lying behaviour and other forms of screen based entertainment with low energy expenditure at the level of 1.0 – 1.5 metabolic equivalent units (METs) (Thorp *et al.* 2011; Tremblay *et al.* 2017; Wilmot *et al.* 2012), with 1MET being equivalent to the amount of energy expended during rest (Ainsworth *et al.* 2000; Jette *et al.* 1990).

Historically, many researchers have typically used the phrase sedentary lifestyle to represent people who are physically inactive but more recently sedentary behavior has been defined as low energy sitting (or reclining) during waking hours (Mark 2012), thus excluding sleep or seated exercise. It is, essentially, 'sitting time' rather than 'lack of exercise'. Thus, it is possible for a person to meet PA guidelines and also spend a large proportion of the day sedentary. For example if someone does 30 min of MVPA in the evening but sits for the rest of the day, they are meeting PA guideline, but also highly sedentary. **Figure 1-1** shows the continuum of PA and sedentary behavior (Dempsey et al. 2014;Saunders et al. 2014). There is evidence which has shown that being sedentary and being inactive are different constructs and have a differential effect on health factors such as cardiovascular disease (CVD), some types of cancer, diabetes and all-cause mortality (Hamilton *et al.* 2008; Lynch 2010; Wilmot *et al.* 2012). Hamilton et al (Hamilton *et al.* 2007) mentioned that the term sedentary includes a sense of "lack of exercise", and is not limited to the original Latin definition of sitting. This has led to the inclusion of standing time with sitting in the classification of sedentary in some studies. However, a systematic review undertaken by Thorp et al (Thorp *et al.* 2011) concluded that, standing should not be assigned as "a sedentary activity", suggesting that the term sedentary should be used to refer only both seated and reclining posture. Thus, standing activity, even at low energy expenditure, can be defined as non-sedentary (Ainsworth *et al.* 2011). The corollary of this is that sedentary behaviour should be studied as a unique behavior that is distinct from physical activity (Dempsey *et al.* 2014; Saunders *et al.* 2014).

However, this view is not unequivocal. Gibbs et al, defined, sedentary behavior by intensity only without an additional posture component with sedentary behaviour characterised as any waking behaviour or activity at the level of ≤ 1.5 (METs), Here, light activity was defined as 1.5 - 2.9 METs, moderate activity as 3.0 - 5.9 METs and vigorous activity as ≥ 6.0 METs. Thus the difference between the definitions is that quiet standing would be included as sedentary behavior by the intensity definition, but not by the posture and intensity definition. Thus, there is debate about which definition should be used and how sedentary behaviour should be assessed. (Gibbs *et al.* 2015). There is also debate on the MET values range that should be ascribed to sedentary behavior. According to Sedentary Behaviour Research Network (Sedentary Behaviour Research Network 2012), sedentary behavior defined as "any waking behavior characterized by an energy expenditure of ≤ 1.5 METs while in a sitting or reclining posture". On the other hand, Ainsworth has coded MET values from 1.0 - 2.5 for time spend in sedentary behavior such as that sitting at a desk, sitting in a vehicle, watching TV, while, standing activities, which are not categorized as sedentary, are coded with a MET value of 1.5 (Ainsworth *et al.* 2011). Moreover, another sitting activity, for example, playing games (often classified as sitting time in self-report questionnaire) categorized to have 4.5 MET values (O'Donovan *et al.* 2012).

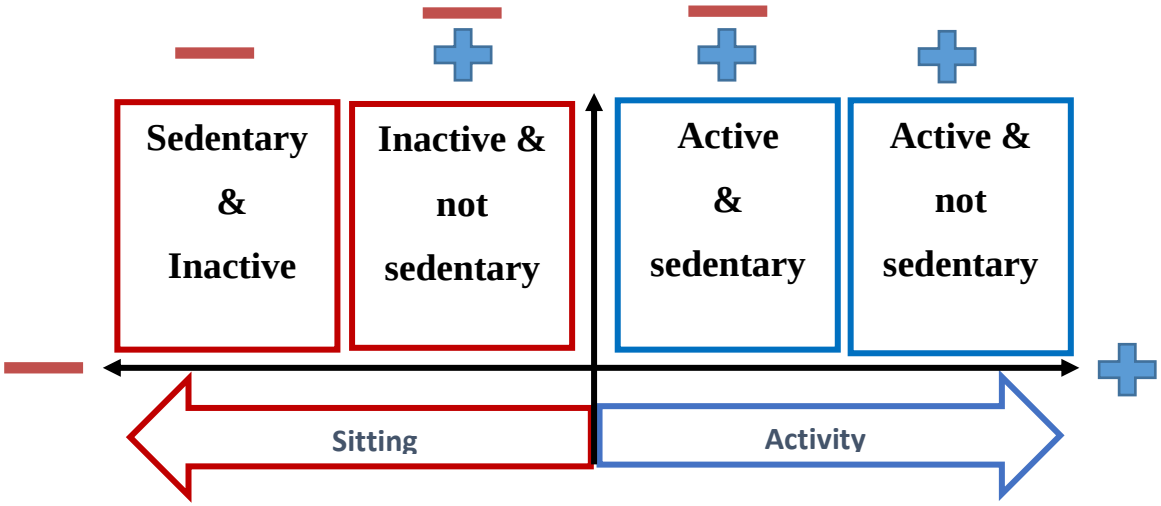


Figure 1-1: Continuum of time spent sitting (left side) and in MVPA (right side) as 2 distinct classes of behaviour. Plus signs = healthier behaviour pattern, minus signs = riskier behaviour pattern. (Dempsey *et al.* 2014;Saunders *et al.* 2014).

1.5 The prevalence of sedentary behaviour

Over the past few decades, the way in which we live our daily lives has changed rapidly. Technological advances and societal changes have significantly influenced the way we

spend our leisure and work time, resulting in considerable proportions of the day spent sedentary. The prevalence of sedentary time has been described in a number of international studies. The average weekday time spent sitting in 32 European countries was 309 minutes per day, equating to 5-6 hours of sitting per day (Bennie *et al.* 2013), similarity, the average time engaged in sedentary was 300 minutes per day across 20 International countries (Bauman *et al.* 2011). Also, Milton et al (Milton *et al.* 2015) observed the prevalence of sedentary time in 27 European countries and the results indicated that the average daily time spent sitting in evaluated countries was 292 minutes per day in 2013. It is important to recognise that these studies measured sedentary time from mostly developed countries, the outcomes therefore cannot be generalised to lower- income nations. Furthermore, in all studies, a self-report questionnaires were used to quantify time spend sitting. However, the IPAQ questionnaire have been reported to underestimate sedentary behaviour and has poor validity (Atkin *et al.* 2012). Estimates from objective monitoring of sedentary behaviour in the US showed that adults spent 55 % - 57 % of their waking day engaging in sedentary pursuits (Healy *et al.* 2008b;Matthews *et al.* 2008), which is substantially higher than the estimates from self-report.

Over the past five decades there has been a significant reduction in the proportion of people who are employed in physically active occupations and on the other hand there has been a growth in the proportion of employees in more sedentary jobs (Church et al. 2011). These type of sitting occupations usually involve prolonged bouts of sitting time at an office desk or driving a vehicle. A recent study in office workers indicated that individuals spent a greater proportion of time in sedentary behaviour during working hours (68% vs 60%) and less time in light-intensity physical activity (28% vs 36%) compared to non-working days. Overall, these adults spent up to 71% of their working days sedentary (Clemes et al. 2014a). In comparison to the international epidemiological studies mentioned above, researchers have demonstated that office workers are sedentary for approximately 10 hours/day (Clemes et al. 2016; Clemes et al. 2014a; Clemes et al. 2014b). This shift towards sedentary occupations may have serious implications for health and well-being (Morris *et al.* 1953; Paffenbarger, Jr. 2000).

1.6 Sedentary behaviours, diabetes, CVD disease, metabolic syndrome and all-cause mortality

In recent years, there has been a large number of observational studies (including prospective cohort studies) investigating the association between sedentary behaviour (sitting) and health outcomes. Studies have shown that individuals can spend 50 – 60% their waking hours in sedentary activities (Edwardson *et al.* 2012; Wilmot *et al.* 2012). Several recent reviews have highlighted the health risks linked to this type of conduct, such as metabolic syndrome (Edwardson *et al.* 2012; Wijndaele *et al.* 2011), type 2 diabetes (Ford *et al.* 2010; Hu *et al.* 2001; Hu *et al.* 2003; Zhang *et al.* 2006), cancer, CVD and all-cause mortality (Katzmarzyk *et al.* 2009; Patel *et al.* 2010; Stamatakis *et al.* 2011). A considerable number of systematic reviews and meta-analyses have been published summarising the available evidence on sedentary behaviour and health outcomes. These studies are summarised in **Table 1-3**. In a meta-analysis including 16 prospective and 2 cross-sectional studies, with a total 794,000 participants from all over the world Wilmot et al (Wilmot *et al.* 2012) reported that individuals spending the greatest time spent sedentary compared to the lowest time spent sedentary had 112% higher relative risk (RR) for diabetes, 147% higher risk of cardiovascular disease, 90% higher risk of cardiovascular mortality and 49% higher of all-cause mortality. Similarly, in a meta-analysis of 10 cross-sectional studies with a total of 21,393 participants Edwardson and colleagues reported that high levels of sedentary behaviour were associated with increased risk of the metabolic syndrome by 73% (OR 1.73, 95% CI 1.55 – 1.94, $p < 0.0001$) (Edwardson *et al.* 2012). Importantly, the results remained largely unchanged when only studies which adjusted for physical activity were included (OR 1.73, 95% CI 1.54 –1.97, $p < 0.0001$).

Some meta-analyses considered specific aspects of sedentary behaviour. In a meta-analysis including 11 prospective (cohort, case-cohort, and nested case-control) studies and at total of 236,700 adults, Grontved and Hu (Grontved and Hu 2011) assessed the association between TV viewing and risk of diabetes, fatal or nonfatal CVD, and all-cause mortality, showing that watching more than 2 hours of TV per day was associated with a 13% higher risk of all-cause mortality, a 15% higher risk of fatal or non-fatal CVD, and a 20% higher risk of diabetes. The pooled relative risks of watching TV for 2 hours per day was 1.13; 95% CI = 1.07-1.18) $P < 0.001$ of all-cause mortality, 1.15; 95% CI = 1.06 - 1.23) $p< 0.001$ of fatal or non-fatal cardiovascular disease, and 1.20; 95% CI, 1.14 - 1.27) $p< 0.001$ of diabetes. The absolute risk differences per every 2 hours of TV viewing per day were 176 cases of

type 2 diabetes, 38 cases of fatal cardiovascular disease, and 104 deaths for all-cause mortality per 100 000 individuals per year. Similarly, Ford and Caspersen (Ford and Caspersen 2012) evaluated the associations between screen time (TV viewing, watching videos and using a computer) and total sedentary time and CVD in a meta-analysis of nine prospective studies, with 496,394 participant, aged ≥18 to 90 years. The summary hazard ratio for CVD risk per 2-hour increase in sitting time was 1.05 (95% CI 1.01-1.09), in contrast the summary hazard ratio for CVD risk per 2-hour increases in TV viewing time substantially higher 1.17 (95% CI 1.13-1.20). Thus, it appears that TV viewing has a stronger association with adverse health outcomes than overall sedentary behaviour.

The study findings suggest that substituting sedentary behaviour with standing or light-intensity physical activity may reduce the risk of chronic disease and mortality, independent of the amount of MVPA undertaken. This implies that reduction of sedentary time may impact prevention of these diseases. This research attempts to show that the risk of sedentary behavior seems to be independent of physical activity. There is an urgent need to further investigate the impact of reducing sedentary time on metabolic syndrome.

A systematic review done by Thorp et al (Thorp *et al.* 2011) examined the relationship between self-reported and device-based measures of prolonged sitting with diabetes, cardiovascular disease, obesity and all-cause mortality in adults across 48 longitudinal studies from 1996 to 2011. All of the participants were aged more than 18 years. This study measured different types of sitting time, such as, TV screen time only, TV screen time and other screen-time behaviour, and TV screen time plus other sedentary behaviour. Sedentary behaviour here refers to sitting during commuting, in the place of work and the household environment, and during free time. Overall, there seems to be some evidence to indicate that prolonged sitting, especially in relation to TV viewing time and other screen-based activities with increased snacking behaviour, is positively associated with increased risk health outcomes such as obesity, cholesterol/lipids, metabolic syndrome and type 2 diabetes. Sitting in front of the TV was strongly associated with the consumption of energy-dense snacks, soft drinks and fast food, and was consistently inversely associated with fruit and vegetable consumption and also can influence the type of food they desire and consume, resulting in a lack of awareness of actual food consumption or overlooking food sign that may lead to overconsumption (Pearson and Biddle 2011).

A recent meta-analyses on 47 prospective cohort studies was undertaken by Biswas et al (Biswas *et al.* 2015). The aim of this paper was to review recent research into the association between sitting time, hospitalizations, all-cause mortality, cardiovascular disease, diabetes, and cancer. It was observed that, greater sedentary time was positively associated with an increased risk for cardiovascular disease (HR, 1.14 [CI, 1.00 to 1.73]), cardiovascular disease mortality (HR, 1.18 [CI, 1.11 to 1.26]), all-cause mortality (HR, 1.24 [95% CI, 1.09 to 1.41]), cancer mortality (HR, 1.17 [CI, 1.11 to 1.24]), cancer (HR, 1.13 [CI, 1.05 to 1.21]), and type 2 diabetes (HR, 1.91 [CI, 1.64 to 2.22]), but the main statistical impact was associated with the risk for type 2 diabetes. Moreover, sitting less than < 8h per day was associated with lower risk of potentially preventable hospitalization by 14% (HR, 0.86 [CI, 0.83 to 0.89]). The multivariate regression model (the technique to estimate a single regression model with more than one input variable), was adjusted for age, sex, education, marital status, income, geographic remoteness of residence, language, health insurance, chronic disease history, previous admission for potentially preventable hospitalization, MVPA, and other health behaviors. The most striking result to emerge from the data is that prolonged sitting time is independently associated with greater risk for all-cause mortality, cardiovascular disease and type 2 diabetes, regardless of PA. However, the risk associated with sedentary time was lower among people who participated in higher levels of PA compared with lower levels.

In 2013, Chau et al (Chau *et al.* 2013) published a paper in which they described the relationship between the daily total sitting and all-cause mortality risk in adults using meta-analysis. Six prospective studies were accepted with 595,086 participants, aged ≥ 18. Sedentary behaviour was measured by using self-report or accelerometer. Each additional hour of daily sitting in intervals 0-3, > 3-7 and > 7 h/day total sitting time is associated with increase the risk of all-cause mortality, the HRs were 1.00 (95% CI: 0.98 - 1.03), 1.02 (95% CI: 0.99 - 1.05) and 1.05 (95% CI: 1.02 - 1.08) respectively, adjusted for MVPA. Sitting for more than 10 h/day had 34% (HR = 1.34, 95% CI: 1.28 - 1.40) and 52% (HR = 1.52, 95% CI: 1.46 - 1.58) increased the risk all-cause mortality with and without adjusting for physical activity, respectively. Physical activity partly attenuated the increased risk associations between prolonged sitting and all-cause mortality, especially in those who spent the most time in sitting.

Another systematic review was undertaken by Van Uffelen et al. The purpose of this paper was to systematically review the recent evidence on association between occupational sitting

and BMI (12 studies); cancer (17 studies); cardiovascular disease (8 studies); diabetes mellitus (4 studies); and mortality (6 studies). Of the 43 papers identified, 21% were cross-sectional, 14% were case control and 65% were prospective cohort studies. Only five cross-sectional studies out of ten confirmed a positive relationship between sitting and BMI, five of the 17 studies showed there was a relationship between sitting and higher risk of cancer. Moreover, there was association between sitting and increase the risk of CVD in four studies. Two prospective and one cross sectional showed a positive association between sitting and diabetes. Finally, four prospective studies found a positive relationship between sitting and mortality (van Uffelen *et al.* 2010).

Shen and colleagues undertook a systematic review and meta-analysis to examine the association between sedentary behavior and incident cancer. A total of 17 prospective studies were identified in the systematic review, including 857,581 participants and 18,553 cases. The present study was determined that time spent in sedentary behaviour was associated with increased risk of cancer (RR = 1.28, 95% CI = 1.08 – 1.53), in endometrial cancer, (RR = 1.30, 95% CI = 1.12 – 1.49), in colorectal cancer, (RR = 1.17, 95% CI = 1.03 – 1.33), in breast cancer, (RR = 1.27, 95% CI = 1.06 – 1.52), in lung cancer. However, there was no association of sedentary behaviour with ovarian cancer (RR = 1.26, 95 % CI = 0.87 –1.82), renal cell carcinoma (RR = 1.11, 95% CI = 0.87 – 1.41) or non-Hodgkin lymphoid neoplasms (RR = 1.09, 95% CI = 0.82 – 1.43) (Shen *et al.* 2014).

One of the most important recent meta-analyses was undertaken by Ekelund and colleagues in 2016 (Ekelund *et al.* 2016). This study aimed to address the following research question: "Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality?". Sixteen prospective studies were identified as potentially relevant, with an overall sample size across the studies of 1,005,791 participants. In all studies, self-report questionnaires were used to assess physical activity and sedentary behaviour. Sitting time and TV-viewing time were categorised into four groups each. In the least active quartile ($\leq$ 2.5 MET-h per week), those sitting > 8 h/day had significantly increased risk of mortality compared with those who sat the least (HR = 1.27, 95% CI = 1.22 – 1.32). However, in the most active quartile (>35 MET-h per week) the hazard ratio associated with sitting > 8 h/day was not statistically significant (HR = 1.04, 95% CI = 0.98 – 1.10). This suggest that physical activity appears to eliminate the excess risk associated with prolonged sitting. In comparison, TV viewing for more than 5h/day was associated with increased risk of mortality at all levels

of physical activity. These results are in agreement with those obtained by Chau and Wilmot (Chau et al. 2013; Wilmot et al. 2012).

Thus, the available evidence suggests that high levels of sedentary behaviour are associated with increased risk of a number of adverse health outcomes. There is also increasing evidence that the pattern of sedentary behaviour as well as the total volume may be associated with some health outcomes. This will be considered in the next section.

Table 1-3: Meta-analyses of epidemiological studies investigating the association between sedentary behaviours and health outcomes.

	Meta-analysis	Sample	Exposure measure	outcome	confounders	Main finding
1	Wilmot et al 2012	16 prospective and 2 cross-sectional studies with a total of 794,577 participants, aged ≥ 18	Self-reported Sedentary time	T2DM, CVD, and all-cause mortality	Adjusted for baseline event rate, BMI or waist circumference	Comparing the highest vs. the lowest sedentary time increased the relative risk of T2DM by 112% (RR 2.12 ; 95 % credible interval [CrI]1.61, 2.78), 147% increase in the risk of cardiovascular disease (RR 2.47 ; 95 % CI 1.44, 4.24), 90% increase in the risk of cardiovascular mortality (HR 1.90; 95% CrI 1.36, 2.66) and 49% increase in the risk of all-cause mortality (HR 1.49; 95% CrI 1.14, 2.03)
2	Grontved and Hu 2011	11 studies (Prospective cohort, Case cohort , and nested case-control designs) with a total of 236,700 participants, aged > 18 years	Self-reported Television viewing	T2DM, fatal or non-fatal CVD, and all-cause mortality	Dietary variables, BMI	Watching TV for >2 hours per day was associated with a 13% increase in the risk of all-cause mortality (RR=1.13; 95% CI=1.07-1.1) $P < 0.001$. 15% increase in the risk of fatal or non-fatal cardiovascular disease (RR1.15; 95% CI = 1.06 - 1.23) $p < 0.001$, and 20% increase in the risk of diabetes 1.20; 95% CI, 1.14 - 1.27) $p < 0.001$.
3	Edwardson et al. 2012	10 Prospective and cross-sectional studies with a total of 21,393 participants, aged > 18 years	Self-report and accelerometer measured sedentary behaviour	Metabolic syndrome	BMI	Sedentary behaviour increased risk of metabolic syndrome by 73% (OR 1.73, 95% CI 1.55 – 1.94, $p < 0.0001$)
4	Ford and Caspersen 2012	9 Prospective studies with a total of 496,394 participants, aged ≥ 18	Screen and sitting time assessed by Self-report, accelerometer and heart rate monitor	Fatal and non-fatal CVD	Adjusted for several cardio metabolic factors	Summary hazard ratio for CVD risk per 2-hour increases in sitting time was 1.05 (95% CI 1.01-1.09). Summary hazard ratio for CVD risk per 2-hour increases in TV viewing time was 1.17 (95% CI 1.13-1.20). Compared with lowest levels of sedentary time, risk estimates for fatal and non-fatal CVD ranged up to 1.68 for the highest level of sedentary time, and 2.25 for the highest level of screen time sitting.

5	Biswas et al 2015	47 Prospective and cross-sectional studies with a total of 2,125,989 participants, aged > 18 years	Self-reported sedentary behaviour	CVD, T2DM, cancer, all-cause mortality	MVPA, age, sex, and other health behaviors	Comparing the highest vs. the lowest sitting time increased the hazard ratio of CVD disease by 14% (HR, 1.14 [CI, 1.00-1.73]),and 18% increase in the risk of cardiovascular disease mortality(HR, 1.18 [CI, 1.11 to 1.26]) 24% increase in the risk of all-cause mortality (HR, 1.24 [95% CI, 1.09 to 1.41]) 17% increase in the risk of cancer mortality (HR, 1.17 [CI, 1.11 to 1.24]) and 13% (HR, 1.13 [CI, 1.05 to 1.21]) for cancer. 91% increase in the risk of type 2 diabetes (HR, 1.91 [CI, 1.64 to 2.22]
6	Chau et al 2013	6 Prospective studies with a total of 595,086 participants, aged > 18 years	Sedentary behaviour assessed by self-report and accelerometer	All-cause mortality	MVPA	Each additional hour of daily sitting in intervals 0-3, > 3-7 and > 7 h/day total sitting time is associated with increase the risk of all-cause mortality, the HRs were 1.00 (95% CI: 0.98 - 1.03), 1.02 (95% CI: 0.99 - 1.05) and 1.05 (95% CI: 1.02 - 1.08) respectively. Sitting for more than 10 h/day had 34% (HR = 1.34, 95% CI: 1.28 - 1.40)
7	Shen et al 2014	17 prospective studies with a total of 857,581 participants, aged >40 years	Self-reported total sitting time, occupational sitting, leisure sitting time or TV viewing	Cancer	BMI, PA and energy intake	Time spent in sedentary behaviour was associated with increased risk of cancer (RR = 1.28, 95% CI = 1.08 – 1.53), in endometrial cancer, (RR = 1.30, 95% CI = 1.12 – 1.49), in breast cancer, (RR = 1.27, 95% CI = 1.06 – 1.52), in lung cancer.
8	Ekelund et al 2016	16 prospective studies with a total of 1,005,791 participants, aged > 18 years	Self-reported TV-viewing time	Mortality	Sex, age and PA	Sitting for <4 h/day with the lowest activity level (<2.5 MET-h per week) had increased the hazard ratio of mortality by 27% (HR = 1.27, 95% CI = 1.22 – 1.32).However, in the most active quartile (>35 MET-h per week) the hazard ratio associated with sitting > 8 h/day was not statistically significant (HR = 1.04, 95% CI = 0.98 – 1.10)

1.7 Breaking-up sedentary time (observation studies)

Another important aspect is the concept that interrupting extended periods of sitting may attenuate a proportion of the association with cardiovascular and metabolic health. Several cross-sectional studies have examined the relationship between sedentary breaks and risk factors associated with cardiovascular and metabolic risk. **Table 1-4** lists observational studies that have examined the associations of breaking up sedentary behaviour and health outcomes in adults. The available evidence from prospective and cross-sectional studies suggested that the number of transitions or breaks sedentary time appear associated with some adverse biomarkers of metabolic health, such as BMI and waist circumference. These studies have all used an intensity-based definition of sedentary behaviour based on accelerometer counts from an Actigraph accelerometer, with ≤ 100 count/min defined as sedentary. Thus breaks in sedentary time could be defined by an increase in accelerometer counts above the >100 counts/min threshold. Accordingly, it is import to note that these studies are unable to distinguish between sitting and quiet standing, so a change from standing quietly to walking, as well as a change from sitting to upright activities, would be classified as a break in sedentary behaviour in these studies. **Figure 1-2** provides an illustration of typical profiles of individuals with long and short bouts of sedentary behaviour over the course of the day (Healy *et al.* 2008a).

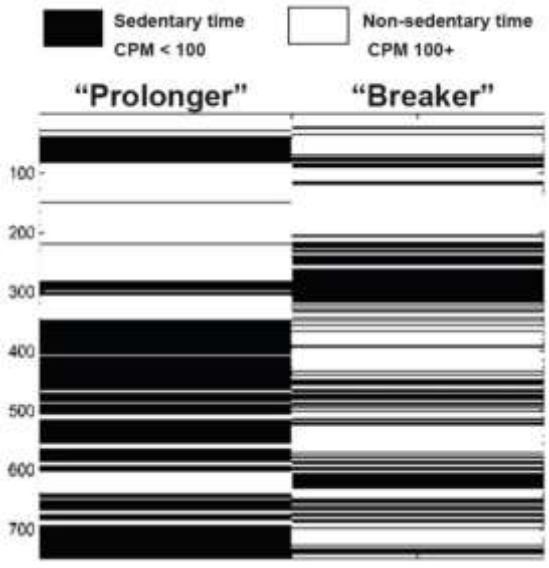


Figure 1-2: Sedentary time < 100 counts per min in the left-hand panel: Break in sedentary > 100 counts per min in the right-hand panel, recorded by accelerometer, but different ways of accumulation (Owen *et al.* 2010a)

Observational studies which have evaluated the association between breaks in sedentary behaviours and biomarkers of health are summarised in **Table 1-4**. In an observational study with 528 participants, Cooper et al (Cooper *et al.* 2012), considered the association of sedentary time and breaks in sedentary time on biomarkers of metabolic health in patients with type 2 diabetes. Waist circumference (WC), fasting HDL-cholesterol, insulin and glucose levels, HOMA-estimated insulin resistance (HOMA-IR), and physical activity (accelerometer) were measured at baseline and at 6 months follow-up. The results found that each hour of sedentary time was associated with larger WC (unstandardised regression coefficient [*B*] [95% CI] (1.89 cm [0.94, 2.83]), higher insulin (*B* = 8.22 pmol/l [2.80, 13.65]), lower insulin sensitivity HOMA-IR (*B* = 0.42 [0.14, 0.70]), and lower HDL cholesterol (*B* = - 0.04 mmol/l [- 0.06 - 0.01]) (*P* < 0.005 for all). The number of daily breaks in sedentary time was associated with lower WC (*B* = - 0.15 cm [- 0.24, - 0.05] *p* = 0.003). Healy et al (Healy et al. 2008a) investigated the association between breaks of sedentary time and metabolic risk. An accelerometer was used to measure their sedentary time, for seven consecutive days, with <100 accelerometer counts per minute being defined as sedentary. An interruption in sedentary time was defined as an increase from < 100 counts per minute to >100 accelerometer counts per minute. As such, participants spent 57% of their waking hours sedentary, 39% in light intensity and 4% in moderate to vigorous activity. This research demonstrated that interruption of sedentary time has a beneficial effect on waist circumference (standardized B = - 0.16, *P* = 0.026), BMI (B = - 0.19, *P* = 0.026), triglycerides (B = - 0.18, *P* = 0.029), and 2-h plasma glucose (B = - 0.18, *P* = 0.025), adjusted for age, gender, employment status, alcohol intake, income, education, smoking status, family history of diabetes, diet, moderate to vigorous activity MVPA, sedentary time, and the average activity intensity during the breaks. Thus these data provide preliminary evidence that more interruptions in sedentary time were beneficially associated with metabolic risk variables independent of total sedentary time and moderate-to-vigorous intensity activity time.

In addition, Healy et al (Healy et al. 2011) analysed cross-sectional data from 4757 adults in the US NHANES study, to observe the relationship between prolonged sitting and interruptions in sitting, on the one hand, and cardio-metabolic and inflammatory biomarkers, on the other. It was found that increased sedentary time negatively affected several biomarkers, whereas increasing breaks, independent of total sedentary time, were associated with reduction of waist circumference and fasting plasma glucose.

Henson et al (Henson *et al.* 2013a) performed a cross-sectional analysis of 878 adults at high risk of type 2 diabetes mellitus, reporting that breaks in sedentary behaviour were strongly and adversely associated with 2 h glucose concentration (β=−0.11 ± 0.055, p = 0.05), waist circumference (β=−0.21 ± 0.05, $p < 0.001$) and BMI (β=−0.15 ± 0.05, p = 0.003). However, further adjustment for BMI reduced the association with 2 h plasma glucose. Similarly, in a study of 1,367 older adults Bankoski et al (Bankoski *et al.* 2011), observed that people with higher sedentary time and fewer sedentary breaks had a large WC, low HDL cholesterol, high triglycerides, and metabolic syndrome (all $p < 0.05$), adjusted for age and sex. Another cross-sectional study by Henson et al (Henson *et al.* 2013b) observed that increased breaks in sedentary time, independent of MVPA, were beneficially associated with IL-6 (β = – 0.09 ± 0.05, p = 0.04) and leptin (β = – 0.07 ± 0.04, p = 0.04), in 552 adults (mean age 63.7 years, (SD 7.7) with high risk of type 2 diabetes mellitus T2DM. However, these relations were reduced when further adjusted for SB and MVPA.

Current findings suggest that prolonged bouts of sitting time are positively related to metabolic risk, independent of physical activity. It is important to minimize sedentary time among older adults, but the most important part is reducing prolonged periods of uninterrupted sedentary time and increasing intermittent movements during sedentary time.

Gupta et al (Gupta *et al.* 2016) used an isotemporal substitution approach in a group of 692 workers to report that replacing 30 minutes/day of sedentary behaviours with 30 minutes/day of MVPA during whole day was significantly inversely associated with waist circumference [B (95% CI); -3.93 (- 6.62 to - 1.23) cm, BMI -1.28 (- 2.2 to - 0.35) kg/m^2, and fat percentage [B (95% CI - 2.38 (-3.7 to - 1.06) %, ($P < 0.05$ for all).

In conclusion, the findings from these studies emphasise that regardless of how long you are sedentary over the day, frequently interrupting sedentary time has a beneficial association on metabolic markers, particularly those related to adiposity variables, even if the interruptions are short and only involve light activity or standing. However, the cross-sectional and observational nature of these data make it impossible to determine the direction of these relationships and potential causality. If these relationships are causal, these findings could have practical applications for interventions to attenuate or eliminate the negative deleterious health consequences of sedentary time as they suggest that small behavioural modifications could potentially have a considerable protective consequence. The beneficial results of breaking prolonged sitting on metabolic markers are best investigated using

controlled laboratory based intervention studies. In such sedentary studies, breaking in sedentary can be directly monitored and the acute or cumulative effects of sedentary on metabolic parameters can be observed. Human data from intervention studies supporting a *causal* role for sedentary behaviour in cardio-metabolic disease risk are particularly needed.

Table 1-4: Cross-sectional observational studies of the association between breaking sedentary behaviour and metabolic risk markers.

	Author(Study)	Design	Sample/ country	Measurement of breaks	Breaks unit	Outcomes	Confounders	Main results
1	(Healy et al. 2008a) Breaks in sedentary time: beneficial associations with metabolic risk.	Cross-sectional	168, adults, age (SD) 53.4 ± 11.8 years BMI(SD) 27.2 ± 4.7 Australia	Actigraph (1-min epoch); *SB calculated as <100 counts/min; *BSB defined as interruption from SB state to active state(≥100 counts/min) for a minimum of 20 min * Non wear, continuous 0 cpm as intervals of 60min	Breaks per recording time (5 -7 days)	Waist circumference, BMI, 2-h glucose, insulin , triglycerides, HDL cholesterol, blood pressure	Age, gender, alcohol intake, employment status, education, household income ,smoking status, family history of diabetes, diet quality, PA time and SB tim	Breaking up sedentary behaviour was associated with lower waist circumference (standardized B = - 0.16, *P* = 0.026), BMI (B = - 0.19, *P* = 0.026), triglycerides (B = - 0.18, *P* = 0.029), , and 2-h plasma glucose (B = - 0.18, *P* = 0.025), independent of total sedentary time and moderate-to-vigorous intensity activity time.
2	(Healy *et al.* 2011) Sedentary time and cardio-metabolic biomarkers in US adults (NHANES03-06)	Cross-sectional	4,757 adults, age ≥ 20 years US	Actigraph(1-minepoch); *SB is <100 counts/min; *LTPA (100 – 1951 cpm) *MVPA (≥1952cpm) *BS(≥100counts/min) was considered as break * Non wear, continuous 0 cpm as intervals of 60min	Breaks per recording time worn on the right hip for 4-7 days	Waist circumference, BMI,2-h plasma glucose, triglycerides, HDL cholesterol, insulin level,	Age, sex, PA, smoking, alcohol intake, fat in diet, energy intake, hypertension, hyperlipidemia, medical history, family history, socio-economic status	Comparing the lowest vs the highest, breaking sedentary time [B (95% CI); 99.2 (97,9 – 100.6) vs [B (95% CI); 95.1 (94.0 – 96.1) was associated with reduction of waist circumference, and fasting plasma glucose [B (95% CI); 5.55 (5.46-5.64) vs [B (95% CI); 5.51(5.40-5.62) respectively,independent of total sedentary time and PA.
3	(Bankoski *et al.* 2011) Sedentary activity associated with metabolic syndrome independent of physical activity	Cross-sectional	1,367, older adults, 665 with metabolic syndrome, age (SD) 71.0 ± 7.4 years 702 without metabolic syndrome, age (SD)71.0 ± 8.0 U.S.	Actigraph (1-min epoch) *SB defined as<100 counts/min; *BSB defined as transition from SB state to active state (≥100 counts/min) * Non wear, continuous 0 cpm as intervals of 60min	Breaks per day 4 days	Waist circumference, HDL cholesterol, triglycerides, fasting glucose, metabolic syndrome	Age, gender, race/ethnicity, education, alcohol consumption, smoking status, BMI, self-reported diabetes and heart disease,PA and SB time	Higher percentage of sedentary time out of total wear time (quartile 2: odds ratio [OR] 1.52 [95% CI 1.04–2.21]; and fewer sedentary breaks (quartile 3: 1.50 [1.02–2.21]) were related to a significantly increased likelihood of metabolic syndrome, independent of physical activity.

	Author(Study)	Design	Sample	Measurement of breaks	Breaks unit	outcomes	Confounders	Main results
4	(Cooper et al. 2012) Sedentary time, breaks in sedentary time and metabolic variables in people with newly diagnosed type 2 diabetes	Cross-sectional and longitudinal	582 adults, age 30 - 80 years, newly diagnosed with type-2 diabetes UK	Actigraph(1-minepoch); *SB defined as<100 counts/min; * BSB defined as transition from SB state to active state(≥100 count/min) *Nonwear time was ≥20 min with continuous 0 values	Breaks per day measured for 5 - 7 days	Waist circumference, HDL cholesterol, glucose levels and HOMA of insulin resistance	Age, gender, current smoking status, family history of diabetes, lipid lowering or diabetes medication, PA and SB time	The number of daily breaks in sedentary time was associated with lower WC (*B* = - 0.15 cm [- 0.24, - 0.05] *p* = 0.003). All associations were independent of levels of MVPA
5	(Henson et al. 2013b) Sedentary time and markers of chronic low-grade inflammation in a high risk population.	Cross-sectional	558 adults, age (SD) 63.6 ±7 .7 BMI (SD) 32.2 ± 5.2 UK	Actigraph GTX3(15-s epoch); *SB defined as < 25 counts / 15s; *BSB defined as transition from SB state to active state (≥25counts / 15s) for a minimum of 15s *MVPA (≥ 488 counts per 15 seconds)	Breaks per day 7consecutive days during waking hours	C-reactive protein, adiponectin, leptin,	Age, gender, smoking status, ethnicity, social deprivation, anti hyper tensive medication, lipid-lowering medication, aspirin, family history of diabetes, PA , and SB time	Breaks in sedentary time were beneficially associated with lower IL-6 (β = – 0.09 ± 0.05, *p* = 0.04) and leptin (β = – 0.07 ± 0.04, *p* = 0.04), in people with high risk of type 2 diabetes mellitus T2DM, independent of total time spent in MVPA.
6	(Henson et al. 2013a) Associations of objectively measured sedentary behaviour and physical activity with markers of cardiometabolic health.	Cross-sectional	878 obese adults with high risk of diabetes, age (SD) 58 ± 13y BMI (SD) 32.5 ± 5.2 kg/m^2 UK	ActigraphGTX3(15-s epoch); *SB defined as < 25 counts / 15s; * BSB defined as transition from SB state to active state (≥ 25 counts /15s) ,activity (≥ 25 to < 488 counts per 15 s) * MVPA (≥488 counts per 15 s)	Breaks per day 4 - 7 consecutive days during waking hours	Waist circumference, BMI, impaired fasting glucose, triglycerides, HDL cholesterol, total	Age, gender, smoking status, ethnicity, social deprivation, family history of type 2 diabetes mellitus, PA and SB time	Significant beneficial association of breaks with waist circumference β=−0.21 ± 0.05, *p* < 0.001, BMI β=−0.15 ± 0.05, p = 0.003 and 2-h plasma glucose β=−0.11 ± 0.055, *p* = 0.05, independent of total time spent in MVPA and sitting time.

	Author(Study)	Design	Sample	Measurement of breaks	Breaks unit	outcomes	Confounders	Main results
7	(Gupta *et al.* 2016) What Is the Effect on Obesity Indicators from Replacing Prolonged Sedentary Time with Brief Sedentary Bouts, Standing and Different Types of Physical Activity during Working Days? A Cross-Sectional Accelerometer-Based Study among Blue Collar Workers	Cross-sectional	692 worker, age (SD) 45.1 ± 9.9 years BMI (SD) 27.5± 4.9 kg/m^2 Sweden	ActigraphGT3X+ *Sedentary time was defined as (≤ 5 min), *Moderate(> 5and ≤ 30 min) *Long (> 30 min)bouts	1– 4 working days	Obesity indicators, BMI(kg/m^2), waist circumference (cm) and fat percentage	Sex, age, smoking, alcohol intake, diet	Replacing sitting time with MVPA was significantly inversely associated with waist circumference [B (95% CI); -3.93 (-6.62 to - 1.23) cm, BMI -1.28 (- 2.2 to - 0.35) kg/m^2, and fat percentage [B (95% CI - 2.38 (-3.7 to - 1.06) %, ($P < 0.05$ for all). Breaking up sitting time with brief activity bouts was associated with lower waist circumference by ~3 – 5%; equivalent to a meaningful amount of ~2.6 – 2.7cm, ~1.4 – 1.6% fat percentage, and ~ 0.8 – 0.9 kg/m^2 BMI per 30min / day.

1.8 Interrupting sedentary time (intervention studies)

A number of studies have evaulated the effects of interventions to reduce or break up in **Table 1-5** and described below.

Dunstan et al (Dunstan *et al*. 2012b) showed that in overweight and obese adults, breaking up periods of sitting with 2-min bouts of light-intensity activity every 20 min, resulted in a 24% reduction in postprandial glucose incremental area under curve iAUC (5.2 mmol/L·h (4.1 - 6.6), $p < 0.01$) and a 23% reduction in insulin iAUC (633.6pmol/L·h (552.4 - 727.1pmol/L·h), $p < 0.01$), while sitting interrupted by moderate-intensity lowered postprandial glucose iAUC and insulin iAUC by 30 % (4.9mmol/L·h (3.8 - 6.1); $p < 0.01$) and 23% (637.6 pmol/L·h (555.5 - 731.9 pmol/L·h), $p < 0.01$) respectively.

Similarity, Peddie et al (Peddie et al. 2013) demonstrated that the regular-activity breaks intervention, walking for 1min 40s, twice per hour over 9h, reduced plasma glucose iAUC by 39%, 18.9 $mmol{\cdot}L^{-1}{\cdot}9hr^{-1}$ (95 % CI: 10.028.0 $mmol.L^{-1}{\cdot}9hr^{-1}$; $p < 0.001$) compared with the prolonged sitting and by 37%, 17.4 $mmol.L^{-1}.9hr^{-1}$ (8.4 - 26.3$mmol.L^{-1}.9hr^{-1}$; $p < 0.001$) compared with the physical activity intervention, which comprised walking for 30 min at the start of the day. Additionally, the regular-activity-break intervention significantly reduced plasma insulin iAUC by 26%, 866.7 $IU{\cdot}L^{-1}.9hr^{-1}$ (506.0 - 1227.5 $IU{\cdot}L^{-1}.9hr^{-1}$; $p < 0.001$) when compared with the long sedentary bouts intervention and by 18%, 542.0 $IU{\cdot}L^{-1}.9hr^{-1}$ (179.9 - 904.2 $IU{\cdot}L^{-1}.9hr^{-1}$; $p= 0.003$, when compared with the physical activity intervention. The effects of the physical activity and regular activity break interventions on plasma triglyceride iAUC were not significantly different from the effects of sitting. The current study highlighted that regularly interrupting sedentary time with short bouts of activity is beneficially associated with lower postprandial glucose and insulin concentrations compared to a single continuous bout of physical activity.

In a study by Nygaard and colleagues, 14 females were asked to complete 3 experimental trials examining the effects of undertaking different durations of walking after consuming a carbohydrate-rich meal containing, 1 g carbohydrate per kilogram body mass (cornflakes: 84 g carbohydrate, 7 g protein, 1 g fat per 100 g; skimmed milk: 4.7 g carbohydrate, 3.3 g protein, and 0.7 g fat er 100 g). Participants sat for two hours, or walked for 15, or 40 minutes, over a 2 hour observation period. The main influence of walking time (15, and 40 minutes) on the 2-hr blood glucose iAUC were 231 ± 31$mmol{\cdot}L^{-1}{\cdot}min$ for control, 205 ±

29mmol·L^{-1}·min for 15 minutes walking and 159 ± 13mmol·L^{-1}·min for 40 minutes walking. Walking for 40 minutes lowered 2-h glucose iAUC by 31.2% compared to sitting p= 0.014, whereas walking for 15 minutes lowered 2-h glucose iAUC by 11% compared to sitting. The difference between walking for 15 min and 40 min was 22.7%, although this observation was not significantly different (p > 0.05) (Nygaard *et al.* 2009).

Thorp et al undertook a study in which 23 office workers, aged 48.2 ± 7.9 and BMI 29.6 ± 4.1 kg/m^2 undertook two 5-d experimental conditions (Thorp *et al.* 2014b). Participants were asked to perform their usual work in a deskbound (seated) work posture over 8 h.d^{-1} in the, control condition. The intervention condition required participants to perform their job swapping between a seated and standing posture every 30-min for 8 h d^{-1} using of an electric, height-adjustable workstation. The result demonstrated that glucose concentration iAUC was reduced by 11.1% after the prolonged bouts of sitting interrupted every 30min by 30min of standing over 8 h observation period, (6.38 mmol/L·h^{-1} (confidence interval 5.04 - 7.71mmol/L·h^{-1})) compared to the prolonged sitting condition (7.18 mmol/L·h^{-1} (confidence interval, 5.85-8.52 mmol/L·h^{-1}). No significant difference was observed between standing vs. sitting conditions on the insulin iAUC (p = 0.41), or triglyceride concentration iAUC (p = 0.45). The study demonstrated that breaking prolonged sitting with short bouts of standing significantly lower postprandial glucose responses in obese office workers.

Using a similar protocol, Thorp and colleagues undertook another study to determine whether increasing standing at work by using an electric, height-adjustable workstation during the workday could improve fatigue levels and lower back discomfort in 23 overweight/obese, aged 28.2 ± 8 years. This study reported that replacing sedentary time with standing every 30 min across the workday led to reduced musculoskeletal discomfort by 32% (p = 0.03) compared to sitting in overweight/obese office workers (Thorp *et al.* 2014a).

Henson et al (2016) undertook a study in twenty two overweight, postmenopausal women at high risk of type 2 diabetes, who underwent trials where they sat continuously for 7.5 h or broke up sitting with 5 minutes of walking at 4 km/h or 5 minutes of standing every 30 minutes (Henson et al. 2016). The postprandial glucose iAUC was reduced by 34% with standing *(P = 0.022)*, and by 28% with walking *(P* = 0.009) and with insulin iAUC reduced by 20% (P = 0.045) and 37% (P = 0.008) by standing and walking, respectively. There was no difference between standing and walking conditions *(P* = 0.398). Moreover, the

observations for glucose (19% and 17% reductions for standing and walking, respectively) and insulin (24% reduction for walking only) persisted into the next day.

In contrast, Miyashita et al (Miyashita *et al.* 2013) examined the effects of the prolonged sitting, standing and walking trials on postprandial lipaemia and glucose in 15 healthy men. Each participant undertook three, 2-day laboratory-based trials. In the sitting trial, participant sat comfortably over 6h observation period. For the standing trial, participants were asked to stand for six, 45-min periods and for the walking trial, participants walked briskly for 30 min at approximately 60 % of maximum heart rate. On day 2, of each trial, participants rested in the laboratory for 6 h and consumed test meals for breakfast and lunch. The result on day 2 showed that serum TG responses were lower by 18% on the walking trial than the sitting (P = 0.031) and standing trials (P = 0.048). Also, the walking intervention significantly reduced the postprandial plasma glucose concentrations compared to the sitting (P = 0.008) but did not differ significantly between sitting and standing trials (P = 0.707) or between standing and walking trials (P = 0.146).

A further study undertaken by Miyashita et al (Miyashita *et al*. 2008) aimed to identify the difference between continuous session 30 minutes of moderate activity and 10 bouts of 3 minutes of moderate physical activity every 30 minutes on postprandial plasma TG concentrations and resting blood pressure in fifteen healthy men, aged 23.4 ± 0.8 years. The main result was that multiple short (3-min) bouts of moderate activity and one session of 30-min brisk walk reduced postprandial plasma TG AUC concentration by 16% compared to sitting (P = 0.005) and resting systolic blood pressure by 6 – 7% throughout day 2 on the both activities condition *(*P = 0.005).

Similarly, Duvivier et al (Duvivier *et al.* 2013) monitored the glucose, insulin and lipid responses in 18 healthy young physically inactive participants who performed three different conditions: a sitting regime (14 h/d of sitting + 1 h/d of walking + 1 h/d of standing); a minimal intensity PA regime (5 h/d of walking + 3 h/d of standing + 8 h/d of sitting); and an exercise regime (1-h MVPA and 13 h/d of sitting + 1h /d of walking + 1 h/d of standing). Participants completed each condition for 4 d and were assessed on the fifth day. The data showed that the minimal intensity PA regime improved the lipid profile and insulin sensitivity when compared with the prolonged sitting condition. There was a significant intervention effect on AUC for insulin during OGTT after the minimal intensity PA regime compared to both sitting and exercise regimes 6727 ± 4329 vs 7752 ± 3014 and 8320 ± 5383

mU.min/ml, respectively. The minimal intensity PA regime significantly reduced TG by 22%, compared to sitting and there was no significant observed in exercise regime, despite the comparable energy expenditure to the light-activity protocol.

Kim et al (Kim *et al.* 2014) observed that interrupting sitting time with either 1-h moderate-intensity exercise (65 % VO_{2max}) or intermittent light-intensity walking (25 % VO_{2max}) for 9 h produced lower triglyceridaemic and glycaemic responses to a high-fat meal on the next day in healthy active participants. Moderate intensity exercise and light intensity significantly lowered TG iAUC by 33.6 % ($P < 0.005$) and 19.8 % ($P < 0.05$), respectively compared to sitting. The authors also showed that moderate intensity exercise significantly reduced TG iAUC by 17.2% ($P < 0.03$) compared to light intensity, and also reduced plasma glucose response and improved fat oxidation compared to light intensity and sitting conditions (for all, $P < 0.05$). Notably, moderate intensity exercise and light intensity reduced postprandial TG responses compared with sitting. However, moderate intensity exercise was more efficacious in reducing postprandial TG compared with light intensity.

Altenburg et al (Altenburg *et al.* 2013) undertook a study of eleven healthy adults, who performed two interventions trials, on a different occasion, prolonged sitting for 8 h and 8 h of sitting interrupted with 8-min of moderate-intensity cycling (40 % – 60 % of the heart rate reserve) per hour. The authors detected that muscle activity during cycling was seven to eight times higher compared with prolonged sitting. Breaking sitting time led to significantly lower postprandial levels of C-peptide (unstandardized regression coefficient = - 0.19; confidence interval = [- 0.35; - 0.03]; P = 0.017) compared with prolonged sitting. Postprandial levels of glucose, triglycerides and cholesterol were not significantly different between conditions.

Van Dijk et al. (van Dijk *et al.* 2013) evaluated twenty adult males with type 2 diabetes who completed a prolonged sitting condition, both a 45-min moderate-intensity continuous exercise (~ 350 kcal expended) and 3× 15-min bouts of light-intensity activity (~175 kcal expended) throughout the day. The average blood glucose concentrations were significantly lower by 0.66 ± 0.1mmol/L ($P < 0.001$) during a single session of moderate-intensity exercise, compared with prolonged sitting. The 35 ± 5% reduction in the cumulative glucose iAUC during the moderate-intensity exercise condition was higher than the 17 ± 6% reduction detected in the light-intensity activity condition, though this intervention did not

differ significantly (P = 0.06). Also, a single session of moderate-intensity exercise significantly reduced the insulin iAUC compared with light-intensity activity condition ($P < 0.001$).

In contrast, Bailey and Locke (Bailey and Locke 2015) did not find any significant effects of breaking sedentary time with 2-min bouts of standing every 20 min on postprandial glucose in 10 normal to overweight participants compared with 5 h of prolonged sitting. Interestingly, compared to sitting condition, the postprandial glucose response was significantly lower with 2-min bouts of light walking every 20 min by 16.7%. The researchers did not observe any positive effects of breaks on lipidemia or blood pressure ($p > 0.05$). Thus, these data suggest that breaking sitting time with frequent brief bouts of light-intensity activity, but not standing, can lead to beneficial postprandial responses that may enhance cardiometabolic health. These outcomes could be importance in the design of practical interventions to minimize the risk of cardiometabolic disease.

John et al (John *et al.* 2011), examined the effects of introducing treadmill desk workstations over a 9 month period for 5 males and 7 females overweight adult office workers in an uncontrolled trial. The authors reported significant increases were seen in standing (146 – 203 min·day^{-1}) and stepping time (52 – 90 min·day^{-1}) and total steps/day (4351 – 7080 steps/day; $P < 0.05$) with reductions in sitting. This resulted in significant reductions in waist (by 5.5 cm) and hip (by 4.8 cm) circumferences, LDL by cholesterol (by 16 mg·dL^{-1}) and total cholesterol (by 15 mg·dL^{-1}), ($P < 0.05$) during the study. Notably, these positive changes were noticed despite no changes in dietary intake. A 3 separate 24-hour dietary recall interviews were recorded at each of the 3 time points: baseline, 3 months, and 9 months, in total of 9 dietary recalls per individual). Participants were asked to recall their dietary intake on randomly selected days of the week.

Alkhajah et al (Alkhajah *et al.* 2012) studied the effects of introducing sit-stand workstations in adult non-obese healthy adult office workers. After 3 months, the intervention group reduced sitting time by more than 2h d^{-1}, which was almost exclusively replaced by standing with minimal changes to stepping time, compared with the control group. The intervention group increased HDL cholesterol by an average of 0.26 mmol/L (95 % CI = 0.10, 0.42). However, no significant differences were observed with other biomarker. It is important to

consider that food intake was not controlled in this study, which may have affected the results.

Buckley et al (Buckley *et al.* 2014) studied the effects of breaking sitting time on adult desk-based office workers, compared to 4 h of seated desk work. The postprandial glucose was reduced by 43% (p = 0.022) with sit-stand desk workstation groups during 4 h. Moreover, energy expenditure AUC for 210min, during an afternoon work was 174 ± 66 kcals (0.83 kcals/min; p = 0.028) greater in standing (487 ± 174 kcals) compared to sitting (313 ± 139 kcals). While, the researchers did not clearly quantify the time of spent sitting and standing in both conditions, these finding recommended that standing could be sufficient to counteract the risk of prolonged bouts of sitting in office workers.

In a further report, Latouche et al (Latouche *et al.* 2013) observed a positive effects of light- and moderate-intensity breaks on postprandial glucose iAUC when compared to sitting, the glucose response was effectively reduced by 24.8% (P = 0.004) and 23.4% (P = 0.015) with light and moderate-intensity breaks, respectively.

Holmstrup et al (Holmstrup *et al.* 2014) showed that breaking up 12 h of prolonged sitting with either 1-h moderate-intensity exercise to vigorous exercise (EX; 60 – 65% VO_{2peak} peak or interrupted hourly by 5min of moderate to vigorous exercise (INT; 60 – 65% VO_{2peak}) induced a significant differences in the 12-h glucose iAUC (P = 0.021) with glucose concentrations highest in the EX group in overweight subjects. The 12-h insulin iAUC was higher ($P < 0.05$) compared to the interrupted of moderate to vigorous exercise and moderate-intensity exercise conditions. However, no significant differences were observed in the 12-h insulin iAUC response between the EX and INT conditions (P = 0.13). The 2-h c-peptide iAUC in a single session exercise and interrupted hourly by 5min of exercise were significantly reduced relative to the sedentary control ($P < 0.05$).

Larsen et al. (Larsen *et al.* 2014) also evaluated the impact of breaking sitting time on blood pressure. The authors focused on 11male and, 12 females and showed that bouts of 2 min of light-intensity walking at 3.2 km/h every 20 min or bouts of 2 min of moderate-intinsity walking at 5.8 and 6.4 km/h every 20 min during 5 h of sitting time. Both conditions significantly reduced systolic blood pressure (light: 120 ± 1mmHg, p = 0.002; moderate: 121 ± 1 mmHg, p = 0.02), compared to sitting condition (123 ± 1mmHg). Also, diastolic blood

pressure was lowered during both of the activity conditions (light: 76 ± 1 mmHg, $p = 0.006$; moderate: 77 ± 1 mmHg, $p = 0.03$) compared to sitting condition (79 ± 1 mmHg).

Swartz et al (Swartz *et al.* 2011) suggested that people who have a desk job, could make a small changes, such as breaking sitting time with five minute of walking every hour, may be helpful to control or loss weight and prevent obesity in developed countries.

Thus there have been a substantial number of studies investigating the metabolic consequences of breaking up periods of prolonged sitting. Taking these data together, there is clear evidence that regularly interrupting sedentary time with multiple short (2-3 min) bouts of light or moderate activity throughout the day could decrease postprandial glucose, insulin and triglyceride responses, and blood pressure, on the same or following day. However, data on whether these benefits could be stimulated by simply breaking up time spent sitting down by standing up, and more limited and equivocal. This knowledge is essential as there are a number of 'standing desk' interventions being undertaken to decrease time spent sitting, but there are only very limited data available to show whether this type of intervention can induce a measurable metabolic benefit (Buckley et al. 2014; Reiff et al. 2012) and whether the pattern of standing and sitting could influence these effects. Furthermore, in all of these studies, the intervention to break up sedentary time also reduced sedentary time by increasing the amount of time spent standing or walking. Thus, it is not clear from the existing literature whether breaking up prolonged sedentary time with small bouts of activity provides any additional benefits over and above simply undertaking the same amount of activity in a single bout either before or after a prolonged sedentary period. There needs to be further research into the consequences of breaking up sedentary time with repeated short bouts of light activity or standing, compared to undertaking the same amount of standing or light activity in a single continuous bout on day-long metabolic responses. This will help understanding of the role of the frequency of breaks in sedentary time per se on metabolic responses.

Table 1-5: Breaking sedentary behaviour (Intervention Studies)

	Author & Study	Design	Sample (n)	Measurements	Results	Study design
1	(Dunstan *et al*. 2012b) Breaking up prolonged sitting reduces postprandial glucose and insulin responses.	1) Uninterrupted sitting for 7 hours (420min) 2) Sitting interrupted every 20min by two min of light-intensity walking (3.2km/h) (14breaks) for 5h 3) Sitting interrupted every 20min by 2min of moderate-intensity walking (5.8-6.4km/h) (14breaks) for 5h.	19 obese adults /11 males Mean (SD) age 53.8 ± 4.9years Mean (SD) BMI 31.2 ± 4.1 kg/m^2	Postprandial response Serum/plasma glucose and insulin	The glucose iAUC which represents the area under the plasma concentration curve without resting period, the plasma (mmol/L).h after both activity-break conditions was reduced (light: 5.2 [4.1 – 6.6]; moderate: 4.9 [3.8 – 6.1]; both $P < 0.01$) compared with uninterrupted sitting (6.9 [5.5–8.7]). Insulin iAUC (pmol/L.h) was also reduced with both activity-break conditions (light: 633.6 [552.4 – 727.1]; moderate: 637.6 [555.5 – 731.9], $P<0.0001$) compared with uninterrupted sitting (828.6 [722.0 – 950.9]), after adjustment for age, sex, weight, period effects.	Randomized crossover trial 6-days washout period
2	(Peddie et al. 2013) Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a	Three conditions: 1) Uninterrupted sitting for 9h. 2) 30min, walked on the treadmill, and then sat continuously for 8 h and 15 min. (physical activity intervention) 3) Sitting interrupted by 18 breaks of 1min 40s bouts of brisk treadmill walking (total of 30 min) over the 9-h period. (regular-activity-break)	70 adults/ 42 males Mean (SD) age 25.9 ± 5.3 years Mean BMI (SD) 23.6 ± 4.0 kg/m^2	Postprandial response Serum/plasma glucose, insulin, and triglycerides.	The regular-activity-break intervention lowered plasma glucose iAUC by 18.9 $mmol.L^{-1}.9h^{-1}$ (95% CI: 10.0, 28.0 $mmol.L^{-1}.9h^{-1}$; $P < 0.001$) compared with the prolonged sitting intervention and by 17.4 $mmol.L^{-1}.9h^{-1}$ (95% CI: 8.4, 26.3 $mmol.L^{-1}$. $9h^{-1}$; $P < 0.001$) compared with the physical activity intervention, walking for 30 min. The effects of the prolonged sitting and physical activity interventions on plasma glucose and insulin iAUC did not differ significantly; ($P = 0.730$), ($P = 0.079$), respectively. The regular-activity-break intervention lowered plasma insulin iAUC by 866.7$IU.L^{-1}$ $.9h^{-1}$ (95% CI: 506.0, 1227.5$IU.L^{-1}.9h^{-1}$; $P<0.001$) when compared with the prolonged sitting intervention and by 542.0 $IU.L^{-1}.9h^{-1}$ (95% CI: 179.9, 904.2 $IU.L^{-1}$. $9h^{-1}$; $P = 0.003$) when compared with the physical activity intervention, adjusted for age, sex, and BMI The mean difference between the physical activity intervention and the prolonged sitting intervention on plasma triglyceride iAUC was 3.8$mmol{\cdot}L^{-1}$ $9hr^{-1}$ ($p = 0.098$) and between the regular activity break and prolonged sitting interventions was 2.4$mmol{\cdot}L^{-1}$ $9hr^{-1}$ ($p = 0.284$).	Randomized crossover trial 6 to 13 d washout period

3	(van Dijk et al. 2013) Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes.	Three conditions: 1) sitting for 11h, 2) 15min of ADL (activities of daily living) (slow-paced strolling,~3 MET) 3) 45min bout of moderate intensity cycling (~6MET)	20 adult males with type 2 diabetes Mean (SD) age 64 ± 1years Mean (SD) BMI 29.5 ± 0.9kg/m^2	Postprandial plasma insulin and glucose	45 min of moderate-intensity exercise significantly reduced blood glucose concentrations by 0.66 ± 0.1mmol/L ($P < 0.001$) compared to the sitting. The 35 ± 5% reduction in the cumulative glucose iAUC during the moderate-intensity exercise condition was greater than the 17 ± 6% reduction observed in the ADL condition, although this observation did not reach statistical significance (P=0.06). The resulting plasma insulin response was 17 ± 5% lower during the ADL condition (214 ± 24 nmol/L/11 h; $P < 0.05$) and 33 ± 4% lower during the moderate condition (170 ± 18 nmol/L/11 h; $P < 0.001$) compared to the sitting (250 ± 23 nmol/L/11 h). The insulin iAUC during the moderate condition also was lower compared with the ADL condition ($P < 0.001$).	Randomized crossover trial 7-d washout period
4	(Holmstrup et al. 2014) Multiple short bouts of exercise over 12-h period reduce glucose excursions more than an energy-matched single bout of exercise	1) (SED) sitting for 12h. 2) (EX) 1h, continuous moderate to vigorous exercise (EX; 60–65% VO_2 peak followed by sitting for11h. 3) (INT) Sitting, interrupted hourly by 5min of moderate to vigorous exercise (12 breaks) at 60 –65% VO_2peak.	11 obese adults / 8 males with impaired glucose tolerance Age (range) 18–35 years, BMI > 30kg/m^2	Postprandial plasma glucose, insulin	There were significant differences in the 12-h glucose iAUC SED vs. EX (5536.9 ± 255.3 vs. 6249.6 ± 286.3 mmol/L*min for 12-h; $P = 0.042$); INT vs. EX (5457.0 ± 238.8 vs. 6249.6 ± 286.3 mmol/L*min for 12-h; $P = 0.048$) No significant differences were observed in the 12-h insulin iAUC response between the EX and INT conditions ($P = 0.13$).	Randomized crossover trial 7-d washout period
5	(Bailey and Locke 2015) Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not	Three conditions: 1) Sitting for 5h (300min). 2) Sitting interrupted every 20min by 2min of standing (14breaks). 3) Sitting interrupted every 20 min by 2 min of light intensity treadmill walking (3.2km/h) (14breaks)	10 obese adults / 7 males Mean (SD) age 24 ± 3 years Mean BMI (SD): 26.5 ± 4.3kg/m^2	Postprandial triglycerides, total cholesterol, glucose, and blood pressure	Glucose area under the curve was lower in the walking-break condition compared to the uninterrupted sitting and standing-break conditions: mean area under the curve 18.5 (95% CI 17, 20mmol L/5-h), 22.0 (20.5, 23.5 mmol L/5-h), and 22.2 (20.7, 23.7 mmol L/5-h), respectively, $p < 0.001$.	Randomized crossover trial 7-d washout period

6	(Thorp *et al.* 2014b) Alternating bouts of sitting and standing attenuates postprandial glucose responses	Two conditions lasting 5 days each 1) Uninterrupted sitting for 8h (480 min) 2) Sitting interrupted every 30 min by 30 min of standing (8 breaks) Each experimental condition was performed for five consecutive workdays (Monday to Friday).	23 Obese adults, /17 males Mean (SD) age 48.2 ± 8 years Mean (SD) BMI 29.6 ± 4 kg/m^2	Postprandial triglycerides, glucose, and insulin	Compared to sitting, breaks lowered plasma glucose iAUC by 11.1% (6.38mmol/L·h^{-1} confidence interval, 5.04 – 7.71) relative to the control condition (7.18mmol/L·h^{-1} confidence interval, 5.85 – 8.52)(P = 0.007), No significant effect on insulin or triglycerides.	Randomized crossover trial 7-d washout period
7	(Altenburg et al. 2013) The effect of interrupting prolonged sitting time with short, hourly, moderate-intensity cycling bouts on cardiometabolic risk factors in healthy, young adults.	1) 8 h prolonged sitting (420min) (SIT) 2) 8 h of sitting, interrupted hourly with, (8min of moderate-intensity cycling at 40%–60% of HRR) (SIT-CYCLE)	11 adult / 6 females Age (range): 18 – 24year BMI (range): 20 – 26 kg/m^2	Postprandial: glucose, TG, LDL chol, T-chol and C - peptide	Muscle activity during cycling was seven to eight times higher compared with sitting. Postprandial levels of other cardiometabolic biomarkers (e.g., glucose, triglycerides, cholesterol, HDL, LDL cholesterol l) were not significantly different between conditions.	Randomized crossover trial 7-d washout period
8	(Buckley *et al.* 2014) Standing-based office work shows encouraging signs of attenuating post-prandial glycaemic excursion	1 – Uninterrupted sitting (240min) 2- Standing (240min)	10 adults /8females Age (range):males 22 - 61years females, 22 – 59 years BMI<30 kg/m^2	Postprandial glucose, energy expenditure	Glucose AUC was attenuated blood by 43% (p = 0.022) following 185 min of standing (143, 95% CI 5.09 to 281.46 mmol/L min) compared to sitting (326; 95% CI 228 to 425 mmol/L min).	Open non randomized crossover trial

9	(Duvivier et al. 2013) Minimal Intensity Physical Activity (Standing and Walking) of Longer Duration Improves Insulin Action and Plasma Lipids More than Shorter Periods of Moderate to Vigorous Exercise (Cycling) in Sedentary Subjects When Energy Expenditure Is Comparable	Participants were instructed to perform three activity regimes of four days each. 1) Sitting regime, 14h.d^{-1} +walking1h.d^{-1}+ standing 1 h.d^{-1} and 8 hr/day sleeping. 2) EX regime: Sitting13h.d^{-1}+walking 1 h.d^{-1}+ standing 1 h.d^{-1}+ MVPA 1h.d^{-1} 3) minimal intensity PA regime: sitting 8h.d^{-1} + walking 5h.d^{-1}+ standing 3 h.d^{-1}	18 healthy adults / 11males Mean (SD) age 21 ± 2years Mean (SD) BMI 22.6 ± 3.6kg/m^2	Postprandial response on the next day: glucose, insulin, TG, HDL-chol, and LDL	Area under the curve for insulin during OGTT was significantly lower after the minimal intensity PA regime compared to both sitting and exercise regimes 6727.3 ± 4329.4 vs 7752.0 ± 3014.4 and 8320.4 ± 5383.7 mU.min/ml, respectively. Triglyceride level improved significantly in the minimal intensity PA regime compared to sitting and showed non-significant trends for improvement compared to exercise.	Randomized crossover trial 10-d washout period
10	(Nygaard *et al.* 2009) Slow postmeal walking reduces postprandial glycemia in middle-aged women	After CHO rich meal the subject completed 3 experimental trials 1) Sitting, for 2 hours 2) Slow walking (15mins) (W15) 3) Slow walking (40 mins) (W40)	14 females, age >50 years Mean (SD) BMI 24 ± 3 kg/m^2	Glucose	The main influence of walking time (0,15, and 40 minutes) on the 2-hr blood glucose iAUC were 231 ± 31mmol·L^{-1} ·min for control, 205 ± 29mmol·L^{-1}·min for 15 minutes walking and 159 ± 13mmol·L^{-1}·min for 40 minutes walking.	Randomised crossover trial 4-30 days washout period
11	(Miyashita *et al.* 2008) Accumulating short bouts of brisk walking reduces postprandial	Subjects completed three 2d trials: On day1 1) sitting for 7h, 2) 10 bouts of 3 minutes of moderate physical activity every 30 minutes 3) One 30-min bout of of moderate physical activity	15 healthy adult males Mean (SD) age 23.4 ± 0.8 years	postprandial triacylglycerol and blood pressure	On day 2, TG AUC was 16% lower on the accumulateing of 30 min of walking 9.98 ± 0.67, continuous 30min walking 9.99 ± 0.76 than control trial 11.90 ± 1.02 mmol. 7h/L, P = 0.005. Resting systolic blood pressure was 6–7% lower throughout day 2 on the accumulated walking 109±1 and continuous walking 110 ± 1 compared with control trial 117 ± 2 mm Hg, P < 0.0005).	Randomized crossover trial 6-d washout period

	plasma triacylglycerol concentrations and resting blood pressure in healthy young men	On day 2, subjects rested and consumed high-fat test meals for breakfast and lunch	Mean (SD) BMI 23.4 ± 0.6 kg/m^2			
12	(Latouche et al. 2013) Effects of breaking up prolonged sitting on skeletal muscle gene expression.	three 5-h interventions were completed in the postprandial state after a standardized test drink 1- Un interrupted sitting (420min)(SIT) 2- Sitting (402min) +2-min LIPA every20min for 5h (3.2km.h^{-1}), 14 breaks, 3- Sitting (402min) +2-min MVPA every20 min for 5h (5.8–6.4km.h^{-1}	8 obese adults/1female Mean (SD) age 55.6 ± 6years Mean (SD) BMI 30.9 ± 2.9kg/m^2	postprandial glucose and insulin	The glucose iAUC was reduced by 24.8% (P = 0.004) after sitting interrupted with LIPA and 23.4% (P = 0.015) after sitting interrupted with MVPA compare to sitting. The insulin-to-glucose ratio incremental area under the curve was 25.1% (P = 0.001) lower after LIPA and 21.9% (P = 0.014) lower after sitting interrupted with MVPA compared with sitting, adjusted for age, sex, body weight.	Randomized crossover trial 6-d washout period
13	(Larsen *et al.* 2014) Breaking up prolonged sitting reduces resting blood pressure in overweight/obese adults.	1) sitting, 5 h 2) Seated with 2-min bouts of LIPA (walking at 3.2 km/h) every 20 min 3) Seated with 2-min bouts of MVPA (walking 5.8 and 6.4 km/h) every 20 min	19 obese/ overweight adults /8 females Mean (SEM) age 53.8 ±1.1years Mean (SEM) BMI 31.2 ± 0.9 kg/m^2	Systolic and diastolic blood pressure	Breaking up prolonged sitting with LIPA and MVPA was lower systolic blood pressure (light: 120 ± 1mmHg, p = 0.002; moderate: 121 ± 1 mmHg, p = 0.02), compared to sitting (123 ± 1 mmHg). Diastolic blood pressure was also significantly lower during both of the activity conditions (light: 76 ± 1 mmHg, p = 0.006; moderate: 77±1mmHg, p=0.03) compared to sitting (79 ± 1 mmHg). Adjusted for sex, age, BMI, fasting blood pressure.	Randomized crossover trial 7-d washout period
14	(Miyashita *et al.* 2013) postprandial lipaemia: effects of sitting,	2-day trials in a random order: Day 1 : 1) sitting 6h, 2) Standing, for six, 45-min periods.	15 healthy males Mean (SD)age 26.8 ± 2.0 years	Postprandial TG concentrations postprandial lipaemia	On day 2 of the intervention, after the consumption of the test meals Walking trial was significantly reduced the total AUC for TG by (8.0 ± 1.6 mmol·6 h/L) than the sitting (9.8 ± 3.7 mmol·6 h/L, P = 0.028), and walking compared to standing (9.7 ± 2.6 mmol·6 h/L, P = 0.043).	Randomized crossover trial 7-d washout period

	standing and walking in healthy normolipidaemic humans	3) Walking briskly for 30 min at 60 % of maximum heart rate. Participants consumed a packed lunch midway through the day were instructed to consume an early evening meal and to rest for the remainder of the evening. On day 2 Of each trial, participants rested and consumed test meals for breakfast and lunch.	Mean (SD) BMI 22.5 ± 1.5 kg/m ^{2}	postprandial plasma glucose, insulin	However, insulin iAUC was not significantly different between conditions, standing (927 ± 347pmol·6 h/L), walking (834 ± 260pmol·6 h/L, or sitting (916 ± 319 pmol·6 h/L)	
15	(Swartz *et al.* 2011) Energy expenditure of interruptions to sedentary behavior	1) Sitting for 30 consecutive minutes. 2) 14 minutes of sitting one minute of walking and 15 minutes of sitting, for a total of 30 minutes 3) 13 minutes of sitting two minutes of walking and 15 minutes of sitting, for a total of 30 minutes. 4) 13 minutes of sitting five minutes of walking and 12 minutes of sitting, for a total of 30 minutes.	20 males and females Mean (SD) age 28.1 ± 5.7 years Mean (SD) BMI 27.8 ± 6.6 kg/m^2	Body composition and resting metabolic rate	Significantly more energy was expended during walking break than sitting (p < 0.05 for all comparisons). On average, participants expended an additional 3.0, 7.4, and 16.5 additional activity kilocalories during activites 2, 3, and 4, respectively compared to sitting.	Randomized crossover trial 14-d washout period
16	(Thorp *et al.* 2014a) Breaking up workplace sitting time with intermittent standing bouts improves fatigue and musculoskeletal discomfort in overweight/obese office workers	Each trial was performed for five consecutive workdays (Monday to Friday) 1) Uninterrupted sitting for 8h. 2) Sitting interrupted every 30 min by 30 min of standing.	23 overweight/ obese adults /17 males Mean (SD) age 48.2 ± 7.9 years Mean (SD) BMI 29.4 ± 1.4 kg/m^2	fatigue, musculoskeletal discomfort	The total fatigue score was significantly higher during the sitting condition (mean 67.8 (95% CI 58.8 to 76.7)) compared with the sit-stand condition (52.7 (43.8 to 61.5); p < 0.001). Lower back musculoskeletal discomfort was significantly lower during the sit-stand condition compared with the sitting condition (31.8% reduction; p = 0.03).	

17	(Kim et al. 2014) Effects of moderate- and intermittent low-intensity exercise on postprandial lipemia	1- Sitting (420min) (CON), 2-Sitting (360min)+60min of running MVPA (65%$V\dot{O}_{2max}$)at the end of sitting (MOD) 3-Sitting (260) min+9breaks intermittent walking exercise at (self-selected walking speed 25% $V\dot{O}_{2max}$ (LOW) but energy matched to the MVPA condition8h	9 healthy males Mean (SD) age: 24.0 ± 4.0 years BMI < 30 kg/m^2	Postprandial response the next day: TG, glucose	MOD and LOW reduced incremental triglyceride (TG) area under the curve (TG AUC*I*) compared with that in CON by 33.6% ($P < 0.005$) and 19.8% ($P < 0.05$), respectively. MOD also reduced TG AUC*I* compared with that in LOW by 17.2% ($P < 0.03$). The reduced TG AUC*I* in MOD was accompanied by reduced plasma glucose response and enhanced fat oxidation compared with those in LOW and CON (for all, $P < 0.05$), respectively. Both MOD and LOW were effective in reducing PPTG compared with CON. However, MOD was more effective in reducing PPTG compared with LOW.	Randomized crossover trial 7-d washout period
18	(Peddie et al. 2013) Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial	1) Sitting (810min) 9 hours (SIT) 2) Sitting (780min)+1bout of Walking for 30-min MVPA (60.5%VO_{2peak}) and then sitting (Physical activity) 3) Sitting (272min) +18 breaks (1min40s total 30min) every 30 minutes (45.6% of VO_{2peak}) (Regular activity breaks)**.**	42 men / 28 females Mean (SD) age: 25.9 ± 5.3 years Mean (SD) BMI 23.6 ± 4.0 kg/m^2	Postprandial response during trial: glucose, insulin, TG,T-chol, HDL-chol, LDL, Glucose, insulin, triglycerides	The plasma iAUC for insulin differed between interventions (overall $p < 0.001$). Regular activity breaks lowered values by 866.7IU·L^{-1}·9h^{-1} ($p < 0.001$) when compared with sitting and by 542.0 IU·L^{-1}·9h^{-1} ($p = 0.003$) when compared with physical activity. Plasma glucose iAUC also differed between interventions (overall $p < 0.001$). Regular activity breaks lowered values by 18.9mmol·L^{-1} ($p < 0.001$) when compared with prolonged sitting and by 17.4 mmol·L^{-1} ($p < 0.001$) when compared with physical activity. Plasma triglyceride iAUC differed between interventions (overall $p = 0.023$). Regular activity breaks were more effective than continuous physical activity at decreasing postprandial glucose and insulin	Randomized crossover trial 6 to 13 - d washout period
19	Alkhajah etal, 2012 Sit-stand workstations: a pilot intervention to reduce office sitting time.	1) Intervention group,used sit-stand work stations. 2) Comparison group,maintain normal work routine	Intervention,n= 18 Mean (SD) age 33.5 ± 8.7 years, Mean (SD) BMI 22.6 ± 2.6 kg/m^2 Comparison,n= 14 Mean (SD) age 39.9 ± 7.2 years Mean (SD) BMI 22.1 ± 2.6 kg/m^2	PA, Fasting levels of HDL- chol, T-chol,TG, glucose	The intervention group compare to the comparison group reduced sitting time at 1-week follow-up by 143 minutes/day at the workplace (95% CI= -184, -102) and 97 minutes/day during all waking time (95% CI= -144, -50). The intervention group increased HDL cholesterol by an average of 0.26 mmol/L (95% CI=0.10, 0.42) compare to comparison group. Other biomarker differences were not significant.	Randomized controlled trial. Measurement at baseline and 3 months

20	(John et al. 2011) Treadmill workstations: a worksite physical activity intervention in overweight and obese office workers.	Treadmill desk workstations (TMWS) were used to replace sitting time with standing or walking The total duration of the study was 9 months	12 obese adults / 7 females Mean (SD) age males 47.2 ±11.8 years females 45.6 ± 7.8 years Mean (SD) BMI males 33.7 ± 5.8 kg/m^2 females 34.0 ± 4.9 kg/m^2	physical activity	A significant increases were obseved in the standing time (146–203 $min \cdot day^{-1}$) and stepping time (52 – 90 $min \cdot day^{-1}$) and total steps/day (4351–7080 steps/day; $P < 0.05$). Correspondingly, the time spent sitting/lying decreased (1238–1150 $min \cdot day^{-1}$; $P < 0$ .05). Using the TMWS significantly reduced waist (by 5.5 cm) and hip circumference (by 4.8 cm), low-density lipoproteins (LDL) (by 16 $mg \cdot dL^{-1}$), and total cholesterol (by 15 $mg \cdot dL^{-1)}$ during the study ($P < 0.05$).	Prospective uncontrolled trial 9-month follow-up
21	Henson et al. 2016 Breaking Up Prolonged Sitting With Standing or Walking Attenuates the Postprandial Metabolic Response in Postmenopausal Women: A Randomized Acute Study	Day 1 1) Sitting 7.5 h 2) Standing for 5min every 30 min 3) Walking at light intensity 4km/h for min every 30 min Day 2 1) Sitting for 7.5 h	22 overweight/ Obese, postmenopausal females Mean (SD) age 66.6 ± 4.7 years Mean (SD) BMI 32.9 ± 4.7 kg/m^2	Glucose, insulin and TG	Breaking sedentary time with standing or walking reduced postprandial glucose iAUC by 34% (3.5 ± 0.8 mmol/L.h and 28% (3.8 ± 0.7 mmol/L. h compared to sitting Standing and walking activities reduced insulin iAUC by 20% (437.2 ± 73.5 mU/L.h) and 37% (347.9 ± 78.7 mU/L.h, respectively, compared to sitting. Standing (6.2 ± 0.8 mmol/L.h) and walking (6.1 ± 0.8 mmol/L.h) significantly reduced the TG iAUC compared with the sitting (5.6 ± 0.7 mmol/L. h). On day 2, the glucose iAUC (standing and walking) and insulin iAUC (walking only) persisted into the next day by (3.9 ± 0.8 mmol/L. h and 4.0±0.7mmol/L.h) and (354.3±57.3mU/L.h). There was no significant difference in triglyceride between three conditions.	Randomized crossover trial 7-d washout period

1.9 Measurement of Physical Activity and Sedentary Time

Today, there is much evidence indicating sedentary behaviour as an independent risk factor for a number of diseases (Edwardson *et al.* 2012;Katzmarzyk *et al.* 2009;Wilmot *et al.* 2012). Accurate quantification of PA and SB is needed to evaluate current and changing physical activity and sedentary behaviour levels. Validity, reliability, easy collection, and cost are the main concerns when choosing a measurement method (Prince *et al.* 2008). Self-report, heart rate monitor, pedometer and accelerometers are the major methods that have been used to quantity PA and SB.

Subjective methods included self-report questionnaires, diaries, interviewer-administered questionnaires, proxy-report questionnaires (Sirard and Pate 2001;Vanhees *et al.* 2005). Self-report is the major commonly method of assessing PA and SB in epidemiological research e.g. the international physical activity questionnaire (IPAQ) (Bauman *et al.* 2011;Craig *et al.* 2003;Prince *et al.* 2008). Subjective methods are reasonably priced, feasible for use and analyze data, appropriate for across large samples. However, these methods can be limited due to reduced levels of validity, particularly recall and report biases and under-estimates or overestimation of levels of activity from participants, also, it is not valid to assess the energy expenditure level. Thus, there is a strong need to accurately and objectively assess physical activity and sedentary behaviour (Atkin *et al.* 2012;Vanhees *et al.* 2005). In a study comparing actual PA utilizing accelerometers, pedometers, etc vs self-reported PA, it was found that both men and women overestimated their PA considerably by 44% and 138%, respectively (Mozaffarian *et al.* 2016). Moreover, a study evaluating the validity of self-report found that watching TV was significantly lower when measured by self-report compared with an objective measurement (Atkin *et al.* 2012). Similarity, the Zutphen questionnaire (modified to include housework questions) was poor for measuring PA compared to Actigraph and pedometer. There was a strong convergent validity between accelerometers and pedometers for counting steps ($R = 0.86$, $P < 0.001$) but the relation was weaker between both accelerometer ($R = 0.34$, $P < 0.001$), pedometer step count ($R = 0.36$, $P < 0.001$) and self-report Zutphen Physical Activity Questionnaire (Harris *et al.* 2009). A further study was undertaken by Dyrstad which compared between the self-administered IPAQ and (ActiGraph GT1M) for measuring total sedentary behavior and physical activity. The results shown that the subjects informed via IPAQ questionnaires additional vigorous PA and less sitting time compared with the accelerometer. The correlation between self-

reported and accelerometer-measured PA decreased with higher activity MVPA and intensity levels (Dyrstad *et al.* 2014).

A number of objective methods for measuring physical activity and sedentary behavior have been used to address some of limitations associated with subjective methods. Objective methods such as pedometers, heart rate monitors, and accelerometer have been utilized positively in adults and children.

Pedometers are the simplest method and inexpensive electronic devices, used to estimate the number of steps taken during ambulatory activity and can therefore be used on large numbers of population. However, they are limited in that they only count the number of steps and do not distinguish between different patterns or intensity of activity such as if someone sprinted 100 steps and another one walked 100 steps, the pedometer would classify register approximately 100 steps for each person (Berlin *et al.* 2006;Sirard and Pate 2001;Vanhees *et al.* 2005). Furthermore, pedometers may underestimate steps taken at slower speeds (i.e., < 0.9 m/s), and do not accurately measure sitting time, or upper-extremity activity, e.g.; pushing, lifting, or carrying objects. The way of measuring steps by utilizing a horizontal acceleration suspended lever arm that moves up and down in response to vertical accelerations of the hip. Another limitation of the pedometers are that they do not have internal clocks, so they are unable to give data on the pattern or period of specific activities. Pedometers have been validated vs accelerometer measure of PA (Berlin *et al.* 2006).

Heart rate monitors can be used to estimate EE according to the relationship between heart rate and oxygen consumption. They can be used to measure the frequency, intensity and duration of physical activity. The association between heart rate and oxygen consumption is linear with moderate or vigorous activity, however, at low levels of activity, the relationship is not linear. This can lead to error because most people spend a large period of their time in sedentary and light activity, and heart rate can be confounded by emotional stress, caffeine, smoking, type of activity undertaken. On the other hand, heart rate moitors are relatively inexpensive (Sirard and Pate 2001;Vanhees *et al.* 2005). Some work has been done to validate the use of heart for EE. In one report, HR was an accurate method for predicting r =0.87 after adjusting for age and fitness (Strath *et al.* 2000). However, it seems clear that further research is required, taking a combined HR and movement sensing measure as staring point to measure sedentary behaviour (Atkin *et al.* 2012).

Sufficient and accurate methods of assessing sedentary time and physical activity which minimise the chance of misclassification are essential to further our understanding the links between sedentary behaviour and disease (Lagerros and Lagiou 2007). Celis-Morales and colleagues compared the impact of objective vs subjective measurements of sitting time and physical activity on the dose-response association with metabolic risk factors (Celis-Morales et al. 2012). The IPAQ significantly over reported physical activity by 55 minutes per day (2.6-fold). Also, for some metabolic risk factors such as triglyceride concentrations, significant trend were exposed between amount of MVPA and the risk factor when activity was measured by accelerometer $p = 0.022$ but not with the IPAQ $p = 0.139$. This study found that a poor method for measuring sedentary behavior or activity leads to misclassification the strength of some associations between activity and risk factors. Accurate assessment of physical activity and sedentary time is required to prevent the risk of health (Celis-Morales *et al.* 2012).

Assessing physical activity and sedentary behavior become more attainable in recent time because of small devices such as accelerometers and inclinometers. Accelerometers are now being widely used in laboratory and non-laboratory conditions (e.g., at home, work and leisure time activities) (Healy *et al.* 2008b;Patel *et al.* 2010). These devices are easy to use and obtain adequate data (Healy *et al.* 2008b;Matthews *et al.* 2008). Accelerometers are categorized as uniaxial, biaxial, and triaxial depending on the number of planes in which movement is observed. Uniaxial devices register vertical acceleration in 1plane, and biaxial devices register acceleration in 2 planes. Triaxial devices register acceleration in 3 planes by 3 different accelerometers positioned internally at 90 degrees from one another, X-axis (vertical), Y axis (mediolateral) and Z axis (anterioposterior) (Berlin *et al.* 2006). Astatic acceleration due to gravity is recorded in the vertical axis; when walking or moving, a dynamic accelerations are superimposed on this and measured in all three planes. The vector magnitude acceleration can be used to summarise overall acceleration values (Stanton et al. 2014).

Accelerometer can measure the frequency, intensity of movement total time spent sedentary and physical activity. It can be used to estimate short incidental breaks in sitting time which might not be practically recorded by self-report measures (Atkin *et al.* 2012). Accelerometer has an internal clock so physical activity can be time stamped which allows to record daily patterns of physical activity and storing for later recall.

While objective measures, such as accelerometers, can quantify activity and sedentary time, the quality of measuring sedentary time using accelerometers might depend on the wear location. One of the most used accelerometers for measuring sedentary behaviour and physical activity is the ActivPAL Professional physical activity monitor (PAL technologies Ltd, Glasgow, UK), (5.4cm x 3.5cm x 0.6cm), which is usually attached to the thigh and integrates a tri-axial sensor to measure acceleration in three different axes (x, y, and z), 0.05 - 2.5 g, and it measures acceleration at a sampling frequency of 20 Hz. From determining the axis through which the static acceleration due to gravity (g) is observed, the orientation of the accelerometer can be determined. As the orientation of the thigh changes between sitting and upright activities, the thigh placement of the ActivPAL enables determination of sitting and upright postures and therefore enables measurement of sedentary behaviour according to the posture-based definition. In addition the device can measure dynamic accelerations due to stepping and can therefore quantify number of steps and stepping rate. The ActivPAL has been validated for use with adults as a measure of physical activity and body posture, for assessing posture during free living activities (Dahlgren *et al.* 2010; Dowd *et al.* 2012; Godfrey *et al.* 2007; Grant *et al.* 2006; Harrington *et al.* 2011; PAL.technologies.Ltd 2006; Ryan *et al.* 2006).

Grant et al determined the validity of the ActivPAL compared to direct observation to measure sitting time in a laboratory environment. The mean percentage difference between sitting time between the accelerometer and direct observation was 0.19% (Grant *et al.* 2006). In another validation study, Kozey-Keadle et al 2012 (Kozey-Keadle *et al.* 2012) determined that the relationship between the ActivPAL and the direct observation for measuring sitting time was high ($R^2 = 0.94$). Consequently, the activPAL is a valid tool to estimate the time spend sitting in adults (Atkin *et al.* 2012; Grant *et al.* 2006; Kozey-Keadle *et al.* 2011). In addition, studies have determined that the ActivPAL was valid for determining the number of transitions between sitting and standing (breaks in ST) in both laboratory (Grant *et al.* 2006) and free-living conditions (Lyden *et al.* 2012). The ActivPAL has also been shown to have better agreement with direct observation of sitting time compared the Actigraph accelerometer (model GT3X) (Dowd *et al.* 2012).

The activPAL has been established as a potentially useful tool for measuring sitting, standing time and step counts. One limitation of the ActivPAL is that it can only provide accurate step counts, but cannot gain any information of different types of activity being undertaken (Atkin *et al.* 2012). The ActivPAL's thigh-based accelerometer position may also provide

advantages for the assessment of physical activity, over other body locations, as accelerations at the thigh must be generated by the person moving their leg. Currently, the ActivPAL generates an output of step counts, based on a proprietary algorithm, but an opportunity exists to develop more sophisticated physical activity output metrics from the acceleration signals generated by thigh movement.

Recently, researchers explored whether acceleration data generated by ActivPAL monitor could be used to adequately discriminate between time sitting or lying. Lyden and colleagues developed and validated a new method to distinguish between sitting and lying by using the acceleration signal from the y-axis of a thigh-placed AP to define rotation of the thigh. The author detected that the algorithm correctly recognized 96.7% of the sedentary time as lying and 92.9% of the time as not lying. This study can assist researchers in understanding the relationship between the actual time spend sitting and health outcomes (Lyden *et al.* 2016).

Another popular device for the academic measurement of physical activity is ActiGraph, which is a small tri-axial monitor accelerometer (size: 38x37x18mm, weight: 27g). It is designed to be worn on the hip by using an adjustable belt, and integrates a tri-axial sensor to measure acceleration in three axes at sampling rates up to 100 Hz, using cut points with traditionally a cut-point of < 100 counts per minute (cpm) applied to estimate sedentary time. Although much progress has been made in the assessment of physical activity with accelerometers, there are several limitations when using hip-based accelerometers to assess sedentary time. Accelerometers do not include an inclinometer for measuring postures and it could not, therefore, distinguish between sitting and standing pattern. As a result, time spent standing is counted as sedentary (Atkin *et al.* 2012). Recent models of the ActiGraph such as GT3X and GT3X+ contain an inclinometer algorithm which can define sitting, lying, standing time and when the device not been worn. However, when the device is worn at the hip, the output between sitting and standing is similar, leading to misclassification of standing as sitting time (Atkin *et al.* 2012;Carr and Mahar 2012;Lyden *et al.* 2012). Further research is needed to examine the validity of this additional feature (Carr and Mahar 2012). Kozey-Keadle et al (Kozey-Keadle *et al.* 2011) tested the validity of an Actigraph accelerometer in quantifying sedentary time using the threshold value of 100 counts per minute. It was found that the Actigraph underestimated sedentary time by 4.9% (SE 3.4 %) compared to direct observation. Similarly Lyden et al (Lyden *et al.* 2012), found that the ActiGraph is not a valid tool to assess breaks in sitting time. However, the ActiGraph is one of the most widely used and extensively validated tools for assessing physical activity

intensity. The Freedson cut-points are one of the common approaches (Freedson *et al.* 1998) that have been used to evaluate time spent in light intensity activity (100 – 1951 cpm) and MVPA (≥ 1952 cpm). Freedson and colleagues (1998) developed the regression equation on a sample of 50 adults (mean age 24.8 years) men and women. Subjects achieved slow (4.8 $km.h^{-1}$) and high walking speeds (6.4 $km.h^{-1}$), and jogging (9.7 $km.h^{-1}$) speeds. The equation was thereafter cross-validated on a random sample of 15 subjects. The result indicated that there was a good correlation between actual and predicted EE from Actigraph using the developed equation (r=0.93, SEE = ± 0.93 $kcal.min^{-1}$, $P < 0.05$). The developed Freedson equation is: $Kcal.min^{-1}$ = (0.00094 x $cnts \cdot min^{-1}$) + (0.1346 x body mass (kg)) – 7.37418 (r^2 = 0.82, SEE = ± 1.40 $kcal \cdot min^{-1}$) (Freedson *et al.* 1998). The Actigraph can also be used to determine step counts. A recent study suggested that step outputs gained from ActiGraph accelerometers at waist and wrist positions are in general not equivalent under both laboratory and free-living conditions (Tudor-Locke *et al.* 2015).

In another study, Steeves et al. (Steeves *et al.* 2015) compared the Actigraph and ActivPAL when worn on the thigh during controlled and free-living conditions. Participants were asked to perform (six sitting, two standing, nine stepping, and one cycling) and writing on a whiteboard with intermittent stepping under laboratory conditions, and under free-living conditions for 3 d. In the laboratory condition, both monitors acceptably quantified 100% of standing time and >95% of the time spent in 4 of 6 sitting postures. Both devices misclassifed sitting on a laboratory (Actigraph 14% vs ActivPAL 95%). ActivPAL misclassified 14% of sitting time with legs elongated; whereas ActiGraph classified this correctly in all cases. Both devices were >95% accurate for stepping rate, while Actigraph was less accurate for descending stairs (86%), ascending stairs (92%), and running at 2.91 $m.s^{-1}$ (93%). The two accelerometers categorised whiteboard writing differently (ActiGraph 85% standing and 15% stepping vs activPAL 98% standing and 2% stepping). ActivPAL categorized 93% of cycling time as stepping, in contrast to the Actigraph categorized <1% of cycling time as stepping. In free-living condition, accelerometers were similarly accurate in correclyu classifying activities (86% observed). The two accelerometers categorized similar amounts of time as sitting (ActiGraph 64% vs ActivPAL 62%). There was variation in time recorded as standing (ActiGraph 21% vs ActivPAL 27%) and stepping (ActiGraph 15% vs ActivPAL 11%).

Berendsen et al (Berendsen et al. 2014) observed the validity of activPAL3, ActiGraphGT3X and CAM under laboratory and free-living conditions. This study presented that ActiGraph

(worn at the waist) correctly classified 33.9% of the time during sitting, lying and upright posture time, whereas the activPAL and CAM were100% accurate. Skotte et al. (Skotte et al. 2014) assessed the validity of triaxial accelerometer ActiGraph GT3X+, placed on the hip and thigh for measuring sitting time through controlled and free-living conditions. Under free living conditions, the thigh position showed improved performance of sensitivity (98%) and specificity (93%) for identify sitting time compared to the hip position (73 and 58% respectively). In another study Carr et al, (Carr and Mahar 2012) evaluated the accuracy of ActiGraph GT1M, ActiGraph GT3X+, and StepWatch for measuring light-intensity activities and various sedentary under controlled conditions. Their findings showed that all three monitors correctly assessed most behaviors.

Another study has done by Judice (Judice *et al.* 2015b) to observe the accuracy of the GT3X and Actiheart for measuring sitting time and break sitting in 10 overweight/obese adults in free living conditions, using the ActivPAL as the criterion reference. Sedentary time was overestimated by GT3X and underestimated by Actiheart (bias = 135min: bias = -156 min respectively), and both devices overestimated time of sedentary breaks (bias = 78min: bias = 235 min respectively).

Another study has done to observe the validity of inclinometer functions of Actigraph (AG) GT3X+ positioned on waist vs wrist and ActivPAL in measuring 3 different postures (sitting, standing and stepping). Sixty two participants were asked to complete 15 activities which included 5 patterns of sitting, 4 patterns of standing, climbing stairs, walking at 2.0, 3.0 mph and walking at 3.0mph and typing at a treadmill-desk (TrekDesk) and running at 4.5mph, 5.5mph. Based on direct observation, ActivPAL seemed to be accurate for measuring sitting and standing compared to waist and wrist AG (An *et al.* 2016).

However, although the available evidence suggests that the thigh-positioned ActivPAL accelerometer provides the gold-standard position for the measurement of sedentary behaviour, the current outputs for physical activity from this device are relatively limited, with outputs limited to step count and stepping rate. This has resulted in a number of researchers using two devices to obtain a complete assessment of sedentary behaviour and physical activity – an ActivPAL for the former and another device, such as the Actigraph with a more comprehensive output of physical activity for the latter (An *et al.* 2016). Thus, there is an opportunity to develop new physical activity outputs from the activPAL, such as walking speed and an estimation of oxygen uptake and energy expenditure which will enable

researchers to use a single device to obtain comprehensive assessment of both sedentary behavoiur and physical activity.

This thesis therefore has two main sections. The first is to determine the effects of interventions which break up sedentary time of metabolic responses which may influence vascular and metabolic risk. The second is to undertake studies to facilitate better measurement of physical activity using a thigh-worn accelerometer device.

The aims of this thesis are therefore:

1) To compared the effects of prolonged sitting, prolonged periods of standing, and the same total amount of standing undertaken in multiple short standing bouts, on metabolic responses over the course of a day. This will help to determine whether, in principle, the number of transitions between sitting and standing influences metabolism independent of total time spent sitting or upright.

2) To determine whether, breaking up prolonged sedentary time by undertaking 'chair squats' repeated sit-to-stand transitions over a short period (sitting and standing 10 times over 30 seconds, every 20 minutes) – provides measureable metabolic benefits. This will help to determine the efficacy of a practical, light touch intervention, which could potentially be used in a real-world intervention.

3) To determine the accuracy of measurement and validate new metrics of physical activity for thigh-worn accelerometers. Specifically, these aims are to:

3a) To compare the accuracy of measurement of directly observed stepping rate with thigh- and hip-placed accelerometers across a range of walking and running speeds.

3b) To determine the relationship between raw accelerations and walking and running speeds for thigh- and hip-placed accelerometers for treadmill-based walking and running.

3c) To determine the relationship between raw accelerations and oxygen uptake for thigh- and hip-placed accelerometers for treadmill-based walking and running.

3d) To compare the relationships between raw accelerations and walking and running speeds for treadmill-based compared with overground walking and running.

3e) To use the information above to develop and validate algorithms to estimate energy expenditure from raw acceleration counts for thigh- and hip-placed accelerometers.

2. General Methods

This chapter provides a description of all general methods that have been implemented in the following experimental chapters. Methods specific to individual chapters will be highlighted separately in each experimental chapter. Methods used for statistical and data analyses are outlined in the relevant study chapters.

2.1 Participants

Participants were recruited from the students and staff of University of Glasgow and residents in the Glasgow area via emails, online advertising and advertisement in the public places. Participants were required to attend for baseline screening at the University to ensure they fulfilled the inclusion criteria of each study. The study was explained in detail and all the questions were answered. The information sheets were provided to describe the aim of the study, the experimental procedures involved and the risk and benefits of participation **(Chapter 3: Appendix A, Chapter 4: Appendix I, Chapter 5: Appendix N)**. Volunteers were also encouraged to ask any questions before agreeing to participate. Each participant completed health screening questionnaire and were asked to sign a consent form to participation in the study, which was approved by Research Ethics Committees of Medical, Veterinary and Life Sciences from the University of Glasgow **(Appendix B)**. Their resting blood pressure measurements were taken using an automated sphygmomanometer (Omron Healthcare, Inc., Illinois, USA), three measurements were taken, of which values were averaged, and fasting finger-prick blood sample were also tested to measure glucose following a 12-hour overnight fast. Common exclusion criteria were used as follows frank diabetes (physician diagnosed or fasting glucose (>7 $mmol.l^{-1}$ on screening), uncontrolled hypertension (>160/90 mmHg on anti-hypertensive medication), previous history of established CHD or current medications known to affect lipid or glucose metabolism, smoker and non-overweight (body mass index < 25 kg/m^2).

2.2 Anthropometric Measurements

2.2.1 *Statuse*

Height was measured using a standard stadiometer (Invictus Plastics Ltd., Leicester, England). Each Participants were asked to stand barefoot, with both feet alongside one another, and with their back of the head, back, buttocks, calves and heels against a stadiometer. The head was positioned in the Frankfort plane. The participant was asked to look straight. This was then immediately followed by recording the last measurement on the on the rule. Measurement was recorded to the nearest 0.1 cm.

2.2.2 *Body Mass*

Body mass was measured in light and minimal clothing (i.e. generally light-weight shorts and t-shirt) without shoes. Measurement was recorded to the nearest 0.01 kg using a balanced-beam scale. Participants were asked to stand in the centre of the platform, facing forward and with arms straight to the sides of their body. Body mass was estimated using the same scale through all the experimental studies. BMI was then calculated as body mass in kilograms divided by the square of height in metres.

2.2.3 *Waist and hip Circumference Measurement*

Hip and waist circumference were measured in touch with the skin using a flexible, steel tape measure (Supralip 160, West Germany). Hip circumference was measured horizontally around the maximum circumference over the trochanters (buttocks), with the participants standing with both feet alongside one another and arm the side. The waist circumference measurement points were noted in precise and exact terms (namely, between the costal margin and iliac crest. The measurements were taken twice and then the average was calculated. If the two readings were inconsistent by more than 0.5 cm, a third reading was taken.

2.3 Expired Air Measurements and Heart Rate Monitoring

2.3.1 Measurement of oxygen uptake and carbon dioxide production

Oxygen uptake and carbon dioxide production in Chapters 3, 4 and 5 were determined at rest before and during exercise. Expired air was collected using the Douglas bag method, which was also used as the gold standard comparison. Prior to all resting measurements, participants achieved a ten-minute run in period to ensure they were comfortable and in a true resting state. Participants were fitted a nose clip, breathing through a rubber mouthpiece connected to a lightweight large 2-way respiratory valve (2700 series, Hans Rudolph Inc. USA), which in turn was connected to a flexible plastic tubing. The tubing was connected to evacuated 100,150 or 50-litre Douglas bag via another two-way valve to control the flow of expired air into the Douglas bag. 50-litre Douglas bag was used for measuring the gas when the expired values were small, for example during 30 second collections of chair squats activity, 100 and 150-litre bags were used at other activities.

Once the gas sample was collected, a small amount of gas was extracted from the used Douglas bag measured by a Servomex Gas Purity Analyser (Analyser Series 1400) to determine the F_EO_2 %, F_ECO_2% in each separate bag. The gas analyser was calibrated prior to each test using certified reference gases (BOC Gases, Surry, UK) with known reference gases (i.e. 100% nitrogen, 16% O_2, 5% CO_2 and room air calibration). The remaining expired sample volume in the bag was extracted out using a dry gas meter (Harvard Apparatus, Kent, UK) to record Gas sample volume and temperature. Barometric pressure was recorded using a standard mercury barometer during each test. These were utilised alongside fractional expired oxygen (F_EO_2) and carbon dioxide concentration (F_ECO_2) to evaluate oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$), expired air fractions and volumes were corrected for standard room temperature and pressure for a dry gas (STPD) (760 mmHg) to indicate $\dot{V}O_2$ (STPD), $\dot{V}CO_2$ (STPD), and respiratory exchange ratio (RER). The respiratory exchange ratio uses the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$ as a marker of substrate oxidation and is typically between 0.7 and 1.0; 0.7 reflecting total fat oxidation and 1.0 total carbohydrate oxidation (Ferrannini, 1988).

2.3.2 Calculation of fat and carbohydrate oxidation, and energy expenditure by indirect calorimetry

Fat and carbohydrate oxidation rates, were calculated via indirect calorimetry using the equations defined by Frayn (Frayn 1983) as described below.

$\dot{V}O_2$ (1.min^{-1}) = 0.746 C + 2.03 F+ 6.04 N (Equation 2-1)

$\dot{V}O_2$ (1.min^{-1}) = 0.746 C+ 1.43 F+ 4.89 N (Equation 2-2)

Where:
C = carbohydrate oxidation in grams per minute
F = fat oxidation in grams per minute
N = urinary nitrogen excreted in grams per minute

No direct measure of urinary nitrogen excretion was performed in any experimental chapter, therefore, a constant rate of nitrogen excretion of 0.00011 g.kg^{-1}.min^{-1} was utilized, a value which has previously used in the literature (Flatt et al. 1985; Melanson et al. 2005).

The constant nitrogen was calculated as the equation below as follow:

N (g.min^{-1}) = 0.00011 x body mass (Equation 2-3)

Therefore, non-protein oxygen consumption (NP $\dot{V}O_2$) and non-protein carbon dioxide production (NP $\dot{V}O_2$) and the non-protein respiratory quotient (NPRQ) can be calculated as follows:

NP $\dot{V}O_2$ (l.min^{-1}) = 0.746 C + 2.03 F- 6.04 N (Equation 2-4)

NP $\dot{V}O_2$ (l.min^{-1}) = 0.746 C + 1.43 F - 4.89 N (Equation 2-5)

NPRQ = NP $\dot{V}CO_2$ / NP $\dot{V}O_2$ (Equation 2-6)

Substrate utilization, was calculated as below based on the protein corrected values from above:

Fat oxidation ($g.min^{-1}$) = (NP $\dot{V}O_2$ - NP $\dot{V}CO_2$) / 0.6 (Equation 2-7)

Carbohydrate oxidation ($g.min^{-1}$) = (NP $\dot{V}O_2$ - 2.03 x Fat ox) / 0.746 (Equation 2-8)

Protein oxidation ($g.min^{-1}$) = N x 6.25 (Equation 2-9)

Total energy expenditure (EE) was calculated by multiplying the amount of substrate oxidised by their appropriate energy density value which were taken from (Brody, 1999; Mottram, 1979):

Energy expenditure (kJ) = (F x 39.0) + (C x 15.5) + (P x 17.0) (Equation 2-10)

Net energy expenditure and energy substrate utilisation rates were calculated by subtracting the baseline rate from the total energy expenditure or substrate utilisation to give the rise above resting values (Brody 1999; Mottram 1979).

2.4 Heart Rate Monitoring

Heart rates were monitored during exercise by a Polar heart rate system which consisted of a heart rate transmitter and a wrist receiver (POLAR, Kempele, Finland).

2.5 Dietary Assessment

2.5.1 2-Day Dietary Record

In Chapters 3 and 4, participants were asked to weigh and record their food intake, and refrain from alcohol on the two days preceding their first main experimental trial and to replicate this for the two days preceding subsequent trials. Scales, record sheet and written instruction were provided to record as detailed as possible each item that they ate or drank, the time that ate it and the quantity in grams **(Appendix D).**

2.5.2 Test meal

In Chapters 3 and 4 participants were given a standardised breakfast and lunch comprising a buttered bagel and strawberries (Complan Foods Ltd, UK) made up with whole milk to

form a strawberries milkshake drink, which provided (~ 8 kcal/kg body weight with ~ 37% energy from fat, ~ 49% from carbohydrate and ~ 14% protein). All participants were asked to consume each test meal within 10 minutes and water was allowed during this time.

2.6 Daily Physical Activity Assessment

Participants were asked to refrain from planned exercise (undertaking only the activities of normal daily living) for 3 days. Physical activity and sedentary behaviour was objectively measured using ActivPAL accelerometers (PAL Technologies Ltd., Glasgow, UK).

For each subject, the number of activities (sitting, standing, walking, steps number and the number of transitions ("breaks") were calculated using the summary formed **Figure 2-1** generated by the activPAL Professional Research Edition software (Version 5.8.2.3). Non-wear time was known from the subject's activity recording sheet **(Appendix E)**.

2.7 Blood Sampling and Analysis

In chapters 3 and 4, blood samples were used for analysis of postprandial metabolites analysis. Subjects arrived at the metabolic suite in the morning on an overnight fast. Subjects were asked to rest in a semi-supine position while a cannula was placed in an antecubital vein, to which a 10 cm three-way stopcock (Connecta plus 3, BD, Sweden) was connected. A baseline sample was collected after 10 min the cannula was kept patent by flushing with a small amount of non-heparinized saline solution 0.9% after each sample collection. A saline waste remaining in the connector tube was taken off by a 2 ml syringe, before each blood samples, then, a blood samples were taken in 10 ml tube containing K3EDTA **(**Becton Drive Vacutainer, New Jersey, USA) during the observation period, as specified in chapter 3 and 4, and placed immediately in ice and centrifuged (GS-6KR, Beckman Instruments, Inc, California, US) within 15 minutes at 4000 revolutions per minute (rpm) at 4 C, 2580 relative centrifugal force (RCF). When the plasma and red blood cells were separated, 3 ml aliquots of plasma were extracted and placed into 200 µl in 0.5 ml labelled tubs (Alpha laboratories, Ltd, UK). All samples were frozen immediately at -80°C.

2.7.1 Insulin Analysis

Insulin was measured in freshly frozen EDTA plasma using commercially available ELISA kits (Mercodia AB, Uppsala, Sweden). All ELISA procedures were based on a 'sandwich' technique which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. The wells of the plates were coated with antibody specific to the protein of interest being measured in plasma. A plasma samples (25 µl) was added to the wells. Then a 100 µl of freshly prepared enzyme conjugate solution was pipetted to each well. The plates were then incubated on a plate shaker for 1 hour at room temperature. During this incubation period, insulin in the samples reacted with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to plate wells. After that, the plates were washed and dried 6 times by automatic washer to remove any unbound enzyme labelled antibody using the provided wash buffer solution. Bound conjugates which remained in the wells were identified by adding 200 µl of 3,3',5,5'-tetramethylbenzidine (TMB). Then, the plates were incubated for 15 minutes at room temperature to allow reaction between substrate TMB and bound conjugates. After incubation, 50 µl of the Stop solution containing 0.5 M sulphuric acid were pipetted to each well to stop the reaction. A yellowish-tint colour developed according to the concentration of conjugate-substrate complex. The optical density of each well was read at 450 nm using a spectrophotometer. All samples were run in duplicate together with the standards ranging from 0 to 200 mU/l. A standard curve was obtained by computerised data reduction of the absorbance for the standards against the concentration using cubic spine regression. The concentration of insulin in the samples was then determined by comparing the optical density of the samples to that of the standard curve for each respective plate. All reagents and samples were brought to room temperature before use. Coefficients of variation for the assay were <5%.

2.7.2 Glucose Analysis

Glucose was measured in fresh EDTA plasma using (YSI 2300 STAT Plus^{TM} Glucose and Lactate Analyser, YSI (UK) Ltd.). EDTA plasma was used after centrifugation for 15 min at 4000 rpm, the Relative Centrifugal Force or G-force 2580 (RCF). The analyser was calibrated prior to each test in the morning, afternoon and after last sample using high and low certified reference. A sample was placed into a manual sample station. The result appeared on a small screen and a printer paper was obtained. Each sample was measured

twice and the average has been taken. All samples within each subject were performed on a single run and in duplicates with coefficients of variation of <3%.

The YSI 2300 STAT PLUS uses a sensor technology with an immobilized enzyme membrane. Glucose in plasma is rapidly oxidized by glucose oxidase enzyme producing hydrogen peroxide (H_2O_2). The hydrogen peroxide, in turn, is oxidized at a platinum anode producing electrtons. The electron flow, which is measured by the senor, is linearly proportional to the concentration of glucose.

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{glucono-}\delta\text{-lactone} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Platinum anode}} 2\,H^+ + O_2 + 2\,e^-$$

2.7.3 Triglyceride Analysis

Plasma TG concentrations were measured by members of staff in the Clinical Biochemistry Department at Glasgow University.

A lipoprotein lipase derived from micro-organisms is used to rapidly and completely hydrolyse TG to glycerol followed by the oxidation of glycerol to dihydoxylacetone phosphate and hydrogen peroxide. The hydrogen peroxide then reacts with 4aminophenazone and 4-chlorophenal under the catalytic action of peroxidase to form a red dyestuff. All samples within each subject were performed on a single run and in duplicates with coefficients of variation of <2%.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{glycerol} + \text{fatty acide}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerokinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{glycerol-3-phosphate oxidase}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4- aminoantipyrine} + \text{p-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneime} + 4\,H_2O$$

2.8 Objective measurement of Physical Activity and Sedentary Behaviour

In chapter 5, participants performed two experimental trials – one involving walking and running on a treadmill and one involving walking and running on an athletics track. For each trial, participants wore activPAL devices (small commercially-available matchbox-sized accelerometer/inclinometers on in a number of locations on the body (lower thigh, upper thigh, and hip on the left and right sides), and Actigraph accelerometers (small commercially-available matchbox sized accelerometers, fixed on the right and left hips, to record body accelerations and posture changes.

2.8.1 ActivPAL (AP) Accelerometer

Sitting, standing, walking and other types of physical activity were measured using the ActivPAL professional monitor (PAL Technologies Ltd., Glasgow, UK) (Firmware: v 5.8.2.3), which is a tri-axial accelerometer and inclinometer (is a single-unit monitor based on a uniaxial) (5.4cm (L) x 3.5cm (W) x 0.6cm (D)), weighing approximately 15g. Typical activPAL devices are shown in **Figure 2-2**. The device is manufactured by PAL technologies Ltd. Glasgow, Scotland. The AP designs to wear midline on the anterior aspect of the thigh **Figure 2-3**, which is attached to the skin using double-sided hydrogel adhesive pads, (PALstickies), and covered with clear adhesive tape. The device produces a signal related to thigh inclination which responds to gravitational accelerations resulting from segmental movement (Dowd *et al.* 2012; Ryan *et al.* 2006), that is recorded the activity by measuring raw accelerations counts in three orthogonal axes, X plane (vertical),Y plane (mediolateral) and Z plane (anterioposterior) (Stanton *et al.* 2014), **Figure 2-4** shows illustration of ActivPAL axis: x, y and z. The activPAL provide outputs including time spent sitting /lying, standing, step count and cadence and has been shown to be valid and reliable measurement to quantify poster, activity of daily living (Dowd *et al.* 2012;Grant *et al.* 2006), step number and cadence in a healthy adult population (Ryan *et al.* 2006). This monitor has a sampling frequency of 20 Hz for each 15 second time interval (epoch), and has the memory of 4 Mb and battery life capacity to record and store data for >8 days. Propriety software (ActivPAL Professional Research Edition) permit the monitor to be initialised for data collection start and end dates and times via the PAL3 USB Dock charging system. There are five stations on the docking cable, four for charging **Figure 2-2** and one for initializing, data

transfer, and charging as well. PAL3 USB Dock Charging System can be used to download the data retrieval to the computer in the form of daily and hourly activity, which is classified as time spent sitting/lying, standing, stepping, step cadence and energy expenditure over 1h and numeric formats can be exported to Microsoft Excel. In addition, proprietary algorithms also, classifies and records posture transitions [sit-to-stand (u) and stand-to-sit (d)] **Figure 2-5**. The result can be obtained per hour, day and week.

Figure 2-2: ActivPAL Monitor

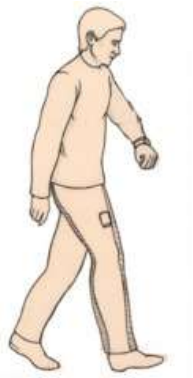


Figure 2-3: ActivPAL Placement

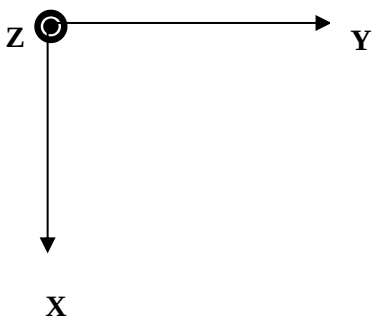


Figure 2-4: Raw Accelerations Counts (X,Y and Z)

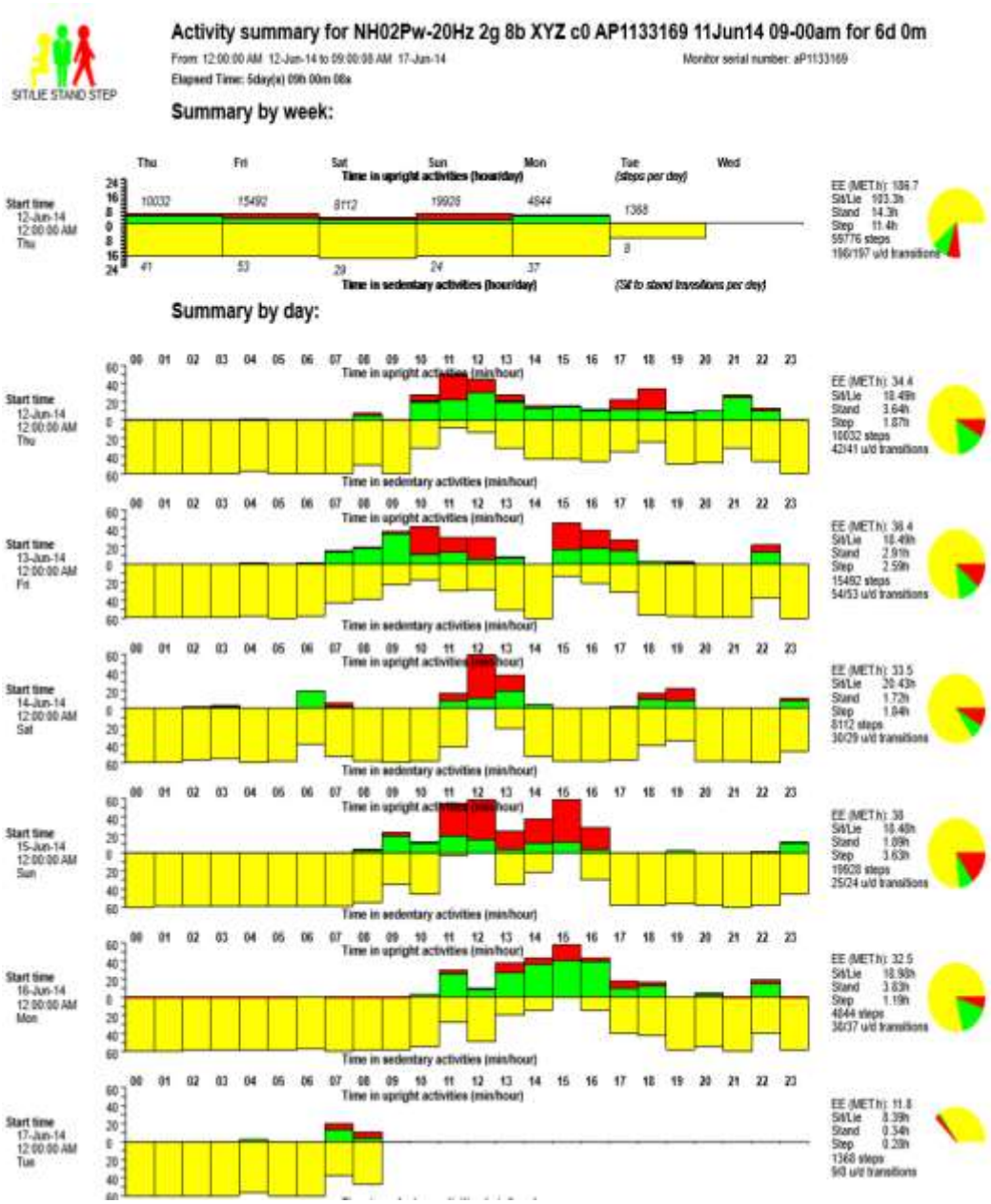


Figure 2-5: ActivPAL output (summarized by hour).

Sit/lie times in yellow, standing time in green, and walk-steps in red. Transitions (sit-to-stand (u), stand-to-sit (d)) are presented at the right side.

2.8.2 ActiGraph (AG) Accelerometer

The second selected type of movement sensing device is Actigraph accelerometer which is used for measuring physical activity (PA). The device is attached to a belt strapped around the waist. The Actigraph GT3X+ (Firmware v 4.1.0) (model 7123: Actigrph, LLC, Fort Walton Beach, Florida) is small (2.0 × 1.6 × 0.6 inches (5.1 × 4.1 × 1.5 cm)) and lightweight (19 grams). AG is a tri-axial accelerometer and can measures acceleration in three individual orthogonal planes using a vertical axis activity acceleration data (Axis 1), horizontal axis activity acceleration data (Axis 2), and perpendicular axis activity acceleration data (Axis 3), indicates whether a subject is standing, sitting or lying down when the device is worn at the hip as well as indicating that a device is not being worn at all has enable to directly identify periods of sitting/lying, standing and stepping. When worn on the hip and perfectly vertical, the y-axis alone should contain the total acceleration due to gravity. As a subject inclines, the offset angle (θy) increases. If the device is not being worn, then one expects the z-axis to reflect the total acceleration due to gravity as the device rest on a table-top for example. Therefore, the addition of the z-axis offset angle (θz) is required to distinguish between lying and off. **Figure 2-6** contains examples of this y-axis offset angle in the standing (top-left), sitting (top-right), lying (bottom-left), and z-offset angle in the off (bottom-right) positions. The AG sample acceleration at rate of 30-100Hz, for each 1 second time interval (epoch), and a memory capacity of 4 GB that allows recording of data in excess of 180 days. The AG interfaces with a windows compatible PC and the software package (ActiLife 6) analyses the activity record using proprietary algorithms. The device also connects with a PC program via a USB to initialize and download the data. The software summarises activity over 10 sec periods in graphical format, the data and graph were saved in Excel file and PDF respectively. (Freedson et al. 1998) cut-points used to define intensity domains (light < 1952 count.min^{-1}; moderate 1952-5724 count.min^{-1}; vigorous >5725count.min^{-1}). A sedentary bout was define as a period of < 100 count.min^{-1}, while non-wear time was defined as intervals of least 60 min of 0 activity counts (Actigraph 2017).

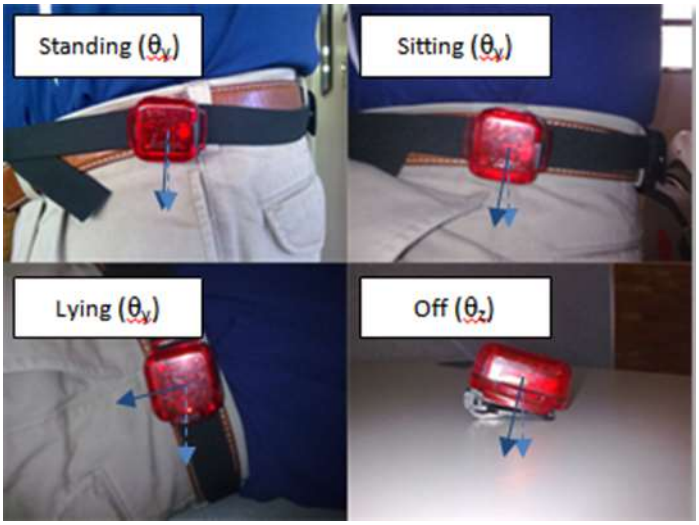


Figure 2-6: Sitting, Standing, Lying and Off Position

3. Frequency of breaks in sedentary time and postprandial Metabolism

3.1 Introduction

There is a large body of observational data showing strong associations between time spent engaged in sedentary behaviour – defined as non-sleeping activities in a sitting or reclining posture with energy expenditure ≤1.5 METS (where 1 MET is resting energy expenditure) (Sedentary Behaviour Research Network 2012) – and a number of adverse health outcomes, including mortality, cardiovascular disease, type 2 diabetes and obesity (Edwardson et al. 2012; Healy et al. 2011; Thorp et al. 2011; Wilmot et al. 2012). These relationships are often independent of time spent engaged in moderate-to-vigorous physical activity (>3 METS) (Edwardson et al. 2012; Healy et al. 2011; Thorp et al. 2011; Wilmot et al. 2012). In addition, recent observational data in almost 700 adults from the AusDiab study, using a postural sensor to objectively monitor time spent sitting, standing and stepping, suggested that isotemporally replacing sitting with standing was associated with favourable changes to glucose and lipid metabolism (Healy *et al.* 2015). There is also observational evidence to suggest that individuals who break up sedentary time more frequently have a more favourable cardio-metabolic risk profile – particularly with respect to adiposity variables – than those who habitually engage in prolonged periods of uninterrupted sedentary time, independent of total time spent sedentary (Cooper *et al.* 2012; Healy *et al.* 2008a; Healy *et al.* 2011). However, the mechanisms by which more frequent breaks in sedentary time may impart these benefits, independent of total sedentary time, are unclear. A number of short-term intervention studies have shown that interrupting sedentary periods with multiple short (≤3 min) bouts of light or moderate activity throughout the day can reduce postprandial glucose, insulin and triglyceride (TG) responses, and blood pressure, on the same or following day (Dunstan *et al.* 2012b; Larsen *et al.* 2015; Miyashita *et al.* 2008; Peddie *et al.* 2013). Other studies have shown that interrupting prolonged sitting with periods of static standing ranging from five minutes every 30 minutes (Henson *et al.* 2016) to 30 minutes every hour (Thorp et al. 2014b), can reduce postprandial glucose concentrations. However, in all of these studies sedentary time was replaced by standing or walking leading to a reduction in total time spent sedentary, so the effects of altering the frequency of breaks in sedentary time, independent of changing total time sedentary, on these metabolic responses

are not known. It is also not known whether altering the frequency of breaks in sedentary time influences metabolic rate and substrate utilisation, which may provide an explanation for the association between frequency of sedentary breaks and adiposity observed in the epidemiological data (Cooper *et al.* 2012; Healy *et al.* 2008a; Healy *et al.* 2011). The aim of this study was therefore to compare the metabolic effects of breaking up sedentary time with prolonged periods of standing *versus* multiple shorter standing bouts with the same total duration to determine whether – in principle – altering the frequency of breaks in sedentary time, influences metabolic responses over the course of the day.

3.2 Methods

3.2.1 Participants

Ten men, aged 33 ± 13 years, with body mass index (BMI) 28.3 ± 2.8 $kg.m^{-2}$, waist circumference 100.2 ± 9.5 cm [mean ± SD], and low levels of habitual physical activity (less than 2 hours per week of moderate-to-vigorous physical activity as assessed by the International Physical Activity Questionnaire), were recruited for this study though personal contacts and local advertising. All participants had BMI >25 $kg.m^{-2}$, were non-smokers, had no known history of CVD or diabetes (and fasting glucose <6.0 $mmol.l^{-1}$ on screening), and were not taking any medications known to affect lipid or glucose metabolism. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the College of Medical, Veterinary and Life Sciences Research Ethics Committee at the University of Glasgow. All participants provided written informed consent.

3.2.2 Study design

Participants each completed three 8-hour experimental trials; uninterrupted sitting (SIT), prolonged standing (PRO-Stand), and intermittent standing (INT-Stand) in a randomised order, with an interval of 1 week between trials (**Figure 3-1**).

Uninterrupted sitting trial (SIT): Participants arrived at the metabolic suite after a 12-hour overnight fast. They sat comfortably for 10 minutes, before two sequential 5-minute expired air samples were collected via a mouthpiece into a Douglas bag to calculate metabolic rate and substrate utilisation using indirect calorimetry (Frayn and Macdonald 1997). The average of these samples was used as the baseline value. A cannula was then inserted into

an antecubital vein for repeated blood sampling, with was kept patent by flushing with saline throughout the day. A baseline fasting blood sample was drawn in K_2EDTA tube and placed immediately on ice. Further blood samples were taken at 30, 60, 120, 180 and 240 minutes after breakfast (see section **2-7** for more details). Four hours after breakfast, participants consumed a test lunch, which was identical to breakfast, and further blood samples were taken 30, 60, 120, 180 and 240 minutes after lunch (i.e. 270, 300, 360, 420 and 480 minutes after breakfast). Expired air samples for the determination of metabolic rate and substrate utilisation were collected at 15-minute intervals every 30 - minute throughout the 8-hour observation period. Participants sat comfortably and continually (reading, watching TV, doing paperwork etc) throughout the observation period and were permitted to drink water throughout the day. Comfort breaks to the toilet (which was ~20 m from the metabolic investigation suite) were permitted using a wheel chair: these were recorded, and as far as possible replicated in subsequent trials.

Prolonged standing trial (PRO-Stand): This was identical to the SIT trial, except that in each 30-minute period throughout the day, participants were asked to sit for 15 minutes and stand stationary for 15 minutes, so that in total they stood for 4 hours and sat for 4 hours, with 16 sit-to-stand and 16 stand-to-sit transitions over the 8-hour observation period but the total time of sitting was consistent at 8 h for all trials. All blood samples were taken during 15-minute sitting periods.

Intermittent standing trial (INT-Stand): This was identical to the SIT trial, except that in each 30-minute period, participants sat for 5 minutes; then undertook 10 cycles of standing for 90 seconds followed by sitting for 30 seconds (20 minutes in total); then sat for 5 minutes. Thus they stood for 15 minutes and sat for 15 minutes every 30 minutes, but the standing occurred in 10 x 90-second blocks, rather than a single 15-minute block. Thus, over the 8-hour observation period they stood for 4 hours and sat for 4 hours, with 160 sit-to-stand and 160 stand-to-sit transitions, ditto previous comment. All blood samples were taken during the 10 minutes of continuous sitting in each 30-minute period. The full protocols can be seen in **Appendix G**. Participants were paid £100 as a token of thanks for completing the study. The study was involved participants spending ~24 hours in the lab over 3 occasions, and we feel that this modest recompense could help us with recruitment.

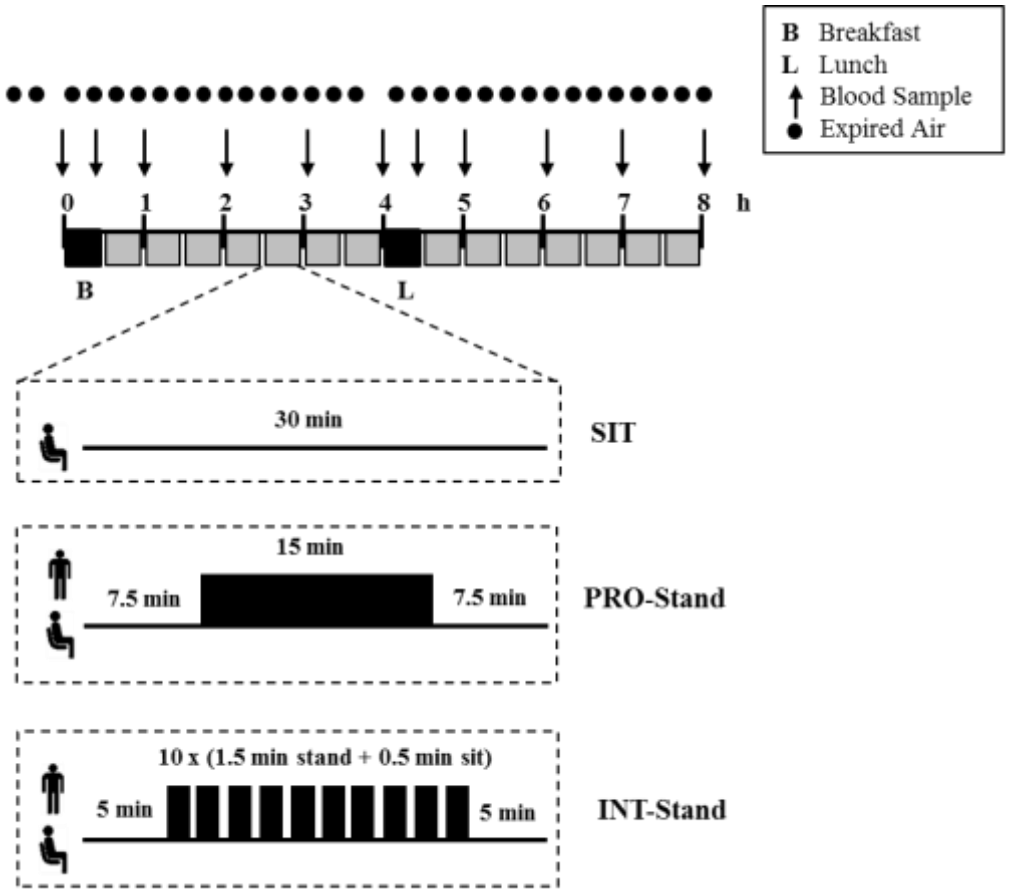


Figure 3-1: Study protocol. The 8-hour observation period for each trial day. Participants completed three trials in random order: Uninterrupted sitting (SIT), Prolonged standing (PRO-Stand), and Intermittent standing (INT-Stand).

The grey boxes represent each 30-minute intervention period throughout the day, with the protocol undertaken during each 30-minute period expanded below.

3.2.3 Standardised Meals

Participants consumed two standardised meals for breakfast and lunch. Each meal consisted of a buttered bagel and a meal replacement drink (Complan Foods Ltd, UK) made up with whole milk, which provided 8 kcal energy per kg body mass (37% energy from fat, 49% carbohydrates, and 14% protein) to match the typical Scottish daily macronutrient intake (Marriott and Buttriss). Energy, protein, lipid, and carbohydrate intake were calculated using nutrient information obtained from respective online sources or food labels. Participants were asked to consume the meal within 10 minutes.

3.2.4 Standardisation of diet and exercise

Standardisation of diet and exercise have previously been described in (see section **2-5**, and **2-6**). Sitting, standing, walking and other types of physical activity were monitored using

the ActivPAL. Participants were instructed to wear the monitors on the right thigh all times, except when showering, swimming and sleeping for 3 days before each trial. At the end of monitoring period, the monitor was returned to the researcher and the output of the activPAL (summarized by hour) was installed by using the activPAL professional software. Total time recorded as sitting, standing and steps for the preceding 3-d period before observation day is clarified in **Figure 3-2**. Data output is expressed as mean time (hours) spent in various level of activity. In this study, a 3 days before each trials were considered to explore the difference in the activity between the participants. Statistical analyses and calculations were conducted using the statistical software version and Microsoft Office Excel 2010. Data were tested for the normality. All data were normal distributed using Shapiro-Wilk. One-way ANOVA was used to compare the physical activity during each trail. There were no significant differences in daily activity across trials $p > 0.05$.

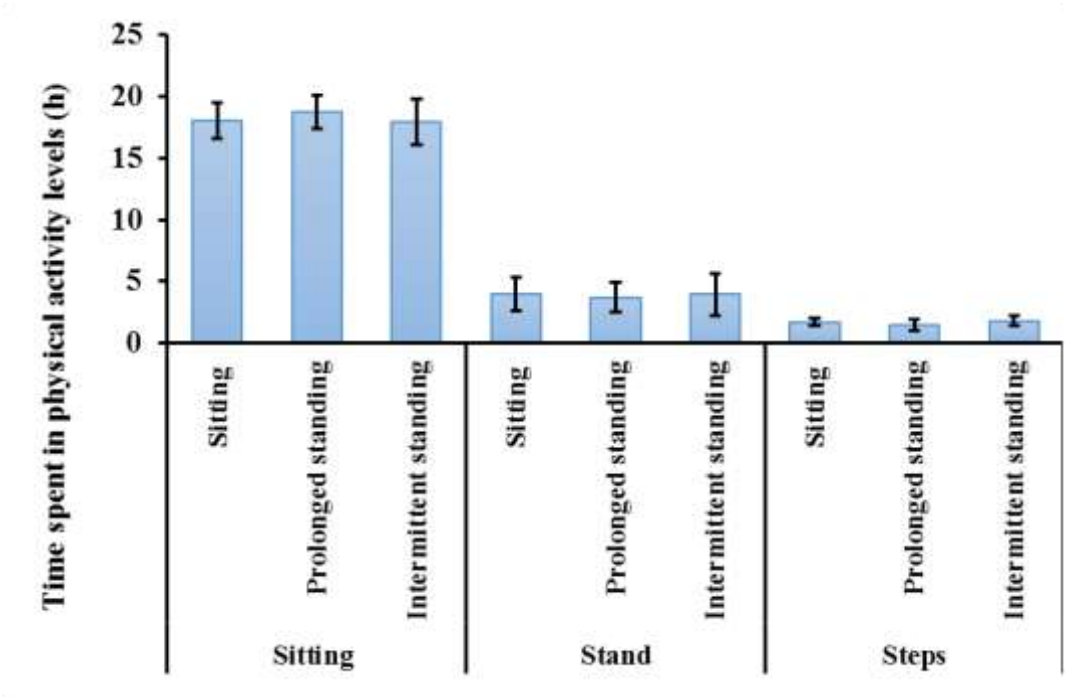


Figure 3-2: Mean time spent in various physical activity levels during the preceding 3-d period in sitting, prlonged standing and intermittent standing trials (n=10). Values are expressed as means, with standard errors represented by vertical bars.

3.2.5 Calculation of energy expenditure and substrate utilization

Fat, carbohydrate oxidation and energy expenditure were calculated using indirect calorimetry (Frayn and Macdonald 1997) (see section **2-3** for more details). For these calculations urinary nitrogen excretion was assumed to be 0.11 $mg.kg^{-1}.min^{-1}$ throughout each trial, based on data from previous studies in the literature (Flatt *et al.* 1985;Melanson

et al. 2005). The first expired air samples were taken twice after 10 min period to determine the metabolic rate, gas samples were collected during the final 4 minutes of each 15 min bout of sitting, intermittent and prolonged standing throughout the 8 hour observation period.

3.2.6 Power calculation

As the most consistent association between frequency of sedentary breaks and health outcomes related to adiposity variables (i.e: BMI and waist circumference) (Cooper et al. 2012; Healy et al. 2008a; Healy et al. 2011), For example, those in the highest quartile of breaks in sedentary time had, on average, a 5.9 cm lower waist circumference than those in the lowest quartile. However, this is an observational study using cross-sectional data, and further investigations are required to determine possible causal associations. (Healy, 2008). Sedentary behaviour can increase the risk of obesity in adulthood. Some evidence also exists for breaks in sedentary time to be associated with a more favourable BMI, and for use of a car to be associated with greater risk of obesity (Biddle *et al.* 2017). We primarily based our sample size on the number of participants needed to detect a difference in overall energy expenditure over the observation period, as this would be the likely mechanism by which changes in sedentary breaks could influence adiposity. Our previous data had shown that the within-person SD for difference in resting oxygen uptake was 6.1% (Farah and Gill 2013). We assumed that the within-person SD for differences in energy expenditure between trials here would be similar. Accordingly, we calculated that ten participants would enable detection of a ~6% difference in energy expenditure between trials with 80% power at $p < 0.05$. In addition, based on our earlier observations that the within-person SD for postprandial glucose, TG and insulin responses were 3.4%, 10.1% and 22.9%, respectively (Gill et al. 2005), our sample would enable detection of respective differences between trials of ~3%, ~10% and ~23%, in glucose, TG and insulin responses.

3.2.7 Statistical analysis

Statistical analyses were performed using Statistica (Version 10, StatSoft, Inc.) and Minitab (Version 14, Mintab Inc.). Data were tested for normality using the Anderson-Darling normality test, and where necessary, data were logarithmically transformed prior to statistical analysis. The area under curve (AUC), calculated using the trapezium rule was used as a summary measure of the postprandial responses for energy expenditure, fat oxidation and carbohydrate oxidation. This provides a measure of total amount of energy expended or substrate used over the observation period. For glucose, insulin and TG

concentrations, the time-averaged AUC (i.e. AUC divided by the duration of the observation period) was used as a summary measure. This provides a measure of the average concentration over the observation period. Comparisons between trials were made using repeated measures ANOVA, with post-hoc Fisher LSD tests used to identify where any differences lay. Cohen's d effect sizes were calculated to describe the magnitude of differences between trials (>0.8 large, 0.5-0.8 medium, <0.5 small, <0.2 trivial) (Cohen 1992). Data are presented as mean ± SEM unless otherwise stated, and $p < 0.05$ was considered significant.

3.3 Results

3.3.1 Baseline values

Baseline values in the three trials are shown in **Table 3-1**. There were no differences in body mass, rates of energy expenditure, carbohydrate oxidation, or plasma glucose, insulin or TG concentrations between the three experimental conditions in the fasted state, before the interventions were commenced, indicating that control of lifestyle in the days preceding the trials was sufficient to ensure that the baseline metabolic state in all trials were similer.

Table 3-1: Baseline values in the fasted state in the three experimental conditions. Values are mean ± SEM, n = 10. [a]statistics performed on log-transformed data.

	SIT	**PRO-Stand**	**INT-Stand**	***p***	***SIT vs PRO-Stand***	***SIT vs INT-Stand***	***POR-Stand vs INT***
					Cohen`s d effect size		
Body mass (kg)	89.9 ± 3.4	89.8 ± 3.4	89.7 ± 3.3	0.92	0.004	0.009	0.004
Resting Energy expenditure ($kJ.min^{-1}$)	5.6 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	0.12	0.20	0.17	0.01
Fat oxidation ($g.min^{-1}$)	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.50	0.31	0.05	0.34
Carbohydrate oxidation ($g.min^{-1}$)	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.18	0.15	0.18	0.36
Plasma glucose ($mmol.l^{-1}$)	5.2 ± 0.2	5.2 ± 0.1	5.4 ± 0.2	0.09	0.04	0.24	0.21
Plasma insulin ($mU.l^{-1}$)	7.4 ± 0.9	8.1 ± 1.4	8.8 ± 1.7	0.26	0.09	0.16	0.07
Plasma TG[a] ($mmol.l^{-1}$)	1.2 ± 0.2	1.2 ± 0.1	1.2 ± 0.2	0.49	0.05	0.01	0.07

3.3.2 Energy expenditure and substrate utilisation during the interventions

Energy expenditure and substrate utilisation over the 8-hour observation period are shown in **Figures 3-2, 3-3 and 3-4** with summary data for these responses shown in **Table 3-2**. Compared to the SIT trial total energy expenditure over the 8 hours was 320 ± 62 kJ (10.7 ± 2.0%) higher in the PRO-Stand trial and 617 ± 76 kJ (20.4 ± 2.3%) higher in the INT-Stand trial: energy expenditure in the INT-Stand trial was 296 ± 78 kJ (9.0 ± 2.3%) higher than the PRO-Stand trial (all $p<0.001$). The Cohen's d effect sizes for all of these differences were large. Total fat oxidation over the observation period was 7.1 ± 1.9 g (20.2 ± 6.7%) greater in the INT-Stand trial than the SIT trial ($p<0.01$), with a large effect size, but the 2.5 ± 2.2 g (7.6 ± 5.4%) difference in fat oxidation between the PRO-Stand and SIT trials was not statistically significant and the effect size was small. Total fat oxidation was 4.6 ± 2.6 g (13.7 ± 7.6%) greater in the INT-Stand trial than the PRO-Stand trial ($p=0.06$), with a large effect size. Compared to the SIT trial, total carbohydrate oxidation was 14.4 ± 5.2 g (30.8 ± 12.6%) higher in the PRO-Stand trial ($p = 0.038$) and 22.0 ± 6.0 g (44.0 ± 12.8%) higher in the INT-Stand trial ($p = 0.008$). The difference in carbohydrate oxidation between the INT-Stand and PRO-Stand trials (7.6 ± 7.8 g; 15 ± 12.4%) was not statistically significant and had a small effect size.

In *post-hoc* observations, it became apparent that the pattern of substrate utilization between trials differed between the post-breakfast (0-240 minute) and post-lunch (240-480 minute) postprandial observation periods. We therefore decided to analyse these periods separately. In the post-breakfast period 19.6 ± 1.5 g, 20.1 ± 1.5 g, and 25.0 ± 1.8 g of fat were oxidised in the SIT, PRO-Stand and INT-Stand trials, respectively. Fat oxidation over this period was significantly higher in the INT-Stand trial than the other two trials ($p < 0.001$ for both), but did not differ significantly between the SIT and PRO-Stand trials ($p = 0.68$). In contrast, fat oxidation over the post-lunch period did not differ significantly between any of the trials (SIT: 18.9 ± 1.4 g; PRO-Stand: 20.8 ± 1.8 g; INT-Stand: 20.5 ± 1.4 g).

In the post-breakfast period, carbohydrate oxidation was significantly higher than SIT (31.2 ± 3.2 g) in the PRO-Stand stand trial (41.0 ± 3.3 g) ($p = 0.007$) and tended to be higher than SIT in the INT-Stand trial (38.0 ± 2.7 g) ($p = 0.055$), but did not differ significantly between the PRO-Stand and INT-Stand trials ($p = 0.36$). Carbohydrate oxidation was significantly higher in the INT-Stand trial (48.1 ± 3.6 g) than both the SIT trial (32.8 ± 3.1 g) ($p = 0.002$)

and the PRO-Stand trial (37.4 ± 3.4 g) ($p = 0.02$) but did not differ significantly between the SIT and PRO-Stand trials ($p = 0.30$). Thus, the increment in energy expenditure in the INT-Stand trial over the PRO-Stand trial was largely mediated by an increase in fat oxidation in the post-breakfast period and an increase in carbohydrate oxidation in the post-lunch period.

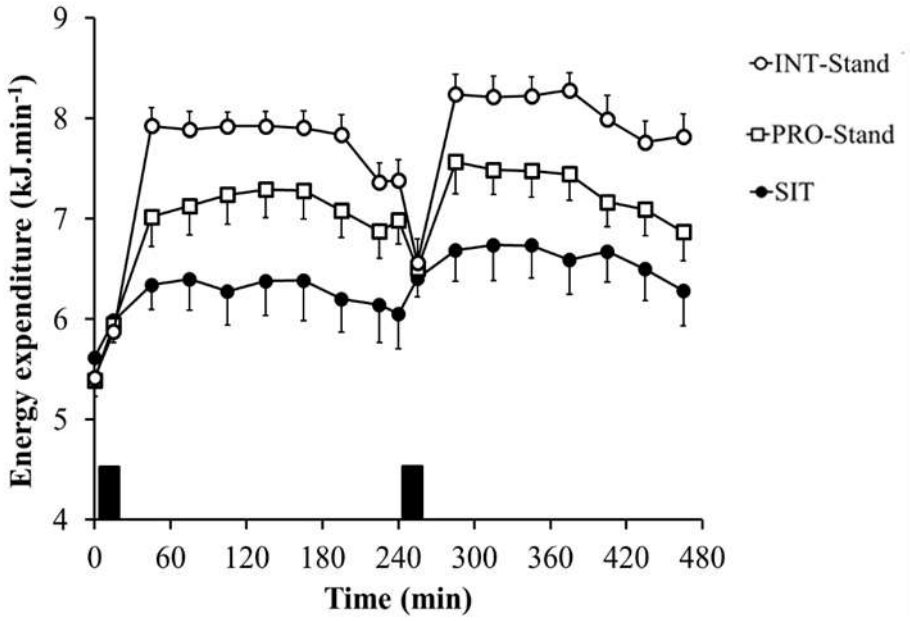


Figure 3-3: Energy expenditure over the 8-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

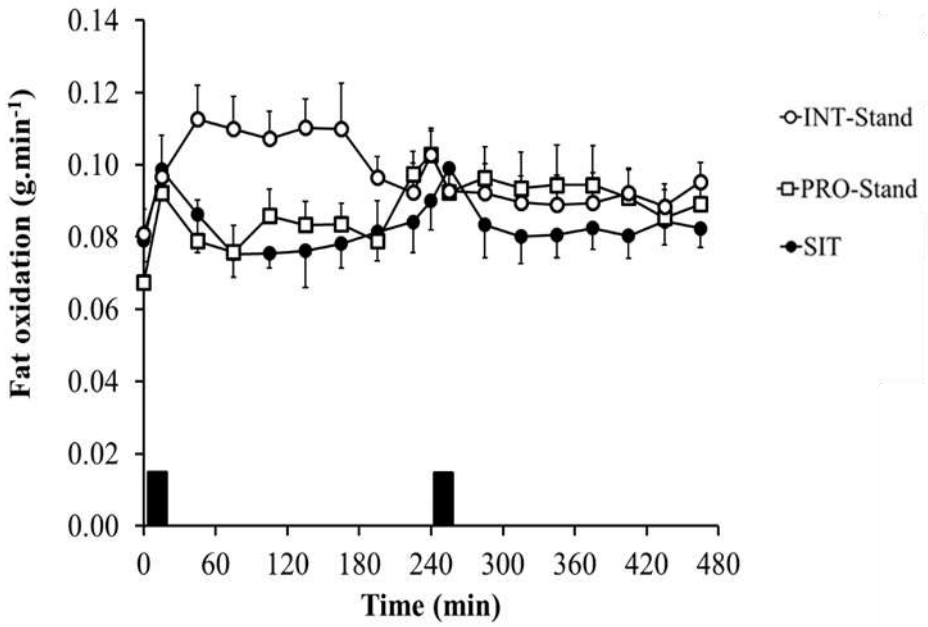


Figure 3-4: Fat oxidation over the 8-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

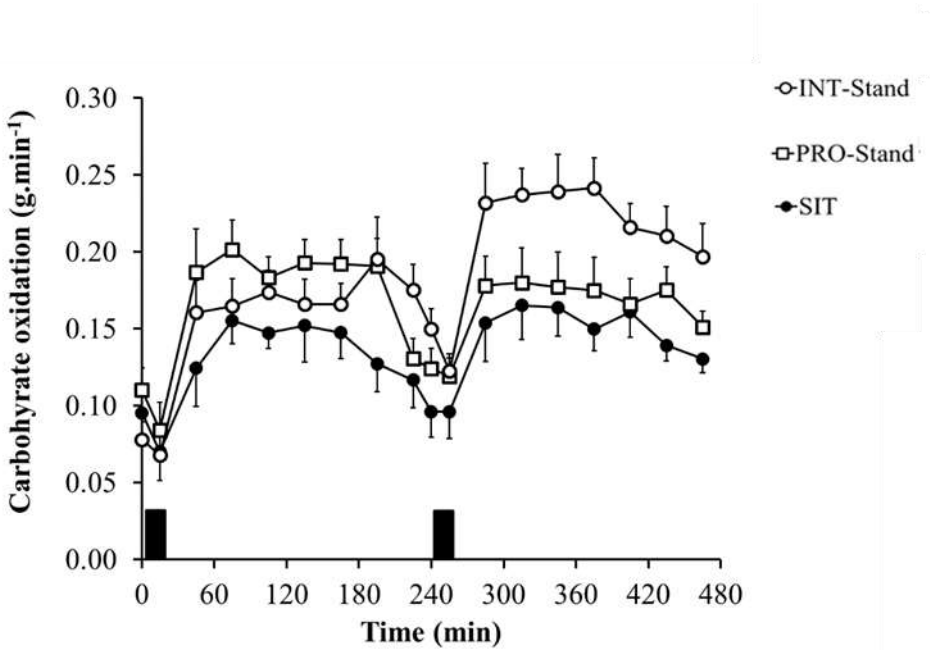


Figure 3-5: Carbohydrate oxidation over the 8-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

Table 3-2: Summary postprandial responses over the 8-hour postprandial observation period in the three experimental conditions.

Values are mean ± SEM, n = 10. $^{\#}$p=0.06, *p < 0.05, **p<0.01, ***p<0.001.

	SIT	**PRO-Stand**	**INT-Stand**	**SIT *vs* PRO-Stand**	**SIT *vs* INT-Stand**	**PRO-Stand *vs* INT-Stand**
	Mean ± SEM			*Effect size*		
Total energy expenditure (kJ)	2980 ± 78	3301 ± 112	3597 ± 139	1.64***	2.56***	1.19***
Total fat oxidation (g)	38.4 ± 2.7	40.9 ± 2.9	45.5 ± 3.0	0.36	1.19**	0.54$^{\#}$
Total carbohydrate oxidation (g)	64.1 ± 5.9	78.4 ± 5.6	86.1 ± 5.5	0.87*	1.17**	0.31

3.3.3 Blood glucose, insulin and TG responses during the interventions

Blood glucose, insulin and TG responses over the 8-hour observation period are shown in **Figure 3-5, 3-6 and 3-7**, with summary data for these responses shown in **Table 3-3.** There were no significant differences between the three trials in glucose, insulin and TG responses. The effect sizes for the differences between trials in the insulin and TG responses were trivial to small. Although not statistically significant, a medium effect size was observed when comparing the glucose response in the PRO-Stand trial with the SIT trial ($p = 0.16$) and the INT-Stand trial ($p = 0.48$).

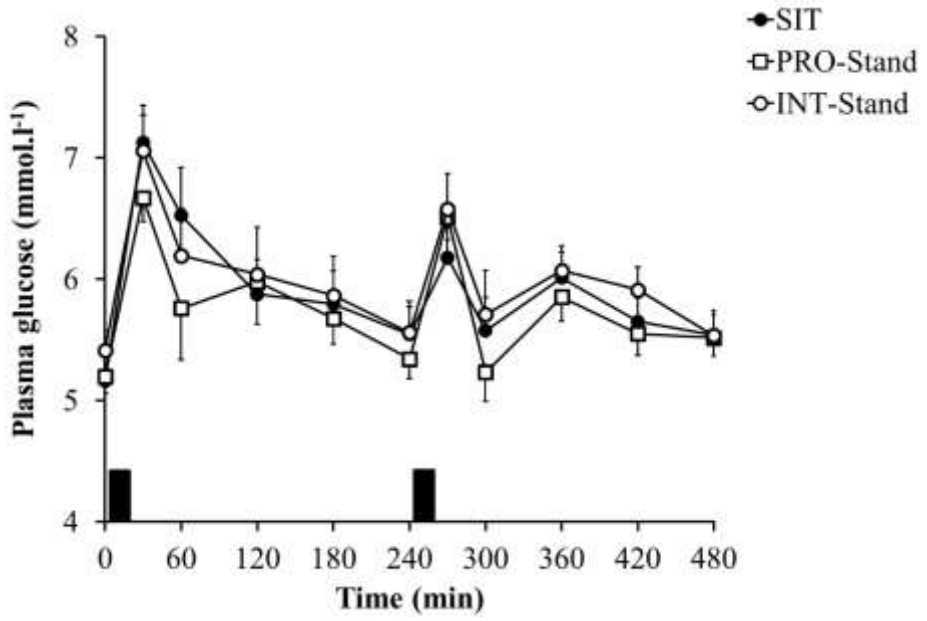


Figure 3-6: Glucose responses over the 8-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

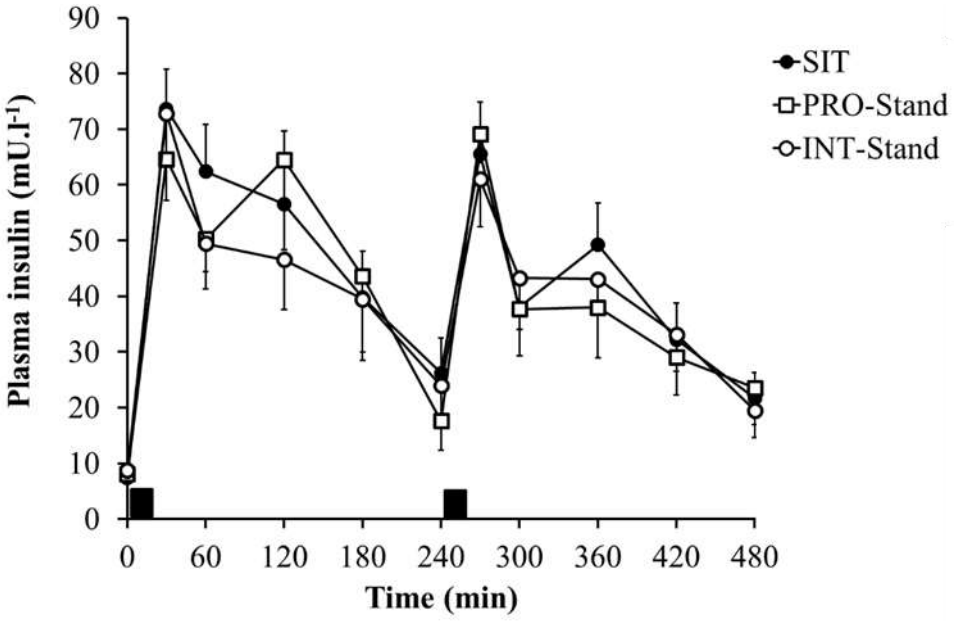


Figure 3-7: Insulin responses over the 8-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

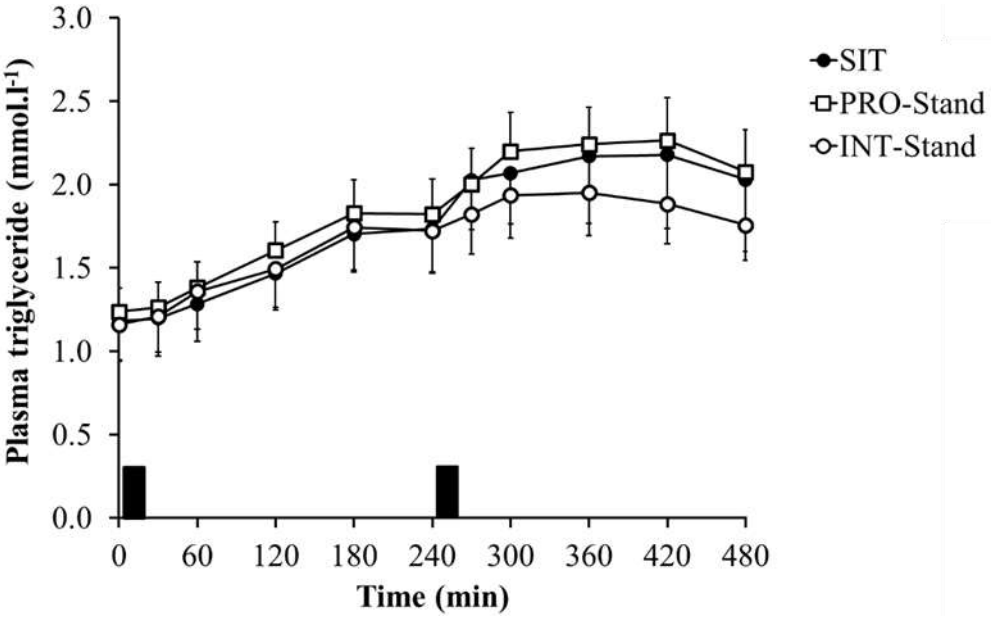


Figure 3-8: Triglyceride responses over the 8-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

Table 3-3: Time-averaged concentrations - AUC/ over the 8-hour postprandial observation period in the three experimental conditions.

Values are mean ± SEM, n = 10. [a]statistics performed on log-transformed data.

	SIT	**PRO-Stand**	**INT-Stand**	**SIT *vs* PRO-Stand**	**SIT *vs* INT-Stand**	**PRO-Stand *vs* INT-Stand**
	Mean ± SEM			*Effect size*		
Plasma glucose AUC ($mmol.l^{-1}$)	5.9 ± 0.2	5.7 ± 0.2	5.9 ± 0.2	0.78	-0.19	-0.61
Plasma insulin AUC ($mU.l^{-1}$)	44.4 ± 5.9	41.9 ± 7.5	41.1 ± 5.7	0.23	0.24	0.06
Plasma TG AUC[a] ($mmol.l^{-1}$)	1.8 ± 0.3	1.9 ± 0.2	1.7 ± 0.2	-0.17	0.23	0.38

3.4 Discussion

The main finding of this study is that increasing the frequency of breaks in sedentary time, while keeping total sedentary time constant, increased energy expenditure and fat oxidation over an 8-hour postprandial observation period. This is the first time that an independent effect of the number of sedentary breaks on day-long metabolic responses has been demonstrated and these findings provide an explanation for the association between frequency of sedentary breaks and adiposity observed in the epidemiological data (Cooper *et al.* 2012; Healy *et al.* 2008a; Healy *et al.* 2011).

A number of studies have reported that energy expenditure during quiet standing is 2-33% higher than observed during sitting (Judice *et al.* 2015a; Levine *et al.* 2000; Reiff *et al.* 2012; Speck and Schmitz 2011). The present findings are consistent with this. In the PRO-Stand condition – where participants alternated 15 minutes of sitting with 15 minutes of standing throughout the observation period – energy expenditure was 10.7% higher than the SIT condition, an absolute increase in expenditure of 320 kJ over 8 hours. In the INT-Stand condition – where participants undertook 10 1.5-minute bout of standing in every half-hour – there was a further increase in energy expenditure of 9.0% (296 kJ), despite participants sitting and standing for the same total duration in both trials. To put these figures into context, if participants replicated the protocol in the trial for 4 weeks, energy expenditure in the PRO-Stand and INT-Stand conditions would be 9.0 MJ and 17.3 MJ higher than the SIT condition. Assuming no change in energy intake, this would equate to ~1.2 kg weight loss, relative to SIT, in the PRO-Stand condition and a ~2.2 kg weight loss in the INT-Stand condition. Interestingly a large proportion of the increase in energy expenditure from increasing the frequency of sedentary breaks was in fat oxidation. Participant oxidised 7.1 g more fat and 7.7 g more carbohydrate in the INT-Stand compared with the PRO-stand trials, which equates to 277 kJ increased fat and 131 kJ increased carbohydrate oxidation in terms of energy. This disproportionate increase in fat oxidation with increasing sit-to-stand transitions may have implications for the long-term regulation of body weight as high levels of fat oxidation have been shown to be protective against long-term weight gain, independent of metabolic rate (Marra *et al.* 1998; Seidell *et al.* 1992; Zurlo *et al.* 1990).

The increased energy expenditure in the INT-Stand compared with the PRO-Stand condition, was likely mediated by the increased concentric and eccentric muscular activity

associated with the larger number of sit-to-stand transitions. A recent study by Judice and colleagues (Judice *et al.* 2015a) attempted to quantify the energy expended in sit-to-stand transitions per se by comparing the energy expended over 10 minutes when participants stood and sat down immediately once per minute for10 minutes with 10 minutes of sitting, reporting the energy cost of a single sit-to-stand transition was ~0.02 kJ per kg body mass. In the present study, participants stood for 4 hours and sat for 4 hours, with 16 sit-to-stand (and 16 stand-to-sit) transitions in the PRO-Stand condition and stood and sat for the same duration but with 160 sit-to-stand (and 160 stand-to-sit) transitions in the INT-Stand condition. Thus, the 296 kJ difference in energy expenditure represents the energy expended in 144 sit-to-stand/stand-to-sit transitions, i.e. ~2 kJ per transition or ~0.02 kJ per kg, in line with Judice et al's calculations. Thus the present findings suggest that the 'snapshot' calculation of the energy expended during short-duration sit-to-stand transitions in the fasted state, can be extended over the course of a day under 'real-life' postprandial conditions.

We found no significant effects of either prolonged or intermittent standing breaks on postprandial incremental glucose, insulin or TG responses. The effect sizes for the difference in incremental insulin and TG responses between trials were trivial to small. Thus the lack of a statistically significant effect of prolonged or intermittent standing on these responses appears to reflect the absence of a physiologically important influence of the standing interventions on these outcomes, rather than a lack of statistical power to detect a clinically relevant effect. The postprandial glucose response was ~3% lower in the PRO-Stand trial, but ~1% *higher* in the INT-Stand, than the SIT trial. Neither of these differences were statistically significant, but there was a medium effect size for the difference between the PRO-Stand and SIT conditions, suggesting that this difference could conceivably be physiologically relevant, but that the study did not have sufficient statistical power to detect it. However, while we cannot definitively exclude a potential glucose-lowering effect of PRO-Stand – albeit a relatively modest one – it is intriguing that a similar pattern was not observed for INT-Stand, where the glucose response was not lower than the SIT condition. This could conceivably be a consequence of the concentric and eccentric muscular activity associated with the repeated sit-to-stand and stand-to-sit transitions in INT-Stand condition, which are essentially equivalent to performing 160 bodyweight squats over the observation period. Thus, the INT-Stand condition could be considered analogous to a session of resistance exercise spread over a number of hours. While resistance exercise training programmes have been shown to improve insulin sensitivity and reduce glucose concentrations over the long-term, particularly in people with type 2 diabetes (Ishiguro *et al.*

2016), there is evidence of a transient increase in plasma glucose concentrations in response to resistance exercise (Fatouros *et al.* 2009; Kraemer *et al.* 2004). Thus, it is conceivable that an acute muscle contraction-mediated glucose-raising effect could have offset any potential glucose-lowering effect of standing *per se* in the INT-Stand condition. Further work is therefore needed to confirm whether this hypothesis is correct and, importantly, to determine whether over the longer-term, adaptations in skeletal muscle in response to such repeated contractions could elicit favourable effects of high frequency breaks in sedentary behaviour on glucose metabolism.

A number of previous reports have demonstrated that breaking up continuous sitting time with ≤3-minute intervals of light or moderate intensity physical activity every 20-30 minutes can reduce postprandial glucose, insulin and TG concentrations (Dunstan *et al.* 2012b; Larsen *et al.* 2015; Miyashita *et al.* 2008; Peddie *et al.* 2013). Studies evaluating the effects of breaking up sitting with static standing on these postprandial blood responses have had more mixed results. Henson and colleagues (Henson *et al.* 2016) recently reported that in postmenopausal women (mean age 66 years) with impaired glucose regulation, breaking up sitting time with 5 minutes of quiet standing every 30 minutes over a 7.5-hour postprandial observation period reduced the glucose and insulin incremental AUCs by 34% and 20%, respectively, with no significant effect on the postprandial TG response. In an intervention by Thorp and colleagues (Thorp et al. 2014b), in which overweight/obese middle-aged participants (mean age 48 years) performed normal work tasks over an 8-hour workday either seated or alternating 30 minutes of sitting and 30 minutes of standing using a sit-to-stand workstation, the incremental glucose response was 11% lower in the sit-to-stand condition, but there was no significant effect of the intervention on insulin or TG responses. In contrast, Bailey and Locke (Bailey and Locke 2015) recently reported that in young (mean age 24 years) non-obese adults, breaking up prolonged sitting with 2 minutes of standing every 20 minutes had no effect on postprandial glucose or TG responses over a 5-hour period, but breaking up sitting with 2 minute breaks of light ambulation (3.2 km/h walking) every 20 minutes reduced glucose (but not TG) responses by ~16%. In the present study we found no significant effects of either prolonged or intermittent standing breaks on postprandial incremental glucose, insulin or TG responses in our group of relatively young (mean age 33 years), overweight/obese, normoglycaemic men, although we could not definitely exclude a modest potential glucose-lowering effect in the PRO-Stand condition. Thus, no intervention study has observed a statistically significant acute effect of standing on postprandial insulin or TG concentrations in normoglycemic adults – in contrast to the

findings of studies where sitting was broken up by light to moderate physical activity (Dunstan *et al.* 2012b; Larsen *et al.* 2015; Miyashita *et al.* 2008; Peddie *et al.* 2013) suggesting that a greater stimulus than standing is needed to positively alter these responses in young to middle-aged adults without pre-existing dysglycaemia. Observational data from AusDiab study of middle-aged and older adults (mean age 57.9 years) reported that reallocation of 2 hours of sitting with 2 hours of standing per day was associated with ~2% lower fasting glucose and ~11% lower fasting TG concentration (Healy *et al.* 2015). While the causality and direction of these associations cannot be confirmed from such a cross-sectional analysis, these data do raise the possibility that metabolic benefits of standing may be more clearly observed in interventions undertaken in an older population. Further study is therefore needed to determine i) whether interventions to replace sitting with standing improve postprandial glucose, insulin and TG metabolism in older individuals, and ii) whether interventions to increasing the frequency of interruptions to sitting might enhance the previously reported benefits of standing breaks on postprandial glucose, insulin and TG metabolism in those with glucose dysregulation (Henson *et al.* 2016).

3.5 Conclusion

In conclusion, this study was designed to determine whether, in principle, the number of transitions between sitting and standing could influence postprandial metabolic responses independent of total time spent sitting and standing. Our data clearly indicate that the frequency of interruptions to sedentary time has a marked independent influence on metabolic rate, which is likely due to the increased energy expended due to muscular contractions in the sit-to-stand and stand-to-sit transitions. Each additional sit-to-stand transition cycle expended ~2 kJ energy, which can help explain the epidemiological observation between sedentary breaks and adiposity (Cooper *et al.* 2012; Healy *et al.* 2008a; Healy *et al.* 2011). While our INT-Stand protocol, with 20 sit-to-stand transition cycles per hour is clearly impractical to implement in 'real world' settings, these findings can help inform the design of practical interventions to reduce sedentary behaviour. For example, performing 4 sit-to-stand transition cycles per hour (i.e. standing then sitting once every 15 minutes) over the course of the waking day would lead to ~100-120 kJ of additional daily energy expenditure over and above the increment in metabolic rate elicited by standing *per se*. We found no evidence that standing, either in prolonged bouts or intermittent bouts could influence postprandial insulin or TG responses in these normoglycaemic participants (although we cannot definitively exclude a potential modest glucose lowering effect of

prolonged standing from the present data) suggesting that it may be necessary to break up sitting with activities of greater intensity than quiet standing to positively influence postprandial metabolism in relatively young, normoglycaemic overweight/obese men.

4. Effects of breaking up sedentary time with `sit/stand` on postprandial metabolism

4.1 Introduction

There is a growing body of epidemiological evidence that high levels of sedentary behaviour (defined as non-sleeping activities in a sitting or reclining posture with energy expenditure ≤1.5 METS (where 1 MET is resting energy expenditure) (Sedentary Behaviour Research Network 2012) are associated with adverse cardio-metabolic biomarker risk profiles and with increased risk of cardiovascular disease, diabetes, metabolic syndrome, obesity and death from any cause, with this effect often independent of time spent engaged in moderate-to-vigorous physical activity (MVPA) (> 3 METS), except when levels of physical activity are very high (Celis-Morales et al. 2012; Edwardson et al. 2012; Healy et al. 2011; Thorp et al. 2011; Wilmot et al. 2012). In addition, observational studies suggest that the *pattern* as well as total amount of sedentary behaviour may be important: it has been reported that individuals who regularly break up their periods of sedentary time have a more favourable cardio-metabolic risk profile, particularly with respect to adiposity-related variables, than those who habitually engage in prolonged periods of uninterrupted sedentary time, independent of total time spent sedentary (Cooper *et al.* 2012; Healy *et al.* 2008a; Healy *et al.* 2011).

Building on these observational findings, data presented in chapter 3 and recently published (Hawari *et al.* 2016) has demonstrated that breaking prolonged sitting with intermittent standing (10 x 1.5 minutes of standing per 30 minutes) had significantly greater effects on metabolic rate (21% vs 11% increase) and fat oxidation (18% vs 7% increase) than breaking up sitting with prolonged standing (1 x 15 minutes per 30 minutes) over an 8-hour observation period in 10 overweight men. Thus, these data provided proof-of-principle that frequency of sedentary breaks influences energy expenditure and substrate utilisation, independent of total time spent sedentary. This provides a potential explanation for the independent effect of frequency of sedentary breaks on indices of adiposity observed in large epidemiological studies (Cooper *et al.* 2012; Healy *et al.* 2008a; Healy *et al.* 2011). Although the intermittent protocol used in that study was clearly not feasible to implement as a practical intervention, it demonstrated that the number of transitions from sitting to standing

had effects on metabolism independent of the total time spent sitting or upright. The aim of the present study was therefore to build on this observation to determine whether, breaking up prolonged sedentary time by undertaking 'chair squats' – repeated sit-to-stand transitions over a short period (sitting and standing 10 times over 30 seconds, every 20 minutes) – provides measureable metabolic benefits. If so, this approach could conceivably be used as a practical intervention to improve metabolic health in individuals who are required to sit for long periods of time.

4.2 Methods

4.2.1 Participant.

Fourteen participants (11 men, 3 women), aged 37 ± 16 years, with body mass index (BMI) 30.5 ± 3.8 $kg.m^{-2}$, waist circumference 102.3 ± 10.7 cm [mean ± SD], and low levels of habitual physical activity (less than 2 hours per week of moderate-to-vigorous physical activity as assessed by the International Physical Activity Questionnaire), were recruited for this study though personal contacts and local advertising. Female participants were all post-menopausal. All participants had BMI > 25 $kg.m^{-2}$, were non-smokers, had no known history of CVD or diabetes (and fasting glucose < 6.0 $mmol.l^{-1}$ on screening), and were not taking any medications known to affect lipid or glucose metabolism. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the College of Medical, Veterinary and Life Sciences Research Ethics Committee at the University of Glasgow. All participants provided written informed consent.

4.2.2 Study design

Participants each completed two 6.5 hours experimental trials; (Sit) and (Sit/stand), in a randomised order, with an interval of 1 week between trials. The experimental protocol is shown in **Figure 4-1** and described below.

a) Uninterrupted sitting trial (sit) – Participants arrived at the metabolic investigation suite at the West Medical Building after 12-hours an overnight fast. Participants sat on a chair and rested for 10 min before two sequential 5-minute expired air samples were collected via a mouthpiece into a Douglas bag to calculate metabolic rate and substrate utilisation using indirect calorimetry (Frayn and Macdonald 1997). The average of these samples was used as the baseline value. A cannula was then inserted in an antecubital vein for repeated blood sampling and was kept patent by flushing with saline throughout the day.

A baseline fasting blood sample was drawn in K_2EDTA tube and placed immediately on ice. Further blood samples were taken at 30, 60, 120, 180, 210 minutes after breakfast (see section **2-7** for more details). Three and a half hours after breakfast, Participants were asked to consume a standardised lunch, which was identical to breakfast, and further blood samples were taken at 240, 270, 330 and 390 minutes after lunch. Expired air samples for the determination of metabolic rate and substrate utilisation were taken at ~10 minute intervals throughout the 6.5-hour observation period. Samples were collected into 100 L or 150 L Douglas bags while participants were fitted with a nose clip and 2-way respiratory value (see section **2-3** for more details). Participants sat comfortably (reading, watching TV, doing paperwork etc) throughout the observation period and were permitted to drink water throughout the day. Comfort breaks to the toilet (which was ~20m from the metabolic investigation suite) were permitted.

b) Sit/stand trial 'chair squats' – This trial was identical to the Sit trial, except that participants were asked to repeatedly sit and stand 10 times over 30 seconds, every 20 minutes, without using their arms to assist them, throughout the 6.5-hour observation period, (except when blood samples were taken or meals were consumed)**.** All details can be seen in **Appendix K.**

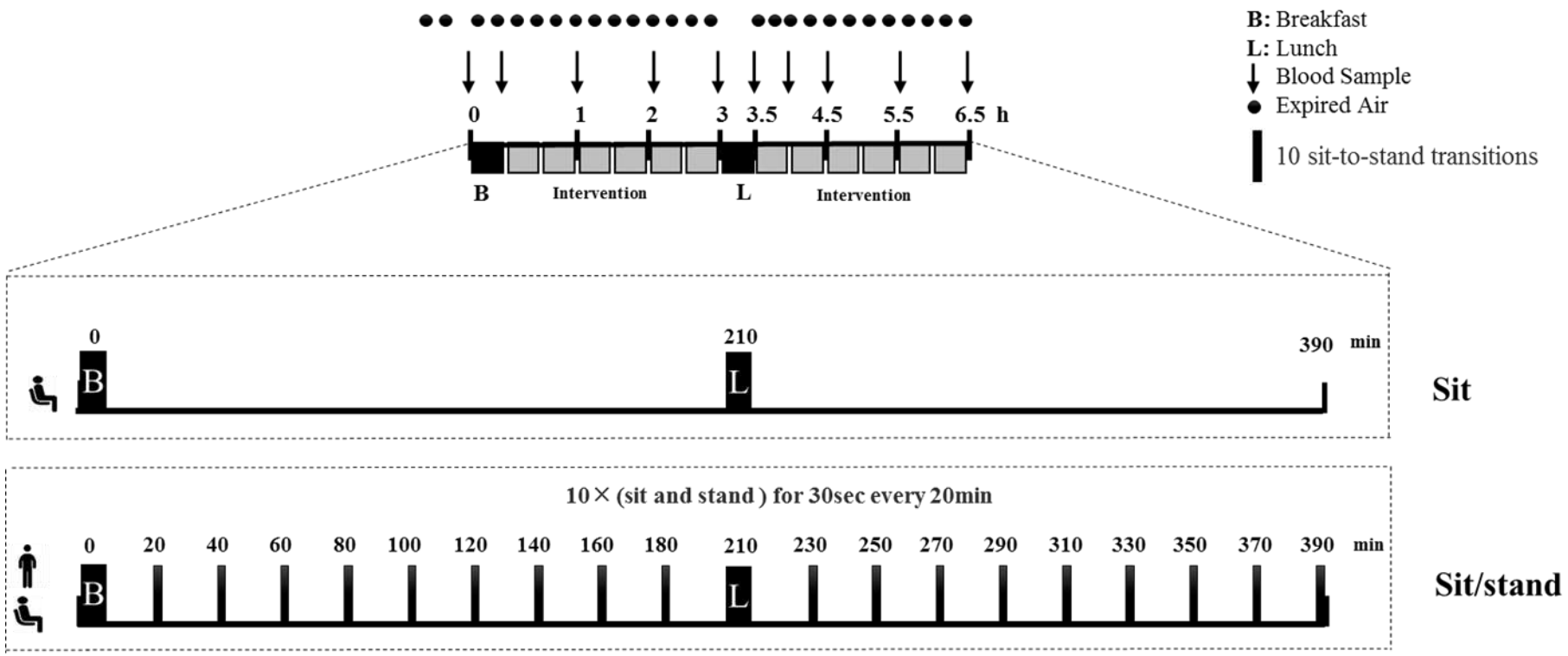


Figure 4-1: Study protocol. Participants completed two trials in random order: Uninterrupted sitting (Sit) and (Sit/stand). The grey boxes represent each 20- minute intervention period throughout the day.

4.2.3 Standardised Meals

Participants consumed two standardised meals for breakfast and lunch. Each meal consisted of a buttered bagel and a meal replacement drink (Complan Foods Ltd, UK) made up with whole milk. The meal was designed to provide 8 kcal.kg^{-1} of body mass with 37 % of energy from fat, 49 % from carbohydrates and 14 % from protein. Participants were asked to consume the meal within 10 minutes.

4.2.4 Standardisation of diet and exercise

Standardisation of diet and exercise have previously been described in (Chapter **2-5**). Sitting, standing, walking and other types of physical activity were monitored using the ActivPAL. Participants were instructed to wear the monitors on the right thigh all times, except when showering, swimming and sleeping for 3 days before and during each trial (Chapter **2-6**). Total time recorded as sitting, standing and steps for the preceding 3-d period before observation day is clarified in **Figure 4-2** Data output is expressed as mean time (hours) spent in various level of activity. In this study, a 3 days before each trials were considered to explore the difference in the activity between the participants. Statistical analyses and calculations were conducted using the minitab software version and Microsoft Office Excel 2010. Data were tested for the normality. All data were normal distributed using. There were no significant differences in any activity between both trials in steps, sitting and standing, p= 0.65, p= 0.91 and p= 0.90 respectively.

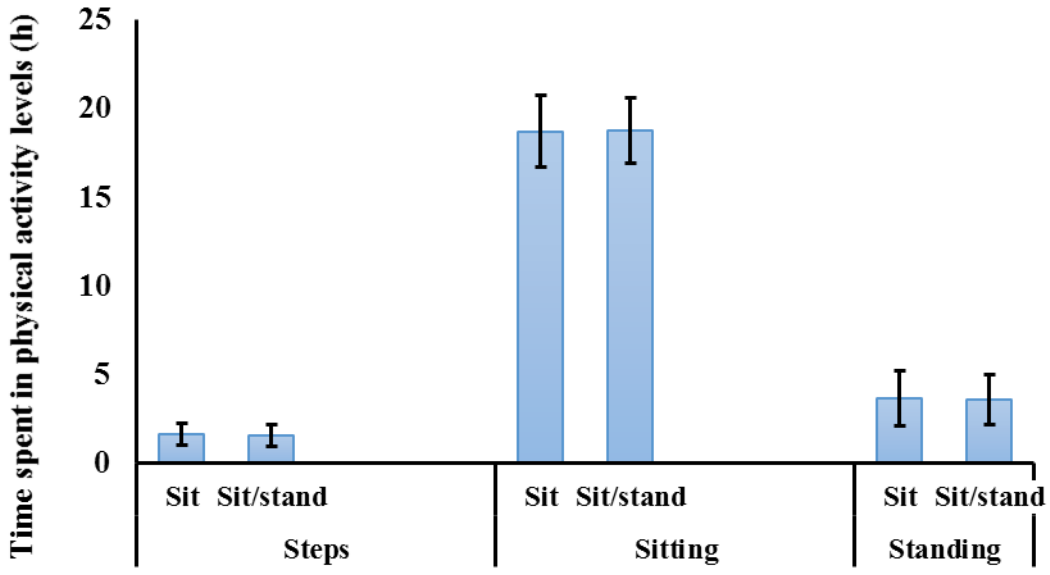


Figure 4-2: Mean time spent in various physical activity levels during the preceding 3-d period in sitting and sit/stand trials (n=14). Values are expressed as means, with standard errors represented by vertical bars.

4.2.5 Calculations of Energy expenditure and substrate utilisation

Fat, carbohydrate oxidation and energy expenditure were calculated using indirect calorimetry (Frayn and Macdonald 1997) (Chapter **2.3**). For these calculations urinary nitrogen excretion was assumed to be 0.11 $mg.kg^{-1}.min^{-1}$ throughout each trial, based on data from previous studies in the literature (Flatt *et al.* 1985; Melanson *et al.* 2005). The first expired air samples were taken twice after 10 min period to determine the metabolic rate. Gas samples were collected for 8 minutes of each 10 min bout of sitting trial and 6 times gas collection every 10 minutes in sit/stand trial throughout the 6.5 hours observation period.

4.2.6 Power calculation

As the most consistent association between frequency of sedentary breaks and health outcomes related to adiposity variables (Cooper *et al.* 2012; Healy *et al.* 2008a; Healy *et al.* 2011), we primarily based our sample size on the number of participants needed to detect a difference in overall energy expenditure over the observation period. Previous data from our lab had shown that the within-person SD for difference in resting oxygen uptake was 6.1 % (Farah and Gill 2013). We assumed that the within-person SD for differences in energy expenditure between trials here would be similar. Accordingly, we calculated that ten participants would enable detection of a ~ 6 % difference in energy expenditure between trials with 80 % power at $p < 0.05$. Based on the previous chapter, where differences between prolonged standing and intermittent standing for EE was 9 %. It would provide sufficient power to detect the likely differences between trials. In addition, based on earlier observations that the within-person SD for postprandial glucose, TG and insulin responses were 3.4 %, 10.1 % and 22.9 %, respectively (Gill *et al.* 2005), this sample size would enable detection of respective differences between trials of ~3 %, ~10 % and ~23 %, in glucose, TG and insulin responses.

4.2.7 Statistical analysis

Statistical analyses and calculations were performed using Minitab (Version 14, Mintab Inc.) and Microsoft® Office Excel 2013. Data were tested for normality using the Anderson-Darling normality test, data were logarithmically transformed prior to statistical analysis. The area under curve (AUC), calculated using the trapezium rule was used as a summary measure of the postprandial responses for energy expenditure, fat oxidation and carbohydrate oxidation. This provides a measure of total amount of energy expended or substrate used over the observation period. For glucose, insulin and TG concentrations, the

time-averaged AUC (i.e. AUC divided by the duration of the observation period) was used as a summary measure. This provides a measure of the average concentration over the observation period. AUC was calculated separately for the post-breakfast (0 to 180 mins) and post-lunch (210-390 mins) as well as the overall observation period. Comparisons of summary measures between trials were made by paired t-test. Where appropriate (i.e. when differences were observed in baseline values between conditions) statistical analyses of postprandial responses were adjusted for fasting values. Cohen's d effect sizes were calculated to describe the magnitude of differences between trials (>0.8 large, 0.5-0.8 medium, <0.5 small, <0.2 trivial) (Cohen 1992). Data are presented as mean ± SEM unless otherwise stated, and $p < 0.05$ was considered significant.

4.2.8 Baseline values

Baseline values in the two trials are shown in **Table 4-1**. There were no differences in body mass, fat oxidation or carbohydrate oxidation, or plasma glucose, insulin or TG concentrations between experimental conditions in the fasted state, before the interventions were commenced, but baseline energy expenditure was ~7% higher in the sit/stand trial than the sit trial.

Table 4-1:Baseline values in the fasted state in the two experimental conditions.
Values are mean ± SEM, n = 14. There were no significant differences in any variable between trials.

	sit	sit/stand	*p*	Cohen`s d effet size
Body mass (kg)	92.5 ± 3.8	92.5 ± 3.8	0.93	0.002
Energy expenditure ($kJ.min^{-1}$)	5.40 ± 0.2	5.85 ± 0.3	0.01	0.22
Fat oxidation ($g.min^{-1}$)	0.1 ± 0.006	0.1 ± 0.005	0.18	0.22
Carbohydrate oxidation ($g.min^{-1}$)	0.1 ± 0.01	0.1 ± 0.01	0.89	0.01
Plasma glucose ($mmol.l^{-1}$)	4.9 ± 0.1	5.0 ± 0.1	0.47	0.09
Plasma insulin ($mU.l^{-1}$)	12.0 ± 1.1	12.6 ± 1.4	0.52	0.10
Plasma TG ($mmol.l^{-1}$)	1.3 ± 0.1	1.2 ± 0.1	0.46	0.02

4.2.9 Energy Expenditure and substrate utilisation during the interventions

Energy expenditure and substrate utilisation over the 6.5-hour observation period are shown in **Figures 4-2, 4-3 and 4-4**, with summary data for these responses shown in **Table 4-2**. Compared to the sit trial total energy expenditure over the 6.5 hours was 410 ± 42 kJ (16.6 ± 1.7%) higher in the sit/stand trial ($p < 0.0001$). This difference remained statistically significant after adjustment for baseline energy expenditure (p = 0.0007). The Cohen's d effect sizes for this difference was 2.55, a large effect. Total carbohydrate oxidation was 21.0 ± 4.5 g (33.9 ± 8.2 %) higher in the sit/stand trial than the sit trial ($p < 0.0005$), and had large effect size 1.17; the difference in total fat oxidation between trial over the 6.5-hour observation period was not statistically significant 2.2 ± 1.3 g (9.7 ± 5.3%) higher in sit/stand, p = 0.11). As we previously observed differences in the effects of standing on postprandial responses in post breakfast and post-lunch observation periods (Hawari, 2016), we decided to analyse these periods separately. Energy expenditure over both the post-breakfast period (0 - 180 mins) (by 219 ± 21 kJ (19.9 ± 1.6%)) and post-lunch period (210 - 390 mins) (by 185 ± 21kJ (16 ± 2 %)) were significantly higher in the sit/stand than the sit trial ($p <0.0001$ for both). The Cohen's d effect sizes was 2.81 (post-breakfast period) and 2.35 (post-lunch period). Similarly, carbohydrate oxidation was higher in the sit/stand than the sit trial over both the post-breakfast (by 9.4 ± 2.2 g (44.1 ± 13.6 %)) and post-lunch (by 10.6 ± 2.3 g (31 ± 7.1 %)) periods (both $p < 0.001$) The Cohen's d effect sizes was 1.15 (post-breakfast period) and 1.22 (post-lunch period). Fat oxidation was higher in the Sit/stand trial than the Sit trial over the post-breakfast period (by 1.9 ± 0.7 g (15.9 ± 5.8 %), $p < 0.01$), but did not differ significantly between trials over the post-lunch period ($p = 0.48$) The Cohen's d effect sizes was 1.87 (post-breakfast period) and 0.20 (post-lunch period).

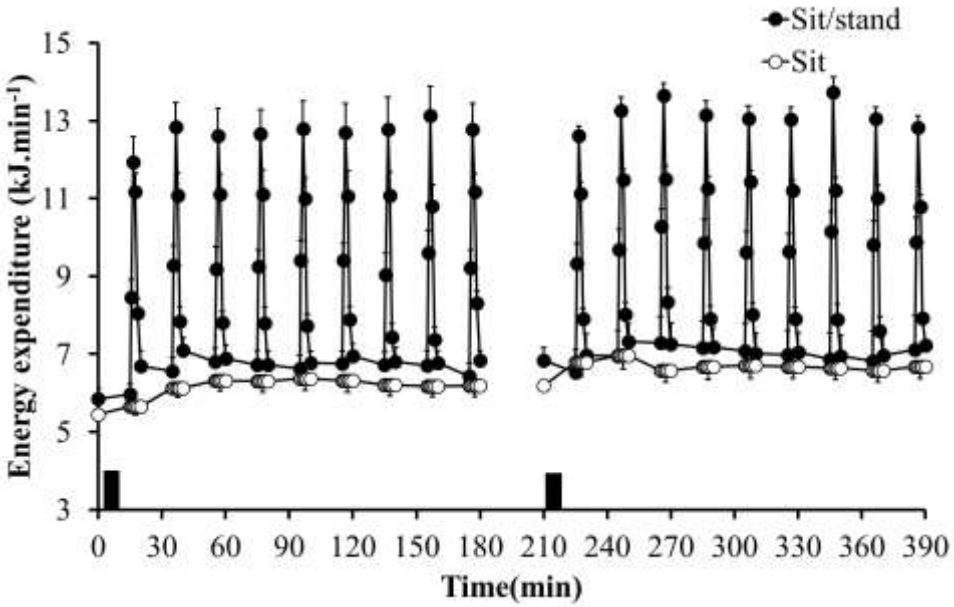


Figure 4-3: Energy expenditure over the 6.5 - h observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

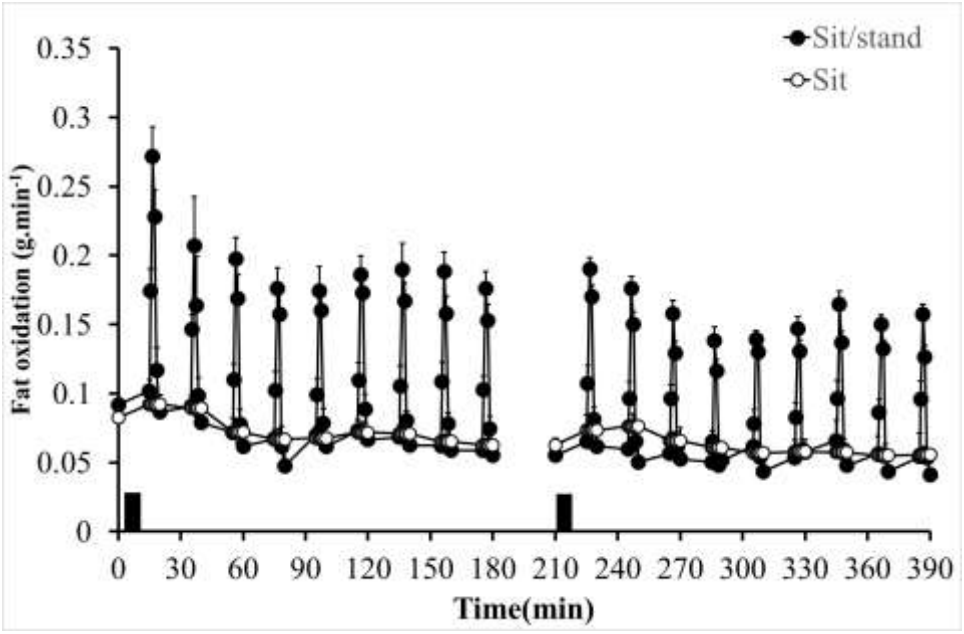


Figure 4-4: Fat Oxidation over the 6.5 - h observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

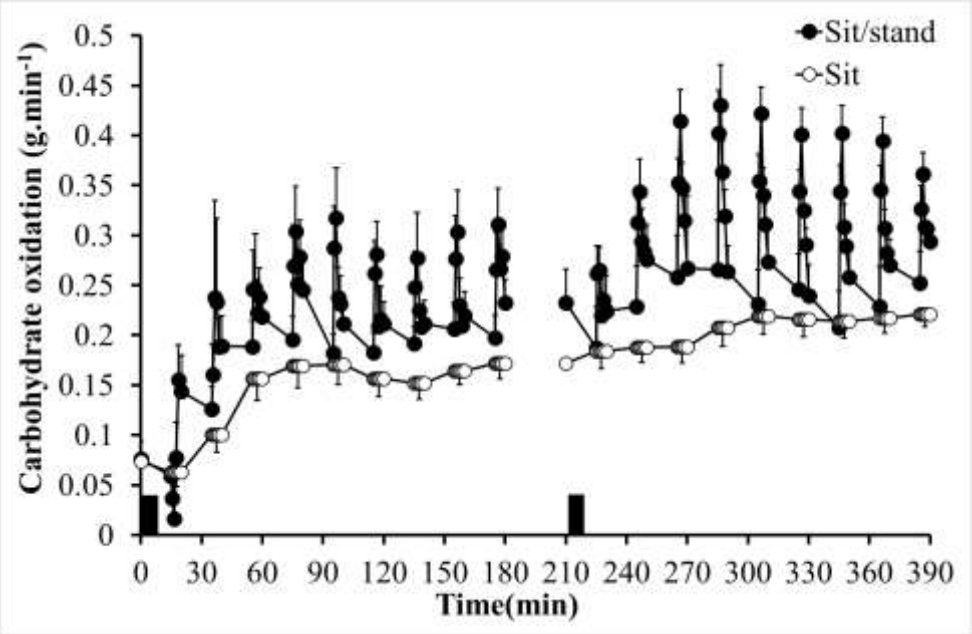


Figure 4-5: CHO Oxidation over the 6.5 - h observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch

Table 4-2: Summary postprandial responses for energy expenditure, fat oxidation and carbohydrate oxidation over the post-breakfast, post-lunch and overall 6.5h observation period in the Sit and Sit/stand conditions. Results are mean ± SEM, n=14. *P* value for the difference between means of the two trials.

	Post-Breakfast period (0-210 mins)			Post-Lunch period (210-390 mins)			Overall (0 to 390 mins)		
	Sit	Sit /stand	*p*	Sit	Sit /stand	*P*	Sit	Sit /stand	*P*
Total Energy Expenditure (kJ)	1105.9 ± 47.3	1325.3 ± 59.4	0.0001	1201.8 ± 51.5	1386.5 ± 57.0	0.00008	2502.5 ± 105.3	2912.3 ± 123.4	0.0001
Total Fat Oxidation (g)	13.4 ± 1.1	15.3 ± 1.3	0.01	11.3 ± 1.0	11.7 ± 1.1	0.48	26.7 ± 2.2	28.9 ± 2.4	0.113
Total CHO Oxidation (g)	25.1 ± 2.9	34.5 ± 3.6	0.001	36.6 ± 2.4	47.2 ± 3.2	0.001	67.1 ± 5.5	88 ± 7.1	0.0005

4.2.10 Blood glucose, insulin and TG responses during the interventions

Blood glucose, insulin and TG responses over the 6.5-hour observation period are shown in **Figures 4-5, 4-6 and 4-7** with summary responses shown in **Table 4-3.** Postprandial insulin concentrations over the post-breakfast period were 10.9 ± 8.4% lower in the sit/stand trial than the sit trial ($p = 0.047$), but the insulin response in the post-lunch period, or when taken over the overall 6.5 hour observation period did not differ significantly between the two trials. There were no significant differences between the two trials in glucose and TG responses.

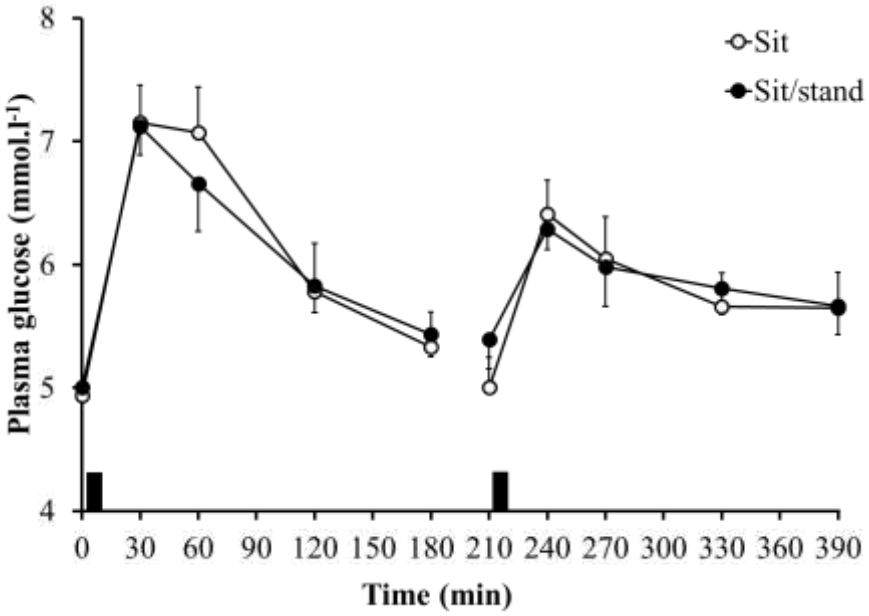


Figure 4-6: Plasma glucose, the Post breakfast and lunch over the 6.5 - h observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

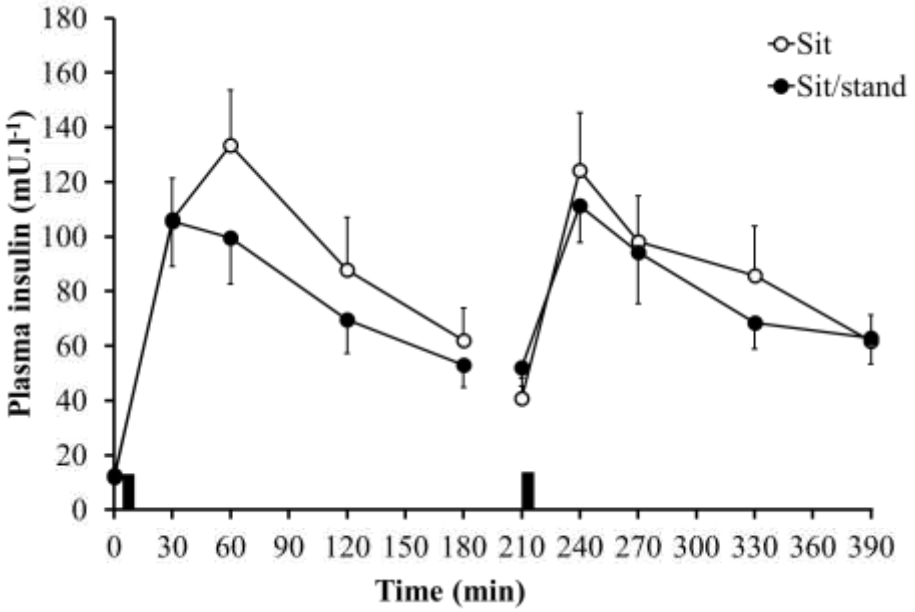


Figure 4-7: Insulin Concentration, the Post breakfast and lunch over the 6.5 - h observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

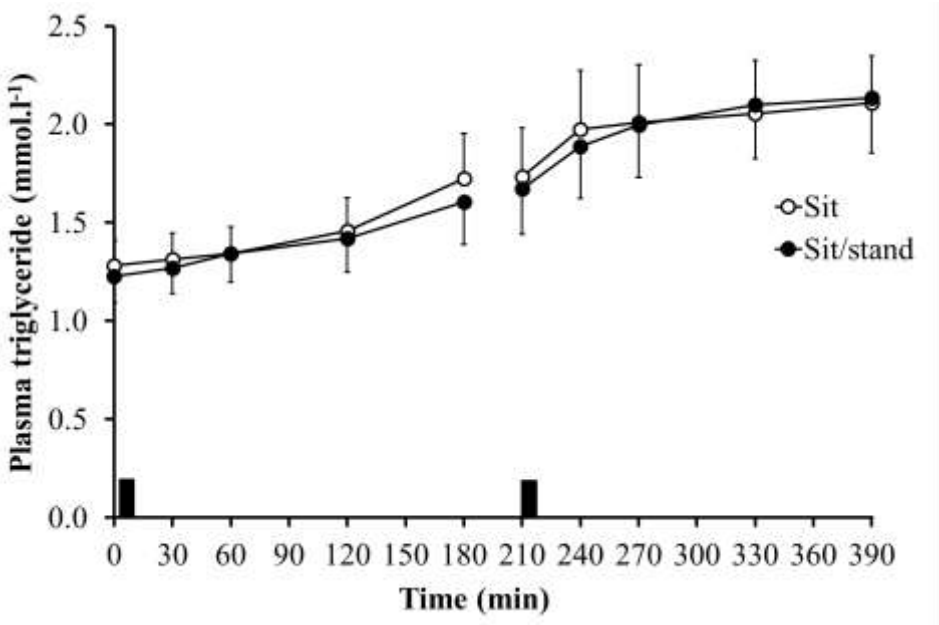


Figure 4-8: TG Concentration, the Post breakfast and lunch over the 6.5 - h observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

Table 4-3: Time-averaged concentrations – AUC/ postprandial responses over the post Breakfast, Lunch and overall 6.5h observation period in the two conditions. Results are mean ± SEM, n=14. *P* value for the difference between means of the two trials.

	Post-Breakfast period (0-210 mins)				Post-Lunch period (210-390 mins)				Overall (0 to 390 mins)			
	Sit	Sit /stand	*p*	Cohen`s d effect size	Sit	Sit /stand	*P*	Cohen`s d effect size	Sit	Sit /stand	*P*	Cohen`s d effect size
Plasma Glucose ($mmol.l^{-1}$)	6.2 ± 0.3	6.1 ± 0.2	0.72	0.10	5.8 ± 0.2	5.9 ± 0.2	0.75	0.09	5.9 ± 0.2	5.9 ± 0.2	0.94	0.05
Plasma Insulin ($mU.l^{-1}$)	91.0 ± 14.5	75.5 ± 10.9	0.047	0.58	87.5 ± 14.6	79.8 ± 11.0	0.21	0.35	86.1 ± 13.8	75.2 ± 10.1	0.10	0.38
Plasma TG ($mmol.l^{-1}$)	1.4 ± 0.2	1.4 ± 0.2	0.53	0.17	2.0 ± 0.3	2.0 ± 0.3	0.98	0.01	1.7 ± 0.2	1.7 ± 0.2	0.71	0.16

4.3 Discussion

The major finding of this study is that breaking up prolonged sedentary time with repeated 'chair squats' transitions for 30 seconds every 20 minutes significantly increased energy expenditure by 16.6% over a 6.5-hour observation period during which a test breakfast and test lunch were consumed. Over the 3 hours following breakfast, post-prandial fat oxidation was 15.9% higher and postprandial insulin concentrations were 10.9% lower, but these changes did not persist in to the post-lunch period. There were no differences between the two trials in postprandial glucose or insulin responses.

These findings build on the work presented in the previous chapter (Hawari *et al.* 2016), which we observed that intermittently standing for 1.5 minutes 10 times every 30 minutes led to 9% higher energy expenditure over an 8-hour postprandial period than standing continuously for 15 minutes every 30 minutes over the same time-frame. The difference between these conditions was the number of sit-to-stand transitions - there were 144 additional sit-to-stand transitions in the intermittent standing condition and 296 kJ additional energy was expended: from this it was possible to calculate that a sit-to-stand transition expended ~2 kJ of energy. The findings from the present study are consistent with this, energy expenditure was 410 kJ higher in the Sit/stand compared with the Sit condition and 180 additional sit-to-stand transitions were undertaken in the former – equivalent to 2.3 kJ energy expenditure per transition. Thus, the present data provide confirmation that the differences in energy expenditure between the two standing conditions in the previous chapter can be fully accounted for by the energy expended in the transition from sitting to standing and taken together these two chapters provide a robust estimation of energy expended in a sit-to-stand transition cycle. Interestingly in both the previous and present chapters, increasing the number of sit-to-stand transitions resulted in an increase in fat oxidation in the postprandial period following breakfast, but not following lunch, where the increase in energy expenditure was accounted for by an increase in carbohydrate oxidation. It is not immediately clear why this was the case, although the consistency to this observation across two different studies suggests that this effect is likely to be real. One potential factor is that the Sit/stand intervention had a larger attenuating effect on postprandial insulin concentrations in the post-breakfast period, which could conceivably have led to reduced suppression of fatty acid release from adipose tissue, increasing availability of fatty acids for oxidation over this time-frame.

There were no differences between trials in the postprandial glucose or TG responses, which is consistent with the observations from the study in Chapter 3, suggesting that the stimulus this volume of sit-to-stand transitions, with or without periods of standing between them, is insufficient to materially affect these aspects of the postprandial metabolic response in normoglycaemic adults. However, in contrast to the earlier observations where sit-to-stand transitions were separated by an interval of 1.5 minutes of standing (Hawari et al, 2016), postprandial insulin concentrations were lower in the post-breakfast period in the Sit/stand trial than the Sit trial, although this did not persist into the post-lunch period. This may reflect the increased frequency of the contractions stimulating contraction-mediated glucose uptake (Krook *et al.* 2004), thereby reducing the requirement for insulin to maintain glucose homeostasis. Indeed, the repeated sit-to-stand transitions over 30 seconds, in effect represents multiple sets of bodyweight squats over the course of the day. Interestingly, Dempsey and colleagues recently reported that breaking up prolonged sitting with 3 minutes of bodyweight resistance exercises every 30 minutes over a 7-hour postprandial observation period reduced postprandial glucose, insulin and TG concentrations in adults with type 2 diabetes (Dempsey *et al.* 2016). This more potent intervention effect in Dempsey's study may reflect two things. First, the volume of resistance exercise undertaken in that study (6 vs 1.5 mins per hour) was substantially higher than in the present study. It may well be that a larger volume of sit-to-stand transitions – for example 60 seconds of 'chair squats', rather than 30 seconds, every 20 minutes – may elicit more substantial effects on postprandial insulin, glucose and TG responses. Secondly, the participants in the present study were normoglycaemic and it may be the case that the stimulus required to positively affect postprandial metabolic responses may be greater in healthy normoglyaemic individuals than those with metabolic dysfunction where there is greater capacity for improvement. For example, lab-based interventions breaking up sitting with standing have been effective at reducing postprandial glucose and insulin concentrations in post-menopausal women with impaired glucose regulation (Henson *et al.* 2016), but this effect has not be replicated in similar interventions in younger, normoglycaemic individuals (Bailey and Locke 2015; Hawari *et al.* 2016; Miyashita *et al.* 2013). Thus, going forward, studies are needed i) to determine whether the present intervention is effective at reducing postprandial glucose, insulin and TG responses in individuals with impaired glucose regulation and ii) to determine whether the metabolic benefits observed here would be enhanced in normoglycaemic individuals with an increased 'dose' of 'chair squats' transitions.

The intervention undertaken in the present study is simple, requires no equipment and little space and only takes 1.5 minutes per hour. Thus, it should be readily implementable in real-world situations, for example, amongst office workers. It increased EE, together with the modest reductions in postprandial insulin concentrations, suggest that pragmatic, low volume, and interventions of this nature may have the potential to elicit benefits to metabolic health. Thus, the present findings provide a rationale for undertaking longer-term randomised controlled trials to determine whether interventions of this nature are acceptable to individuals and sustainable in practice and whether they induce long-term benefits to metabolic health.

This study does have some limitations. Firstly, although it had sufficient power to clearly detect an effect of the intervention on energy expenditure, with 14 participants, it may have been underpowered to detect clear effects on the postprandial insulin response in the post-lunch period. Secondly, we did not consider different doses of sit-to-stand transitions to determine the nature of the dose-response relationship. Further research is required to define whether effects can be generalised to other population such as the non-obese and patients with impaired glucose regulation or type 2 diabetes.

4.4 Conclusion

In conclusion, this study demonstrated that a simple, unobtrusive intervention of performing 10 ‘chair squats’ transitions over 30 seconds every 20 minutes over a 6.5-hour observation period increased energy expenditure by over 400 kJ, a 16.6% increase over prolonged sitting on normoglycaemic overweight and obese men and women. The intervention also reduced insulin concentrations in the post-prandial period following breakfast. Further study is needed to determine whether larger ‘doses’ would induce greater metabolic benefits and whether this approach can be translated into an effective longer-term intervention.

5. Development and validation of algorithms to objectively assess activity using an accelerometer/inclinometer device

5.1 Introduction

A large body of evidence has shown that physical activity (PA) associated with reduced risk of several illnesses such as cardiovascular disease (CVD) and diabetes (Gill and Cooper 2008; Nocon *et al.* 2008; Warburton *et al.* 2010). Conversely, high levels of sedentary behaviour are associated with increasing the risk of these adverse health conditions (Edwardson *et al.* 2012; Marshall and Ramirez 2011; Owen *et al.* 2010a; Wilmot *et al.* 2012). Most of the evidence evaluating the strength and dose-response relationship between physical activity and sedentary behaviour with prospective health outcomes – including the evidence underpinning guidelines for physical activity (Department of Health 2011; Haskell *et al.* 2007; World Health Orgnisation 2010) has been based on estimates of physical activity and sedentary behaviour from self-reported questionnaires. However, such questionnaire provide relatively crude markers of activity status (Craig *et al.* 2003; Hagstromer *et al.* 2006; Rosenberg *et al.* 2008; Shephard 2003; van Poppel *et al.* 2010), and this measurement error can lead to underestimation of the strength of the relationship between activity and disease risk (Celis-Morales *et al.* 2012). This highlights the need to accurately and objectively assess physical activity and sedentary behaviour. In recent years, accelerometers - small devices which, by measuring accelerations in one or three axes, can be used to detect motion changes - have been used to accurately quantify physical activity behaviours. Objective measurement of physical activity in this manner leads to stronger associations being observed between physical activity and biomarkers of cardio-metabolic disease risk (Celis-Morales *et al.* 2012). One such accelerometer device - the ActivPAL – is worn on the front of the thigh and thus by measuring changes in the axis through which the static acceleration due to gravity is felt, it is able to distinguish between sitting and upright postures. Other placement positions for accelerometers - often the hip, but increasingly the wrist, - are more commonly used for assessment of physical activity, but recent validation studies have demonstrated that these positions are inferior to the thigh for determination of sedentary behaviour (Edwardson *et al.* 2016; Koster *et al.* 2016; Lyden *et al.* 2012). However, ActivPAL's thigh-based

accelerometer position may also provide advantages for assessment of physical activity over other body locations, as accelerations at the thigh during locomotion are greater than other body locations such as the hip, so it is possible that low intensity incidental activities, such as very slow walking, may be more accurately determined in the thigh-based position. Currently, the ActivPAL generates an output of step counts based on a proprietary algorithm; however, it is possible to develop more sophisticated physical activity output metrics from acceleration signals generated by thigh movement. This would enable use of a single thigh-based accelerometer to be used for the robust and detailed assessment of both sedentary behaviour and physical activity. The aims of this study are therefore to compare thigh and hip positions for accelerometer placement for the measurement of step-based physical activity and to develop an algorithm for the estimation of walking or running speed and energy expenditure from acceleration outputs from a thigh-based accelerometer. Specific objectives of the study are:

1) To assess the reproducibility of stepping rate outputs for ActivPAL and Actigraph accelerometers in thigh and hip positions across a range of walking and running speeds by comparing outputs from devices worn on the left and right sides of the body.

2) To assess the accuracy of measurement of stepping rate of ActivPAL and Actigraph accelerometers in thigh and hip positions in comparison to directly observed stepping rate across a range of walking and running speeds.

3) To assess the reproducibility of vector magnitude acceleration outputs for ActivPAL and Actigraph accelerometers in thigh and hip positions across a range of walking and running speeds by comparing outputs from devices worn on the left and right sides of the body.

4) To determine the relationship between vector magnitude acceleration outputs and oxygen uptake for thigh- and hip-placed accelerometers across a range of walking and running speeds on a treadmill.

5) To compare the relationships between vector magnitude acceleration outputs for treadmill compared with overground walking and running across a range of walking and running speeds.

6) To use the information above to develop and validate algorithms to estimate oxygen uptake (and therefore metabolic exercise intensity) from vector magnitude acceleration outputs for thigh- and hip-placed accelerometers.

5.2 Methodology

5.2.1 Subjects

A total of 40 healthy adults (20 female), aged 26.6 ± 5.7 years, with body mass index (BMI) 23.43 ± 4.5 kg.m^{-2}, [mean ± SD], were recruited for this study though personal contacts and local advertising. All participants had no known history of CVD or uncontrolled hypertension (>160/95 mm Hg on anti-hypertensive medication), and did not having any conditions such as arthritis or injuries that alter gait and/or limit ability to walk or run on a treadmill. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the College of Medical, Veterinary and Life Sciences Research Ethics Committee at the University of Glasgow. All participants provided written informed consent.

5.2.2 Study Design

Forty participants completed a treadmill experimental trial. Moreover, to address aim number 4 in this study, 15 out of 40 participants completed both treadmill and overground trials, with an interval of at least three days.

Participants who completed treadmill and overground trials were asked to meet with the researchers on three occasions and two occasions for participants who completed treadmill trial. On the first occasion, participants attended the lab in west medical building for baseline screening. The study was explained in more detail and all the questions were answered. If the participant was still interested to take part she\ he was asked to complete a health screening questionnaire. Body measurements were taken including blood pressure, body mass and height, from which the body mass index (BMI) was calculated.15 participants performed two experimental trials – one involving walking and running on a treadmill (treadmill) and one involving walking and running on a (track) and 40 participants performed one experimental trial which involved walking and running on a treadmill (treadmill). For each trial, subject's wore ActivPAL devices at some locations on their body (lower thigh, upper thigh and hip, on the left and right sides). The subjects also wore Actigraph accelerometers on the right and left hips **Figure 5-1,** to record body accelerations and posture changes. The specific location of these devices were described as listed:

Lower Thigh: 10 cm above mid-line of the knee on the Lower Left and Right Thigh.

Upper Thigh: 20 cm above mid-line of the knee on the Upper Left and Right Thigh.

Hip: at the highest point of the iliac crest of the Left and Right Hip.

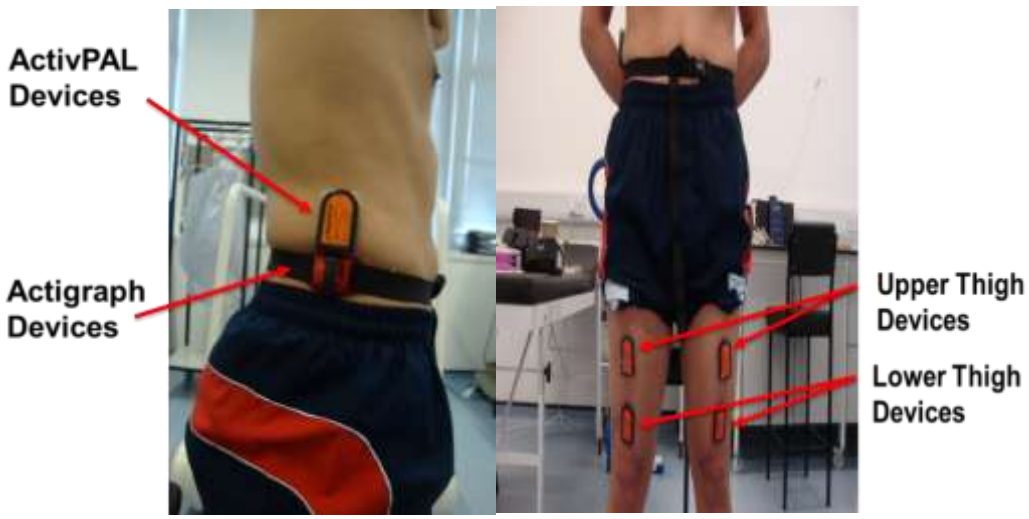


Figure 5-1: 6 (ActivPAL) and 2 (Actigraph) were attached on the body.

5.2.3 Treadmill trials

The subjects undertook the treadmill test in West Medical Building, University of Glasgow. For the trial, subjects initially sat for 10 minutes. Thereafter, 5 minutes standing; after which, they undertook 5 minutes-stages of walking on the treadmill at 1, 2, 3, 4, 5, 6, 7 and 8 km/h and running on the treadmill at 7, 8, 9, 10 and 11 km/h **Figure 5-2**. The treadmill sat at a 0% incline for the period of the testing. Expired air samples were collected during both sitting and standing; and 2 minutes gas collection for each speed between 3-5 minutes by using Douglas bags that were connected to a mouthpiece via a 2 way non-rebreathing valve and tubing to determine oxygen uptake **Figure 5-3.** Heart rate was measured by short-range telemetry. The trial was video-recorded using (Coolpix S6300, Nikon) to count the stepping for each speed and comparison with the values from ActivPAL and Actigraph devices. Subjects could have a break at any time for as long a period that they needed during the protocol. Subject was also able to stop testing at any time and had the decision to return on a different day to complete testing if he or she felt unable to carry out the entire protocol in one session. At the end of the test, a cool down period on the treadmill was carried out.

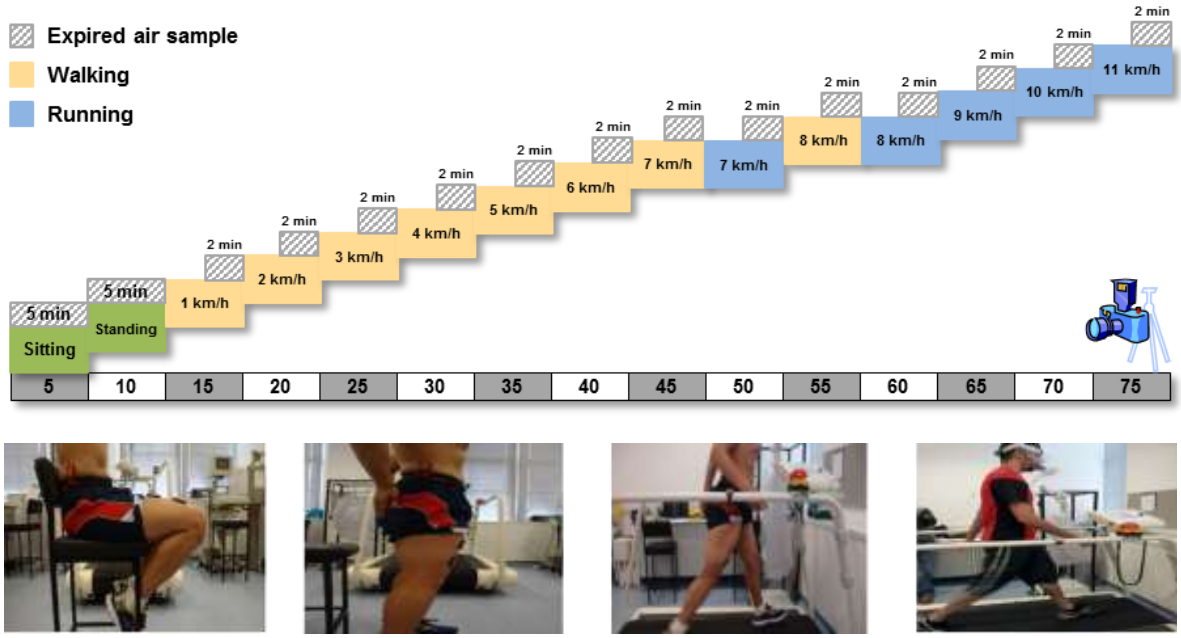


Figure 5-2: Study Design.

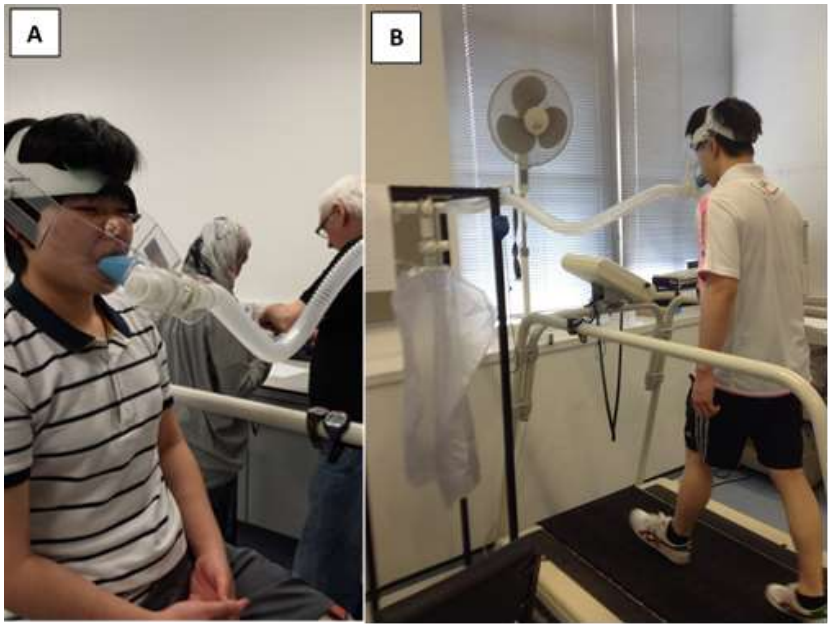


Figure 5-3: Oxygen consumption measurement during the experimental trial: a) sitting on a treadmill, b) walking on treadmill.

5.2.4 Gas Analysis, Heart Rate Monitoring and Step count

Expired air samples were collected during the treadmill protocol using Douglas bags. The fractional oxygen uptake (FeO_2%) and fractional carbon dioxide production ($FeCO_2$%), the volume and temperature of the expired air was measured using a Servomex Gas Purity

Analyser (Analyser Series 1400) and a Harvard Dry Gas Meter. These values were used to calculate the corresponding oxygen consumption $\dot{V}O_2$ and carbon dioxide production $\dot{V}CO_2$ values for each speed (Chapter **2-3**). Heart rate was monitored (Monitor FTI, Polar UK) and recorded throughout testing (Chapter **2-4**). The exercise testing was stopped when the heart rate exceeded 90% of the subject's predicted age–related maximum heart rate (220-age). All the speeds completed by the subject on the treadmill below the 90% heart rate cutoff point were matched in the track protocol. A camera (Coolpix S6300, Nikon) was set up on a tripod next to the treadmill and at the end of the track to record the feet of the subjects at each speed completed, allowing step count for each speed to be analysed post testing, using Movie Maker, version 2012(Build 16.43503.0728, Samsung) and a hand tally counter. Steps count as measured by each ActivPAL and Actigraph devices were also recorded.

5.2.5 Track trials

The track trial was performed at Scotstoun Stadium in Glasgow **Figure 5-4**. The testing protocol involved participants walking at 1, 2, 3, 4, 5, 6, 7 and 8 km/h and running at 7, 8, 9, 10 and 11 km/h round the indoor track for 100 m. Subjects were asked to walk and run three times for each speed. The aim of this trial was therefore to compare the relationships between raw accelerations and walking and running speeds for treadmill-based compared with overground walking and running. On one side of the track, every 1 metre interval was marked by a trundle wheel, using adhesive index tabs. Subjects required to achieve 40 m distances for walking speed, 1 km/h, 2 km/h, 3 km/h, 4 km/h, 5km/m, and 6 km/h. Each speed was done 3 times. A chair was placed at the end of this 40m. The subject sat on the chair before each time that achieved a distance.

Figure 5-4: Scotstoun Stadium Indoor Track.

With 7 km/h, 8 km/h walking and running at 7 km/h, 8 km/h, 9 km/h, 10 km/h and 11km/h, the chair was were then repositioned to use a 90m distance. In addition, the protocol was done as same as walking protocol. To ensure that participants were walking and running at required target speed, a spotter walked and ran beside the participant in the adjacent lane to reduce any differences. A flags and metronome were used to enable the subject to walk and run close to the target speed. Flags placed depend on which speed want to achieve and each beats of the metronome (Metronome Beats for Android devices, Version 2.2, Stonekick) the subject and spotter had to reach the flag **Figure 5-5**. One beat was equal 17 beat per minute (bpm). For example, to walk 1km/h, a flag was placed at every 1m mark and to walk at 2 km/h a flag was placed every 2 m marks, and so on. A stopwatch was using to record the time that took to complete each attempt that led to know the actual time of each speed of the subject to be calculated in case it changed from the target speed. The trials were being video-recorded to enable stepping rates to be counted after completion of the experiment and compared to calculated values from the ActivPAL and Actigraph devices. HR was monitored, using AG heart rate belt. Participants were able to have a rest between stages as required.

Figure 5-5: Track Protocol.

5.3 Data Analysis

5.3.1 Steps analysis

The activPAL software (PALTechnologies) classified data (i.e., sitting/lying, standing, stepping) by proprietary algorithms and it can be saved as csv files in numerous formats. A 15-s epoch summary file shows the number of seconds spent in various activities, number of steps and sit-to-upright transitions occurring during that 15-s time window over 24 hours. In the treadmill protocol, the average over the four 15s epochs in the third minute of stepping

activity in each five-min period was selected to calculate the number of steps/min for each speed. In the track protocol, the average of two 15s epochs acquired in each attempt, this was averaged for the three attempts to calculate the stepping rate.

The Actigraph software classified data (i.e., sitting, lying, standing, stepping, and non-wear) by proprietary algorithms and that were downloaded to a computer in the form of csv files. A summary file shows the number of seconds spent in various activities occurring during that 1-s time window over 24 hours. In the treadmill protocol, the third minute of stepping activity in the middle of the five-min period was selected for each speed. In the track protocol, the average stepping rate over each attempt was calculated, and the average of the three attempts was used to calculate the stepping rate.

Participants were video-recorded during the trials to enable their actual stepping rates to be determined and compared with the values recorded by the ActivPAL and Actigraph devices. For the treadmill trials, only the lower body of participants was videotaped, however, for the track trials, it is likely that some identifying shots was taken. The stepping time was compared to the direct observation data to examine the accuracy of stepping activity for the AP and AG. Video recordings were analysed by a researcher categorising time as sitting, standing and steeping and classified speed and steps taken. From the video the timing of the third min of stepping activity in the middle of the five-min period on the treadmill was selected. The total number of steps observed on video within the third min period was used as the gold standard measure. The time synchronisation was achieved between the video record and the activity monitors by identifying the first stride of walking commencing in the video records. This time synchronisation was used across the whole activity. All descriptive data are presented as mean ± SD. For each participant, the steps number for each treadmill and outdoor walk and run were calculated by a researcher.

5.3.2 Decision Rules

ActivPAL device measures raw acceleration of X, Y and Z axis count at 20Hz frequency. The ActivPAL accelerometer which uses the static acceleration (due to gravity) acting on X, Y and Z axis to determine orientation of the thigh and therefore distinguish between time spent sitting/lying and standing, and uses dynamic acceleration (due to body movement) to determine stepping rate. This is illustrated in **Figure 5-6**. The monitor produces a 10 bit output with a range from 0 to 1024 in each of the three axes, to cover the a 16 g acceleration

range, with 0 = - 8 g and 1024 = + 8 g, where g is gravitational force, equivalent to 9.81 m/s² (g = 9.81 m/s²). This is illustrated in **Figure 5-7**.

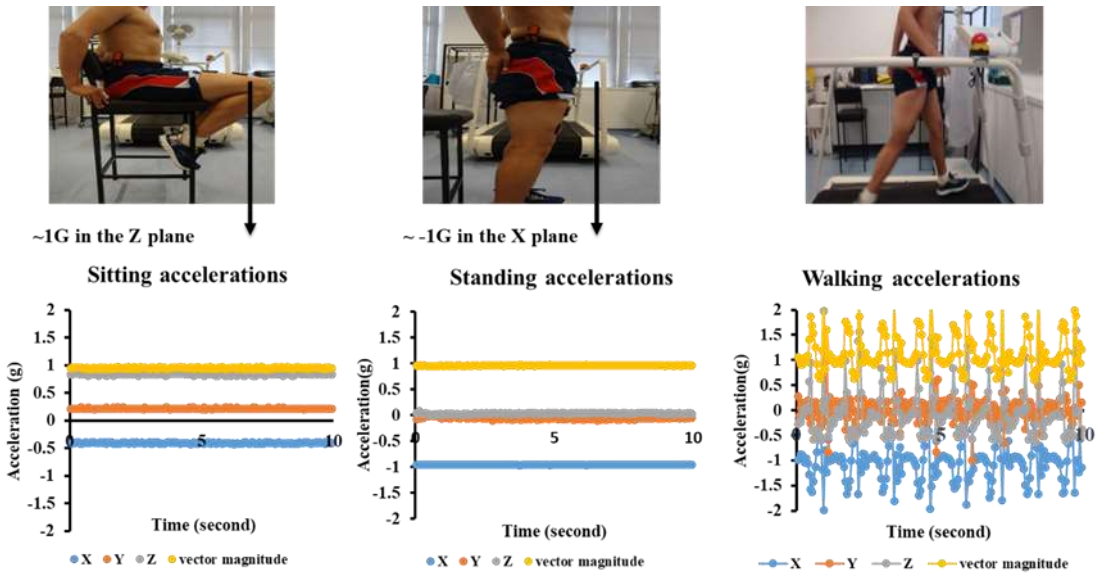


Figure 5-6: ActivPAL acceleration (x, y and z axis), during sitting, standing and walking.

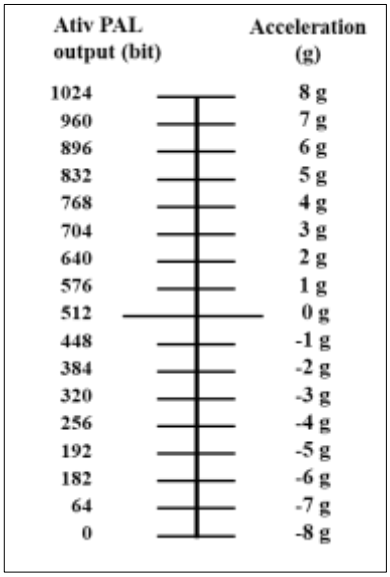


Figure 5-7 : Raw data output, expressing data acceleration due to gravity.

The ActivPAL measurs raw acceleration in three different planes (X, Y and Z) at a frequency of 20 Hz. An example of raw data outputs for the x-axis during stepping activity is shown on the top panel of **Figure 5-9**. The magnitude of the difference between each acceleration value was then summed over one second, expressing the value as the sum of changes in acceleration as illustrated in **Figure 5-8**. The magnitude of the differences is shown on the

second panel in **Figure 5-9** and a rolling average of these summed over one second are shown on the third panel. To further smooth this signal to obtain a relatively stable single value which could summarise the acceleration output for that intensity, a rolling average of these one second summed values was calculated. This is shown in the bottom panel of **Figure 5-9**. This process was undertaken for each of the X, Y, Z and the vector magnitude (VM) accelerations were calculated summarise the acceleration profile for each speed, using the equation, VM= $\sqrt{(x^2 + y^2 + z^2)}$ as illustrated in **Figure 5-10**.

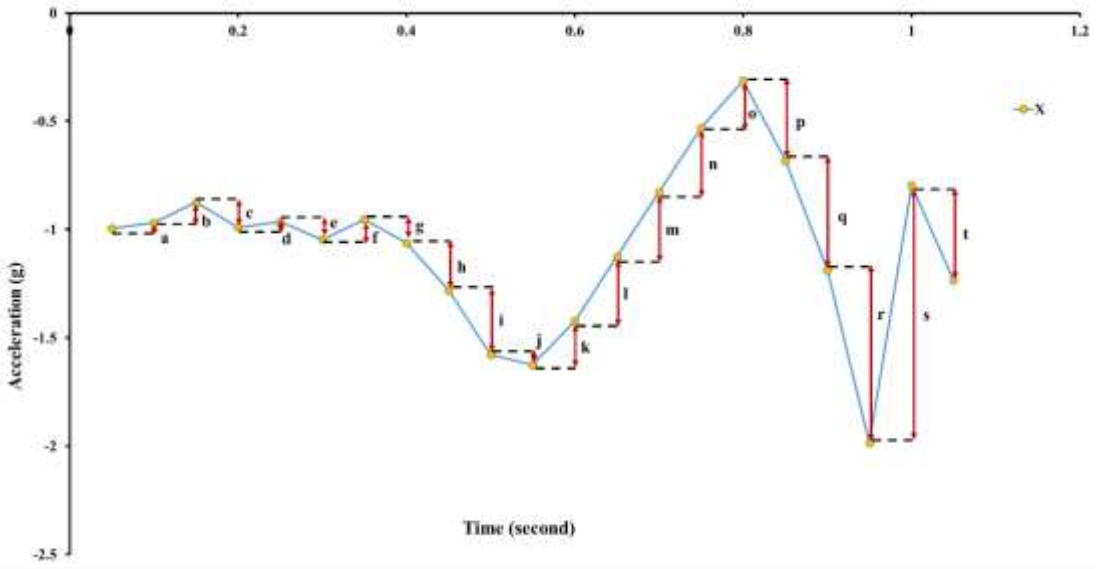
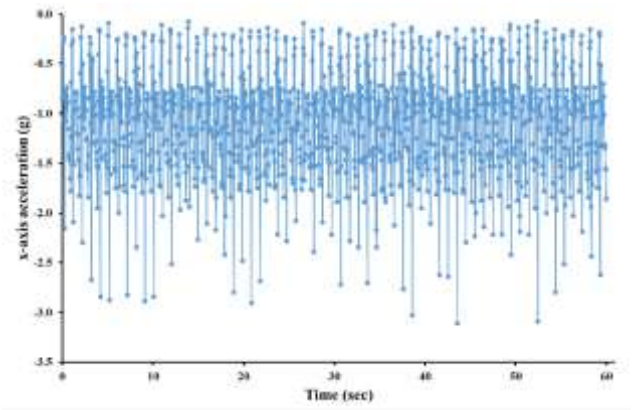
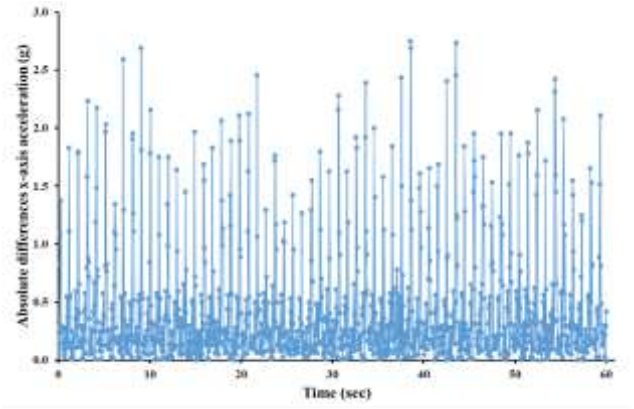


Figure 5-8: The differences of 20 Hz in X acceleration values summed over one second. X acceleration = a + b + c + d ….. etc.



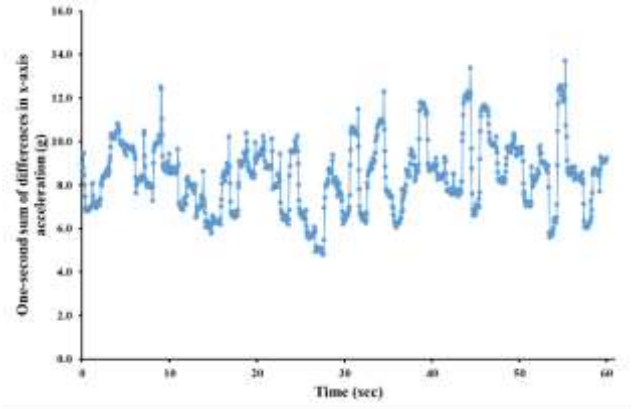
Raw acceleration data over 1 min.

X-axis accelatations (g)



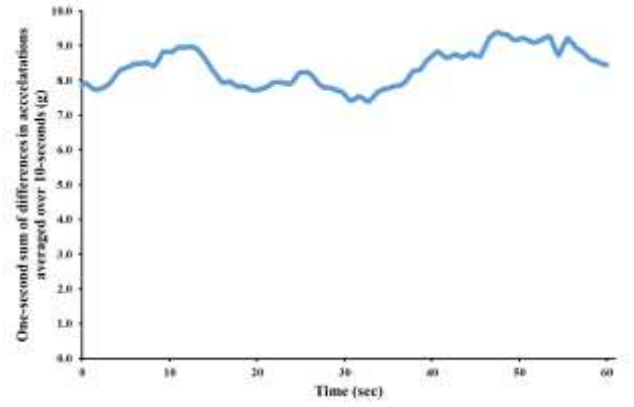
Raw acceleration data over 1 min

Absolute difference between successive points



Sum of differences over 1 second

The values were cumulated over second.



Sum of differences averaged over 10 seconds

The average over 10 second was presented for each speed, in x, y, z and vm accelerations.

Figure 5-9: ActivPAL raw acceleration data analysis during walking and running speed.

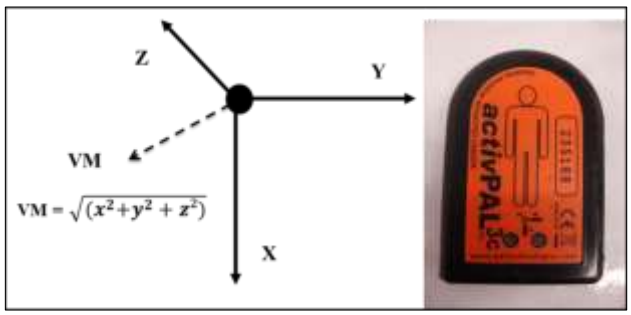


Figure 5-10:ActivPAL axis acceleration and VM acceleration which calculated as VM= $\sqrt{(x^2 + y^2 + z^2)}$.

5.3.3 Statistical Analysis

To address the first aim in this chapter, stepping rates from ActivPAL and Actigraph devices worn in comparable positions on the left and right sides of the body were plotted against each other and the proximity of this relationship to the line of equality was assessed. This analysis was performed on data collected from the treadmill-based trials.

To address aim two, the mean of left and right side values for ActivPAL and Actigraph derived stepping rates from each position were plotted against directly measured stepping rates and compared with the line of equality. This analysis was performed on data collected from the treadmill-based trials.

To address aim three, the vector magnitude acceleration outputs from ActivPAL and Actigraph devices worn in comparable positions on the left and right sides of the body were plotted against each other and the R^2 value for the linear regression between these variables and the proximity of this relationship to the line of equality were determined. This analysis was performed on data collected from the treadmill-based trials.

To address aim four, the linear regression (and R^2 value) and vector between vector magnitude accelerations and $\dot{V}O_2$ was assessed over a range of walking and running speeds. To assess whether a linear relationship provided the best fit, the R^2 values for higher order regressions (quadratic, cubic) were also assessed. This analysis was performed on data collected from the treadmill-based trials.

To address aim five, the linear regression and proximity to the line of equality was assessed for the comparison of vector magnitude acceleration outputs for treadmill-based and overground walking and running at a range of speeds.

To address aim six, the study population was randomly divided 1:1 into derivation and validation groups. In the derivation group, the linear regression between vector magnitude accelerations and $\dot{V}O_2$ was assessed for each accelerometer position. The equation of the regression line was then used in the validation group to predict $\dot{V}O_2$ based on the vector magnitude acceleration. This predicted $\dot{V}O_2$ value was then compared with the actual directly measured $\dot{V}O_2$ value. The validity of the $\dot{V}O_2$ prediction was assessed from the R^2 values between predicted and actual $\dot{V}O_2$ values and the standard error of the estimate (SEE) for the predicted compared with actual $\dot{V}O_2$ values.

5.4 Results

5.4.1 Reproducibility of measurement of stepping rate outputs

The relationship between stepping rates from accelerometers worn on the left and right sides of the body at a range of walking speeds from 1 km/h to 8 km/h and running speeds from 7 km/h to 11 km/h was assessed for the ActivPAL accelerometer worn in lower thigh, upper thigh and hip positions and the Actigraph accelerometer worn in the hip position. These data are shown in **Figures 5-11, 5-12, 5-13 and 5-14**. Observation of these figures shows that stepping rate outputs for left and right sides closely follow the line of equality across the range of walking and running speeds, suggesting that the reproducibility of stepping rate outputs is good for both accelerometers across all positions tested.

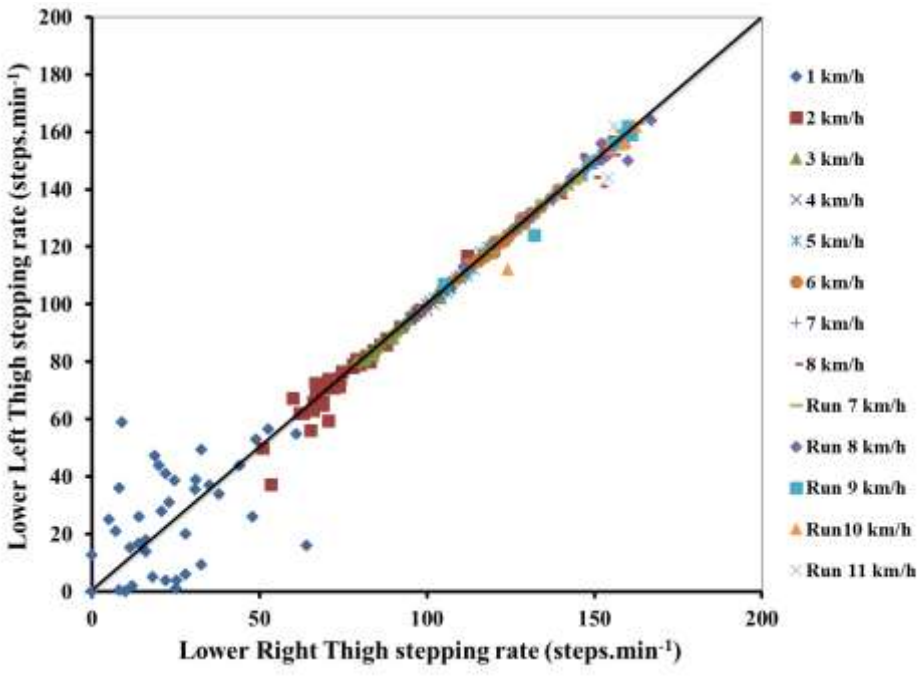


Figure 5-11: Mean Lower Left Thigh steps in relation to mean Lower Right Thigh ActivPAL steps during walking and running on treadmill testing.

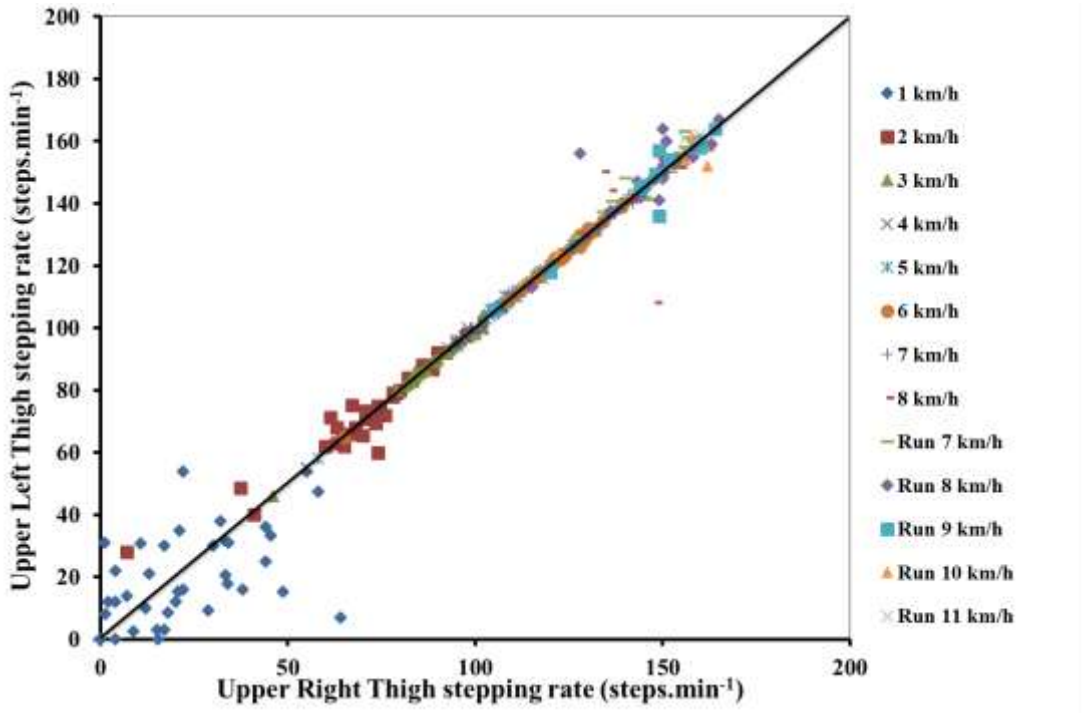


Figure 5-12: Mean Upper Left Thigh steps in relation to mean Upper Right Thigh ActivPAL steps during walking and running on treadmill testing.

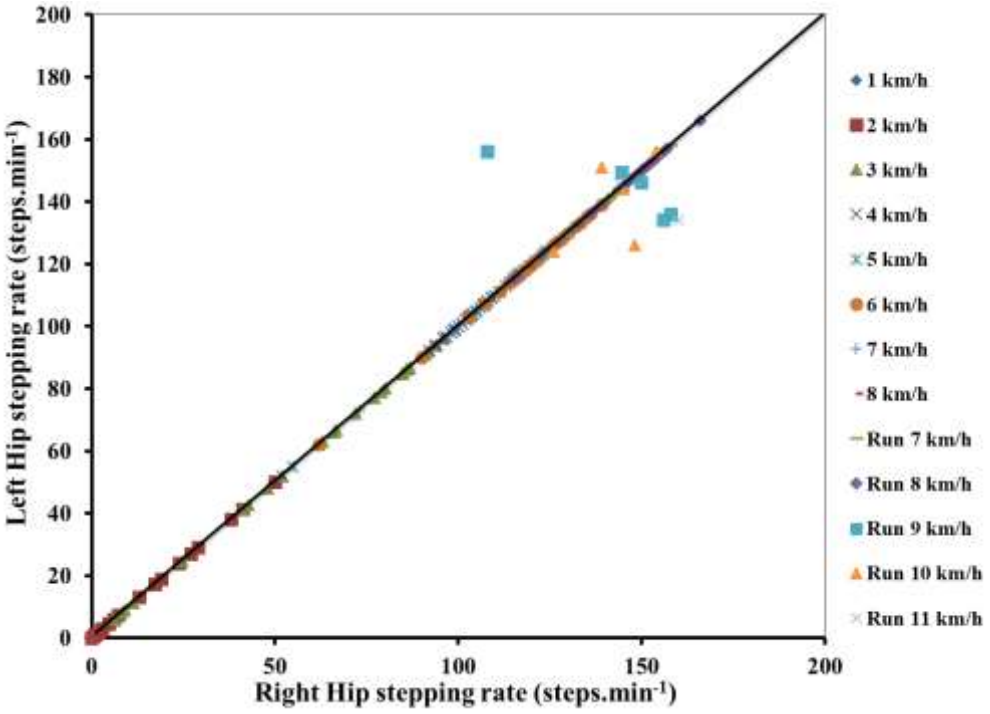


Figure 5-13: Mean Left Hip steps in relation to mean Right Hip ActivPAL steps during walking and running on treadmill testing.

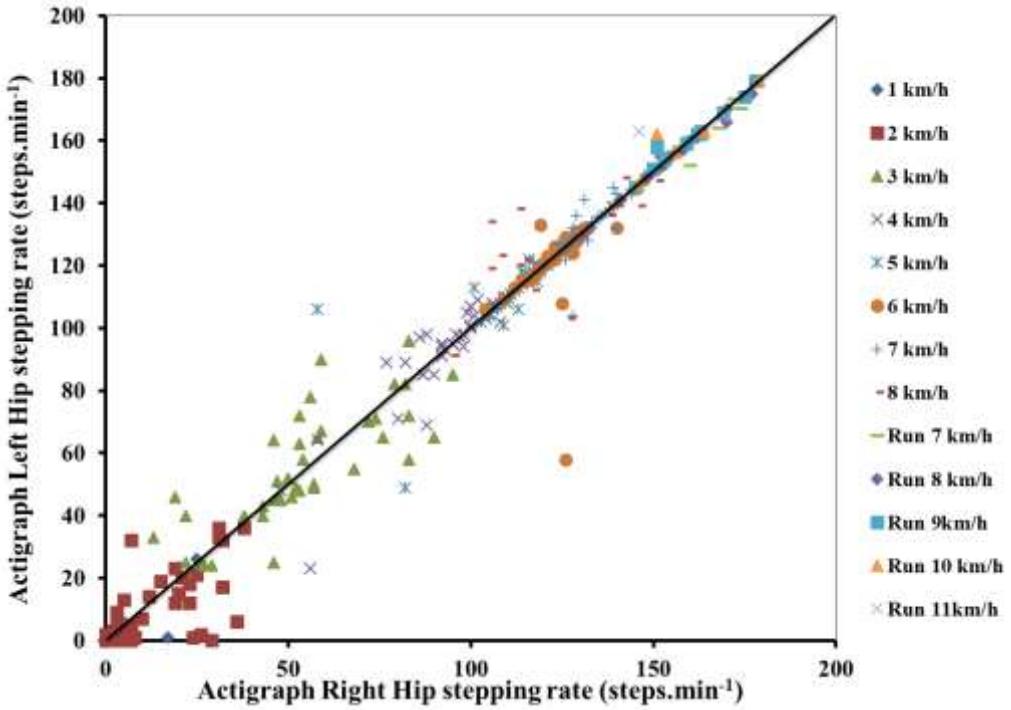


Figure 5-14: Mean Actigraph Left Hip steps in relation to mean Right Hip steps during walking and running on treadmill testing.

5.4.2 Accuracy of measurement of stepping rate of ActivPAL and Actigraph accelerometers

Figures 5-15, 5-16, 5-17 and **5-18** show the relationship between accelerometer-derived stepping rates and actual directly-measured stepping rates for the ActivPAL in lower thigh, upper thigh and hip positions and the Actigraph in the hip position across a range of walking and running speeds, with the line of equality plotted. Observation of the data shows that for the two thigh positions, ActivPAL-derived stepping rates agree closely with the actual stepping rates down to a walking speed of 2 km/h, or a stepping rate of ~60 steps per minute, but below this speed the ActivPAL systematically under-reports the stepping rate. In contrast, observation of the data show that the ActivPAL and Actigraphs worn on the hip were only able to accurately assess stepping rates down to a walking speed of ~ 4 km/h, or a stepping rate of ~100 steps per minute, underestimating stepping rate below this speed.

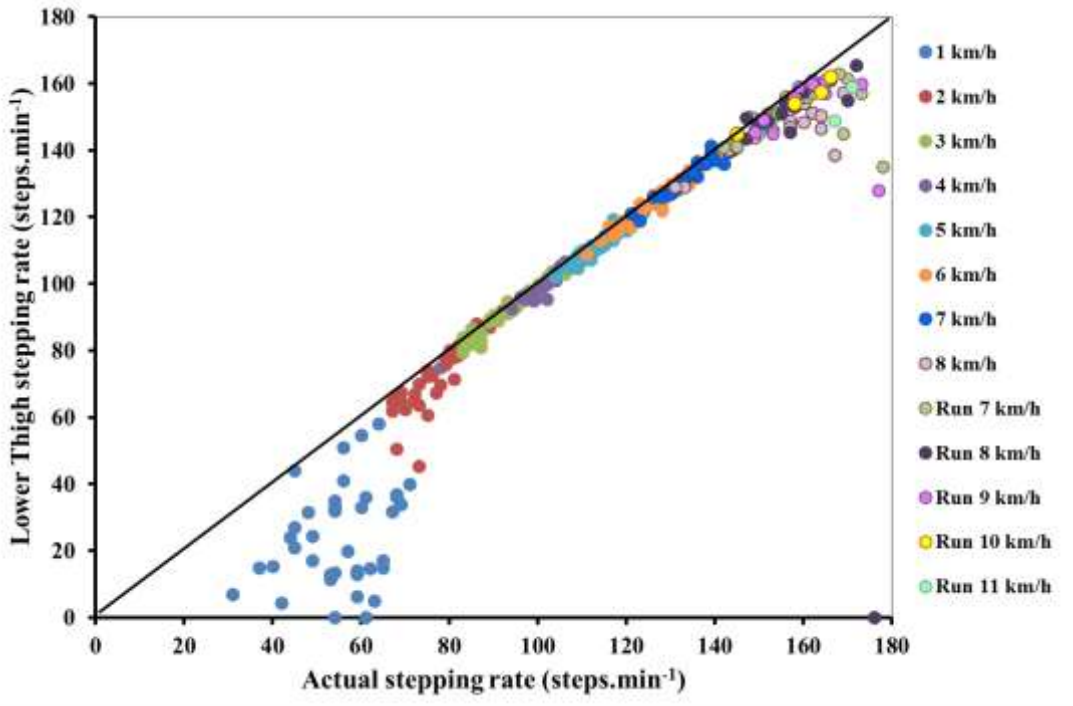


Figure 5-15: Mean Actual steps in relation to mean Lower Thigh ActivPAL steps during walking and running on treadmill testing.

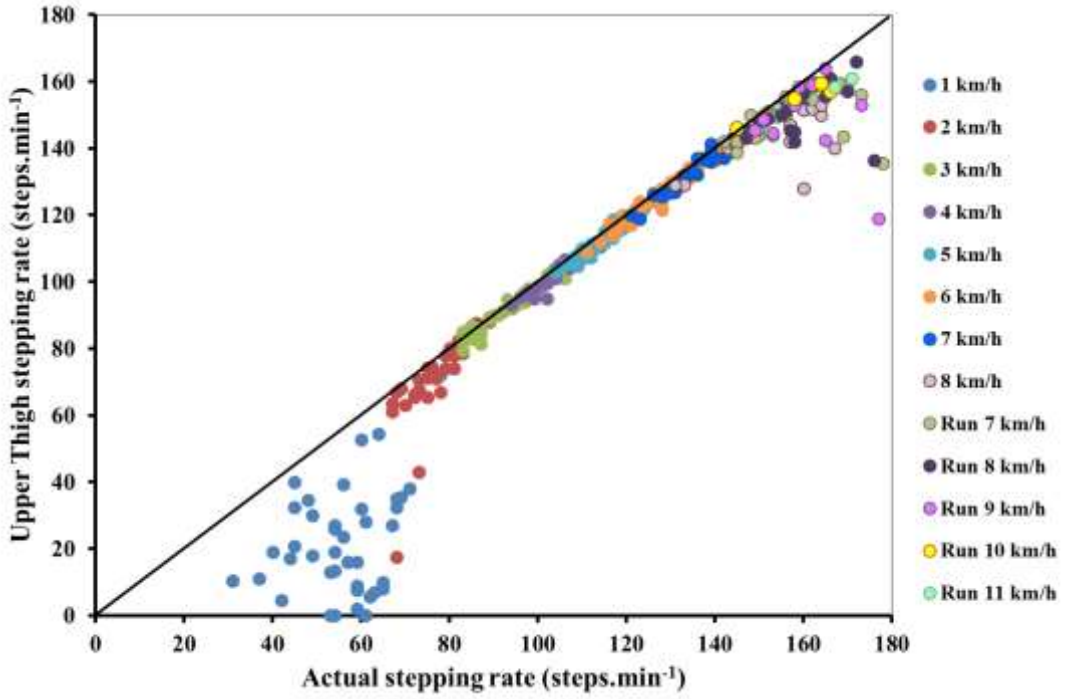


Figure 5-16: Mean Actual steps in relation to mean Upper Thigh ActivPAL steps during walking and running on treadmill testing.

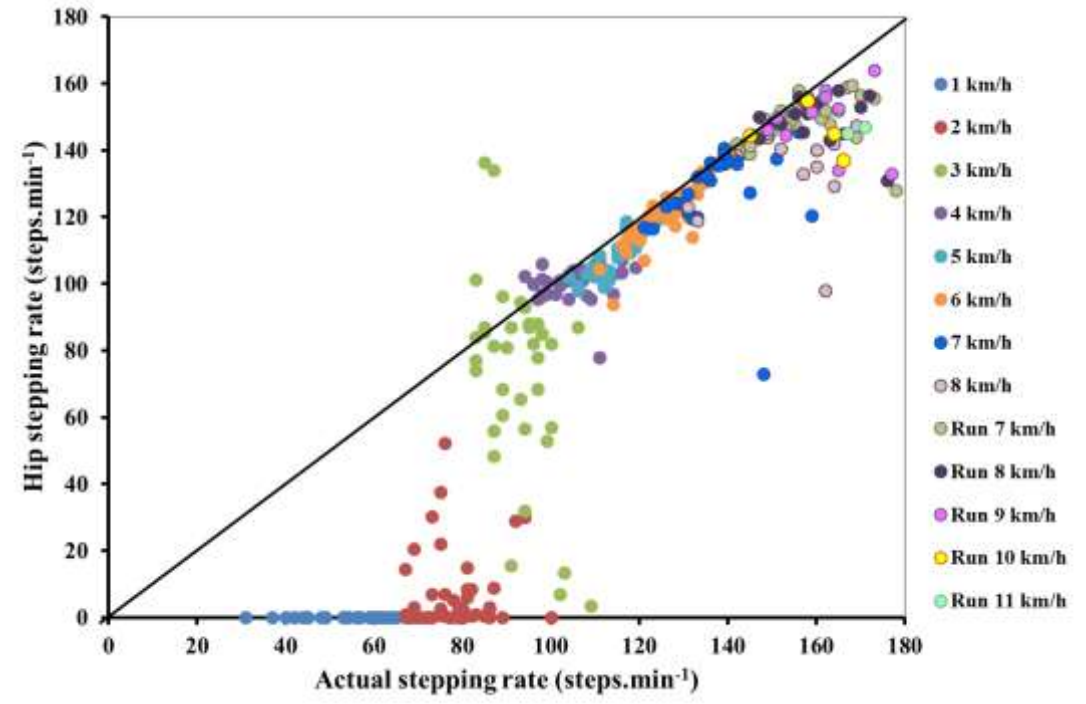


Figure 5-17: Mean Actual steps in relation to mean Hip ActivPAL steps during walking and running on treadmill testing.

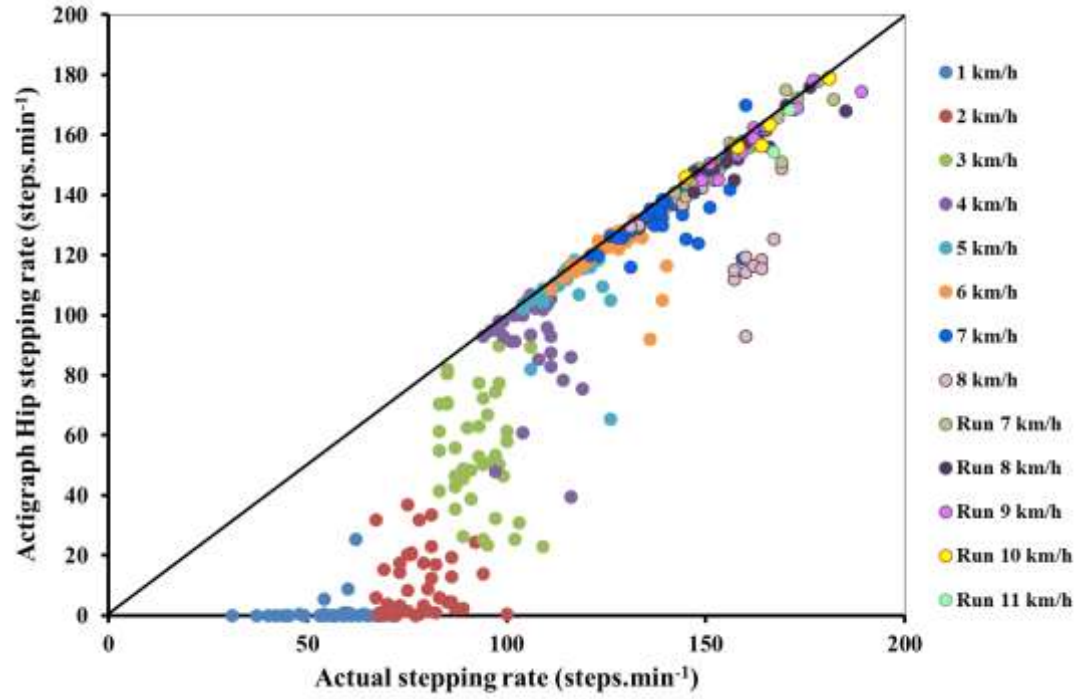


Figure 5-18: Mean Actual steps in relation to mean Hip Actigraph steps during walking and running on treadmill testing.

5.4.3 Reproducibility of vector magnitude acceleration outputs

To determine the reproducibility of the vector accelerometer outputs, data collected from left and right sides for each ActivPAL position (lower thigh, upper thigh and hip) and for the Actigraph in the hip position were compared. These data are shown in **Figures 5-19, 5-20, 5-21 and 5-22.** For all positions, the strength of the relationship between vector magnitude acceleration outputs between the left and right sides was very high, with R^2 values > 0.098 and the relationships closely followed the line of equality, indicating that reproducibility of vector magnitude acceleration outputs was very good.

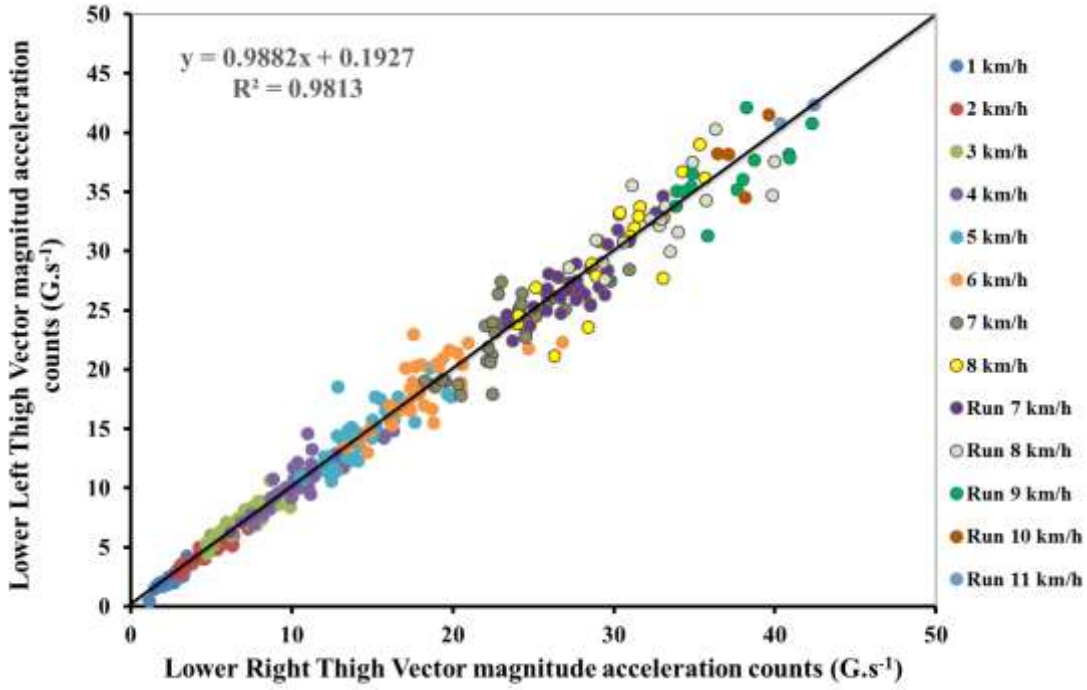


Figure 5-19: Mean Lower Left Thigh Vector magnitude accelerations in relation to mean Lower Right Thigh ActivPAL vector magnitude accelerations during walking and running on treadmill testing.

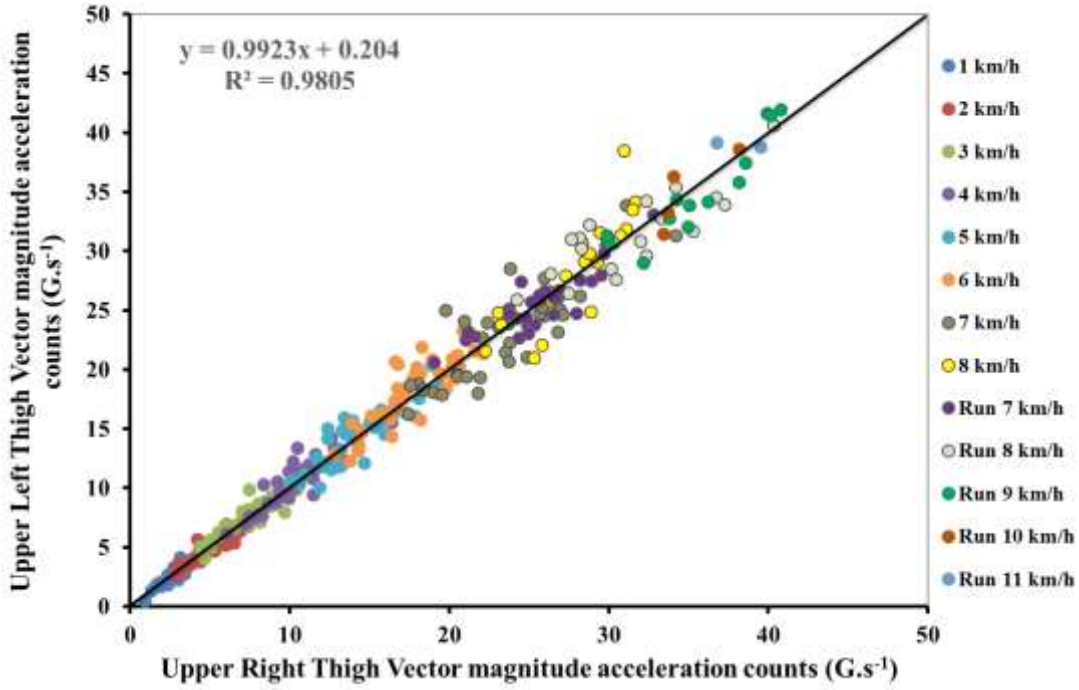


Figure 5-20: Mean Upper Left Thigh Vector magnitude accelerations in relation to mean Upper Right Thigh ActivPAL Vector magnitude accelerations during walking and running on treadmill testing.

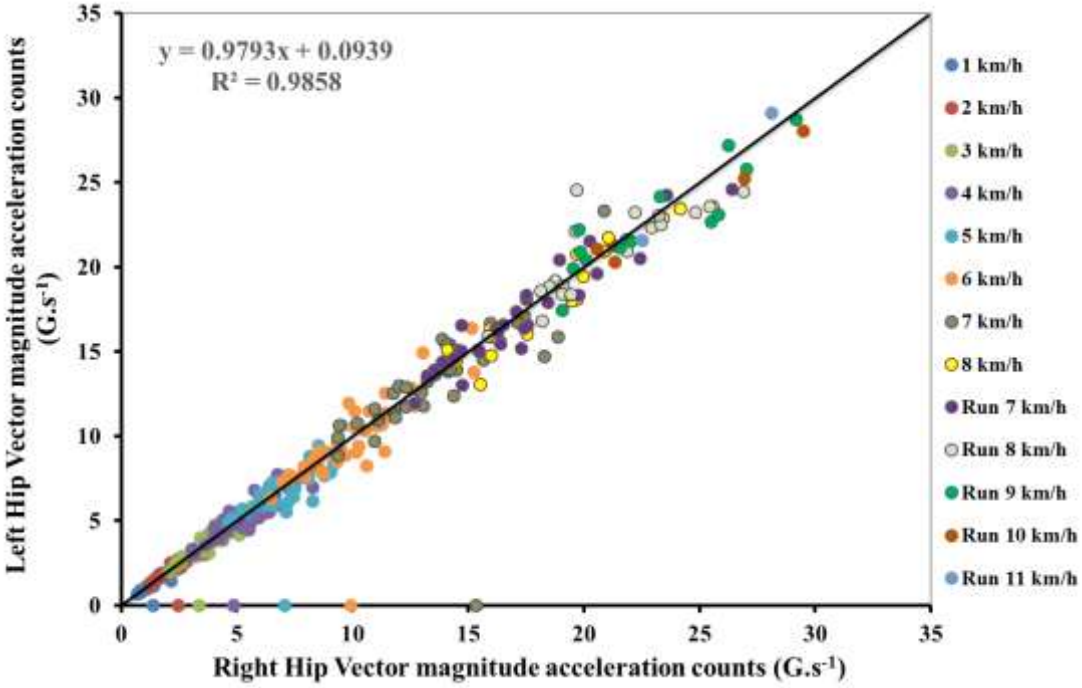


Figure 5-21: Mean Left Hip vector magnitude accelerations in relation to mean Right Hip ActivPAL Vector magnitude accelerations during walking and running on treadmill testing.

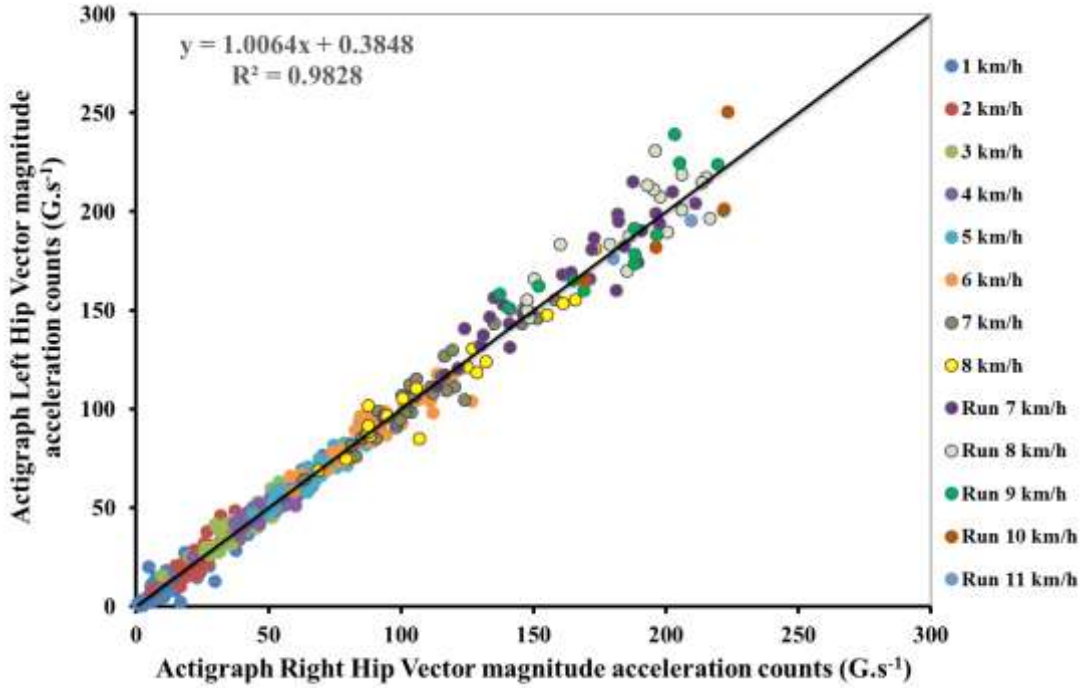


Figure 5-22: Mean Left Hip Vector magnitude accelerations in relation to mean Right Hip Actigraph Vector magnitude accelerations during walking and running on treadmill testing.

5.4.4 Relationship between vector magnitude acceleration outputs and $\dot{V}O_2$

The relationships between vector magnitude acceleration outputs (mean of left and right side values) and $\dot{V}O_2$ across a range of walking and running speeds for the ActivPAL accelerometer worn on the lower thigh, upper thigh and the hip and the Actigraph worn on the hip are shown in **Figures 5-23, 5-24, 5-25 and 5-26.** These data indicate that R^2 value for the relationship was very high for the ActivPAL (0.87 to 0.90) across all positions. The R^2 for the relationship between vector magnitude acceleration output and $\dot{V}O_2$ was slightly less strong for the hip-worn Actigraph. Fitting quadratic and cubic regression equations did not improve the R^2 values (data not shown) indicating that these relationships were best described using a linear model. The coefficient of regression equation differed between the three ActivPAL positions, with a given $\dot{V}O_2$ value corresponding to the highest vector magnitude acceleration value at the lower thigh and lowest at the hip.

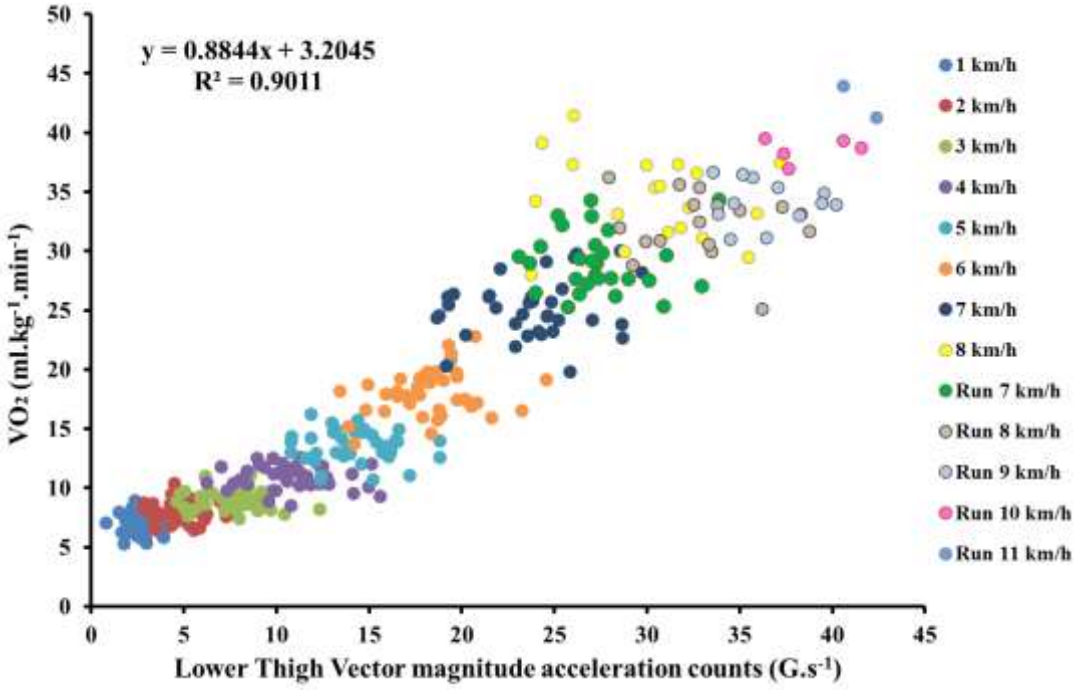


Figure 5-23: Mean $\dot{V}O_2$ in relation to mean Lower Thigh ActivPAL Vector magnitude acceleration counts during walking and running on treadmill testing.

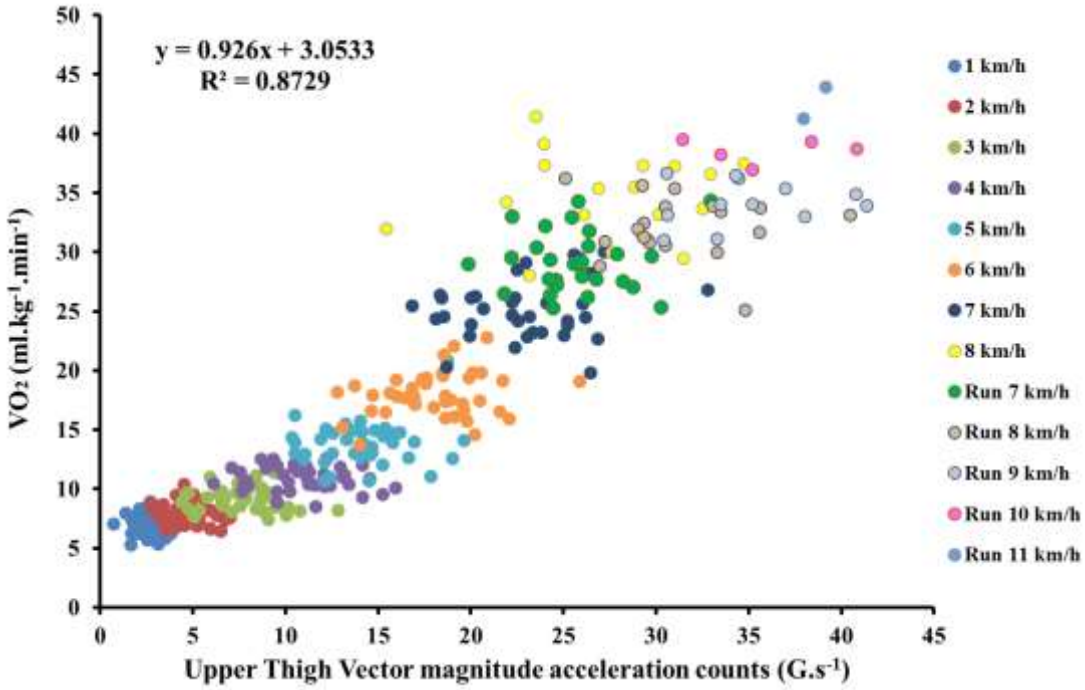


Figure 5-24: Mean $\dot{V}O_2$ in relation to mean Hip Actigraph Vector magnitude acceleration counts during walking and running on treadmill testing.

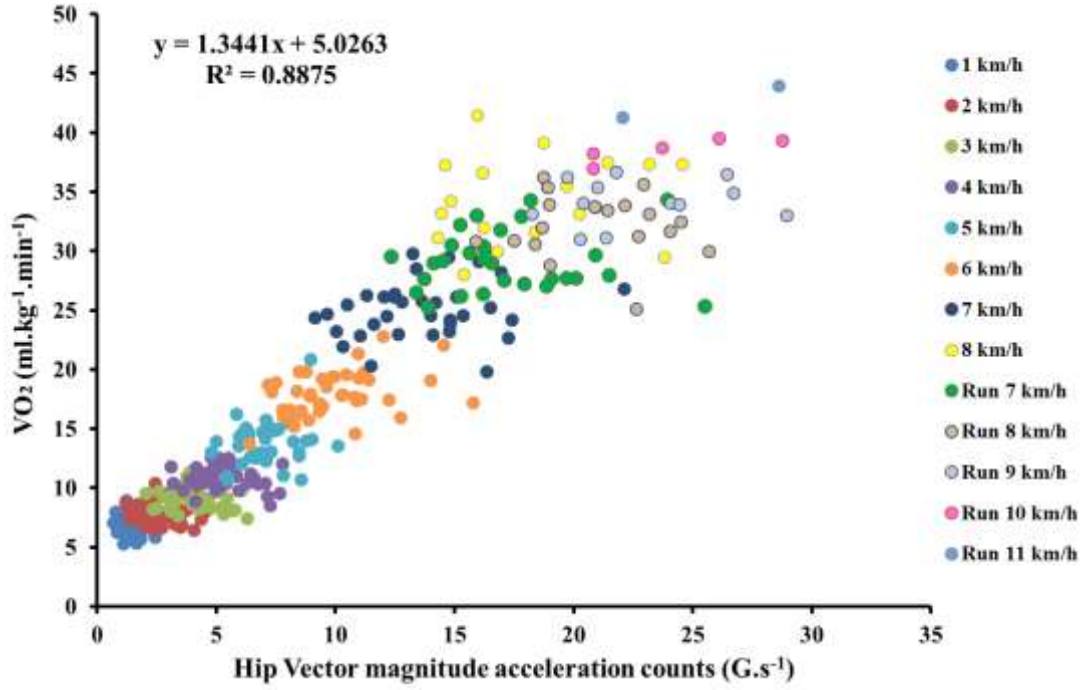


Figure 5-25: Mean $\dot{V}O_2$ in relation to mean Hip ActivPAL Vector magnitude acceleration counts during walking and running on treadmill testing.

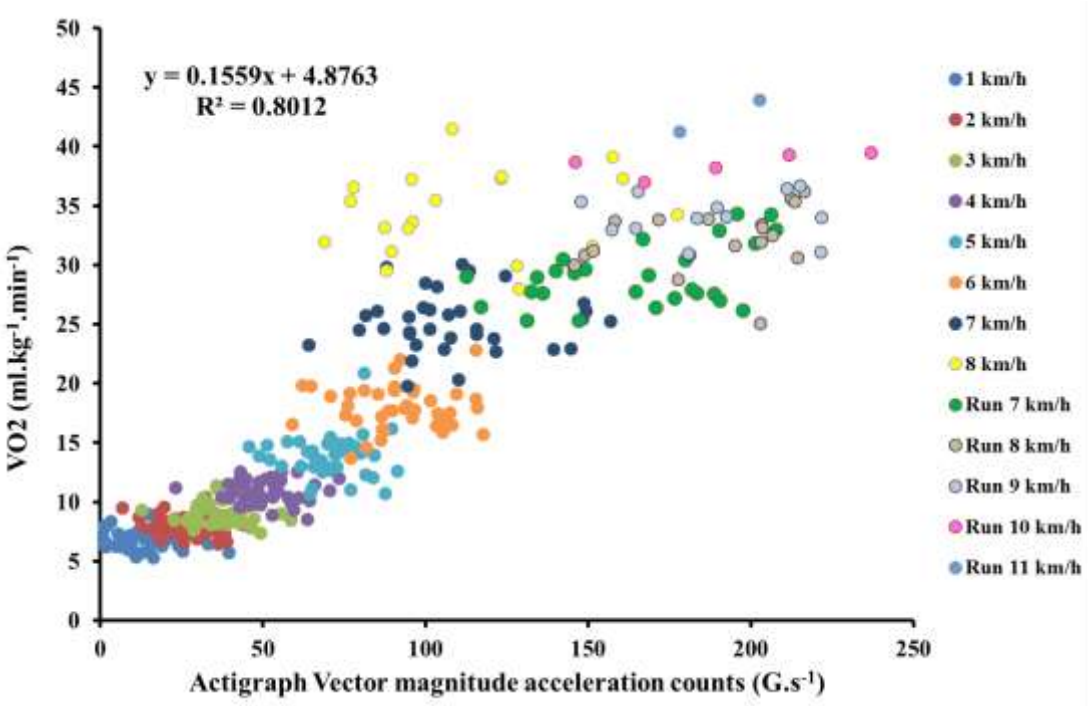


Figure 5-26: Mean $\dot{V}O_2$ in relation to mean Hip Actigraph Vector magnitude acceleration counts during walking and running on treadmill testing.

5.4.5 Relationship between vector magnitude acceleration outputs during treadmill vs overground walking and running

This analysis was undertaken in 15 adults (9 female), aged 29.1 ± 6.7 years, with body mass index (BMI) 24.8 ± 5.4 kg.m^{-2}, [mean ± SD], who undertook trials on both the treadmill and track, in which they walked and ran at the same speeds under both conditions. **Figures 5-27, 5-28, 5-29, and 5-30** show the relationship between vector magnitude acceleration outputs between treadmill and overground (track) walking and running at a range of speeds for ActivPAL devices worn in the lower thigh, upper thigh and hip positions and the Actigraph worn on the thigh positions. These data reveal a very strong relationship between treadmill and track accelerometer outputs for the ActivPAL placed in thigh positions (R^2 ~ 0.97) with the relationship strong but slightly weaker for the hip-placed ActivPAL or the Actigraph (R^2 ~ 0.93). For the thigh-worn devices the outputs lay close to the line of equality, with a slight deviation to higher acceleration outputs for the thigh-based devices. This indicates that, particularly for accelerometers worn on the thigh, there is a broad equivalence in outputs between treadmill-based and overground walking and running, suggesting that it is reasonable to extrapolate data obtained from track-based trials to the broader 'real life', overground walking and running situations.

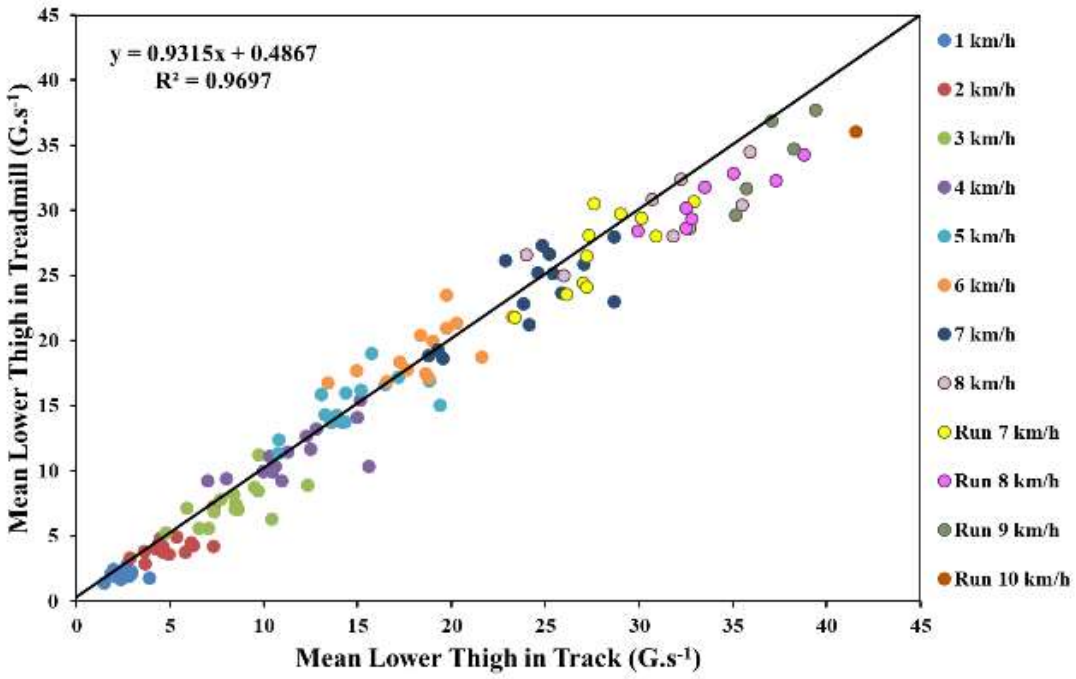


Figure 5-27: Mean Lower Vector magnitude in Treadmill in relation to mean Lower, Upper Thigh and Hip Vector magnitude in Track during walking and running , with line of equality plotted.

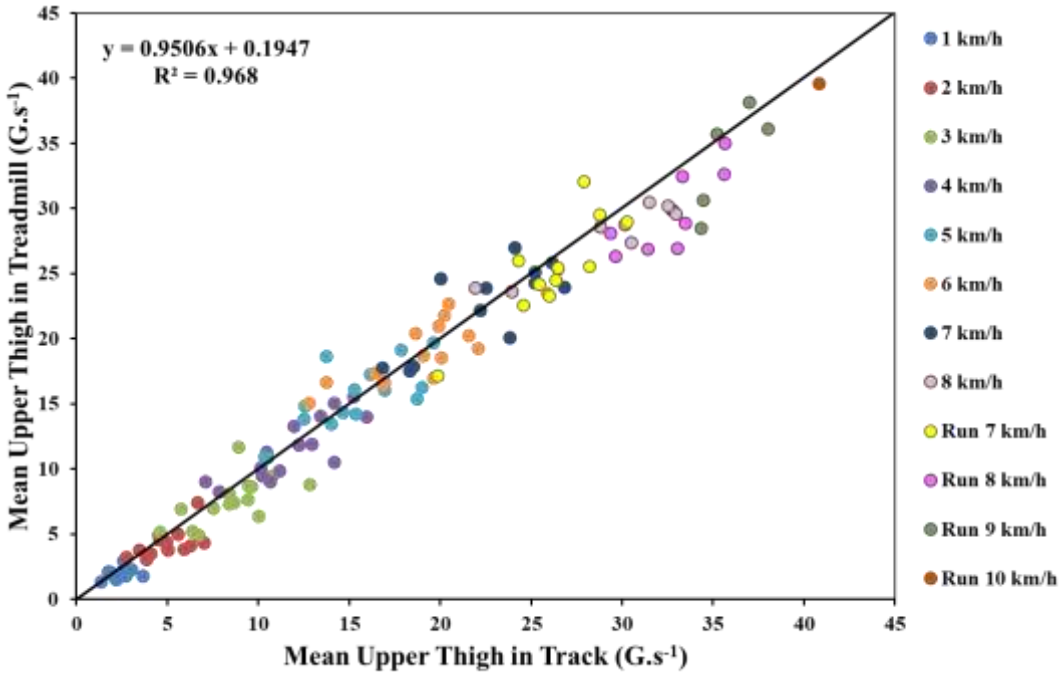


Figure 5-28:Mean Upper Thigh Vector magnitude in Treadmill in relation to mean Lower, Upper Thigh and Hip Vector magnitude in Track during walking and running , with line of equality plotted.

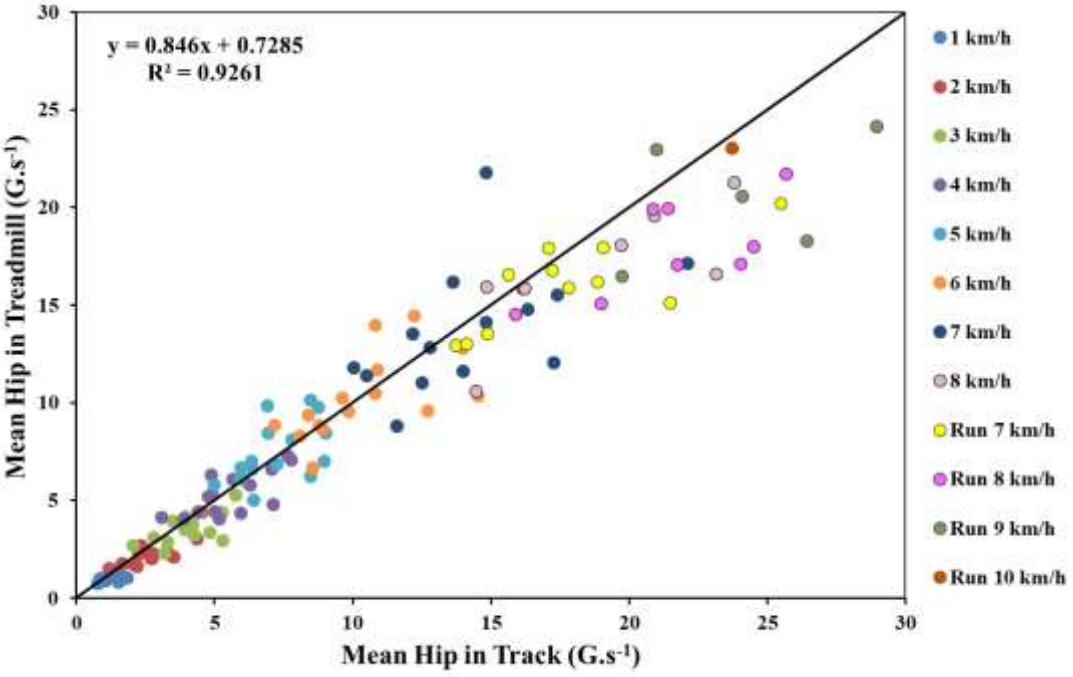


Figure 5-29: Mean Hip Vector magnitude in Treadmill in relation to mean Lower, Upper Thigh and Hip Vector magnitude in Track during walking and running , with line of equality plotted.

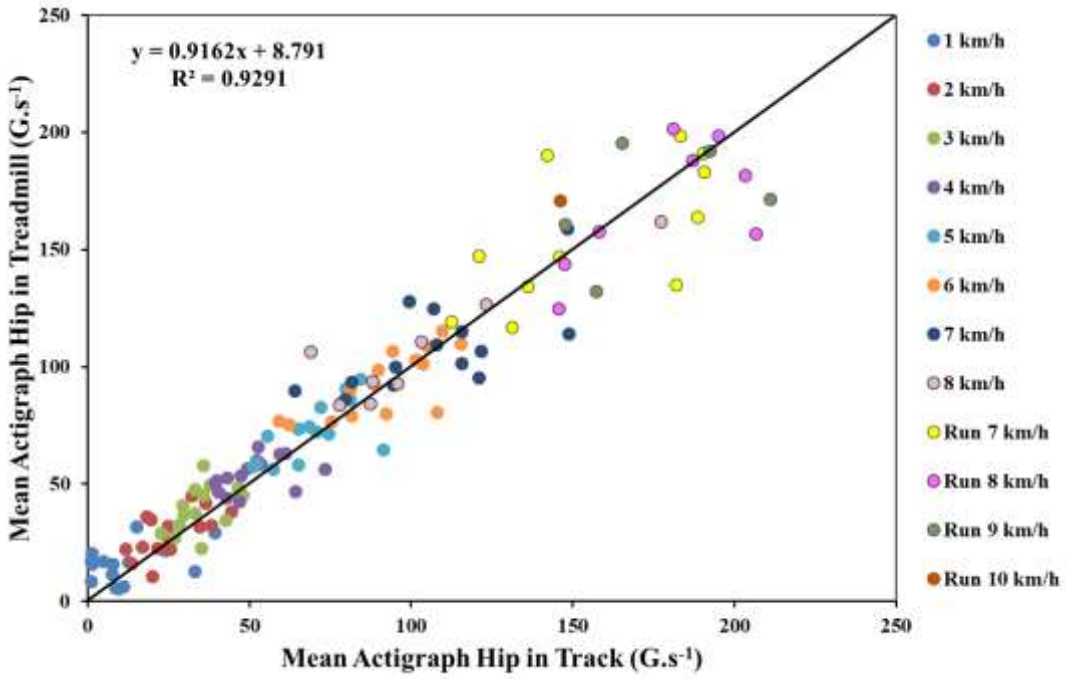


Figure 5-30: Mean Actigraph Hip Vector magnitude in Treadmill in relation to mean Lower, Upper Thigh and Hip Vector magnitude in Track during walking and running , with line of equality plotted.

5.4.6 Development and validation of algorithms to estimate $\dot{V}O_2$ from vector magnitude acceleration outputs

Characteristics of the derivation and validation groups are shown in **Table 5-1**. **Figure 5-31** shows the linear regression relationships between vector magnitude acceleration outputs and VO_2 for the ActivPAL in lower thigh, upper thigh and hip positions and for the Actigraph in the thigh position in the derivation group, with the equation of the regression line and the R^2 for the relationship displayed.

Table 5-1: Characteristics of Derivation and Validation group. Values are mean ± SD, n = 40.

	Derivation group	**Validation group**
Age (year)	28 ± 6.3	25 ± 4.5
Sex	9 F 11M	9F 11M
Body mass (kg)	69.3 ± 16.7	65.9 ± 16.6
BMI	23.4 ± 4.2	23.3 ± 4.8

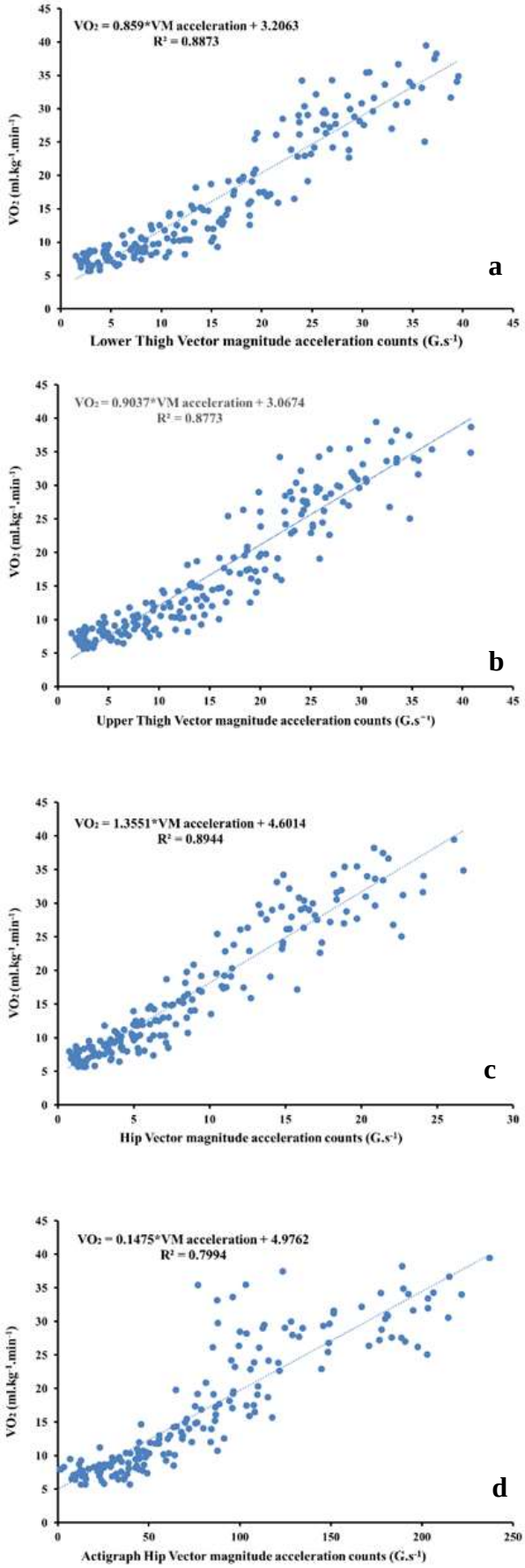
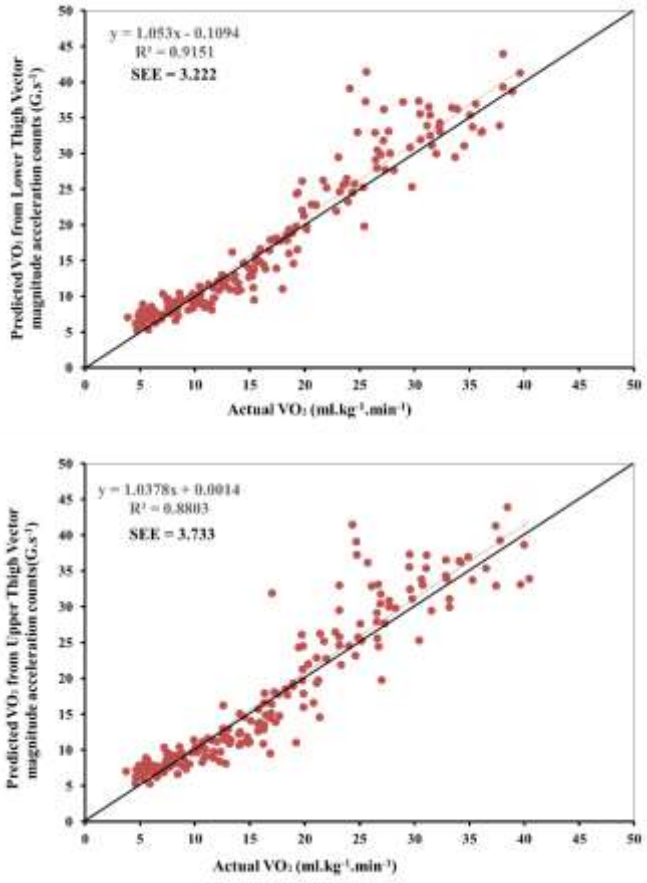


Figure 5-31: The linear correlation between mean Lower, Upper Thigh, Hip and Actigraph Vector magnitude in relation to mean oxygen uptake in Treadmill during walking and running , with the regression line and R^2 value shown.

Figures 5-32 shows $\dot{V}O_2$ values predicted from the regression equations displayed in **Figure 5-31** plotted against actual $\dot{V}O_2$ values in the validation group. The prediction oxygen uptake values in relation to the actual oxygen uptake values observed on treadmill for different positions. For the ActivPAL accelerometer, the R^2 for the relationship between predicted and actual $\dot{V}O_2$ was high (0.88-0.92) in all positions. The R^2 for the relationship between predicted and actual $\dot{V}O_2$ for the Actigraph was slightly lower at 0.81. In all cases the relationship between predicted and actual $\dot{V}O_2$ was close to the line of equality and the standard error of the estimate was <4 $ml.kg^{-1}.min^{-1}$ for all of the ActivPAL positions and <5 $ml.kg^{-1}.min^{-1}$ for the Actigraph.



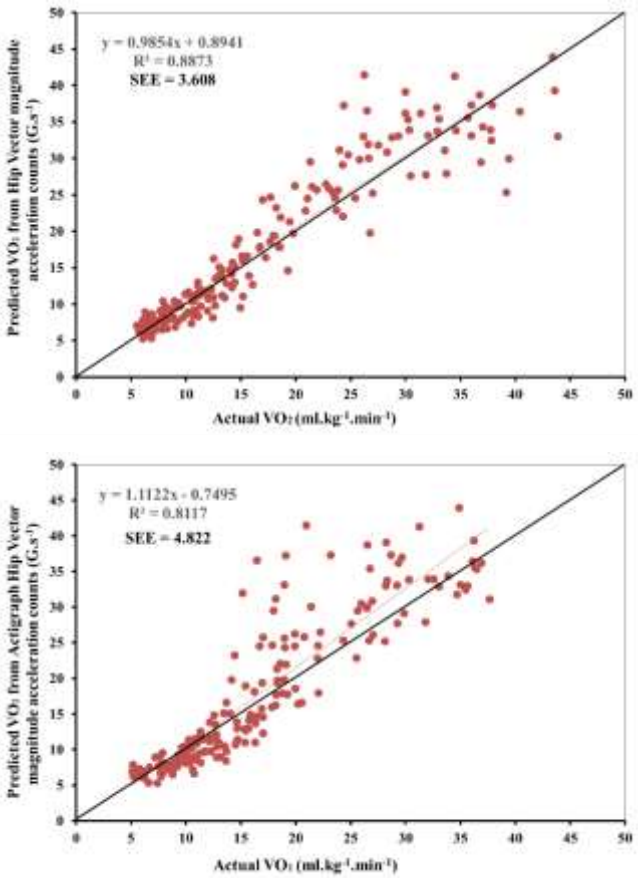


Figure 5-32: Predicted Oxygen uptake, using Lower, Upper Thigh, Hip and Actigraph Vector magnitude acceleration in relation to actual oxygen uptake in Treadmill during walking and running , with the linear regression line, R^2 value and standard error of the estimate shown. Solid black line is the line of equality.

5.4.7 Equations to predicted $\dot{V}O_2$ and Energy expenditure from vector magnitude acceleration values

Thus, the equations displayed in **Figure 5-32** can be used to predict $\dot{V}O_2$ (in $ml.kg^{-1}.min^{-1}$) across a range of walking and running speeds based on accelerometer outputs. The equations for each accelerometer position are shown below **Equations 5-1, 5-5, 5-9 and 5-13**. In addition it is possible to use this information to express exercise intensity in METs. During rest, the $\dot{V}O_2$ is approximately 3.5 $ml.kg^{-1}.min^{-1}$. This is defined as 1 MET. Thus, METs can be calculated by dividing $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$) by 3.5. The equations for predicting exercise intensity in METs for each accelerometer position are shown below **Equations 5-2, 5-6, 5-10 and 5-14**. Furthermore, as each litre of O_2 consumed by the individual is associated with an energy expenditure of approximately ~ 5 kcal of energy (Frayn and Macdonald 1997), it is possible to further derive rate of energy expenditure in $kcal.kg^{-1}.min^{-1}$ **Equations 5-3, 5-7, 5-11 and 5-15** and in $kcal.min^{-1}$ by further multiplying by body mass **Equations 5-4, 5-8, 5-12 and 5-16**.

ActivPAL Lower Thigh

The linear regression equations from **Figure 5-32 (a)** was used to predict oxygen uptake for the ActivPAL in the lower thigh position as described below:

Oxygen uptake ($ml.kg^{-1}.min^{-1}$) = 0.859* VM + 3.2063 **Equation 5-1**

METs can be obtained by dividing the oxygen uptake ($ml.kg^{-1}.min^{-1}$) by 3.5 as below:

METs = $\dot{V}o_2$ ($ml.kg^{-1}.min^{-1}$) / 3.5

METs = 1/3.5*(0.859* VM + 3.2063) **Equation 5-2**

To calculate energy expenditure, each L/min of O_2 consumed equalled 5 kcal/min. To convert ml/min to L/min (divide by 1000), and then (multiply by 5) to convert $\dot{V}o_2$L/min to kcals min (L/min*5) as described below:

Energy expenditure ($kcal.kg^{-1}.min^{-1}$) = $\dot{V}o_2$ (L/min)*5 or $\dot{V}o_2$ (ml/kg/min)/1000*5.

Energy expenditure ($kcal.kg^{-1}.min^{-1}$) = 5/1000*(0.859* VM + 3.2063) **Equation 5-3**

Total energy expenditure ($kcal.min^{-1}$) = bodymass* $\dot{V}o_2$ (L/min)*5.

Or $\dot{V}o_2$ (ml/kg/min)*bodymass » $\dot{V}o_2$ (ml.min)/1000*5.

Total energy expenditure ($kcal.min^{-1}$)= bodymass*(0.0043*VM+ 0.0160) **Equation 5-4**

ActivPAL Upper Thigh

The linear regression equation from **Figure 5-32 (b)** was used to predict oxygen uptake for the ActivPAL in the upper thigh position are described below:

Oxygen uptake ($ml.kg^{-1}.min^{-1}$) = 0.9037*VM +3.0674 **Equation 5-5**

METs = 1/3.5*(0.9037*VM +3.0674) **Equation 5-6**

Energy expenditure ($kcal.kg^{-1}.min^{-1}$) = 5/1000*(0.9037*VM +3.0674) **Equation 5-7**

Energy expenditure ($kg.min^{-1}$) = bodymass*(0.0045*VM + 0.0153) **Equation 5-8**

ActivPAL Hip

The linear regression equation from **Figure 5-32 (c)** was used to predict oxygen uptake for the ActivPAL in the hip position are described below:

Oxygen uptake ($ml.kg^{-1}.min^{-1}$) = 1.3551*VM + 4.6014 **Equation 5-9**

METs = 1/3.5*(1.3551*VM + 4.6014) **Equation 5-10**

Energy expenditure ($kcal.kg^{-1}.min^{-1}$) = 5/1000*(1.3551*VM + 4.6014) **Equation 5-11**

Energy expenditure ($kg.min^{-1}$) = bodymass*(0.0067*VM + 0.023) **Equation 5-12**

Actigraph Hip

The linear regression equation from **Figure 5-32 (d)** was used to predict oxygen uptake for the Actigraph in the hip position are described below:

Oxygen uptake ($ml.kg^{-1}.min^{-1}$) = 0.1475*VM + 4.9762 **Equation 5-13**

METs = 1/3.5*(0.1475*VM + 4.9762) **Equation 5-14**

Energy expenditure ($kcal.kg^{-1}.min^{-1}$) = 5/1000*(0.1475*VM + 4.9762) **Equation 5-15**

Energy expenditure ($kg.min^{-1}$) = bodymass*(0.00074*VM + 0.0248) **Equation 5-16**

Using **equations 5-2, 5-6, 5-10 and 5-14**, it is also possible to derive ranges of vector magnitude accelerations which correspond to light (1.5 – 2.9 METs), moderate (3.0 – 5.9 METs) and vigorous (≥ 6.0 METs) physical activities; these values are shown in **Table 5-2**. By using these MET ranges, it would be possible to characterise the amount of time and number of steps taken in each intensity domain from vector magnitude acceleration putputs.

Table 5-2: Vector magnitude values for light, moderate and vigorous activities.

	MET value	$\dot{V}O_2$ ($ml.kg.min^{-1}$)	VM acceleration values ($g.s^{-1}$)			
			Lower thigh	Upper thigh	Hip	AG Hip
Light	1.5-2.9	5.2 – 10.5	2.3 – 8.4	2.4 – 8.2	0.4 – 4.3	1.8 – 37.4
Moderate	3.0-5.9	10.5 – 21.0	8.4 – 20.7	8.2 – 19.84	4.3 – 12.1	37.4 – 108.6
Vigorous	≥6.0	≥21.0	>20.71	>19.84	>12.10	>108.63

5.5 Discussion

The aim of this study was to develop novel algorithms using the raw acceleration outputs generated by ActivPAL and Actigraph accelerometers, to allow the accurate assessment of physical activity over a wide range of exercise intensities. This study has achieved the initial and crucial steps towards the development of these algorithms, establishing the steps required to organise raw acceleration counts generated by the accelerometers devices, and beginning the process of developing and validating these algorithms from regression models.

The outcomes of this study indicate that there is a relatively consistent relationship between right and left side step counts during walking and running speeds. The equivalence of stepping rate outcomes between Right and Left accelerometer positions has been shown in **Figure 5-11, 5-12, 5-13 and 5-14**. The results of this study further illustrate the utility of the thigh as a highly accurate placement site for activity. The mean of Right and Left side steps was calculated for each position and compared with the actual steps **Figures 5-15, 5-16, 5-17 and 5-18**. The equivalence of stepping rate outcomes between the accelerometer and actual steps demonstrated that thigh-based ActivPAL was capable of determining stepping activity well at and above 2 $km.h^{-1}$, whereas hip-based ActivPAL accelerometer underestimated count steps below 4 $km.h^{-1}$. Below 3 $km.h^{-1}$, the ability of the Actigraph monitor to detect steps declined rapidly. Recent evidence suggests that the ActivPAL monitor does not have a high level of validity at slow speed of walking, the percentage of steps identified was over 90% for walking speeds at or ≥ 0.5 m/s and cadence at or ≥ 69 steps/min. Although, below these speed, steps count reduced rapidly with zero steps detected at 0.1 m/s and at or below 24 steps/min (Stansfield *et al.* 2015). Given previous work showing high accuracy for measuring sedentary behavior and ambulatory activities with thigh-based accelerometers (Grant *et al.* 2006; Maddocks *et al.* 2010; Ryan *et al.* 2006; Skotte *et al.* 2014). Harrington et al (Harrington et al. 2011) also reported strong relationships between Actigraph, Activpal and step rate function. Both the ActivPAL and Actigraph step rate functions were accurate at moderate walking speeds compared to video recorded step rate, although the ActivPAL was more accurate at the slowest walking speed. It is likely that the ActivPAL provided better estimates due to the position on the thigh as opposed to the hip. The result was consistent with Oliver et al 2011 who assessed the validity of the step count function in ActivPal. The mean relative percentage differences between direct observation and ActivPAL step counts was -1.9%, ICC = 0.998, with high degree of

accuracy and reliability for all walking speed (Oliver *et al.* 2011). Another study investigated the validity and reliability of the activPAL physical activity monitor in measuring steps. Participants walked on a treadmill at five different speeds and outdoors at three self-selected speeds (slow, normal, and fast). At all speeds, the activPAL was reliable and excellent for both step number and cadence (ICC (2, 1) ≥0.99). The absolute percentage error for the activPAL was < 1.11% for step number and cadence regardless of walking speed (Ryan *et al.* 2006). Testing the accuracy of the ActivPAL step record over a wide range of speeds is required. Recent evidence providing strong agreement over a narrow range of exercise activities (Dowd *et al.* 2012; Ryan *et al.* 2006), however, the accuracy during fast walking and running speeds were less consistent (Aminian and Hinckson 2012). The finding of this study suggested that thigh-based accelerometer placement provides advantages over hip-based placement for quantification of stepping rate at slow speeds. This may have implications for step counting during light incidental activities of daily living. However, when using raw acceleration profiles to quantify speed and exercise intensity, hip and thigh accelerometer placement was comparable.

There was a strong linear relationship between vector magnitude acceleration and speed in all positions, it has been noted that the equivalence of vector magnitude acceleration between Right and Left accelerometer was good, which determine there was no difference between both sides **Figure 5-19, 5-20, 5-21 and 5-22**. The relationship between Upper and Lower thigh-based and oxygen uptake was very strong $R^2 = 0.90$, $R^2 = 0.87$ **Figure 5-23 and 5-24,** however, the value slightly lower with Actigraph hip-based compared to ActivPAL hip-based. Hip-based placement had lower acceleration compared to thigh-based placement which will be expected due to the greater distance of axis rotation in the thigh **Figure 5-25 and 5-26**.

Montoye (Montoye A *et al.* 2016) compared the accuracy of accelerometers placed on the hip, thigh, and wrists, for measurement of Physical activity intensity, LTPA, MVPA and breaks in SB, using two Actigraph GT3X+ accelerometers were placed on the thigh and hip, and two GENEActiv accelerometers were placed on the wrist. Direct observation was utilized as a criterion measure of activities. They found that thigh-based was greater than wrist- or hip-based for estimating time spent in PA and breaks in SB, by sensitivities and specificities > 99%. Sensitivity and specificity were 87–95%, 93–97% for the hip- based respectively. Sensitivity and specificity for the left wrist-based were > 97% for estimating SB and LPA and 91–95% for MVPA. More recently, Florez-Pregonero et al assessed the

validity of ActiGraph GT3X+; activPAL, and SenseWear 2 in estimating energy expenditure during SB and LTPA, compared to indirect calorimetry (oxygen uptake, VO_2). The activPAL showed the lowest amount of percentage error compared with the other Wearable monitors 14.9%, and 9.3 % for SB and LPA, respectively. Thus, none of the wearable monitors in this study were comparable for assessing sedentary-to-light activities. Moreover, the ability of accelerometers in estimating EE during SB and LTPA, is less well known (Florez-Pregonero Alberto *et al.* 2016). For example, the estimated metabolic equivalent (MET) values from the activPAL at various speeds (2–4mph) are significantly different ($p <$ 0.0001) from the criterion of oxygen uptake (Harrington et al. 2011). Recent result shown that hip and thigh accelerometer placement was comparable for measuring oxygen uptake when using raw acceleration profiles.

It was not possible to determine oxygen uptake for the track-based measurement thus to ensure oxygen uptake value obtain from treadmill was likely to be relevant to free-iving conditions, a comparison between accelerometer and speed between treadmill and over ground was made this demonstrated that the relationship was similar and all the measurements were done in the treadmill-based probably reflected to free-based- living condition **Figures 5-27, 5-28, 5-29 and 5-30.** Based on relationships between acceleration outputs for treadmill and track were similar

Half of the group were used as derivation set and equation tested on the other half of group **Figure 5-31**. The result indicated that the linear regression equation to obtain oxygen uptake from accelerometer was valid in all ActivPAL`s positions with SEE approximately between 3.2 to 3.7 $ml.kg^{-1}.min^{-1}$ **Figure 5-32**, however, actigraph hip–based was somewhat less accurate with SEE = 4.8 $ml.kg.min^{-1}$. Possible explanations for the measurement miscalculation observed in the current study are the difference between Actigraph and ActivPAL is likely due to the use Actigraph propriatory algorithms to determine acceleration count rather than use raw gravitation unit. In addition, a small range of motion for the hip while walking at slow speeds on the treadmill may cause the ActiGraph to misclassify some activities. A study was done to examine the validity of published regression equations designed to predict energy expenditure from Actigraph accelerometer compared to indirect calorimetry, over a wide range of activities. Fifteen previously published equations were used to estimate energy expenditure. The result demonstrated that all equations significantly underestimated vigorous and most other activities ($p < 0.05$) and overestimated walking and sedentary activities. However, Freedson kcal equation was not significantly different from

actual time spent in light and moderate activities. Accurate regression equation is essential for the prediction of energy expenditure over a wide range of activities (Crouter *et al.* 2006). Another study was done to examine the validity of published regression equations to predict energy expenditure from Actigraph accelerometer compared to indirect calorimetry, in treadmill and activities of daily living. The researcher has demonstrated that the Freedson met equation under predicted energy expenditure for all daily living (bias -2.0 METs: 95% CI – 2.1, -1.9) and treadmill activities (bias -0.8 METs: 95% CI -0.8, -0.7). The freedson MET model appears to be most accurate for estimating EE for level treadmill activities (root mean squared error (RMSE) range 0.6 to 1.8 METs) and light intensity daily living that require minimal lower body movement (washing dishes, dusting and laundry) (RMSE range 0.6-0.9 METs) (Lyden *et al.* 2011).

Ward (Ward *et al.* 2005) explained that `one of the most challenging aspects of using accelerometers to measure physical activity behaviour is managing and understanding the vast amount of data collected`, while (Lee and Shiroma 2014) also recognised that procedures to reduce and process these data are not well developed. This study has overcome this challenge by establishing a process to organise the raw acceleration counts generated by the ActivPAL and Actigraph accelerometers into a compatible form will allow accurate and informative assessment of physical activity behaviour in future research.

In this sutdy, the ActivPAL device is worn on Right and Left sides, however it would be expected there was no significant differences in acceleration records in both left and right positions. John et al agreed with that the mean activity counts obtained from the Actigraph in left and right sides were comparable (John *et al.* 2010)

With this knowledge we were able to determine algorithms to calculate acceleration ranges equivalent to light, moderate and vigorous physical activity. This would enable determination of the amount of time and steps in each intensity domain. It also is possible to estimate energy expenditure from the vector magnitude acceleration values. Further work is needed to validate this estimation under free-living conditions, ideally in using doubly labelled water as a gold-standard comparison.

It would be worth developing separate regression models to predict VO_2 dependent on walking and running speed to understand if these produced more accurate results. Other aspects that may be worth including in this study to see if it improved the estimates would

be weight, height / leg length and / or BMI. Age would be another aspect considering since research such as by Ostrosky et al (Ostrosky *et al.* 1994) has shown that gait differs between adult and elderly people. Also worth developing would be separate regression models for males and female, since gait patterns can be different between genders (Senden *et al.* 2009).

There were several strengths to this study. A key strength of study was used a wide range of low and high speed this enable determination accuracy of measurement at very low intensity reflect of everyday activity. Further advantage a treadmill and free-living activity was determined and both was comparable. In this study, we also able to compare between left and right sides in each position. It was clear that value from right and left were comparable indicating the steps counts and acceleration.

This study does have some limitations. A walking and running linear relationship was only considered. However, light activity that people may undertake in free-living should be measured. However, as these activities generally include stepping and thigh-based has been shown as a good position for detecting stepping counts. It is likely that the data generated with thigh would be transferred to everyday activity. However, this require information in further study. Thigh or hip-based placement will underestimate energy expenditure of activity which are using main body and to fully quantify activity from acceleration will be needed. For single accelerometer thigh-based is likely to be better position due to the sensitivity to pick activity and distinguish between sitting and upright activity.

5.6 Conclusion

In conclusion, based on the results of this study, it appears that the use of accelerometer-based data collected from the thigh and hip-based present unique challenges in classifying stepping counts and activities into type and intensity categories and estimating EE. Thigh-based was highly accurate placement site for determining activity and detecting stepping counts. This study has made the challenging and initial steps towards developing algorithms for ActivPAL to estimate oxygen uptake and energy expenditure from the acceleration output. While further research is needed to test the accuracy of these algorithms in a wide range of free-living activities, this work has made an important contribution which will facilitate the use of a single thigh-worn accelerometer for the accurate and detailed quantification of both sedentary behaviour and physical activity.

6. General Discussion

6.1 Summary

As mentioned in the literature review, the implications of the detrimental effect on health of prolonged time sitting are well demonstrated (Biddle *et al.* 2016; Biswas *et al.* 2015; Same *et al.* 2016; Wilmot *et al.* 2012; Young *et al.* 2016). Thus, interventional research is urgently required to reverse the current trend towards lower physical activity levels and increased sitting time (Dunstan *et al.* 2011; Proper *et al.* 2011; Vandelanotte *et al.* 2013). Findings from this thesis describe the effects of different patterns of breaking up sedentary time on postprandial metabolic responses. In addition, novel indices were developed and validated for the estimation of intensity of physical activity and energy expenditure from acceleration outputs from a thigh-worn accelerometer, which may facilitate future use of a single thigh-worn accelerometer for the comprehensive assessment of both sedentary behaviour and physical activity from a single device. Accelerometers provide the potential for accurate objective measurement of physical activity, which is important for epidemiological assessment of activity levels and the association with disease risk and for quantification of changes in activity behaviour in response to interventions. The hip and thigh are commonly used locations for accelerometer placement. However, it is unclear whether these two locations are comparable in terms of measurement of stepping rate, speed and exercise intensity. In long term trials as well as observational research it is essential to be able to measure sedentary behaviour and physical activity. Poor measurement led to misunderstanding the relationship of physical activity, sedentary behaviour and health outcome. Some accelerometers are usually worn on the hip or wrist which measure physical activity intensity. However, these monitors are not able to distinguish between sitting and standing. An accelerometer which able to measure sitting, standing, physical activity intensity, and low intensity steps count is needed to butter understand the differences and risk between sitting and upright activity and measure low intensity steps which is reflect to our normal daily activity.

The main findings of this thesis suggest that duration of bouts of sedentary behaviour appears to influence indices of metabolic health – principally energy expenditure – independent of total time spent or physical activity. Previous observational studies have reported associations between high volumes of sitting and a number of health outcomes,

such as metabolic syndrome (Edwardson *et al.* 2012; Wijndaele *et al.* 2011) type 2 diabetes (Hu et al. 2003) (Ford *et al.* 2010; Hu *et al.* 2001; Zhang *et al.* 2006) cancer, CVD and all-cause mortality (Katzmarzyk *et al.* 2009; Patel *et al.* 2010; Stamatakis *et al.* 2011). The findings presented in chapter 3 are supported by previous observational studies which demonstrate that interrupting periods of sitting by standing has featured a meaningful change in metabolic rate but not with glucose, insulin and TG regulation (Gupta et al. 2016). Moreover, the present findings in chapter 4 suggest that breaking up sedentary time with sit/stand activity might induce a number of positive effects on postprandial metabolism. There are important issues regarding the accurate measurement of sitting behaviour in observational studies which are currently unresolved (Celis-Morales *et al.* 2012). The research reported in Chapter 5 was, to the author's knowledge, the first to evaluate the accuracy of acceleration for measuring step counts and physical activity intensity with thigh-placed accelerometers across a range of walking and running speeds and to develop and validate algorithms to estimate energy expenditure from raw acceleration counts for a thigh-placed accelerometer.

The first aim of this thesis was to compare the metabolic effects of breaking up sedentary time with prolonged periods of standing *versus* multiple shorter standing bouts with the same total duration to determine whether – in principle – altering the frequency of breaks in sedentary time, influences metabolic responses in 10 overweight/obese, over the course of the day. This aim was addressed in chapter three which determined that increasing the frequency of breaks in sedentary time by 10 1.5-minute bout of standing in every half-hour, while keeping total sedentary time constant, increased energy expenditure by 9%, $p < 0.001$, compared with 15 minutes of sitting and 15 minutes of standing over an 8-hour postprandial observation period. The present findings seem to be consistent with other research which found that breaking up prolonged sitting with standing increased energy expenditure by 2-33% compared to sitting (Judice et al. 2015a; Levine et al. 2000; Reiff et al. 2012; Speck and Schmitz 2011). However, there was no significant effects of either prolonged or intermittent standing breaks on postprandial incremental glucose, insulin or TG responses. This also accords with an earlier intervention study, which showed that breaking up sitting time with 2 minutes of standing every 20 minutes had no effect on postprandial glucose or TG responses over a 5-hour period (Bailey and Locke 2015). Although, these results differ from some published studies (Dunstan et al. 2012b;Larsen et al. 2015;Myashita et al. 2008;Peddie et al. 2013) who demonstrated that breaking up prolonged sitting time with ≤3-minute bouts of light or moderate intensity physical activity every 20-30 minutes can lower

postprandial glucose, insulin and TG concentrations. Thus, these data provide proof-of-principle that the number of transitions between sitting and standing influences energy expenditure and substrate utilisation, independent of total time spent sedentary. This provides a potential explanation for the independent effect of frequency of sedentary breaks on indices of adiposity observed in large epidemiological studies (Cooper *et al.* 2012; Healy *et al.* 2008a; Healy *et al.* 2011). However, the intermittent protocol used is clearly not feasible to implement as a practical intervention. The aim of chapter four was to therefore investigate whether undertaking a large number of sit/stand transitions in a more practically feasible format – repeatedly standing and sitting 10 times over 30 seconds every 20 minutes – could induce similar metabolic benefits in inactive, overweight/obese adults.

Epidemiological studies have shown that high level of sedentary behaviour is associated with increased risk of obesity (Thorp et al., 2011). It is conceivable that this may be mediated, at least in part, by the low energy expenditure associated with sitting. A number of experimental studies have shown that replacing sitting with standing increases energy expenditure over the course of the day (Reiff et al., 2012; Speck & Schmitz, 2011). Building on this work, we recently observed that intermittently standing for 1.5 minutes 10 times every 30 minutes led to 9% higher energy expenditure over an 8-hour postprandial period than standing continuously for 15 minutes every 30 minutes over the same time-frame (Hawari et al., 2016), indicating that the number of transitions between sitting and standing influenced energy expenditure independently of the overall amount of time spent sitting and standing. In that study there were 144 additional sit-to-stand transitions in the intermittent standing condition and 296 kJ additional energy was expended: from this it was possible to calculate that a sit-to-stand transition expended ~2 kJ of energy. The findings from the present study are consistent with this, energy expenditure was 410 kJ higher in the SIT/STAND compared with the SIT condition and 180 additional sit-to-stand transitions were undertaken in the former – equivalent to 2.3 kJ energy expenditure per transition. Thus, the present data provide confirmation that previously observed differences in energy expenditure between continuous and intermittent standing (Hawari et al., 2016) can be fully accounted for by the energy expended in the transition from sitting to standing and taken together these independent observations provide a robust estimation of energy expended in a sit-to-stand transition cycle.

Previous investigations of the effects of breaking up prolonged sitting with standing have had equivocal results in terms of alterations in glucose and insulin metabolic responses with

some (Thorp et al., 2014; Henson et al., 2016; Buckley et al., 2014), but not all (Bailey & Locke, 2015; Hawari et al., 2016) studies observing favourable changes when sitting is replaced by standing. In studies which have assessed postprandial TG responses, replacing sitting with standing has generally not resulted in significant changes (Henson et al., 2016; Hawari et al., 2016). In the present study, we observed that breaking up prolonged sitting by with 10 chair-squats every 20 minutes reduced insulin concentrations in the post-breakfast period, although this did not persist into the post-lunch period. This could conceivably be mediated by the skeletal muscle contractions needed to move between sitting and standing stimulating contraction-mediated glucose uptake (Krook, Wallberg-Henriksson, & Zierath, 2004), thereby reducing the requirement for insulin to maintain glucose homeostasis. Indeed, the repeated sit-to-stand transitions over 30 seconds, in effect represents multiple sets of bodyweight squats over the course of the day. However, the chair-squat intervention did not significantly affect postprandial glucose or TG concentrations. Interestingly, Dempsey and colleagues recently reported that breaking up prolonged sitting with 3 minutes of bodyweight resistance exercises every 30 minutes over a 7-hour postprandial observation period reduced postprandial glucose, insulin and TG concentrations in adults with type 2 diabetes (Dempsey et al., 2016). This more potent intervention effect in Dempsey's study may reflect two things. First, the volume of resistance exercise undertaken in that study (6 vs 1.5 mins per hour) was substantially higher than in the present study. It may well be that a larger volume of sit-to-stand transitions – for example 60 seconds of 'chair squats', rather than 30 seconds, every 20 minutes – may elicit more substantial effects on postprandial insulin, glucose and TG responses. Secondly, the participants in the present study were normoglycaemic, and it may be the case that the stimulus required to positively affect postprandial metabolic responses may be greater in healthy normoglyaemic individuals than those with metabolic dysfunction where there is greater capacity for improvement. For example, lab-based interventions breaking up sitting with standing have been effective at reducing postprandial glucose and insulin concentrations in post-menopausal women with impaired glucose regulation (Henson et al., 2016), but this effect has not been replicated in similar interventions in younger, normoglycaemic individuals (Bailey & Locke, 2015; Hawari et al., 2016; Miyashita et al., 2013). Thus, going forward, studies are needed i) to determine whether the present intervention is effective at reducing postprandial glucose, insulin and TG responses in individuals with impaired glucose regulation and ii) to determine whether the metabolic

benefits observed here would be enhanced in normoglycaemic individuals with an increased 'dose' of 'chair squats'.

The intervention undertaken in the present study is simple, requires no equipment and little space and only takes 1.5 minutes per hour. The additional 410 kJ of energy expended over the course of the trial, would equate to 8.2 MJ over 4 weeks if the intervention was carried out on 5 days of the week, which is equivalent to over 1 kg weight loss. This, together with the modest reductions in postprandial insulin concentrations, suggest that pragmatic, low volume, interventions of this nature may have the potential to elicit benefits to metabolic health. Thus, the 'chair squat' approach used in the present study could potentially be developed into an alternative strategy which would be used as an alternative to, or in combination with, other interventions, such as standing desks, to break up periods of prolonged sitting in individuals, such as office workers, to who need to work at a desk throughout the day. This would require substantial further development, and the present findings provide a rationale for undertaking longer-term randomised controlled trials to determine whether interventions of this nature are acceptable to individuals and sustainable in practice and whether they induce long-term benefits to metabolic health.

Nevertheless, the data in chapter 4 suggests that this approach may be an effective way of reducing the adverse effects of sedentary time during working hours or leisure time, and this occurred without inducing a major reduction in total daily sitting time. Such findings add considerably to the existing literature and are important as they suggest that changing between sitting and standing postures more frequently in adults could be important for positive health outcomes. Targeting such facets of behaviour in obese adults, who are likely to be the most susceptible to the health risks associated with prolonged sitting (van Uffelen *et al.*2010).

Chapter 3 and 4 have examined the effect of breaking prolonged sitting time with standing on metabolic health. Whilst the observational evidence supports the relationship between sedentary behaviour and health, intervention level evidence in humans is limited, particularly for the benefits standing without ambulation. More research is therefore required in order to establish the nature of the causal link between sedentary behaviour and metabolic health and the independent effects of standing and light ambulation.

Accurate monitoring for measuring physical activity and sedentary behaviour is needed to better assess the level of physical activities, sedentary behaviour and to quantify the dose response of activity, sedentary and health outcome. People spent relatively small proportion of day undertaking moderate or vigorous physical activity (Dunstan *et al.* 2012a; Loyen *et al.* 2016a). However, people spend most of the day in sedentary or low activity in daily activity level (Bennie *et al.* 2013; Loyen *et al.* 2016a; Loyen *et al.* 2016b; Milton *et al.* 2015; Owen *et al.* 2010b). Thus, accurate quantification of low intensity physical activity and sedentary behaviour is important. The ActivPAL is a gold standard for measuring sedentary and upright posture, and is widely used by researchers. However, the output of this device for physical activity intensity have been relatively limited with only stepping counts and rates being provided. Therefore it was very important to develop approaches to obtain better estimates of physical activity intensity using this device to facilitate the use of a single device to comprehensively monitor both sedentary time and physical activity.

In Chapter 5 the new algorithms were developed and validated to quantify oxygen uptake, energy expenditure and step counts from accelerometer output of the ActivPAL devices worn on the thigh and on this hip. Participants undertook a wide range of walking and running activities, including walking at very low intensities, and validity was assessed under controlled conditions using direct observation as the criterion measure. The findings in Chapter 5 showed that the upper and lower thigh-worn ActivPAL accelerometer are accurate for detecting step counts during walking and running tasks (R^2 = 0.86 for all). Hip-based ActivPAL and Actigraph underestimated steps count at speeds below ~ 3-4 $km.h^{-1}$. Also, there was a strong linear relationship between vector magnitude acceleration and speed in all positions. The relationship between ActivPAL accelarations and oxygen uptake was very strong for both the thigh and hip positions ($R^2 \approx 0.90$), ($R^2 \approx 0.88$) respectively.

An important finding in chapter 5 is that the data generated from treadmill-based walking was applicable with free-living walking, the finding detected that the relationships between mean vector magnitude and speed on the treadmill and on the track were very closely correlated. This means that the results observed during treadmill testing including VO_2 measurements, can be applied to free-living situation.

This finding is of considerable potential interest to researchers who are interested in quantifying sedentary behaviour and physical activity intensity, and for researcher designing

interventions to address these behaviours, as it provides opportunity for comprehensive measurement of both sedentary behaviour and physical activity using a single device.

6.1.1 Conclusions

Thus, overall this thesis has provided novel information show the frequency breaking sedentary has influences metabolic responses, independent of total time spent sitting or standing and showing that practically feasible intervention using, chair-squats to break sedentary time may be a promising approach. Further study is needed to establish whether increasing the number of sit-to-stand transitions per cycle would augment the potential benefits and longer-term intervention studies are needed to determine whether this approach is feasible and effective in 'real world' settings. Targeting such facets of behaviour in adults, especially obese people, holds great potential for behaviour change strategies which could have a large impact on public health.

In addition, this thesis has developed novel algorithms which will facilitate the use of a single thigh-worn accelerometer for comprehensive assessment of both sedentary behaviour and physical activity, which will be of use to researchers working in this domain. Further validation work over a wide-range of free-living activities is now needed to confirm the utility of these new physical activity measurement tools.

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8. Appendices

Appendix A: Volunteer Information Sheet and concent forms – Chapter 3

University of Glasgow | College of Medical, Veterinary & Life Sciences

VOLUNTEER INFORMATION SHEET

Title: Metabolic responses to breaking up sitting time

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?
Spending large amounts of time sitting down increases risk of heart disease, diabetes and obesity. This risk may be reduced by breaking up periods of prolonged sitting with periods of standing up. However, it is unclear whether different patterns of breaking up sitting time (i.e. with many short periods of standing, or a smaller number of longer periods of standing) have different influences on fat and sugar metabolism in the body. This study will compare fat and sugar responses over the course of a day of prolonged sitting, a day when sitting is broken up by relatively long-periods of standing, and a day when sitting is broken up by more frequent shorter periods of standing.

Why have I been chosen?
You have been chosen because you are a healthy man or a postmenopausal women aged between 18-65 years, who is currently relatively physically inactive.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to participate, you will be given this information sheet to keep and be asked to sign a consent form. If you do this you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?
Screening procedures
In the first instance you will be asked to attend for a screening visit in which we will:

- discuss with you and complete confidential questionnaires regarding your health, family history and physical activity level

- measure your blood pressure
- take your height, weight and waist measurements
- take a small blood samples to check the sugar level in your blood.
- provide an opportunity for you to ask questions

These preliminary procedures will enable us to determine whether you fall into the group of people we wish to study and will also ensure that it is perfectly safe for you to take part.

Experimental procedures
We will ask you to undergo 3 main experimental trials. Each trial will run approximately 1-2 weeks apart, in random order.

a) Sitting all day

We will ask you to come to the University after an overnight fast (i.e. having eaten nothing for 12 hours) and spend the day with us (~8 hours). We will then take a breath sample to measure how many calories and how much fat you are burning, ask you to answer some questions to determine your memory and problem solving capacity, and take a small blood sample from a tiny plastic tube called a 'cannula' placed in a vein in your forearm. This is no more painful than a simple blood test. We will then ask you to sit comfortably for about 8 hours (comfort breaks to go to the toilet are allowed), during which time you can read, watch TV or use a computer. We will provide you with a test breakfast and test lunch over the course of the day and throughout the day we will take further small blood samples and breath samples and ask memory and problem solving questions. A total of about 120 ml (about a quarter of a blood donation) of blood will be taken over the course of the day.

b) Prolonged standing

This trial will be identical to the Sitting all day trial, except that we will ask you to stand up continuously for 15 minutes out of each 30 minutes throughout the 8-hour observation period.

c) Intermittent standing

This trial will be identical to the Sitting all day trial, except that we will ask you to repeatedly stand 2 minutes and sit for 2 minutes throughout the 8-hour observation period.

What do I have to do?
Other than the specific tasks described above, we ask you to maintain your usual lifestyle (i.e. don't change your diet or exercise habits) for the duration of this study. We also ask you to weigh and record everything that you eat and drink for the two days before your first main experimental trial (we will provide you with scales and record sheets to do this) and not to undertake any planned exercise or drink alcohol on these days. We will ask you to wear a small matchbox-sized device called an accelerometer during these days, and during the days of the trials themselves, so we can monitor your level or physical activity and sitting. We will ask you to repeat your diet and activity pattern for the two days before your second and third experimental trials.
What are the possible disadvantages and risks of taking part?

- Blood sampling via the cannula may cause minor bruising or an inflammation of the vein. Good practice, however, minimises this risk. Some people may feel faint when they give blood.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high cholesterol or high blood pressure. If such a problem is revealed, we will ask your permission to inform your GP to ensure that you receive appropriate treatment.

What are the possible benefits of taking part?
There may be no immediate benefits to you personally, but as a result of being involved in this study you will receive health information about yourself including a dietary assessment and information about your cholesterol and blood sugar levels. You will also receive £100 as a token of thanks for participating. This study will help us to determine how reducing time spent sitting down can improve risk factors for heart disease, diabetes and obesity. The findings of this study will be published in scientific journals so that understanding about how reducing sitting can help people to improve their cardiovascular health and better control of their weight. This information may contribute towards improving physical activity guidelines.

We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain results and discuss the implications with you.

What if something goes wrong?
The chances of something going wrong are extremely small. We have conducted several similar projects over the past 15 years, with many hundreds of participants, and have never had any problems. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay the costs of such action.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the University or hospital will have your name and address removed so that you cannot be recognised from it. In addition, your records, samples and results will be identified by a number and not your name.

What will happen to the results of the research study?
The results from this study will be presented at scientific meetings and published in scientific journals. The results will also form part of Mrs Nabeha Hawari's PhD thesis. A copy of the published results will be sent to you upon request. You will not be identifiable in any of the data presented or published from this study.

What will happen to my samples after the study has finished?
The blood samples that you provide for this study may be useful for future research into the prevention and treatment of diabetes and heart disease; this may involve investigating new biochemical markers that are not yet identified. Samples will be analysed anonymously and will require a new ethics application before they would be used for future research. If you do not wish your samples to be used for future research, please indicate this on the consent form.

Who has reviewed the study?

This study has been reviewed and approved by the College of Medial Veterinary and Life Sciences Ethics Committee at the University of Glasgow.

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting one of the investigators below:

Mrs Nabeha Hawari
E-mail: n.hawari.1@research.gla.ac.uk
Tel: 0141 3303475 (office) or (mobile) 07919182743

Dr Iqbal AlShayji
E-mail: Iqbal.alshayji@glasgow.ac.uk
Tel: 07799353689

Dr Jason Gill
E-mail: jason.gill@glasgow.ac.uk
Tel: 0141 3302916

You will be given a copy of this information sheet and a signed consent form to keep for your records.

University of Glasgow | College of Medical, Veterinary & Life Sciences

Volunteer Identification Number for this trial: _____

CONSENT FORM

Title: Metabolic responses to breaking up sitting time

Name of Researcher: _____

Please initial box

1. I confirm that I have read and understand the information sheet dated 01.10.2014 for the above study and have had the opportunity to ask questions. ☐

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

3. I agree to take part in the above study. ☐

4. I agree for my samples to be used for future research int prevention and treatment of diabetes and heart disease. This involve analysis of new biochemical markers not yet identified
   Yes ☐
   No ☐

_____	_____	_____
Name of Subject	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	**Date**	**Signature**
_____	_____	_____
Researcher	**Date**	**Signature**

Copy for participant
Copy for researcher

Appendix B: Health Screen – Chapter 3, 4 & 5

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name: …….

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, **do you have any health problem for which you are:**

(a) on medication, prescribed or otherwise	**yes [ ]**	**no [ ]**
(b) attending your general practitioner	**yes [ ]**	**no [ ]**
(c) on a hospital waiting list	**yes [ ]**	**no [ ]**

2. **In the past two years,** have you had any illness which required you to:

(a) consult your GP	yes [ ]	no [ ]
(b) attend a hospital outpatient department	yes [ ]	no [ ]
(c) be admitted to hospital	yes [ ]	no [ ]

3. **Have you ever** had any of the following:

(a) Convulsions/epilepsy	yes [ ]	no [ ]
(b) Asthma	yes [ ]	no [ ]
(c) Eczema	yes [ ]	no [ ]
(d) Diabetes	yes [ ]	no [ ]
(e) A blood disorder	yes [ ]	no [ ]
(f) Head injury	yes [ ]	no [ ]
(g) Digestive problems	yes [ ]	no [ ]
(h) Hearing problems	yes [ ]	no [ ]
(i) Problems with bones or joints	yes [ ]	no [ ]
(j) Disturbance of balance/co-ordination	yes [ ]	no [ ]
(k) Numbness in hands or feet	yes [ ]	no [ ]
(l) Disturbance of vision	yes [ ]	no [ ]
(m) Thyroid problems	yes [ ]	no [ ]
(n) Kidney or liver problems	yes [ ]	no [ ]
(o) Chest pain or heart problems	yes [ ]	no [ ]
(p) Any other health problems	yes [ ]	no [ ]

4. **Have any of your family (parents, grandparents, brothers, sisters, children, aunts, uncles, cousins)** ever had any of the following: (if yes please give details including age of first diagnosis)

(a) Any heart problems	yes [ ]	no [ ]
(b) Diabetes	yes [ ]	no [ ]
(c) Stroke	yes [ ]	no [ ]
(d) Any other family illnesses	yes [ ]	no [ ]

4. **For females only** – Are you postmenopausal?* yes [ ] no [ ]
(*at least 2 years since last menstrual bleeding)

6. Do you currently smoke yes [ ] no [ ]
Have you ever smoked yes [ ] no [ ]

If so, for how long did you smoke and when did you stop? ……

7. How many units of alcohol do you typically drink in a week? …….

If YES to any question, please describe briefly if you wish (e.g. to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

……

……

……

Name and address of GP

……

……

Blood pressure measured at screening: mm Hg

Fasting plasma glucose measured at screening: mmol/l

Appendix C: Volunteer`s Information – Chapter 3

Date		**Subject No. & Trial**	
Name			☐ **M** ☐ **F**
Address			
Telephone			
Email			
Date of Birth		**Age**	
Ethnicity			

Height (cm)			**Body Mass (kg)**		
Waist (cm)			**Hip (cm)**		
BMI (kg/m^2)			**WHR**		
BP				**Glucose (mmol/l)**	
ActivPAL*					

* ActivPAL will be fixed on the lower right thigh

Appendix D: Food Instructions – Chapter 3 & 4

University of Glasgow | College of Medical, Veterinary & Life Sciences

Preparation for the Study Day

1. For **three** days before each trial, please refrain from planned or strenuous exercise, other than for personal transportation.

2. Weigh and record your food and drink for two days before your first test. You will have to repeat this **EXACTLY** prior to the second test, so it is advisable to eat meals that you will easily be able to repeat. You will be provided with kitchen weighing scales and record sheets. No alcohol should be consumed on these days.

3. If possible, please try to have the same amount of sleep prior to each test and wake at the same time on the morning of each test.

4. For second day of the trial, please arrive at the laboratory after a 12-hour fast, *i.e.* if your test is at 8 am then your last food and drink should be taken by 8 pm the evening before. Ensure that you drink plenty of water during the evening to prevent dehydration.

5. Please come to the laboratory warm. This will help with blood collection. Wear warm clothing with loose sleeves that can easily be pulled up.

6. Please come to the West Medical Building by car. If this is a problem, please contact us beforehand and we will arrange transport for you.

7. Remember to bring CDs, videos, books, work etc to keep you occupied during the day.

If you have any queries or worries concerning the experiment, please contact
Nabeha Hawari n.hawari.1@research.gla.ac.uk (e-mail).

Food Inventory Instructions

It is important that you weigh and record everything that you eat and drink for the **two** days prior to experiment. Please do not take any alcohol on these days. Your last food and drink should be taken 12 hours before your trial day.

Please (i) start a separate page for each day.
(ii) start a separate line for each item.

Column 1
Record meal and time and place of eating.

Column 2
Describe each item as accurately as possible, stating where relevant:

i. type and brand
ii. whether food is fresh, dried, canned, frozen, salted, smoked, etc.
iii. whether food is cooked, if so give method of cooking e.g. fried, baked, etc.

Column 3
Record the weight of each item after cooking:

i. place scales on a level surface
ii. place plate or container on top of scales
iii. press 'ON/Reset' button to turn on scales
iv. once zero appears, add first item of food
v. record weight displayed
vi. press reset button before weighing next item

Wherever possible, record weights in grams. If this is not possible, record weights in household measures (e.g. sugar or jam in teaspoons, stating whether level, rounded, or heaped).

Column 4
Record the weight of any leftovers, such as food remaining on plate, weight of container in which food has been weighed, apple cores, etc.

Columns 5
Please leave blank.

If food consists of several items, please list each on a separate line i.e. instead of writing 'one cheese sandwich', record separately the weights of bread margarine, cheese, etc.

Please remember to record all drinks, as well as food, giving weights where possible, or volumes if these are known. Record separately the weights of added milk and sugar.

An example is shown overleaf.

Food Inventory - Example

Name_____ **Date** _____

1. Time/Place	2. Description of food/drink	3. Weight of food/drink (g)	4. Weight of container/ leftovers (g)	Leave Blank
Breakfast	Cornflakes (Kelloggs)	28		
8:30am	Milk (Sainsbury's virtually fat-free)	48		
Home	Bread (Mothers Pride, large white	76		
	sliced, toasted)			
	Flora margarine	7		
	Robinsons lemon marmalade	12		
	Coffee (instant)	2		
	Milk (whole pasteurised)	10		
Lunch	Cheese (Cheddar)	55		
1:00pm	Bread (white, crusty)	76		
Pub	Butter	4		
	Chutney (2 teaspoons)			
Snack	Coffee (instant)	2		
3:30pm	Coffee-mate	6		
Office	Mars Bar	35		
	Apple	76	8 (core)	
Dinner	Turkey Fillet (frozen, grilled)	102		
6:30pm	Potatoes, old, boiled	320	74	
Home			(leftover)	
	Peas (Birds Eye, frozen, boiled)	50		
	Heinz tomato ketchup	14		
	Yoghurt (Ski strawberry thick and	162	10	
	creamy)		(carton)	
	Coffee, filter	148		
	Milk (Sainsbury's virtually fat-free)	8		
Snack	Banana	107		
7:45pm	Orange Tango (can)	330		
Home				

Food Inventory

Name____________________________________ **Date** ________________

1. Time/Place	2. Description of food/drink	3. Weight of food/drink (g)	4. Weight of container/ leftovers (g)	Leave Blank

Appendix E: Accelerometers Instructions – Chapter 3 & 4

University of Glasgow | College of Medical, Veterinary & Life Sciences

Accelerometers Instructions

1. You will be fitted with two accelerometers (one on your right hip and one on your lower right thigh). Please **KEEP THE ACCELEROMETERS ON YOUR PERSON AT ALL TIMES, EVEN DURING SLEEPING**.

2. The **ActivPAL** (on your right thigh) will flash green when it is activated.

3. You will be provided with extra stickers and adhesives in case you needed to refit **ActivPAL**.

4. Please take off the **Actigraph** (the belt around your hip) before you shower as it is **NOT** waterproof. Kindly fit it back on your RIGHT side afterwards.

5. Please use the attached sheet to record the days and times whenever you take off the **Actigraph** and when you put it back on.

	ActivPAL	**Actigraph**
Position	Right thigh (10 cm from the middle of the knee)	Right side of the hip
Accessories	Additional stickers	belt

If any of the accelerometers is flashing RED, please contact Nabeha Hawari
n.hawari.1@research.gla.ac.uk (mobile:* 07919182743*).

Sedentary Time Study: accelerometers 7-Day Record

Volunteer Name:			**Actigraph** (Belt)
Day	**Date**	**Time OFF**	**Time ON**

Volunteer Name:			**ActivPAL** (Knee)
Day	**Date**	**Time OFF**	**Time ON**

Notes

……

……

……

Appendix F: Check List Form – Chapter 3 & 4

Sedentary Study Check List

ALWAYS REMEMBER TO BOOK THE MET SUITE & EXERCISE LAB

A Week Pre-Trial

For subject

- ☐ ActivPAL
- ☐ Actigraph
- ☐ Accelerometers sheet
- ☐ Copy food diary to repeat

A Day Pre-Trial

- ☐ Protocol
- ☐ Charge and initiate an Actigraph and an ActivPAL → for Trial Day
- ☐ Charge and initiate an Actigraph and an ActivPAL → for Next Trial Week
- ☐ Accelerometers sheet
- ☐ Adhesives
- ☐ Self-sealed bags x3 → previous week + trial day + next week accelerometers

Met Suite:

- ☐ Label blood tubes → 1 EDTA + 1 Serum for each time point (**x11 timepoints**)
- ☐ Saline
- ☐ Blood collection sets
  - 2-ml & 5-ml syringes + connector + luer adaptor + tissue/gauze + tray + gloves
- ☐ Cannulation set
  - green cannula + swabs + 3-way stopcock + tourniquet + tape
- ☐ Test tube rack
- ☐ Ice box
- ☐ Sharps bin
- ☐ Seating + Douglas bag rack
- ☐ Douglas bags x2
- ☐ Mouth piece/Valves x2
- ☐ Stopwatches x4 → Blood + Expired Air + Protocol + Backup

Molecular Lab:

- ☐ Label Apex tubes (0.5 ml) → 6 EDTA + 4 Serum for each time point
- ☐ Plastic Boxes for Apex tubes

Trial Day

- ☐ Evacuate Douglas bags, if necessary.
- ☐ Ice
- ☐ Tissues for subject (after expired air collection)

Molecular Lab:

- ☐ Turn on centrifuge
- ☐ Change YSI mode to RUN
- ☐ Run controls on YSI

When the subject arrives:

- ☐ **WEIGH SUBJECT**
- ☐ **BAROMETRIC PRESSURE**
- ☐ Calculate test meal
- ☐ Weigh test meal food and store in fridge (do not toast the bagel)
- ☐ Take food diary and copy for subject to repeat.
- ☐ Replace accelerometers

Post-Trial

- ☐ Clean Expired air equipment:
    - Rinse and soak valves/mouthpieces/nose clips in Trigene (for 3 hours)
    - Wash tube with trigene and leave to dry
    - Blow up Douglas bags
    - Turn off analyser pump at the end of the day
    - Sign sheet behind the door
- ☐ Clean Met Suite
    - Sign sheet behind the door
- ☐ Clean Kitchen
    - Sign sheet behind the door

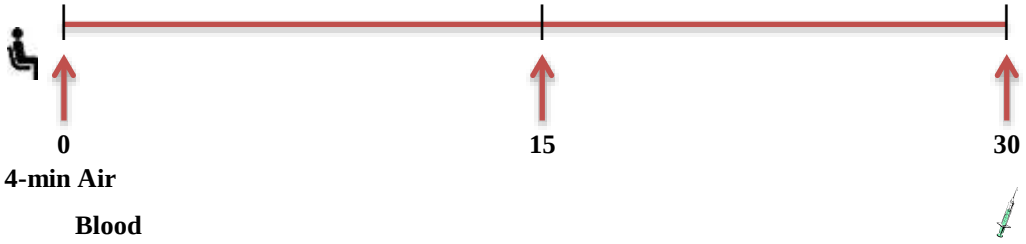
Appendix G: Sitting, prlonged standing and Intermittent Standing Protocols-Chapter 3

SITTING PROTOCOL

Date		Time		Weight (kg)		Subject	

Protocol	Time	Position	Notes
Weigh Subject	On arrival		
Remove accelerometers			Download data and charge
Place new accelerometers			- ActivPAL: lower right thigh - Actigraph: right hip
Rest for 10 mins		**Sit**	
4-min Resting air sample 1		Sit	
Analyse		Sit	☐ OK ☐ Repeat
4-min Resting air sample 2		Sit	
Analyse		Sit	☐ OK ☐ Repeat
Cannulation	-10 min	Sit	Rest for 10 min
Fasting blood sample	0 min	Sit	
Breakfast	**00:00**	**Sit**	**Within 5-10 min**
Start Stopwatch	00:00		**GO TO NEXT PAGE**

Half-hourly Protocol



Date		**1 – 2 HOURS**	**Subject**	

Protocol	Time	Position	Notes
Breakfast	00:00	**Sit**	
Mouthpiece & nose clip	13:00	**Sit**	
4-min air collection	15:00		
Stop air collection	19:00		
Mouthpiece & Nose clip	28:00		
4-min air collection	30:00	**Sit**	Blood Sample **(30 min)**
Stop air collection	34:00		
Mouthpiece & nose clip	43:00		
4-min air collection	45:00		
Stop air collection	49:00		
Mouthpiece & nose clip	58:00		
4-min air collection	1:00:00	**Sit**	Blood sample (**60 min**)
Stop air collection	1:04:00		
Mouthpiece & nose clip	1:13:00		
4-min air collection	1:15:00		
Stop air collection	1:19:00		
Mouthpiece & nose clip	1:28:00		
4-min air collection	1:30:00	**Sit**	
Stop air collection	1:34:00		
Mouthpiece & nose clip	1:43:00		
4-min air collection	1:45:00		
Stop air collection	1:49:00		
Mouthpiece & nose clip	1:58:00		

Date		**2 – 4 HOURS**	**Subject**	

Protocol	Time	Position	Notes
4-min air collection	2:00:00	**Sit**	Blood sample (**120 min**)
Stop air collection	2:04:00		
Mouthpiece & nose clip	2:13:00		
4-min air collection	2:15:00		
Stop air collection	2:19:00		
Mouthpiece & nose clip	2:28:00		
4-min air collection	2:30:00	**Sit**	
Stop air collection	2:34:00		
Mouthpiece & nose clip	2:43:00		
4-min air collection	2:45:00		
Stop air collection	2:49:00		
Mouthpiece & nose clip	2:58:00		
4-min air collection	3:00:00	**Sit**	Blood sample (**180 min**)
Stop air collection	3:04:00		
Mouthpiece & nose clip	3:13:00		
4-min air collection	3:15:00		
Stop air collection	3:19:00		
Mouthpiece & nose clip	3:28:00		
4-min air collection	3:30:00	**Sit**	
Stop air collection	3:34:00		
Mouthpiece & nose clip	3:43:00		
4-min air collection	3:45:00		
Stop air collection	3:49:00		
Blood	4:00:00		Blood sample (**240 min**)

Date		**4 – 6 HOURS**	**Subject**	

Protocol	Time	Position	Notes
Lunch	4:00:00	**Sit**	
Mouthpiece & nose clip	4:13:00	**Sit**	
4-min air collection	4:15:00		
Stop air collection	4:19:00		
Mouthpiece & Nose clip	4:28:00		
4-min air collection	4:30:00	**Sit**	Blood Sample **(270 min)**
Stop air collection	4:34:00		
Mouthpiece & nose clip	4:43:00		
4-min air collection	4:45:00		
Stop air collection	4:49:00		
Mouthpiece & nose clip	4:58:00		
4-min air collection	5:00:00	**Sit**	Blood sample (**300 min**)
Stop air collection	5:04:00		
Mouthpiece & nose clip	5:13:00		
4-min air collection	5:15:00		
Stop air collection	5:19:00		
Mouthpiece & nose clip	5:28:00		
4-min air collection	5:30:00	**Sit**	
Stop air collection	5:34:00		
Mouthpiece & nose clip	5:43:00		
4-min air collection	5:45:00		
Stop air collection	5:49:00		
Mouthpiece & nose clip	5:58:00		

Date		**6 – 8 HOURS**	**Subject**	

Protocol	**Time**	**Position**	**Notes**
4-min air collection	6:00:00	**Sit**	Blood sample (**360 min**)
Stop air collection	6:04:00		
Mouthpiece & nose clip	6:13:00		
4-min air collection	6:15:00		
Stop air collection	6:19:00		
Mouthpiece & nose clip	6:28:00		
4-min air collection	6:30:00	**Sit**	
Stop air collection	6:34:00		
Mouthpiece & nose clip	6:43:00		
4-min air collection	6:45:00		
Stop air collection	6:49:00		
Mouthpiece & nose clip	6:58:00		
4-min air collection	7:00:00	**Sit**	Blood sample (**420 min**)
Stop air collection	7:04:00		
Mouthpiece & nose clip	7:13:00		
4-min air collection	7:15:00		
Stop air collection	7:19:00		
Mouthpiece & nose clip	7:28:00		
4-min air collection	7:30:00	**Sit**	
Stop air collection	7:34:00		
Mouthpiece & nose clip	7:43:00		
4-min air collection	7:45:00		
Stop air collection	7:49:00		
Mouthpiece & nose clip	7:58:00		
4-min air collection	8:00:00		Blood sample (**480 min**)
Stop air collection	8:04:00		
Remove ActivPAL and Actigraph			Download data
Fit ActivPAL and Actigraph for next week			

Sedentary Behaviour Study

Expired Air Samples (1)

Date		Weight (kg)	Lab Temp (ºC)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
							S
Bag	**Time (min)**	**Time (0:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (ºC)**
		Resting 1					
		Resting 2					
		Resting 3					
Breakfast							
	15	**0:15**					
	30	**0:30**					
	45	**0:45**					
	60	**1:00**					
	75	**1:15**					
	90	**1:30**					
	105	**1:45**					
	120	**2:00**					
	135	**2:15**					
	150	**2:30**					
	165	**2:45**					
	180	**3:00**					
	195	**3:15**					
	210	**3:30**					
	225	**3:45**					

*Sampling time: 4 mins (240 sec)

Researcher: _____

Sedentary Behaviour Study

Expired Air Samples (2)

Date		Weight (kg)	Lab Temp (oC)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
							S
Bag	**Time (min)**	**Time (0:00)**	**Sampling Time (sec)***	**FECO2%**	**FEO2%**	**Volume (L)**	**Temp (oC)**
Lunch							
	255	**4:15**					
	270	**4:30**					
	285	**4:45**					
	300	**5:00**					
	315	**5:15**					
	330	**5:30**					
	345	**5:45**					
	360	**6:00**					
	375	**6:15**					
	390	**6:30**					
	405	**6:45**					
	420	**7:00**					
	435	**7:15**					
	450	**7:30**					
	465	**7:45**					
	480	**8:00**					

*Sampling time: 4 mins (240 sec)

Researcher: _____

Sedentary Behaviour Study

Blood Sampling and Glucose Measurements

Date		Subject	
			S

Time (0:00)	Timepoint (min)	✓	Protocol	Glucose (mmol/l)		Notes
0:00	**0**		**Fasting**			
0:30	**30**					
1:00	**60**					
2:00	**120**					
3:00	**180**					
4:00	**240**		**Lunch**			
4:30	**270**					
5:00	**300**					
6:00	**360**					
7:00	**420**					
8:00	**480**					

Controls	Lot No:		**High** value (mmol/l):		**Low** value (mmol/l):
Control (Start)	L	L	H	H	mmol/l
Control (Middle)	L	L	H	H	mmol/l
Control (End)	L	L	H	H	mmol/l

Important notes:

- **TWO** blood samples Serum + EDTA
- EDTA samples should be placed on ice immediately.
- EDTA samples should be spun and aliquoted within **5-10 min** (6 aliquots)
    - Centrifuge should be set at 4000 rpm for 15 mins (programme #4)
- SERUM samples should be left to clot for ~1h before separating and aliquoting (4 aliquots)
- All aliquots should be at least 0.5 ml.

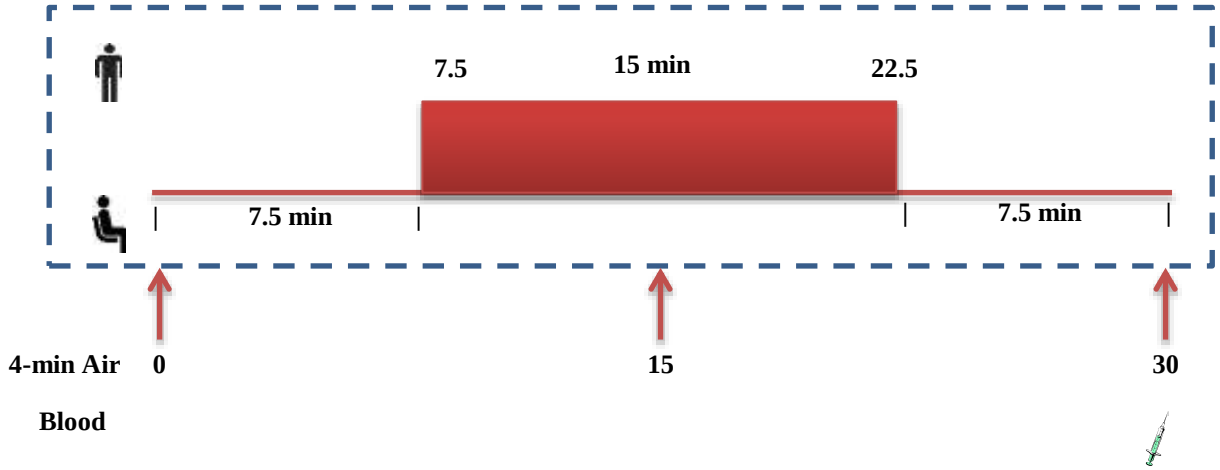
Researcher: _____ Glucose Analysis: _____

PROLONGED STANDING PROTOCOL

Date		**Time**		**Weight (kg)**		**Subject**	

Protocol	Time	Position	Notes
Weigh Subject	On arrival		
Remove accelerometers	On arrival		Download data and charge
Place new accelerometers			- ActivPAL: lower right thigh - Actigraph: right hip
Rest for 10 mins			
4-min Resting air sample 1		Sit	
Analyse		Sit	☐ OK ☐ Repeat
4-min Resting air sample 2		Sit	
Analyse		Sit	☐ OK ☐ Repeat
Cannulation	-10 min	Sit	Rest for 10 min
Fasting blood sample	0 min	Sit	
Breakfast	**00:00**	**Sit**	**Within 5-10 min**
Start Stopwatch	00:00		**GO TO NEXT PAGE**

Half-hourly Protocol



Date		**PART 1**	**Subject**	

Protocol	Time	Position	Notes
Breakfast	00:00	**Sit**	
Mouthpiece & nose clip	13:00	**Sit**	
4-min air collection	15:00		
Stop air collection	19:00		
Mouthpiece & Nose clip	28:00		
4-min air collection	**30:00**	**Sit**	Blood Sample **(30 min)**
Stop air collection	**34:00**		
	37:30	STAND	
Mouthpiece & nose clip	43:00		
4-min air collection	45:00	STAND	
Stop air collection	49:00		
	52:30	**Sit**	
Mouthpiece & nose clip	**58:00**		
4-min air collection	**1:00:00**	**Sit**	Blood sample (**60 min**)
Stop air collection	**1:04:00**		
	1:07:30	STAND	
Mouthpiece & nose clip	1:13:00		
4-min air collection	1:15:00	STAND	
Stop air collection	1:19:00		
	1:22:30	**Sit**	
Mouthpiece & nose clip	**1:28:00**		
4-min air collection	**1:30:00**	**Sit**	
Stop air collection	**1:34:00**		
	1:37:30	STAND	
Mouthpiece & nose clip	1:43:00		
4-min air collection	1:45:00	STAND	
Stop air collection	1:49:00		

Date		**PART 2**	**Subject**	

Protocol	Time	Position	Notes
	1:52:30	**Sit**	
Mouthpiece & nose clip	**1:58:00**		
4-min air collection	**2:00:00**	**Sit**	Blood sample (**120 min**)
Stop air collection	**2:04:00**		
	2:07:30	STAND	
Mouthpiece & nose clip	2:13:00		
4-min air collection	2:15:00	STAND	
Stop air collection	2:19:00		
	2:22:30	**Sit**	
Mouthpiece & nose clip	**2:28:00**		
4-min air collection	**2:30:00**	**Sit**	
Stop air collection	**2:34:00**		
	2:37:30	STAND	
Mouthpiece & nose clip	2:43:00		
4-min air collection	2:45:00	STAND	
Stop air collection	2:49:00		
	2:52:30	**Sit**	
Mouthpiece & nose clip	**2:58:00**		
4-min air collection	**3:00:00**	**Sit**	Blood sample (**180 min**)
Stop air collection	**3:04:00**		
	3:07:30	STAND	
Mouthpiece & nose clip	3:13:00		
4-min air collection	3:15:00	STAND	
Stop air collection	3:19:00		
	3:22:30	**Sit**	
Mouthpiece & nose clip	**3:28:00**		

Date		**PART 3**	Subject	

Protocol	Time	Position	Notes
4-min air collection	**3:30:00**	**Sit**	
Stop air collection	**3:34:00**		
	3:37:30	STAND	
Mouthpiece & nose clip	3:43:00		
4-min air collection	3:45:00	STAND	
Stop air collection	3:49:00		
	3:52:30	**Sit**	
	3:58:00		
Blood sample then **Lunch**	**4:00:00**	**Sit**	Blood sample (**240 min**)
Mouthpiece & nose clip	4:13:00	**Sit**	
4-min air collection	4:15:00		
Stop air collection	4:19:00		
Mouthpiece & Nose clip	4:28:00		
4-min air collection	**4:30:00**	**Sit**	Blood Sample **(270 min)**
Stop air collection	**4:34:00**		
	4:37:30	STAND	
Mouthpiece & nose clip	4:43:00		
4-min air collection	4:45:00	STAND	
Stop air collection	4:49:00		
	4:52:30	**Sit**	
Mouthpiece & nose clip	**4:58:00**		
4-min air collection	**5:00:00**	**Sit**	Blood sample (**300 min**)
Stop air collection	**5:04:00**		
	5:07:30	STAND	
Mouthpiece & nose clip	5:13:00		
4-min air collection	5:15:00	STAND	
Stop air collection	5:19:00		

Date		**PART 4**	Subject	

Protocol	Time	Position	Notes
	5:22:30	**Sit**	
Mouthpiece & nose clip	**5:28:00**		
4-min air collection	**5:30:00**	**Sit**	
Stop air collection	**5:34:00**		
	5:37:30	STAND	
Mouthpiece & nose clip	5:43:00		
4-min air collection	5:45:00	STAND	
Stop air collection	5:49:00		
	5:52:30	**Sit**	
Mouthpiece & nose clip	**5:58:00**		
4-min air collection	**6:00:00**	**Sit**	Blood sample (**360 min**)
Stop air collection	**6:04:00**		
	6:07:30	STAND	
Mouthpiece & nose clip	6:13:00		
4-min air collection	6:15:00	STAND	
Stop air collection	6:19:00		
	6:22:30	**Sit**	
Mouthpiece & nose clip	**6:28:00**		
4-min air collection	**6:30:00**	**Sit**	
Stop air collection	**6:34:00**		
	6:37:30	STAND	
Mouthpiece & nose clip	6:43:00		
4-min air collection	6:45:00	STAND	
Stop air collection	6:49:00		
	6:52:30	**Sit**	
Mouthpiece & nose clip	**6:58:00**		

Date		**PART 5**	Subject	

Protocol	Time	Position	Notes
4-min air collection	**7:00:00**	**Sit**	Blood sample (**420 min**)
Stop air collection	**7:04:00**		
	7:07:30	STAND	
Mouthpiece & nose clip	7:13:00		
4-min air collection	7:15:00	STAND	
Stop air collection	7:19:00		
	7:22:30	**Sit**	
Mouthpiece & nose clip	**7:28:00**		
4-min air collection	**7:30:00**	**Sit**	
Stop air collection	**7:34:00**		
	7:37:30	STAND	
Mouthpiece & nose clip	7:43:00		
4-min air collection	7:45:00	STAND	
Stop air collection	7:49:00		
	7:52:30	**Sit**	
Mouthpiece & nose clip	**7:58:00**		
4-min air collection	**8:00:00**		Blood sample (**480 min**)
Stop air collection	**8:04:00**		
Remove ActivPAL and Actigraph			Download data
Fit ActivPAL and Actigraph for next week			

Sedentary Behaviour Study

Expired Air Samples (1)

Date		Weight (kg)	Lab Temp (ºC)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
							P
Bag	**Time (min)**	**Time (0:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (ºC)**
		Resting 1					
		Resting 2					
		Resting 3					
Breakfast							
	15	**0:15**					
	30	**0:30**					
	45	**0:45**					
	60	**1:00**					
	75	**1:15**					
	90	**1:30**					
	105	**1:45**					
	120	**2:00**					
	135	**2:15**					
	150	**2:30**					
	165	**2:45**					
	180	**3:00**					
	195	**3:15**					
	210	**3:30**					
	225	**3:45**					

*Sampling time: 4 mins (240 sec) *Researcher:* _____

Sedentary Behaviour Study

Expired Air Samples (2)

Date		Weight (kg)	Lab Temp (ºC)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
							P
Bag	**Time (min)**	**Time (0:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (ºC)**
Lunch							
	255	**4:15**					
	270	**4:30**					
	285	**4:45**					
	300	**5:00**					
	315	**5:15**					
	330	**5:30**					
	345	**5:45**					
	360	**6:00**					
	375	**6:15**					
	390	**6:30**					
	405	**6:45**					
	420	**7:00**					
	435	**7:15**					
	450	**7:30**					
	465	**7:45**					
	480	**8:00**					

*Sampling time: 4 mins (240 sec) *Researcher:* _____

Sedentary Behaviour Study

Blood Sampling and Glucose Measurements

Date		Subject	
			P

Time (0:00)	Timepoint (min)	✓	Protocol	Glucose (mmol/l)		Notes
0:00	**0**		**Fasting**			
0:30	**30**					
1:00	**60**					
2:00	**120**					
3:00	**180**					
4:00	**240**		**Lunch**			
4:30	**270**					
5:00	**300**					
6:00	**360**					
7:00	**420**					
8:00	**480**					

Controls	Lot No:		**High** value (mmol/l):		**Low** value (mmol/l):
Control (Start)	L	L	H	H	mmol/l
Control (Middle)	L	L	H	H	mmol/l
Control (End)	L	L	H	H	mmol/l

Important notes:

- **TWO** blood samples Serum + EDTA
- EDTA samples should be placed on ice immediately.
- EDTA samples should be spun and aliquoted within **5-10 min** (6 aliquots)
    - Centrifuge should be set at 4000 rpm for 15 mins (programme #4)
- SERUM samples should be left to clot for ~1h before separating and aliquoting (4 aliquots)
- All aliquots should be at least 0.5 ml.

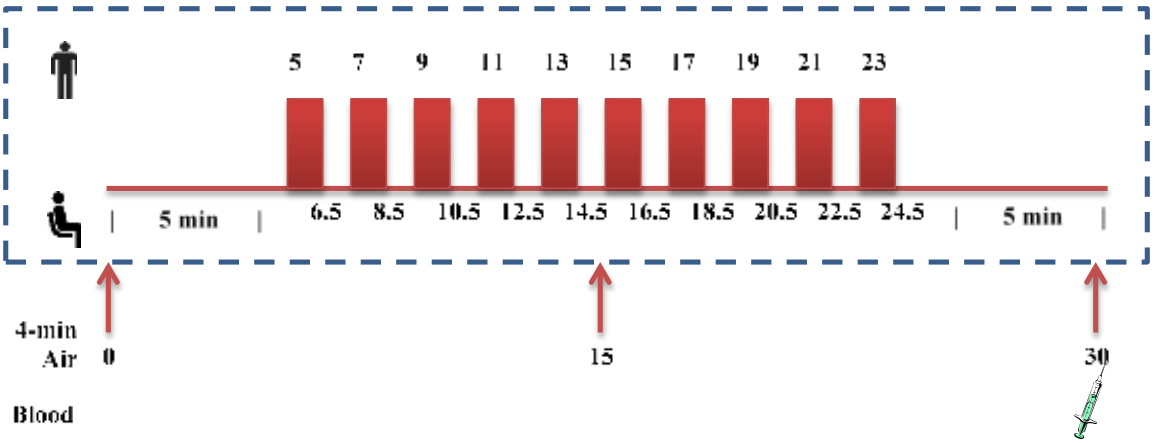
Researcher: _____ *Glucose Analysis:*_____

INTERMITTENT STANDING PROTOCOL

Date		**Time**		**Weight (kg)**		**Subject**	

Protocol	**Time**	**Position**	**Notes**
Weigh Subject	On arrival		
Remove accelerometers			Download data and charge
Place new accelerometers			- ActivPAL: lower right thigh - Actigraph: right hip
Rest for 10 mins		**Sit**	
4-min Resting air sample 1		Sit	
Analyse		Sit	☐ OK ☐ Repeat
4-min Resting air sample 2		Sit	
Analyse		Sit	☐ OK ☐ Repeat
Cannulation	-10 min	Sit	Rest for 10 min
Fasting blood sample	0 min	Sit	
Breakfast	**00:00**	**Sit**	**Within 5-10 min**
Start Stopwatch	00:00		**GO TO NEXT PAGE**

Half-hourly Protocol



Date		**FIRST HOUR**	Subject	

Protocol	Time	Position	Notes
Breakfast	00:00	Sit	
Mouthpiece & nose clip	13:00	Sit	
4-min air collection	15:00		
Stop air collection	19:00		
Mouthpiece & Nose clip	28:00		
4-min air collection	30:00	Sit	Blood Sample **(30 min)**
Stop air collection	34:00		
	35:00	STAND	
	36:30	**Sit**	
	37:00	STAND	
	38:30	**Sit**	
	39:00	STAND	
	40:30	**Sit**	
	41:00	STAND	
	42:30	**Sit**	
Mouthpiece & nose clip	43:00	STAND	
	44:30	**Sit**	
4-min air collection	45:00	STAND	
	46:30	**Sit**	
	47:00	STAND	
	48:30	**Sit**	
Stop air collection	49:00	STAND	
	50:30	**Sit**	
	51:00	STAND	
	52:30	**Sit**	
	53:00	STAND	
	54:30	**Sit**	
Mouthpiece & nose clip	58:00	Sit	

Date		1 – 1.5 HOUR	Subject	

Protocol	Time	Position	Notes
4-min air collection	1:00:00	Sit	Blood Sample **(60 min)**
Stop air collection	1:04:00		
	1:05:00	STAND	
	1:06:30	**Sit**	
	1:07:00	STAND	
	1:08:30	**Sit**	
	1:09:00	STAND	
	1:10:30	**Sit**	
	1:11:00	STAND	
	1:12:30	**Sit**	
Mouthpiece & nose clip	1:13:00	STAND	
	1:14:30	**Sit**	
4-min air collection	1:15:00	STAND	
	1:16:30	**Sit**	
	1:17:00	STAND	
	1:18:30	**Sit**	
Stop air collection	1:19:00	STAND	
	1:20:30	**Sit**	
	1:21:00	STAND	
	1:22:30	**Sit**	
	1:23:00	STAND	
	1:24:30	**Sit**	
Mouthpiece & nose clip	1:28:00	Sit	
4-min air collection	1:30:00	Sit	

Date		1.5 – 2 HOUR	Subject	

Protocol	Time	Position	Notes
Stop air collection	1:34:00	Sit	
	1:35:00	STAND	
	1:36:30	**Sit**	
	1:37:00	STAND	
	1:38:30	**Sit**	
	1:39:00	STAND	
	1:40:30	**Sit**	
	1:41:00	STAND	
	1:42:30	**Sit**	
Mouthpiece & nose clip	1:43:00	STAND	
	1:44:30	**Sit**	
4-min air collection	1:45:00	STAND	
	1:46:30	**Sit**	
	1:47:00	STAND	
	1:48:30	**Sit**	
Stop air collection	1:49:00	STAND	
	1:50:30	**Sit**	
	1:51:00	STAND	
	1:52:30	**Sit**	
	1:53:00	STAND	
	1:54:30	**Sit**	
Mouthpiece & nose clip	1:58:00	Sit	

Date		2 – 2.5 HOUR	Subject	

Protocol	Time	Position	Notes
4-min air collection	2:00:00	Sit	Blood Sample **(120 min)**
Stop air collection	2:04:00		
	2:05:00	STAND	
	2:06:30	**Sit**	
	2:07:00	STAND	
	2:08:30	**Sit**	
	2:09:00	STAND	
	2:10:30	**Sit**	
	2:11:00	STAND	
	2:12:30	**Sit**	
Mouthpiece & nose clip	2:13:00	STAND	
	2:14:30	**Sit**	
4-min air collection	2:15:00	STAND	
	2:16:30	**Sit**	
	2:17:00	STAND	
	2:18:30	**Sit**	
Stop air collection	2:19:00	STAND	
	2:20:30	**Sit**	
	2:21:00	STAND	
	2:22:30	**Sit**	
	2:23:00	STAND	
	2:24:30	**Sit**	
Mouthpiece & nose clip	2:28:00	Sit	
4-min air collection	2:30:00	Sit	

Date		2.5 - 3 HOUR	Subject	

Protocol	Time	Position	Notes
Stop air collection	2:34:00	Sit	
	2:35:00	STAND	
	2:36:30	**Sit**	
	2:37:00	STAND	
	2:38:30	**Sit**	
	2:39:00	STAND	
	2:40:30	**Sit**	
	2:41:00	STAND	
	2:42:30	**Sit**	
Mouthpiece & nose clip	2:43:00	STAND	
	2:44:30	**Sit**	
4-min air collection	2:45:00	STAND	
	2:46:30	**Sit**	
	2:47:00	STAND	
	2:48:30	**Sit**	
Stop air collection	2:49:00	STAND	
	2:50:30	**Sit**	
	2:51:00	STAND	
	2:52:30	**Sit**	
	2:53:00	STAND	
	2:54:30	**Sit**	
Mouthpiece & nose clip	2:58:00	Sit	

Date		3 – 3.5 HOUR	Subject	

Protocol	Time	Position	Notes
4-min air collection	3:00:00	Sit	Blood Sample **(180 min)**
Stop air collection	3:04:00		
	3:05:00	STAND	
	3:06:30	**Sit**	
	3:07:00	STAND	
	3:08:30	**Sit**	
	3:09:00	STAND	
	3:10:30	**Sit**	
	3:11:00	STAND	
	3:12:30	**Sit**	
Mouthpiece & nose clip	3:13:00	STAND	
	3:14:30	**Sit**	
4-min air collection	3:15:00	STAND	
	3:16:30	**Sit**	
	3:17:00	STAND	
	3:18:30	**Sit**	
Stop air collection	3:19:00	STAND	
	3:20:30	**Sit**	
	3:21:00	STAND	
	3:22:30	**Sit**	
	3:23:00	STAND	
	3:24:30	**Sit**	
Mouthpiece & nose clip	3:28:00	Sit	
4-min air collection	3:30:00		

Date		3.5 - 4 HOUR	Subject	

Protocol	Time	Position	Notes
Stop air collection	3:34:00	Sit	
	3:35:00	STAND	
	3:36:30	**Sit**	
	3:37:00	STAND	
	3:38:30	**Sit**	
	3:39:00	STAND	
	3:40:30	**Sit**	
	3:41:00	STAND	
	3:42:30	**Sit**	
Mouthpiece & nose clip	3:43:00	STAND	
	3:44:30	**Sit**	
4-min air collection	3:45:00	STAND	
	3:46:30	**Sit**	
	3:47:00	STAND	
	3:48:30	**Sit**	
Stop air collection	3:49:00	STAND	
	3:50:30	**Sit**	
	3:51:00	STAND	
	3:52:30	**Sit**	
	3:53:00	STAND	
	3:54:30	**Sit**	
Blood Sample & **Lunch**	4:00:00		Blood Sample **(240 min)**

Date		**FIFTH HOUR**	Subject	

Protocol	Time	Position	Notes
Lunch	4:00:00	Sit	
Mouthpiece & nose clip	4:13:00	Sit	
4-min air collection	4:15:00		
Stop air collection	4:19:00		
Mouthpiece & Nose clip	4:28:00		
4-min air collection	4:30:00	Sit	Blood Sample **(270 min)**
Stop air collection	4:34:00		
	4:35:00	STAND	
	4:36:30	**Sit**	
	4:37:00	STAND	
	4:38:30	**Sit**	
	4:39:00	STAND	
	4:40:30	**Sit**	
	4:41:00	STAND	
	4:42:30	**Sit**	
Mouthpiece & nose clip	4:43:00	STAND	
	4:44:30	**Sit**	
4-min air collection	4:45:00	STAND	
	4:46:30	**Sit**	
	4:47:00	STAND	
	4:48:30	**Sit**	
Stop air collection	4:49:00	STAND	
	4:50:30	**Sit**	
	4:51:00	STAND	
	4:52:30	**Sit**	
	4:53:00	STAND	
	4:54:30	**Sit**	
Mouthpiece & nose clip	4:58:00	Sit	

Date		5 – 5.5 HOUR	Subject	

Protocol	Time	Position	Notes
4-min air collection	5:00:00	Sit	Blood Sample **(300 min)**
Stop air collection	5:04:00		
	5:05:00	STAND	
	5:06:30	**Sit**	
	5:07:00	STAND	
	5:08:30	**Sit**	
	5:09:00	STAND	
	5:10:30	**Sit**	
	5:11:00	STAND	
	5:12:30	**Sit**	
Mouthpiece & nose clip	5:13:00	STAND	
	5:14:30	**Sit**	
4-min air collection	5:15:00	STAND	
	5:16:30	**Sit**	
	5:17:00	STAND	
	5:18:30	**Sit**	
Stop air collection	5:19:00	STAND	
	5:20:30	**Sit**	
	5:21:00	STAND	
	5:22:30	**Sit**	
	5:23:00	STAND	
	5:24:30	**Sit**	
Mouthpiece & nose clip	5:28:00	Sit	
4-min air collection	5:30:00	Sit	

Date		5.5 – 6 HOUR	Subject	

Protocol	Time	Position	Notes
Stop air collection	5:34:00	Sit	
	5:35:00	STAND	
	5:36:30	**Sit**	
	5:37:00	STAND	
	5:38:30	**Sit**	
	5:39:00	STAND	
	5:40:30	**Sit**	
	5:41:00	STAND	
	5:42:30	**Sit**	
Mouthpiece & nose clip	5:43:00	STAND	
	5:44:30	**Sit**	
4-min air collection	5:45:00	STAND	
	5:46:30	**Sit**	
	5:47:00	STAND	
	5:48:30	**Sit**	
Stop air collection	5:49:00	STAND	
	5:50:30	**Sit**	
	5:51:00	STAND	
	5:52:30	**Sit**	
	5:53:00	STAND	
	5:54:30	**Sit**	
Mouthpiece & nose clip	5:58:00	Sit	

Date		6 – 6.5 HOUR	Subject	

Protocol	Time	Position	Notes
4-min air collection	6:00:00	Sit	Blood Sample **(360 min)**
Stop air collection	6:04:00		
	6:05:00	STAND	
	6:06:30	**Sit**	
	6:07:00	STAND	
	6:08:30	**Sit**	
	6:09:00	STAND	
	6:10:30	**Sit**	
	6:11:00	STAND	
	6:12:30	**Sit**	
Mouthpiece & nose clip	6:13:00	STAND	
	6:14:30	**Sit**	
4-min air collection	6:15:00	STAND	
	6:16:30	**Sit**	
	6:17:00	STAND	
	6:18:30	**Sit**	
Stop air collection	6:19:00	STAND	
	6:20:30	**Sit**	
	6:21:00	STAND	
	6:22:30	**Sit**	
	6:23:00	STAND	
	6:24:30	**Sit**	
Mouthpiece & nose clip	6:28:00	Sit	
4-min air collection	6:30:00	Sit	

Date		6.5 – 7 HOUR	Subject	

Protocol	Time	Position	Notes
Stop air collection	6:34:00	Sit	
	6:35:00	STAND	
	6:36:30	**Sit**	
	6:37:00	STAND	
	6:38:30	**Sit**	
	6:39:00	STAND	
	6:40:30	**Sit**	
	6:41:00	STAND	
	6:42:30	**Sit**	
Mouthpiece & nose clip	6:43:00	STAND	
	6:44:30	**Sit**	
4-min air collection	6:45:00	STAND	
	6:46:30	**Sit**	
	6:47:00	STAND	
	6:48:30	**Sit**	
Stop air collection	6:49:00	STAND	
	6:50:30	**Sit**	
	6:51:00	STAND	
	6:52:30	**Sit**	
	6:53:00	STAND	
	6:54:30	**Sit**	
Mouthpiece & nose clip	6:58:00	Sit	

Date		7 – 7.5 HOUR	Subject	

Protocol	Time	Position	Notes
4-min air collection	7:00:00	Sit	Blood Sample **(420 min)**
Stop air collection	7:04:00		
	7:05:00	STAND	
	7:06:30	**Sit**	
	7:07:00	STAND	
	7:08:30	**Sit**	
	7:09:00	STAND	
	7:10:30	**Sit**	
	7:11:00	STAND	
	7:12:30	**Sit**	
Mouthpiece & nose clip	7:13:00	STAND	
	7:14:30	**Sit**	
4-min air collection	7:15:00	STAND	
	7:16:30	**Sit**	
	7:17:00	STAND	
	7:18:30	**Sit**	
Stop air collection	7:19:00	STAND	
	7:20:30	**Sit**	
	7:21:00	STAND	
	7:22:30	**Sit**	
	7:23:00	STAND	
	7:24:30	**Sit**	
Mouthpiece & nose clip	7:28:00	Sit	
4-min air collection	7:30:00		

Date		7.5 – 8 HOUR	Subject	

Protocol	Time	Position	Notes
Stop air collection	7:34:00	Sit	
	7:35:00	STAND	
	7:36:30	**Sit**	
	7:37:00	STAND	
	7:38:30	**Sit**	
	7:39:00	STAND	
	7:40:30	**Sit**	
	7:41:00	STAND	
	7:42:30	**Sit**	
Mouthpiece & nose clip	7:43:00	STAND	
	7:44:30	**Sit**	
4-min air collection	7:45:00	STAND	
	7:46:30	**Sit**	
	7:47:00	STAND	
	7:48:30	**Sit**	
Stop air collection	7:49:00	STAND	
	7:50:30	**Sit**	
	7:51:00	STAND	
	7:52:30	**Sit**	
	7:53:00	STAND	
	7:54:30	**Sit**	
Mouthpiece & nose clip	7:58:00	Sit	
4-min air collection	8:00:00	Sit	Blood sample (**480 min**)
Stop air collection	8:04:00		**END**
Remove ActivPAL and Actigraph			Download data
Fit ActivPAL and Actigraph for next week			

Sedentary Behaviour Study

Expired Air Samples (1)

Date		Weight (kg)	Lab Temp (°C)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
							I
Bag	**Time (min)**	**Time (0:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (°C)**
		Resting 1					
		Resting 2					
		Resting 3					
Breakfast							
	15	**0:15**					
	30	**0:30**					
	45	**0:45**					
	60	**1:00**					
	75	**1:15**					
	90	**1:30**					
	105	**1:45**					
	120	**2:00**					
	135	**2:15**					
	150	**2:30**					
	165	**2:45**					
	180	**3:00**					
	195	**3:15**					
	210	**3:30**					
	225	**3:45**					

*Sampling time: 4 mins (240 sec) *Researcher:* _____

Sedentary Behaviour Study

Expired Air Samples (2)

Date		Weight (kg)	Lab Temp (ºC)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
							I
Bag	**Time (min)**	**Time (0:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (ºC)**
Lunch							
	255	**4:15**					
	270	**4:30**					
	285	**4:45**					
	300	**5:00**					
	315	**5:15**					
	330	**5:30**					
	345	**5:45**					
	360	**6:00**					
	375	**6:15**					
	390	**6:30**					
	405	**6:45**					
	420	**7:00**					
	435	**7:15**					
	450	**7:30**					
	465	**7:45**					
	480	**8:00**					

*Sampling time: 4 mins (240 sec) *Researcher:* _____

Sedentary Behaviour Study

Blood Sampling and Glucose Measurements

Date		Subject	
			I

Time (0:00)	Timepoint (min)	✓	Protocol	Glucose (mmol/l)		Notes
0:00	**0**		**Fasting**			
0:30	**30**					
1:00	**60**					
2:00	**120**					
3:00	**180**					
4:00	**240**		**Lunch**			
4:30	**270**					
5:00	**300**					
6:00	**360**					
7:00	**420**					
8:00	**480**					

Controls	Lot No:		**High** value (mmol/l):		**Low** value (mmol/l):
Control (Start)	L	L	H	H	mmol/l
Control (Middle)	L	L	H	H	mmol/l
Control (End)	L	L	H	H	mmol/l

Important notes:

- **TWO** blood samples Serum + EDTA
- EDTA samples should be placed on ice immediately.
- EDTA samples should be spun and aliquoted within **5-10 min** (6 aliquots)
    - Centrifuge should be set at 4000 rpm for 15 mins (programme #4)
- SERUM samples should be left to clot for ~1h before separating and aliquoting (4 aliquots)
- All aliquots should be at least 0.5 ml.

Researcher: _____ *Glucose Analysis:* _____

Appendix H: Appointments Sheet – Chapter 3 & 4

Name: _____

NH01

Date	Time	Test	Place	Duration	Instructions
2/11/14					• No exercise
3/11/14					• No exercise • No alcohol • **Weigh and record dietary intake**
4/11/14					• No exercise • No alcohol • **Weigh and record dietary intake** • **NOTHING TO EAT OR DRINK (EXCEPT WATER) AFTER** 8:30 pm • Sleep well
5/11/14	08:30 am	**Sitting**	University *of* Glasgow	8 hours	• Fasting for 12 hours • Wear warm clothes (loose sleeves) • Bring a DVD, CD, work or a book to read

Name: _____

NH01

Date	Time	Test	Place	Duration	Instructions
9/11/14					• No exercise
10/11/14					• No exercise • No alcohol • ***Repeat diet EXACTLY as consumed on*** _____3/11/14_____
11/11/14					• No exercise • No alcohol • ***Repeat diet EXACTLY as consumed on*** _____4/11/14_____ • **NOTHING TO EAT OR DRINK (EXCEPT WATER) AFTER** ___8:30 pm_____ • Sleep well
12/11/14	08:30 am	**Prolonged Standing**	University *of* Glasgow	8 hours	• Fasting for 12 hours • Wear warm clothes (loose sleeves) • Bring a DVD, CD, work or a book to read

Name: _____

NH01

Date	Time	Test	Place	Duration	Instructions
16/11/14					• No exercise
17/11/14					• No exercise • No alcohol • ***Repeat diet EXACTLY as consumed on*** 3/11/14
18/11/14					• No exercise • No alcohol • ***Repeat diet EXACTLY as consumed on*** 4/11/14 • **NOTHING TO EAT OR DRINK (EXCEPT WATER) AFTER** 8:30 pm • Sleep well
19/11/14	08:30 am	**Intermittent Standing**	University *of* Glasgow	8 hours	• Fasting for 12 hours • Wear warm clothes (loose sleeves) • Bring a DVD, CD, work or a book to read

Appendix I: Volunteer Information Sheet and Consent Form - Chapter 4

VOLUNTEER INFORMATION SHEET

Title: Effects of breaking prolonged sitting with intermittent 'chair squats' on day-long metabolic responses.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

Spending large amounts of time sitting down increases risk of heart disease, diabetes and obesity. This risk may be reduced by breaking up periods of prolonged sitting with periods of standing up. We have recently shown that increasing the number of times a person moves from sitting to standing during the day increases metabolic rate and the amount of fat the body burns. This study will investigate the effects of breaking up prolonged sitting with 10 'chair squats' (repeatedly standing up and sitting down 10 times over 30 seconds) performed every 20 minutes over the course of a day on fat and sugar metabolism in the body.

Why have I been chosen?

You have been chosen because you are a healthy man or a postmenopausal women aged between 18-65 years, who is currently relatively physically inactive. We are planning to include 20 people in this study.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to participate, you will be given this information sheet to keep and be asked to sign a consent form. If you do this you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

Screening procedures

In the first instance you will be asked to attend for a screening visit in which we will:

- discuss with you and complete confidential questionnaires regarding your health, family history and physical activity level
- measure your blood pressure
- take your height, weight and waist measurements
- take a small blood samples to check the sugar level in your blood.
- provide an opportunity for you to ask questions

These preliminary procedures will enable us to determine whether you fall into the group of people we wish to study and will also ensure that it is perfectly safe for you to take part.

Experimental procedures

We will ask you to undergo 2 main experimental trials. Each trial will run approximately 1-2 weeks apart, in random order.

a) Sitting all day

We will ask you to come to the University after an overnight fast (i.e. having eaten nothing for 12 hours) and spend the day with us (~7 hours). We will then take a breath sample to measure how many calories and how much fat you are burning, and take a small blood sample from a tiny plastic tube called a 'cannula' placed in a vein in your forearm. This is no more painful than a simple blood test. We will then ask you to sit comfortably for about 6.5 hours (comfort breaks to go to the toilet are allowed), during which time you can read, watch TV or use a computer. We will provide you with a test breakfast and test lunch over the course of the day and throughout the day we will take further small blood samples and breath samples and ask memory and problem solving questions. A total of about 120 ml (about a quarter of a blood donation) of blood will be taken over the course of the day.

d) Sit/stand

This trial will be identical to the Sitting trial, except that participants will be asked to repeatedly sit and stand 10 times over 30 seconds (chair squats), every 20 minutes, throughout the 6.5-hour observation period.

Recording diet and physical activity

We will ask you to weigh and record everything you eat and drink for two days before your first main trial and to repeat this diet before your second main trial. We will provide you with weighing scales and diet sheets to do this. We will also ask you to wear a small matchbox-sized device called an accelerometer during these days, and during the days of the trials themselves, so we can monitor your level or physical activity and sitting.

What do I have to do?

Other than the specific tasks described above, we ask you to maintain your usual lifestyle (i.e. don't change your diet or exercise habits) for the duration of this study. We also ask you to weigh and record everything that you eat and drink for the two days before your first main experimental trial (we will provide you with scales and record sheets to do this) and not to undertake any planned exercise or drink alcohol on these days. We will ask you to wear a small matchbox-sized device called an accelerometer during these days, and during the days of the trials themselves, so we can monitor your level or physical activity and sitting. We will ask you to repeat your diet and activity pattern for the two days before your second and third experimental trials.

What are the possible disadvantages and risks of taking part?

- Blood sampling via the cannula may cause minor bruising or an inflammation of the vein. Good practice, however, minimises this risk. Some people may feel faint when they give blood.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high cholesterol or high blood pressure. If such a problem is revealed, we will ask your permission to inform your GP to ensure that you receive appropriate treatment.

What are the possible benefits of taking part?

There may be no immediate benefits to you personally, but as a result of being involved in this study you will receive health about yourself including a dietary assessment and information about your cholesterol and blood sugar levels. Please let us know if you would prefer not to receive any of this information. You will also receive £100 as a token of thanks for participating. This study will help us to determine how reducing time spent sitting down can improve risk factors for heart disease, diabetes and obesity. The findings of this study

will be published in scientific journals so that understanding about how reducing sitting can help people to improve their cardiovascular health and better control of their weight. This information may contribute towards improving physical activity guidelines.

We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain results and discuss the implications with you.

What if something goes wrong?
The chances of something going wrong are extremely small. We have conducted several similar projects over the past 15 years, with many hundreds of participants, and have never had any problems. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the University or hospital will have your name and address removed so that you cannot be recognised from it. In addition, your records, samples and results will be identified by a number and not your name.

What will happen to the results of the research study?
The results from this study will be presented at scientific meetings and published in scientific journals. The results will also form part of Mrs Nabeha Hawari's PhD thesis. A copy of the published results will be sent to you upon request. You will not be identifiable in any of the data presented or published from this study.

What will happen to my samples after the study has finished?
The blood samples that you provide for this study may be useful for future research into the prevention and treatment of diabetes and heart disease; this may involve investigating new substances in the blood that are not yet identified. Samples will be analysed anonymously and will require a new ethics application before they would be used for future research. If you do not wish your samples to be used for future research, please indicate this on the consent form.

Who has reviewed the study?
This study has been reviewed and approved by the College of Medial Veterinary and Life Sciences Ethics Committee at the University of Glasgow.

Contact for Further Information
Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting one of the investigators below:

Mrs Nabeha Hawari
E-mail: n.hawari.1@research.gla.ac.uk
Tel: 0141 3303475 (office) or (mobile) 07919182743

Dr Jason Gill
E-mail: jason.gill@glasgow.ac.uk
Tel: 0141 3302916

You will be given a copy of this information sheet and a signed consent form to keep for your records.

University of Glasgow | College of Medical, Veterinary & Life Sciences

Volunteer Identification Number for this trial: _____

CONSENT FORM

Title: Effects of breaking prolonged sitting with intermittent 'chair squats' on day-long metabolic responses.

Name of Researcher: _____

Please initial box

1. I confirm that I have read and understand the information sheet Version 2 dated 22.10.15 for the above study and have had the opportunity to ask questions. ☐

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

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

7. I agree for my samples to be used for future research into prevention and treatment of diabetes and heart disease. This may involve analysis of new biochemical markers not yet identified. Yes ☐ No ☐

_____	_____	_____
Name of Subject	**Date**	**Signature**
_____	_____	_____
Name of Person taking consent (if different from researcher)	**Date**	**Signature**
_____	_____	_____
Researcher	**Date**	**Signature**

Copy for participant
Copy for researcher

Appendix J: Announcement 3 - Chapter 4

University of Glasgow | College of Medical, Veterinary & Life Sciences

Would you like to help out with a research looking into how breaking up sitting time can affect your

We will investigate the effects of breaking up prolonged sitting with 10 'chair squats' over the course of a day on fat and sugar metabolism in the body.

If you are a healthy man (18 – 65 years) or postmenopausal woman, who is heavier than your ideal weight, you may be able to help us.

Exclusion criteria will include uncontrolled hypertension, diabetes or a previous history of heart disease.

Participation will involve 3 visits to our lab (including 30-min screening visit) over a period of 3 weeks. All participants will receive detailed feedback on their blood Pressure, blood glucose, energy expenditure, and your weekly activity.

Participants will also receive payment to compensate for the inconvenience of taking part

If you are interested, please contact

Mrs Nabeha Hawari **n.hawari.1@research.gla.ac.uk**

Thank You ☺

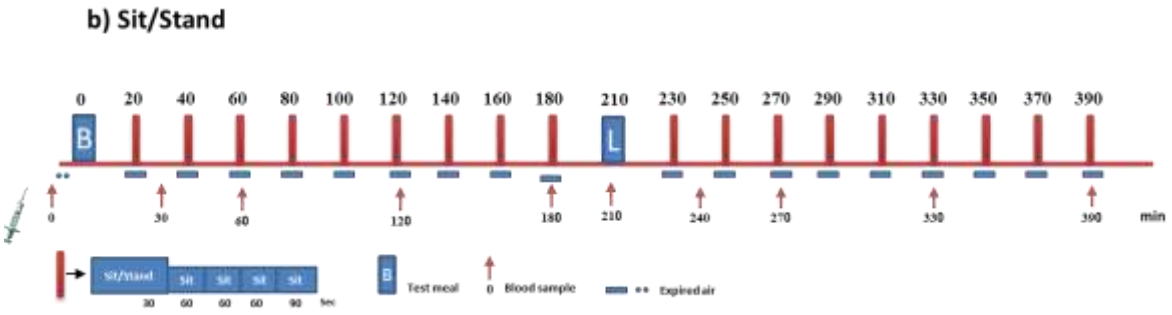
Appendix K: Sit & Sit/Stand Protocols-Chapter 4

Sit/Stand Protocol

Date		Time		Weight (kg)		Subject	

Protocol	Time	Position	Notes
Change accelerometers	On arrival		Download data and charge
Place new accelerometers			- ActivPAL on lower right thigh
4-min Resting air sample 1		Sit	
Analyse		Sit	☐ OK ☐ Repeat
4-min Resting air sample 2		Sit	
Analyse		Sit	☐ OK ☐ Repeat
Cannulation	-10 min	Sit	Rest for 10 min
Fasting blood sample	0 min	Sit	
Breakfast	00:00	Sit	Within 5-10 min
Start Stopwatch	00:00		GO TO NEXT PAGE

Protocol



Date		**PART 1**	**Subject**	

Protocol	Time	Position	Notes
Breakfast	00:00	Sit	
Mouthpiece & nose clip	10:00	Sit	
Open bag 1 (B1)	12:00	Sit	
Stop air collection in B1 (3-min collection) **switch on** to bag (B2)	**15:00**	**Sit/stand 10 times**	
Stop air collection in (B2) (30sec) **switch on** to bag (B3)	15:30	Sit	
Stop air collection in (B3) (60-sec collection) **switch on** to bag (B4)	16:30	Sit	
Stop air collection in (B4) (60 sec air collection) **switch on** to bag (B5)	17:30	Sit	
Stop air collection in (B5) (60 sec air collection) **switch on** to bag (B6)	18:30	Sit	
Stop air collection in (B6) (90 sec air collection)	20:00	Sit	
Mouthpiece & nose clip	30:00	Sit	Blood Sample **(30 min)**
Open bag 1 (B1)	32:00	Sit	3-min collection
Stop air collection in B1 **switch on** to bag (B2)	35:00	Sit/stand 10 times	
Stop air collection in (B2) **switch on** to bag (B3)	35:30	Sit	Gas collection
Stop air collection in (B3) **switch on** to bag (B4)	36:30	Sit	
Stop air collection in (B4) **switch on** to bag (B5)	37:30	Sit	
Stop air collection in (B5) **switch on** to bag (B6)	38:30	Sit	
Stop air collection in (B6)	40:00	Sit	
Mouthpiece & nose clip	50:00	Sit	
Open bag 1 (B1)	52:00	Sit	
Stop air collection in B1 switch on to bag (B2)	55:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	55:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	56:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	57:30		

Date		**PART 2**	Subject	

Protocol	Time	Position	Notes
Stop air collection in (B5) switch on to bag (B6)	58:30	Sit	
Stop air collection in (B6)	01:00:00	Sit	Blood Sample (60 min)
Mouthpiece & nose clip	01:10:00	Sit	
Open bag 1 (B1)	01:12:00	Sit	
Stop air collection in B1 switch on to bag (B2)	01:15:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	01:15:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	01:16:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	01:17:30	Sit	
Stop air collection in (B5) switch on to bag (B6)	01:18:30	Sit	
Stop air collection in (B6)	01:20:00	Sit	
Mouthpiece & nose clip	01:30:00	Sit	
Open bag 1 (B1)	01:32:00	Sit	
Stop air collection in B1 switch on to bag (B2)	01:35:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	01:35:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	01:36:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	01:37:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	01:38:30	Sit	
Stop air collection in (B6)	01:40:00	Sit	
Mouthpiece & nose clip	01:50:00	Sit	
Open bag 1 (B1)	01:52:00	Sit	
Stop air collection in B1 switch on to bag (B2)	01:55:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	01:55:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	01:56:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	01:57:30	Sit	

Date		**PART 3**	Subject	

Protocol	Time	Position	Notes
Stop air collection in (B5) switch on to bag (B 6)	01:58:30	Sit	
Stop air collection in (B6)	02:00:00	Sit	Blood Sample (120 min)
Mouthpiece & nose clip	02:10:00	Sit	
Open bag 1 (B1)	02:12:00	Sit	
Stop air collection in B1 switch on to bag (B2)	02:15:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	02:15:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	02:16:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	02:17:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	02:18:30	Sit	
Stop air collection in (B6)	02:20:00	Sit	
Mouthpiece & nose clip	02:30:00	Sit	
Open bag 1 (B1)	02:32:00	Sit	
Stop air collection in B1 switch on to bag (B2)	02:35:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	02:35:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	02:36:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	02:37:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	02:38:30	Sit	
Stop air collection in (B6)	02:40:00	Sit	
Mouthpiece & nose clip	02:50:00	Sit	
Open bag 1 (B1)	02:52:00	Sit	
Stop air collection in B1 switch on to bag (B2)	02:55:00	Sit	
Stop air collection in (B2) switch on to bag (B3)	02:55:30	Sit/stand 10 times	Gas collection
Stop air collection in (B3) switch on to bag (B4)	02:56:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	02:57:30	Sit	

Date		**PART 4**	Subject	

Protocol	Time	Position	Notes
Stop air collection in (B5) switch on to bag (B 6)	02:58:30	Sit	
Stop air collection in (B6)	03:00:00	Sit	Blood Sample (180 min)
LUNCH (210min)	03:30:00	Sit	Blood Sample (210min)
Mouthpiece & nose clip	03:40:00		
Open bag 1 (B1)	03:42:00		
Stop air collection in B1 switch on to bag (B2)	03:45:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	03:45:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	03:46:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	03:47:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	03:48:30	Sit	
Stop air collection in (B6)	03:50:00	Sit	
Mouthpiece & nose clip	04:00:00	Sit	Blood Sample (240 min)
Open bag 1 (B1)	04:02:00	Sit	
Stop air collection in B1 switch on to bag (B2)	04:05:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	04:05:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	04:06:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	04:07:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	04:08:30	Sit	
Stop air collection in (B6)	04:10:00	Sit	
Mouthpiece & nose clip	04:20:00		
Open bag 1 (B1)	04:22:00	Sit	
Stop air collection in B1 switch on to bag (B2)	04:25:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	04:25:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	04:26:30	Sit	

Date		**PART 5**	Subject	

Protocol	Time	Position	Notes
Stop air collection in (B4) switch on to bag (B5)	04:27:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	04:28:30	Sit	
Stop air collection in (B6)	04:30:00	Sit	Blood sample (270 min)
Mouthpiece & nose clip	04:40:00	Sit	
Open bag 1 (B1)	04:42:00	Sit	
Stop air collection in B1 switch on to bag (B2)	04:45:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	04:45:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	04:46:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	04:47:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	04:48:30	Sit	
Stop air collection in (B6)	04:50:00	Sit	
Mouthpiece & nose clip	05:00:00	Sit	
Open bag 1 (B1)	05:02:00	Sit	
Stop air collection in B1 switch on to bag (B2)	05:05:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	05:05:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	05:06:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	05:07:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	04:08:30	Sit	
Stop air collection in (B6)	05:10:00	Sit	
Mouthpiece & nose clip	05:20:00	Sit	
Open bag 1 (B1)	05:22:00	Sit	
Stop air collection in B1 switch on to bag (B2)	05:25:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	05:25:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	05:26:30	Sit	

Date		**PART 6**	Subject	

Stop air collection in (B4) switch on to bag (B5)	05:27:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	05:28:30	Sit	
Stop air collection in (B6)	05:30:00	Sit	Blood sample (330 min)
Mouthpiece & nose clip	05:40:00	Sit	
Open bag 1 (B1)	05:42:00	Sit	
Stop air collection in B1 switch on to bag (B2)	05:45:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	05:45:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	05:46:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	05:47:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	05:48:30	Sit	
Stop air collection in (B6)	05:50:00	Sit	
Mouthpiece & nose clip	06:00:00	Sit	
Open bag 1 (B1)	06:02:00	Sit	
Stop air collection in B1 switch on to bag (B2)	06:05:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	06:05:30	Sit	Gas collection
Stop air collection in (B3) switch on to bag (B4)	06:06:30	Sit	
Stop air collection in (B4) switch on to bag (B5)	06:07:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	06:08:30	Sit	
Stop air collection in (B6)	06:10:00	Sit	
Mouthpiece & nose clip	06:20:00	Sit	
Open bag 1 (B1)	06:22:00	Sit	
Stop air collection in B1 switch on to bag (B2)	06:25:00	Sit/stand 10 times	
Stop air collection in (B2) switch on to bag (B3)	06:25:30	Sit	Gas collection

Date		**PART 7**	Subject	

Stop air collection in (B3) switch on to bag (B4)	06:26:30		
Stop air collection in (B4) switch on to bag (B5)	06:27:30	Sit	
Stop air collection in (B5) switch on to bag (B 6)	06:28:30	Sit	
Stop air collection in (B6)	06:30:00	Sit	Blood sample (390 min)
Fit ActivPAL for next week			Download data

Chair Squats Study

Expired Air Samples (1)

Date		Weight (kg)	Lab Temp (°C)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
Bag	**Protocol**	**Time (00:00:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (°C)**
		Resting 1					
		Resting 2					
		Resting 3					
Breakfast							
B1	15	**15:00**					
B2	15.5	**15:30**					
B3	16.5	**16:30**					
B4	17.5	**17:30**					
B5	18.5	**18:30**					
B6	20	**20:00**					
B1	35	**35:00**					
B2	35.5	**35:30**					
B3	36.5	**36:30**					
B4	37.5	**37:30**					
B5	38.5	**38:30**					
B6	40	**40:00**					
B1	55	**55:00**					
B2	55.5	**55:30**					
B3	56.5	**56:30**					
B4	57.5	**57:30**					

B5	58.5	**58:30**					
B6	60	**01:00:00**					
B1	75	**01:15:00**					
B2	75.5	**01:15:30**					
B3	76.5	**01:16:30**					
B4	77.5	**01:17:30**					
B5	78.5	**01:18:30**					
B6	80	**01:20:00**					
B1	95	**01:35:00**					
B2	95.5	**01:35:30**					
B3	96.5	**01:36:30**					
B4	97.5	**01:37:30**					
B5	98.5	**01:38:30**					
B6	100	**01:40:00**					
B1	115	**01:55:00**					
B2	115.5	**01:55:30**					
B3	116.5	**01:56:30**					
B4	117.5	**01:57:30**					
B5	118.5	**01:58:30**					
B6	120	**02:00:00**					
B1	135	**02:15:00**					
B2	135.5	**02:15:30**					
B3	136.5	**02:16:30**					
B4	137.5	**02:17:30**					
B5	138.5	**02:18:30**					
B6	140	**02:20:00**					

B1	155	**02:35:00**					
B2	155.5	**02:35:30**					
B3	156.5	**02:36:30**					
B4	157.5	**02:37:30**					
B5	158.5	**02:38:30**					
B6	160	**02:40:00**					
B1	175	**02:55:00**					
B2	175.5	**02:55:30**					
B3	176.5	**02:56:30**					
B4	177.5	**02:57:30**					
B5	178.5	**02:58:30**					
B6	180	**03:00:00**					

Researcher: _____

Sit/Stand Study

Expired Air Samples (2)

Date		Weight (kg)	Lab Temp (°C)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
Bag	**Time (min)**	**Time (0:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (°C)**
Lunch							
B1	225	**03:45:00**					
B2	225.5	**03:45:30**					
B3	226.5	**03:46:30**					
B4	227.5	**03:47:30**					
B5	228.5	**03:48:30**					
B6	230	**03:50:00**					
B1	245	**04:05:00**					
B2	245.5	**04:05:30**					
B3	246.5	**04:06:30**					
B4	247.5	**04:07:30**					
B5	248.5	**04:08:30**					
B6	250	**04:10:00**					
B1	265	**04:25:00**					
B2	265.5	**04:25:30**					
B3	266.5	**04:26:30**					
B4	267.5	**04:27:30**					
B5	268.5	**04:28:30**					
B6	270	**04:30:00**					
B1	285	**04:45:00**					

B2	285.5	**04:45:30**					
B3	286.5	**04:46:30**					
B4	287.5	**04:47:30**					
B5	288.5	**04:48:30**					
B6	290	**04:50:00**					
B1	305	**05:05:00**					
B2	305.5	**05:05:30**					
B3	306.5	**05:06:30**					
B4	307.5	**05:07:30**					
B5	308.5	**05:08:30**					
B6	310	**05:10:00**					
B1	325	**05:25:00**					
B2	325.5	**05:25:30**					
B3	326.5	**05:26:30**					
B4	327.5	**05:27:30**					
B5	328.5	**05:28:30**					
B6	330	**05:30:00**					
B1	345	**05:45:00**					
B2	345.5	**05:45:30**					
B3	346.5	**05:46:30**					
B4	347.5	**05:47:30**					
B5	348.5	**05:48:30**					
B6	350	**05:50:00**					
B1	365	**06:05:00**					
B2	365.5	**06:05:30**					
B3	366.5	**06:06:30**					

B4	367.5	**06:07:30**					
B5	368.5	**06:08:30**					
B6	370	**06:10:00**					
B1	385	**06:25:00**					
B2	385.5	**06:25:30**					
B3	386.5	**06:26:30**					
B4	387.5	**06:27:30**					
B5	388.5	**06:28:30**					
B6	390	**06:30:00**					

Researcher: _____

Sit/Stand Study

Blood Sampling and Glucose Measurements

Date		Subject	
			Sit/Stand

Time (0:00)	Time point (min)	✓	Protocol	Glucose (mmol/l)		Notes
0:00	0		Fasting			
0:30	30					
1:00	60					
2:00	120					
3:00	180					
3:30	210		Lunch			
4:00	240					
4:30	270					
5:30	330					
6:30	390					

Controls	Lot No:		**High** value (mmol/l):		**Low** value (mmol/l):
Control (Start)	L	L	H	H	mmol/l
Control (Middle)	L	L	H	H	mmol/l
Control (End)	L	L	H	H	mmol/l

Important notes:

- **TWO** blood samples Serum + EDTA
- EDTA samples should be placed on ice immediately.
- EDTA samples should be spun and aliquoted within **5-10 min** (6 aliquots)
    - Centrifuge should be set at 4000 rpm for 15 mins (programme #4)
- SERUM samples should be left to clot for ~1h before separating and aliquoting (4 aliquots)
- All aliquots should be at least 0.5 ml.

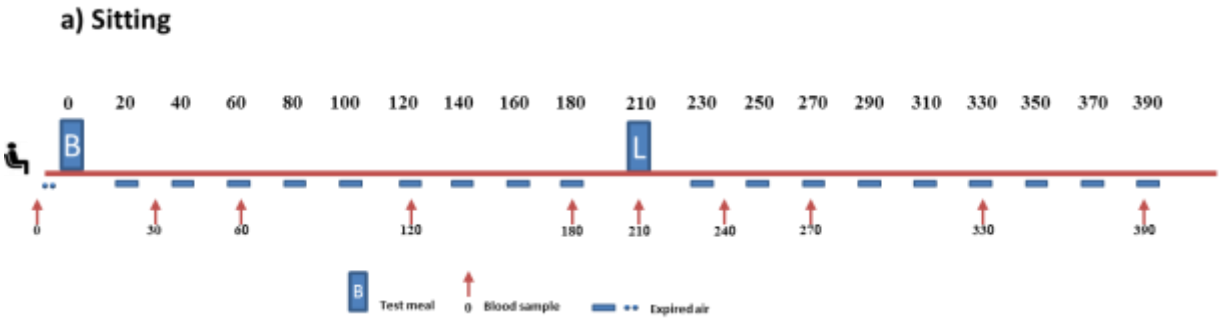
Researcher: _____ *Glucose Analysis:* _____

SIT PROTOCOL

Date		**Time**		**Weight (kg)**		**Subject**	

Protocol	**Time**	**Position**	**Notes**
Change accelerometers	On arrival		Download data and charge
Place new accelerometers			- ActivPAL on lower right thigh
4-min Resting air sample 1		Sit	
Analyse		Sit	☐ OK ☐ Repeat
4-min Resting air sample 2		Sit	
Analyse		Sit	☐ OK ☐ Repeat
Cannulation	-10 min	Sit	Rest for 10 min
Fasting blood sample	0 min	Sit	
Breakfast	**00:00**	**Sit**	**Within 5-10 min**
Start Stopwatch	00:00		**GO TO NEXT PAGE**

Protocol



Date		**PART 1**	**Subject**	

Protocol	Time	Position	Notes
Breakfast	00:00	Sit	
Mouthpiece & nose clip	10:00	Sit	
8-min air collection in (B1)	12:00	Sit	Gas collection
Stop air collection	20:00	Sit	
Mouthpiece & nose clip	30:00	Sit	Blood Sample **(30 min)**
8-min air collection in (B2)	32:00	Sit	Gas collection
Stop air collection	40:00	Sit	
Mouthpiece & nose clip	50:00	Sit	
8-min air collection in (B1)	52:00	Sit	
Stop air collection	01:00:00	Sit	Gas collection & Blood Sample **(60 min)**
Mouthpiece & nose clip	01:10:00	Sit	
8-min air collection in (B2)	01:12:00	Sit	Gas collection
Stop air collection	01:20:00	Sit	
Mouthpiece & nose clip	01:30:00	Sit	
8-min air collection in (B1)	01:32:00	Sit	Gas collection
Stop air collection	01:40:00	Sit	
Mouthpiece & nose clip	01:50:00	Sit	
8-min air collection in (B2)	01:52:00	Sit	
Stop air collection	02:00:00	Sit	Gas collection & Blood Sample **(120 min)**
Mouthpiece & nose clip	02:10:00	Sit	
8-min air collection in (B1)	02:12:00	Sit	Gas collection
Stop air collection	02:20:00	Sit	
Mouthpiece & nose clip	02:30:00	Sit	
8-min air collection in (B2)	02:32:00	Sit	Gas collection
Stop air collection	02:40:00	Sit	

Date		**PART 2**	**Subject**	

Protocol	Time	Position	Notes
Mouthpiece & nose clip	02:50:00	Sit	
8-min air collection in (B1)	02:52:00	Sit	
Stop air collection	03:00:00	Sit	Gas collection & Blood Sample **(180 min)**
LUNCH (210min)	03:30:00	Sit	Blood Sample **(210 min)**
Mouthpiece & nose clip	03:40:00		
8-min air collection in (B2)	03:42:00		Gas collection
Stop air collection	03:50:00	Sit	
Mouthpiece & nose clip	04:00:00	Sit	Blood Sample **(240 min)**
8-min air collection in (B1)	04:02:00	Sit	Gas collection
Stop air collection	04:10:00	Sit	
Mouthpiece & nose clip	04:20:00	Sit	
8-min air collection in (B2)	04:22:00	Sit	
Stop air collection	04:30:00		Gas collection &Blood sample (**270 min**)
Mouthpiece & nose clip	04:40:00	Sit	
8-min air collection in (B1)	04:42:00	Sit	Gas collection
Stop air collection	04:50:00	Sit	
Mouthpiece & nose clip	05:00:00	Sit	
8-min air collection in (B2)	05:02:00	Sit	Gas collection
Stop air collection	05:10:00	Sit	
Mouthpiece & nose clip	05:20:00	Sit	
8-min air collection in (B1)	05:22:00	Sit	
Stop air collection	05:30:00	Sit	Gas collection & Blood sample (**330 min**)
Mouthpiece & nose clip	05:40:00	Sit	

Date		**PART 3**	**Subject**	

8-min air collection in (B2)	05:42:00	Sit	Gas collection
Stop air collection	05:50:00	Sit	
Mouthpiece & nose clip	06:00:00	Sit	
8-min air collection in (B1)	06:02:00	Sit	Gas collection
Stop air collection	06:10:00	Sit	
Mouthpiece & nose clip	06:20:00	Sit	
8-min air collection	06:22:00	Sit	
Stop air collection in (B2)	06:30:00	Sit	Gas collection & Blood sample (**390 min**)
Fit ActivPAL for next week			Download data

Chair squats Study

Expired Air Samples (1)

Date		Weight (kg)	Lab Temp (°C)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
Bag	**Time (min)**	**Time (00:00:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (°C)**
		Resting 1					
		Resting 2					
		Resting 3					
Breakfast							
B1	20	**20:00**					
B2	40	**40:00**					
B1	60	**01:00:00**					
B2	80	**01:20:00**					
B1	100	**01:40:00**					
B2	120	**02:00:00**					
B1	140	**02:20:00**					
B2	160	**02:40:00**					
B1	180	**03:00:00**					

Researcher: _____

Chair squats Study

Expired Air Samples (2)

Date		Weight (kg)	Lab Temp (°C)	Bar Press (mmHg)	Flow Rate (ml/min)	Subject	
Bag	**Time (min)**	**Time (0:00)**	**Sampling Time (sec)***	**F_ECO_2%**	**F_EO_2%**	**Volume (L)**	**Temp (°C)**
Lunch							
B1	230	**03:50:00**					
B2	250	**04:10:00**					
B1	270	**04:30:00**					
B2	290	**04:50:00**					
B1	310	**05:10:00**					
B2	330	**05:30:00**					
B1	350	**05:50:00**					
B2	370	**06:10:00**					
B1	390	**06:30:00**					

Researcher: _____

Chair squats Study

Blood Sampling and Glucose Measurements

Date		Subject	
			Sitting

Time (0:00)	Time point (min)	✓	Protocol	Glucose (mmol/l)		Notes
0:00	**0**		**Fasting**			
0:30	**30**					
1:00	**60**					
2:00	**120**					
3:00	**180**					
3:30	**210**		**Lunch**			
4:00	**240**					
4:30	**270**					
5:30	**330**					
6:30	**390**					

Controls	Lot No:		**High** value (mmol/l):		**Low** value (mmol/l):
Control (Start)	L	L	H	H	mmol/l
Control (Middle)	L	L	H	H	mmol/l
Control (End)	L	L	H	H	mmol/l

Important notes:

- **TWO** blood samples Serum + EDTA
- EDTA samples should be placed on ice immediately.
- EDTA samples should be spun and aliquoted within **5-10 min** (6 aliquots)
    - Centrifuge should be set at 4000 rpm for 15 mins (programme #4)
- SERUM samples should be left to clot for ~1h before separating and aliquoting (4 aliquots)
- All aliquots should be at least 0.5 ml.

Researcher: _____ *Glucose Analysis:* _____

Appendix L: Participants Feedback – Chapter 3 & 4

University of Glasgow | College of Medical, Veterinary & Life Sciences

Metabolic responses to breaking up sitting time

RESULTS FEEDBACK

Name:	***First and Last names***
DOB:	dd/mm/yyy
Address:	Address1
	Address2
	City and Postcode
Study Start Date:	dd/mm/yyyy
Study End Date:	dd/mm/yyyy

Body Composition Measurements

1. Height and Weight

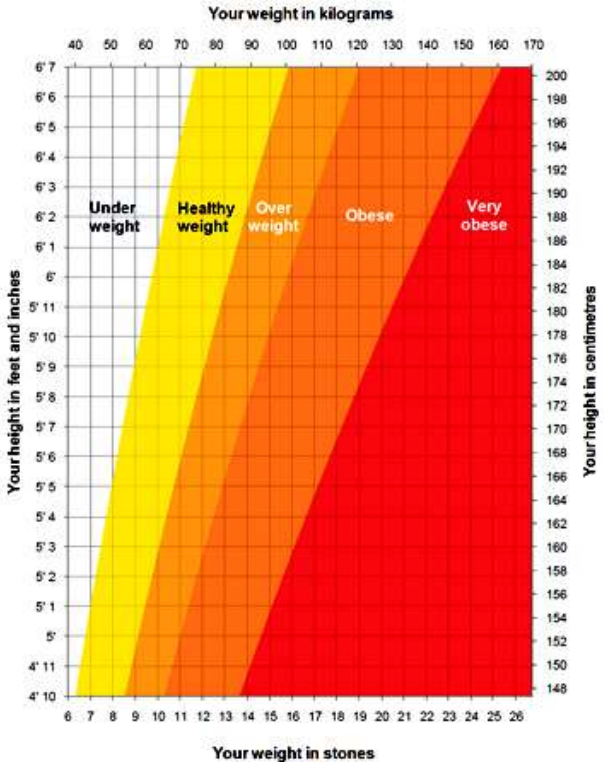
Height and body mass are widely used to measure body fatness. An index called the '**Body mass index**' or '**BMI**' can be used to determine whether somebody is the correct weight for his or her height. Usually, a BMI value of 20 to 25 is normal, 25 to 30 is overweight, and 30+ is classed as obese (BMI is calculated by dividing body mass in kg by height in metres squared, i.e. kg/m^2). However, this index is of limited value, as it does not take into account an individual's build and does not distinguish between fat and muscle mass. (In fact a number of athletes would be classed as overweight by this index, due to their large muscle mass.)

Your height: **xx.x m** (x' xx")

Your weight: **xxx kg** (xx stn xx lbs)

Your body mass index (BMI): **xxx kg/m²**

The following graph shows the ideal body mass for adults (18 years and older) based on their height. **According to your height, your body mass should be between about xx.x – xx.x kg (xx.x – xx.x stones).**



2. Waist Circumference

The waist circumference (or girth) is perhaps of greater importance than BMI in determining risk of metabolic disorders such as diabetes and heart disease. This is because abdominal fat is thought to be in a position anatomically (i.e. near to the liver and other internal organs) where it could potentially cause a lot of harm. **The risk for certain metabolic complications is higher when the waist circumference is greater than 99 cm (39 in) for men and 88.9 cm (35 in) for women.**

Your Waist Circumference (WC): xxx cm (xx.x in)

3. Waist-to-Hip Ratio (WHR)

The pattern of body fat distribution is recognised as an important indicator of heath and prognosis. The more fat on the trunk (also called abdominal fat), the higher the risk of hypertension, diabetes and other metabolic complications. The wait-to-hip ratio (WHR) is the circumference of the waist divided by the circumference of the hip (buttocks/hips). It has traditionally been used as a simple method for assessing body fat distribution and identifying individuals with higher amounts of abdominal fat. Health risk increases as WHR increases and the standards for risk vary with age and sex. **The WHR should be below 0.95 for young men and 0.86 for young women. For individuals aged 60-69 years, the cutoff values are less than 1.03 for men and less than 0.90 for women.**

Your Waist-to-Hip Ratio (WHR): xxx

Health Screening Results

1. Blood Pressure

Your Blood Pressure: xxx/xx mm Hg

The target for the general population is to have a blood pressure **below 140/90 mmHg**. The systolic pressure (**xxx mm Hg**) indicates how hard the heart is working and the force that is blood exerts when blood is pumped from the heart. The diastolic pressure (**xx mm Hg**) tells us what resistance there is to blood flow and therefore how easily blood flows through the blood vessels.

2. Fasting Glucose

Fasting glucose level is used to determine whether you have diabetes or not. The normal range of fasting glucose is 3.5-5.5 mmol/l and a value of greater than 7 mmol/l suggests diabetes.

Your Fasting Glucose: x.x mmol/l (**normal**)

Physical Activity

1. Weekly Analysis

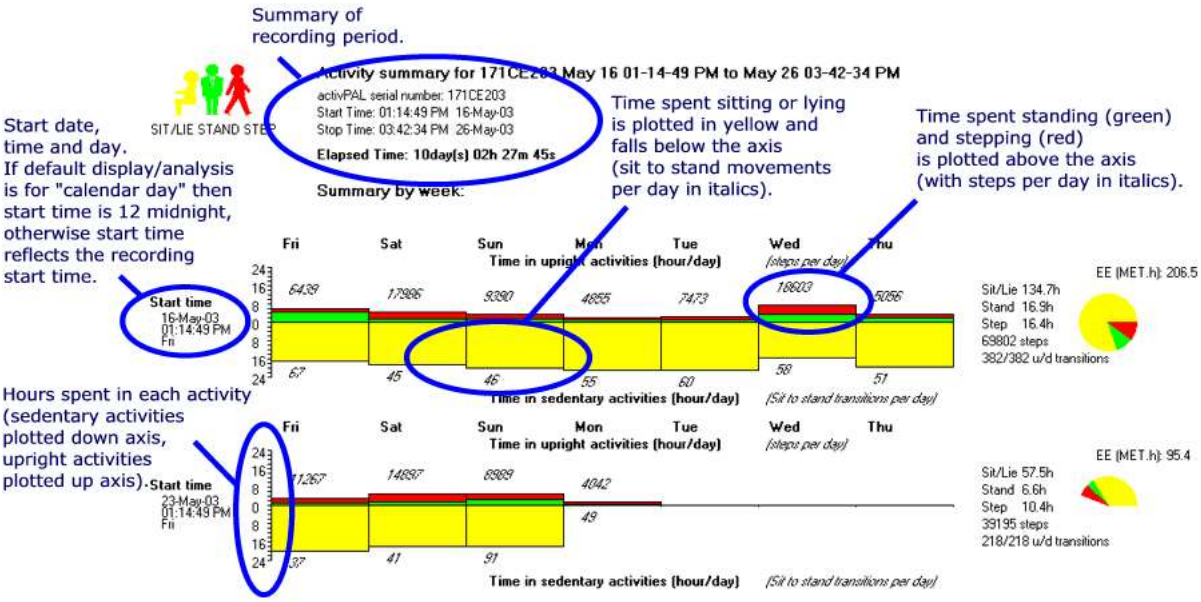
During the three weeks you have participated in the study, you were asked to wear two accelerometers to measure your physical activity. One of these accelerometers is called the *activPAL*, which was fitted on the right thigh. It moved as you moved, generating totals for the periods spent sitting, standing and stepping. The result of your weekly activity is presented by week, day and hour (*see next figure*), and the following parameters were calculated:

- Time sitting/lying (hours)
- Time quiet standing (hours)
- Time stepping (hours)
- Step count (steps)
- Sit to stand transfers (number of)
- Energy expenditure (MET.h)
- Walking frequency (cadence) (number of steps taken at 10steps/minute intervals)

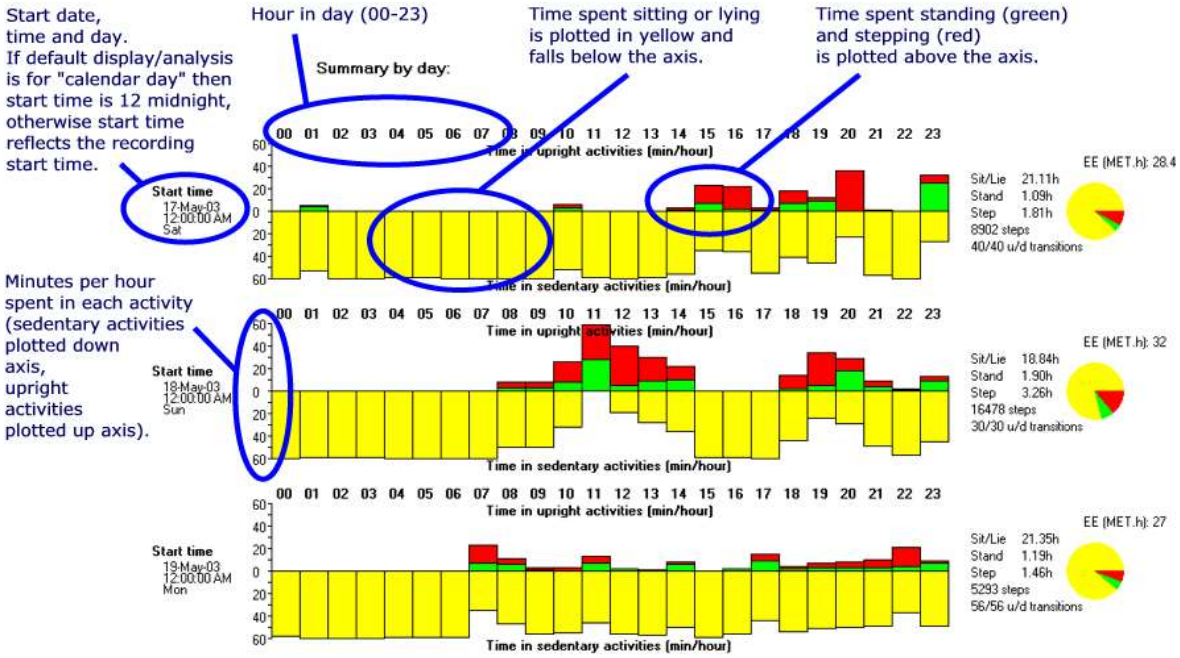
Your weekly results are attached at the back of this report.

Sample of the Weekly Activity Analysis – Explained.

Summary by Week



Summary by Day



- ***Please note that this is <u>not</u> your result. Your weekly analyses for the three weeks are attached at the back.***

2. Energy Expenditure

The body requires energy for every physical activity which is dependent on the duration and type of activity and the body's age and gender. Energy is measured in calories (cal) and is obtained from the body stores or the food we eat, namely carbohydrates, fat and protein. The longer and harder the exercise is, the more calories you burn in order to sustain it. In order to lose 1 pound of fat, you need to burn 3500 kcal (7700 kcal for 1 kg). Your energy expenditure for the three trials are as follows:

Your Energy Expenditure:

Sitting trial:	**xxxx**	**kcal**
Prolonged Standing trial:	**xxxx**	**kcal**
Intermittent Standing trial:	**xxxx**	**kcal**

3. Exercise Recommendations

The UK Physical Activity guidelines recommend that you perform a total of 150 minutes of moderate-intensity activity or 75 minutes of vigorous-intensity activity per week. This physical activity should be spread across the week. For example, you can do *at least* 30 minutes of moderate-intensity activity on 5 or more days of the week. This 30-minutes period does not need to be continuous - you could split it up into a number of shorter exercise periods (each of at least 10 minutes). This amount of exercise is the ideal, but taking any exercise at all will be beneficial. In addition, everyday activities such as walking to the shops can all count towards your daily exercise. It is important to note that for adults who are already overweight or obese and achieving the recommended weekly amount of activity (30 minutes x 5 times a week or 150 minutes per week) will gain multiple health benefits even if they did not lose weight.

Type of activity	Examples
Moderate intensity	Brisk walking, bike riding, dancing, swimming, active travel
Vigorous intensity	Running, playing sport, taking part in aerobic exercise classes, using cardiovascular gym equipment.

ꕥ Thank you for your time and participation ꕥ

Appendix M: Participant Information Sheet and Consent Form - Chapter 5

University of Glasgow | College of Medical, Veterinary & Life Sciences

PARTICIPANT INFORMATION SHEET

Development and testing of methods to measure human movement using a movement sensor positioned on the thigh or in a pocket.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The purpose of this study is to develop and test new methods of measuring human movement using a movement sensor device positioned on the thigh or in your pocket. This information will help us to better understand how people move throughout the day which will help research into understanding of how movement influences risk of diseases such as heart disease and diabetes and help us to understand how we can get people to move more.

Why have I been chosen?

You have been chosen because you are a healthy adult aged between 18-60 years.

Do I have to take part?

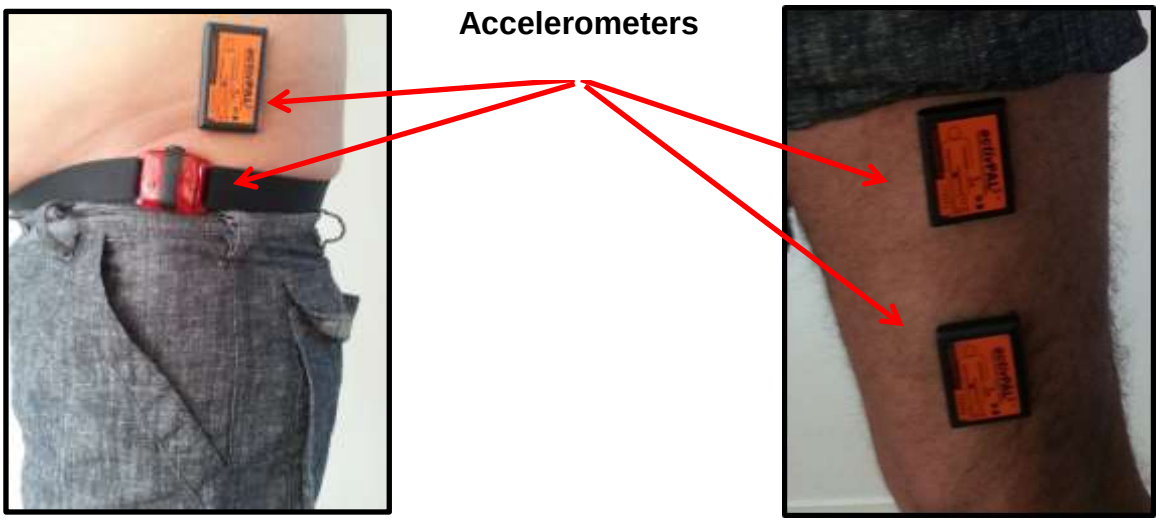
It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

Before enrolling in the study we will ask you to attend for a screening visit in which we will ask you to complete a confidential questionnaire about your health, measure your blood pressure, measure your height and weight and provide an opportunity for you to ask questions.

These screening procedures will enable us to determine whether you fall into the group of people we wish to study and will also ensure that it is perfectly safe for you to participate in this study.

We will then ask you to perform two trials, on different days, involving walking and running at different speeds from very slow walking (1 km/h) to fairly fast running (12 km/h). In one trial, we will ask you to walk and run on a treadmill. In the other trial we will ask you to walk and run around an athletics track. In both trials, we will ask you to wear a number of motion detection devices, called accelerometers, under your clothes on the fronts of both of your thighs (stuck on using a special double-sided adhesive gel), in your trouser or short pockets, and attached to your hips.



The photographs below show the placement of the accelerometer devices. During the treadmill session, we will ask you to breathe through a mouthpiece while you are walking and running to enable us to collect the air that you breathe out to measure the amount of oxygen your body is using. We will also videotape you while you are walking and running during these trials to enable us to count how many steps that you took and compare this to the values recorded on the accelerometer devices. For each trial we will ask you to walk at up to 8 different speeds, and run at up to 6 different speeds in total. You will have the chance to rest between the different walking and running speeds if you need to. Each trial will take about 90-120 minutes in total and we can schedule them at your convenience.

What do I have to do?

We will ask you to perform the trials described above. No special preparation is needed for these trials.

What are the possible disadvantages and risks of taking part?

The walking and running that we are asking you to do will not be at a maximal level but the possibility exists that, very seldom, certain changes may occur during or shortly after the tests. They include abnormal blood pressure, fainting or a change in the normal rhythm of the heartbeat. We will monitor your heart rate throughout the exercise session and will stop the test if your heart rate reaches 85% of your maximum heart rate.

What are the possible benefits of taking part?

There may be no direct benefits to you but the findings will help research into how we can measure human movement better. The findings of this study will be presented at scientific conferences and published in scientific journals and will help us to better understand how movement influences risk of diseases such as heart disease and diabetes and how we can get people to move more. We will also be delighted to explain our findings and discuss their implications with you.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. You will be identified by an ID number, and any information about you will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

The findings of this study will be presented at scientific conferences and published in scientific journals and will help us to better understand how movement influences risk of

diseases such as heart disease and diabetes and how we can get people to move more. You will not be identified in any publication or presentation of this work.

Who is organising and funding the research?

This work forms part of Nabeha Hawari's PhD. She is funded by a scholarship from the Government of Saudi Arabia.

Who has reviewed the study?

This study has been reviewed and approved by the College of Medical, Veterinary and Life Sciences Ethics committee at the University of Glasgow.

Contact for Further Information

Any questions about the procedures used in this study are encouraged. You will be given a copy of this information sheet and a signed consent form to keep for your records. If you have any doubts or questions, please ask for further explanations by contacting either:

Nabeha Hawari on 07919182743 (email : n.hawari.1@research.gla.ac.uk)

Dr Jason Gill on 0141 3302916 (email : Jason.Gill@glasgow.ac.uk)

Thank you for your interest in this study!

14 May 2013 (Version 1)

College of MVLS, Ethics Committee

University of Glasgow | College of Medical, Veterinary & Life Sciences

Participant Identification Number for this trial:

CONSENT FORM

Title of Project: Development and testing of methods to measure human movement using a movement sensor positioned on the thigh or in a pocket.

Name of Researcher(s): Dr Jason Gill, Mrs Nabeha Hawari

Please initial box

I confirm that I have read and understand the information sheet dated 14 May 2013 (version 1) for the above study and have had the opportunity to ask questions. ☐

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

I agree to take part in the above study. ☐

Name of subject	**Date**	**Signature**
Name of Person taking consent (if different from researcher)	**Date**	**Signature**
Researcher	**Date**	**Signature**

(1 copy for subject; 1 copy for researcher)

14 May 2013 (Version 1)

College of MVLS

Ethics Committee

Appendix N: Announcement - Chapter 5

University of Glasgow | College of Medical, Veterinary & Life Sciences

Would you like to help us to better understand how people move throughout the day which will help research into understanding of how movement influences risk of diseases such as heart disease and diabetes and help us to understand how we can get people to move more?

We are testing and development methods to measure human movement using a movement sensor positioned on the thigh or in a pocket

If you are a healthy (man or women), aged between 18 – 60 years. Exclusion criteria will include uncontrolled hypertension, a previous history of established CHD, or conditions such as arthritis or injuries that alter gait and/or limit ability to walk or run on a treadmill, you may be able to help us.

Participants will perform two experimental trials – one involving walking and running on a treadmill and one involving walking and running on an athletics track. For each trial, participants will wear ActivPAL devices in a number of locations on the body (lower thigh, upper thigh, hip and pocket, on the left and right sides), and Actigraph accelerometers on the right and left hips, to record body accelerations and posture changes. The treadmill and track trials will be undertaken in random order.

Nabeha Saleh Hawari 07919182743(Mobile) (Email) n.hawari.12@research.gla.ac.uk Dr Jason Gill	Nabeha Saleh Hawari 07919182743(Mobile) (Email) n.hawari.12@research.gla.ac.uk Dr Jason Gill	Nabeha Saleh Hawari 07919182743(Mobile) (Email) n.hawari.12@research.gla.ac.uk Dr Jason Gill	Nabeha Saleh Hawari 07919182743(Mobile) (Email) n.hawari.12@research.gla.ac.uk Dr Jason Gill

Appendix O: Subject`s Information - Chapter 5

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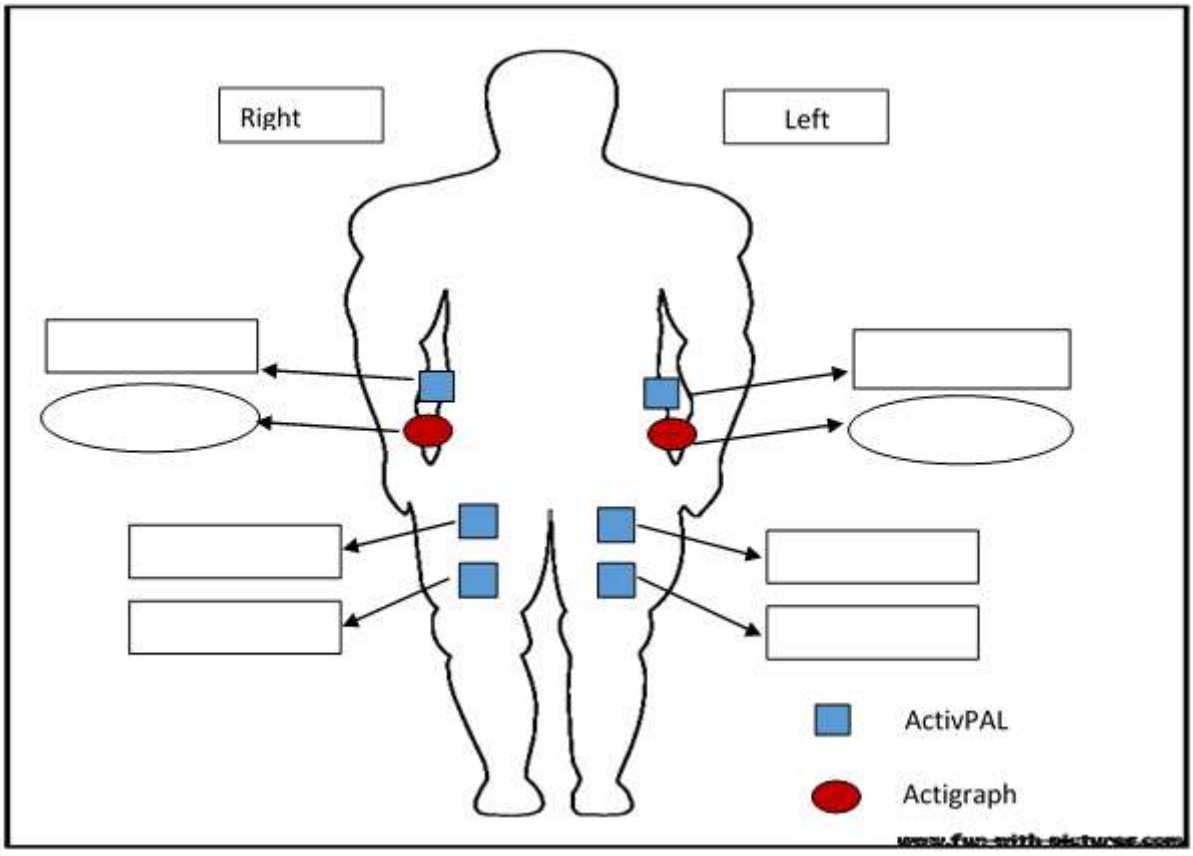
General information

Subject Number :	**Date of screening:** **Time:**
Name:	**Address:**
Date of Birth:	**Email:**
Age:	**Telephone number:**
Height:	**Weight:**
BMI:	**Blood Pressure:**



Appendix P: Gas Collection Duration - Chapter 5

Gas Collection Duration

Condition	Duration (min)	Sampling Time (min)
Sitting	0 - 8	3 – 8
Standing	8 - 13	8 – 13
1 km/h	13 - 18	16 – 18
2 km/h	18 - 23	21 – 23
3 km/h	23 - 28	26 – 28
4 km/h	28 - 33	31 – 33
5 km/h	33 - 38	36 - 38
6 km/h	38 - 43	41 - 43
7 km/h (walking)	43 - 48	46 - 48
7 km/h (running)	48 - 53	51 - 53
8 km/h (walking)	53 - 58	56 - 58
8 km/h (running)	58 - 63	61 - 63
9 km/h (running)	63 - 68	66 - 68
10 km/h (running)	68 - 73	71 - 73
11 km/h (running)	73 - 78	76 - 78
12 km/h (running)	78 - 83	81 - 83

Appendix Q: Treadmill protocol - Chapter 5

University of Glasgow | College of Medical, Veterinary & Life Sciences

Gas Collection on Treadmill

Date	**Name**	**Weight (kg)**	**Height (cm)**	**Lab Temp (°C)**	**Bar Press. (mmHg)**
Bag	**Sample time**	**F_ECO_2 %**	**F_EO_2 %**	**Volume (L)**	**Temp (°C)**
Sitting					
Standing					
1km/h					
2km/h					
3km/h					
4km/h					
5km/h					
6km/h					
7km/h Walking					
7km/h Running					
8km/h Walking					
8km/h Running					
9km/h					
10km/h					
11km/h					
12km/h					

University of Glasgow | College of Medical, Veterinary & Life Sciences

Treadmill Based Running Tests

Subject No: _____ Start Time: _____

Date:_____

Initial Sitting Rest Duration: _____

nitial Standing Rest Duration: _____

P_B = _____ mmHg

Walking

Speed (km/h)	Time of Day (24hr)	Average Heart Rate (bpm)	Video File Name	Douglas Bag Data Collection					
				Collection Time (min)	Initial Reading (L)	Final Reading (L)	Expired Air Temp. (℃)	F_EO_2 (%)	F_ECO_2 (%)
1									
2									
3									
4									
5									
6									
7									
8									

Running

Speed (km/h)	Time of Day (24hr)	Average Heart Rate (bpm)	Video File Name	Douglas Bag Data Collection					
				Collection Time (min)	Initial Reading (L)	Final Reading (L)	Expired Air Temp. (°C)	F_EO_2 (%)	F_ECO_2 (%)
7									
8									
9									
10									
11									

Rest Period Recordings

Speed before rest (km/h)	Duration of Rest (mins & secs)	Time of Day (am/pm)	Additional Notes

Note:

Appendix R: Track Protocol - Chapter 5

University of Glasgow | College of Medical, Veterinary & Life Sciences

Track Protocol

Field Based Walking Tests

Subject No: Start Time: Date

Target Speed (km/h)	Target Time (secs)	Attempt no.		Distance (m)	Actual time (seconds)	Time of Day (am/pm)	Average Heart Rate (bpm)	Video File Name
1	144	1	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		2	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		3	Sitting	-			-	
			Standing	-			-	
			Walking	40				
2	72.0	1	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		2	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		3	Sitting	-			-	
			Standing	-			-	
			Walking	40				
3	48.0	1	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		2	Sitting	-			-	
			Standing	-			-	
			Walking	40				

		3	Sitting	-			-	
			Standing	-			-	
			Walking	40				
4	36.0	1	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		2	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		3	Sitting	-			-	
			Standing	-			-	
			Walking	40				
5	28.8	1	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		2	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		3	Sitting	-			-	
			Standing	-			-	
			Walking	40				
6	24.0	1	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		2	Sitting	-			-	
			Standing	-			-	
			Walking	40				
		3	Sitting	-			-	
			Standing	-			-	
			Walking	40				
7	46.3	1	Sitting	-			-	
			Standing	-			-	
			Walking	90				
		2	Sitting	-			-	

			Standing	-			-	
			Walking	90				
		3	Sitting	-			-	
			Standing	-			-	
			Walking	90				
8	40.5	1	Sitting	-			-	
			Standing	-			-	
			Walking	90				
		2	Sitting	-			-	
			Standing	-			-	
			Walking	90				
		3	Sitting	-			-	
			Standing	-				
			Walking	90				

Field Based Running Tests

Subject No: Start Time: Date

Target Speed (km/h)	Target Time (secs)	Attempt no.		Distance (m)	Actual time (seconds)	Time of Day (24hr)	Average Heart Rate (bpm)	Video File Name
7	46.3	1	Sitting	-			-	
			Standing	-			-	
			Running	90				
		2	Sitting	-			-	
			Standing	-			-	
			Running	90				
		3	Sitting	-			-	
			Standing	-			-	
			Running	90				
8	40.5	1	Sitting	-			-	
			Standing	-			-	
			Running	90				
		2	Sitting	-			-	
			Standing	-			-	
			Running	90				
		3	Sitting	-			-	
			Standing	-			-	
			Running	90				
9	36.0	1	Sitting	-			-	
			Standing	-			-	
			Running	90				
		2	Sitting	-			-	
			Standing	-			-	
			Running	90				
		3	Sitting	-			-	
			Standing	-			-	
			Running	90				
10	32.4	1	Sitting	-			-	
			Standing	-			-	
			Running	90				
		2	Sitting	-			-	

			Standing	-			-	
			Running	90				
		3	Sitting	-			-	
			Standing	-			-	
			Running	90				
11	29.5	1	Sitting	-			-	
			Standing	-			-	
			Running	90				
		2	Sitting	-			-	
			Standing	-			-	
			Running	90				
		3	Sitting	-			-	
			Standing	-			-	
			Running	90				

Appendix S: Conference Posters - Chapter 5

University of Glasgow | College of Medical, Veterinary & Life Sciences

BASES

Determining Stepping Rate, Speed and Exercise Intensity Using a Triaxial Accelerometer: *Effect of Accelerometer Position*

Nabeha S.A. Hawari, Lauren McMichan, Gillian Martin, Waris Wongpipit, Jason M.R. Gill

Introduction

Accelerometers provide the potential for accurate objective measurement of physical activity, which is important for epidemiological assessment of activity levels and the association with disease risk and for quantification of changes in activity behaviour in response to interventions (1). The hip and thigh are commonly used locations for accelerometer placement. However, it is unclear whether these two locations are comparable in terms of measurement of stepping rate, speed and exercise intensity.

Aim

To compare the effects of hip and thigh accelerometer placements on the measurement of stepping rate, speed and exercise intensity at a range of walking speeds.

Results

The **hip-placed** accelerometer was unable to count steps at 1 km·h^{-1} (100 ± 0% underestimation (mean ± *s*), and underestimated stepping rate by 91 ± 15% and 17 ± 11% at 2 and 3 km·h^{-1}, respectively. For speeds ≥4 km·h^{-1}, the hip-based accelerometer underestimated stepping rate by 7 ± 10% (**Figure 2a**). The **thigh-placed** accelerometer underestimated stepping rate by 49 ± 28% at 1 km·h^{-1}, but at speeds ≥2 km·h^{-1} underestimated stepping rate by 4 ± 11% (**Figure 2b**). Quadratic regressions were used to quantify the relationship between acceleration profile, and speed/oxygen uptake for the hip and thigh positions. Model fits were comparable for both positions, with acceleration profile explaining ~99% of the variance in speed and oxygen uptake for both hip and thigh accelerometer placement (**Figure 3**).

Methods

With ethical approval, 23 apparently healthy adults (13 female), aged 19-42 years (13 F, 19-42 years) participated.

ActivPAL accelerometers (Pal Technologies, UK) were affixed to participants' **left thigh** (10 cm above the knee) and **left hip** to record body accelerations. Participants walked on a treadmill for 5 min at each 8 different speeds (1-8 km·h^{-1}).

Expired air samples were collected for determination of oxygen uptake and participants were video recorded to enable actual stepping rates to be determined. **ActivPAL** professional software (v7.1.25) was used to calculate stepping rates. The vector magnitude of sum of changes raw accelerations in the x, y and z directions per second, smoothed over a 10 s interval, was used to summarise the acceleration profile for each speed.

Figure 1 : Study design

Conclusion

Thigh-based accelerometer placement provides advantages over hip-based placement for quantification of stepping rate at slow speeds. This may have implications for step counting during light incidental activities of daily living. However, when using raw acceleration profiles to quantify speed and exercise intensity, hip and thigh accelerometer placement was comparable.

Figure 2: Relationship between ActivPAL (a: Hip; b: thigh) and actual steps at different stepping rates. Line shown is line of equality.

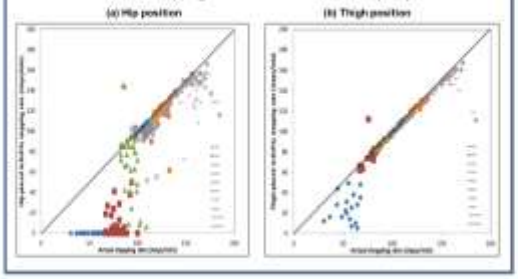
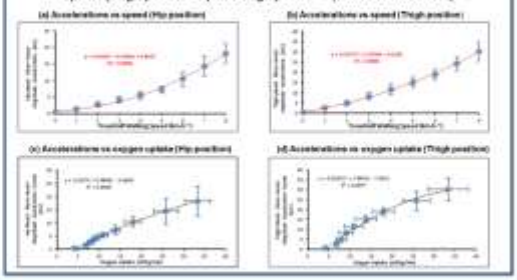


Figure 3: The relationship between vector magnitude accelerations and oxygen uptake (VO_2)/speed for hip and thigh positions (values are mean ± *s*)



Reference

1) Celis-Morales, C., Perez-Bravo, F., Ibanez, L., Salas, C., Bailey, M.E.S., and Gill, J.M.R. (2012) **Objective vs. self-reported physical activity and sedentary time: effects of measurement method on relationships with risk biomarkers.** PLoS ONE, 7 (5), e36345. ISSN 1932-6203

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PALtechnologies PROVIDING THE EVIDENCE

Validation of a novel pocket-based device to measure and provide feedback on physical activity and sedentary behaviour

Jason M.R. Gill[1], **Nabeha S.A. Hawari**[1], Douglas Maxwell[2] for the EuroFIT Consortium
[1]University of Glasgow, Glasgow, UK, [2]PAL Technologies Ltd, Glasgow, UK

Introduction

- Too much sitting is a risk factor for a number of adverse health outcomes, distinct from the risk associated with insufficient moderate-to-vigorous physical activity.
- Pedometers are an effective tool for increasing physical activity, but equivalent consumer devices to measure and provide feedback on sitting time are not available.
- The sitFIT is a pocket-based 'sitting pedometer' which provides real-time feedback on sedentary behaviour and physical activity (Figure 1).
- The pocket position has advantages of enabling detection of movement from sitting to upright from the associated change in device orientation, as well as providing easy access for delivery of feedback to users.
- However, unlike other accelerometers which are generally fixed to the body, the sitFIT can move and change orientation in the pocket, which could conceivably affect accuracy.
- The aim of this study was therefore to validate the sitFIT device for the measurement of sitting/upright time and physical activity.

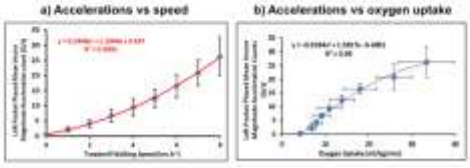
Part 1

This was a lab-based validation of the pocket accelerometer position for determination of speed and activity energy expenditure.

Methods: Forty participants wore activPAL accelerometers in left and right pockets, while sitting, standing and walking at speeds from 1 to 8 km/h on a treadmill. Expired air was collected throughout to measure oxygen uptake (and thus energy expenditure). Vector magnitude accelerations were measured at each intensity and related to walking speed and oxygen uptake. Quadratic regressions were used to quantify the relationship between acceleration profile, and speed/oxygen uptake.

Results: Vector magnitude accelerations explained ~99% of the variance in speed and oxygen uptake (**Figure 2**).

Figure 2. The relationship between vector magnitude accelerations and (a) walking speed and (b) oxygen uptake for a pocket-based accelerometer. N = 40, values are mean (SD).



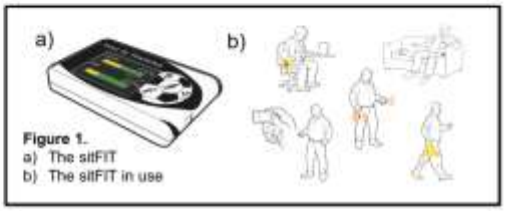


Figure 1.
a) The sitFIT
b) The sitFIT in use

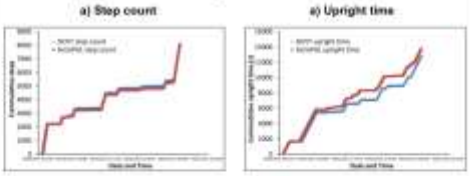
Part 2

This is an ongoing field-based validation of the sitFIT for the measurement of sitting/upright time and step-count in a free-living environment.

Methods: Approximately 20 participants will wear sitFIT devices in their pocket and activPAL accelerometers on their thigh for up to seven days. A novel algorithm to determine sitting/upright time and stepping for pocket placement has been developed, and agreement between the pocket and (gold-standard) thigh position for activity behaviours in the free-living setting will be compared.

Results: Data collection is ongoing (due for completion by end of June 2015). Preliminary analysis of data over a ~10 hour period in one participant suggests that agreement between the pocket-based sitFIT and gold-standard activPAL is good for step count and upright time (i.e. non-sitting time) (**Figure 3**). Formal data analysis will be undertaken when data collection is complete.

Figure 3. Cumulative step count (a) and upright time (b) over a ~10 hour period measured by sitFIT and activPAL.



Conclusion

Our preliminary data confirm the viability of the pocket placement for measurement of walking speed and energy expenditure of stepping activities. Further analysis will determine the viability of the pocket position for the measurement of step count and upright/sitting time in a free-living setting.

EuroFIT

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 602170. www.eurofitp7.eu