The short-term effects of physical inactivity and diet on cardiovascular and metabolic responses in healthy humans

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This PhD thesis is dedicated to my loving parents,

Ali and Bahia,

Without your tremendous support, encouragement and love

this thesis would not be possible

Abstract

Introduction: reduced physical activity is a major predisposing factor for the development of cardiovascular and metabolic disorders. Current lifestyle changes, such as increased intake of high-calorie foods in combination with physical inactivity are considered to play an important role in cardiometabolic disorders worldwide. **Aims:** the overall aim of this thesis was to examine the short-term effects of physical inactivity and energy balance on cardiometabolic responses in healthy humans. Two studies were performed under free-living and standardised laboratory conditions. Study 1 (Chapter 3) aimed to assess the effects of a 2-day reduced physical activity with and without a proportional decrease in energy intake (creating neutral or positive energy balance, respectively), on cardiovascular responses, insulin sensitivity and energy metabolism in eight healthy men and women. Study 2 (Chapter 4) aimed to examine brachial artery endothelial function using the flow-mediated vasodilatation (FMD) technique before and after a 5-day reduced physical activity with a proportional decrease in energy intake to create a neutral energy balance in eight healthy men. **Methods:** cardiovascular responses were measured using the Finometer and venous occlusion plethysmography. Insulin sensitivity was evaluated using the Matsuda index and the Homeostatic Model Assessment for Insulin Resistance. Energy metabolism was assessed by indirect calorimetry. FMD responses were measured using the Duplex-Doppler Ultrasound. The continuous glucose monitoring system, FreeStyle Libre Abbott, performance was compared against the Medtronic iPro2 and arterialised blood glucose measurements using the yellow spring instrument (YSI). **Results:** reduced physical activity with positive energy intake was associated with an increase

in 24-hour glucose concentrations (p = .035 with large effect of η^2_p = 0.38), an increase in postprandial heart rate (p < .001 with large effect size of η^2_p = 0.19) and an increase in insulin incremental area under the curve (p = .051 with large effect η^2_p = 0.35) in response to a mixed meal. These changes improved when energy intake was reduced to match energy expenditure (Study 1). No significant changes in brachial FMD response were detected following short-term inactivity (Study 2). Glucose measurements by the FreeStyle Libre Abbott tended to be lower compared to the Medtronic iPro2 (p < .001 with large effect of η^2_p = 0.33) but has a good agreement with the arterialised blood glucose measurements (Abbott vs YSI MARD 12.69% and iPro2 vs YSI MARD 16.15%). **Conclusions:** physical inactivity with energy surplus is associated with unfavourable cardiovascular and metabolic changes. As a secondary objective, the FreeStyle Libre Abbott is potentially a useful tool to assess the daily glucose profile in healthy normo-glycaemic individuals.

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Table of Contents

Chapter 1: Introduction 1
1.1. Introduction 1
1.2. Behavioural components 2
1.2.1. Definition 2
1.2.2. Prevalence 4
1.2.3. Adverse effects of physical inactivity 5
1.3. Overfeeding 28
1.3.1. Definition 28
1.3.2. Prevalence 29
1.3.3. Adverse effects of overfeeding 29
1.4. Previous human experimental studies 37
1.5. Objectives 66
Chapter 2: General materials and methods 67
2.1. Ethical approval and recruitment 67
2.2. Medical screening 68
2.3. Inclusion and exclusion criteria 71
2.4. Blinding and randomisation 72
2.5. Physical activity monitoring 75
2.5.1. Accelerometers 75
2.6. Blood Glucose measurements 78
2.6.1. Continuous glucose monitoring systems (CGM) 79
2.6.2. Yellow spring instrument (YSI) 81
2.6.3. Meal Tolerance Test (MTT) 82
2.7. Blood analysis 82
2.7.1. Radioimmunoassay (RIA) 83
2.7.2. Enzymatic colour test 83
2.8. Insulin sensitivity 84
2.8.1. Matsuda index 84
2.8.2. HOMA-IR 85
2.9. Cardiovascular Function 86
2.9.1. Finometer 86
2.9.2. Venous occlusion plethysmography (VOP) 88
2.9.3. Duplex-Doppler Ultrasound 89
2.10. Energy metabolism 92
2.11. Maximal handgrip strength test 94
2.12. Statistical analysis 96

Chapter 3: The effects of two days of physical inactivity on cardiovascular responses, insulin sensitivity and energy metabolism in healthy humans 98
3.1. Introduction 98
3.2. Materials and Methods 108
3.2.1. Power calculation 116
3.2.2. Data handling 116
3.2.3. Statistical analysis 120
3.3. Results 122
3.3.1. Day 1 and Day 2 of the intervention 122
3.3.2. Assessment day (Day 3) 129
3.3.3. Secondary analysis 145
3.4. Discussion 153
3.4.1. Cardiometabolic effects of physical inactivity 153
3.4.2. Head-to-head comparison between two continuous glucose monitoring systems 161
Chapter 4: The effects of five days of physical inactivity on endothelial function in healthy humans 166
4.1. Introduction 166
4.2. Materials and methods 169
4.2.1. Power calculation 179
4.2.2. Data handling 180
4.2.3. Statistical analysis 182
4.3. Results 183
4.3.1. Energy intake and physical activity patterns during the 5-day intervention 185
4.3.2. Brachial FMD measurements 188
4.4. Discussion 191
Chapter 5: General discussion 196
References 204
Appendices 232

List of Figures

Figure 1.1. Illustration of the metabolic alterations in adipose tissue and skeletal muscle caused by physical inactivity....6
Figure 1.2. Hypothesised causation between insulin resistance and hypertension mediated by sympathetic nervous system (SNS) activation. 8
Figure 1.3. General features of insulin signalling pathways and interacting mechanisms of glucose uptake and vascular tone under healthy conditions.. 14
Figure 1.4. Under pathological settings of glucotoxicity, lipotoxicity and inflammation, parallel degradation in PI3K-dependent insulin-signalling pathways contributes to a reciprocal interaction of insulin resistance and endothelial dysfunction 18
Figure 1.5. Overview of peripheral hormonal and centrally mediated neuronal appetite regulation .23
Figure 1.6. Insulin and leptin actions in response to energy imbalances....27
Figure 1.7. Schematic diagram explaining the potential metabolic–cardiovascular relationship associated with increased food intake. 35
Figure 3.1. Flow chart summarising participant recruitment and randomisation. 110
Figure 3.2. Schematic diagram of the 2-day study design and the sampling intervals for laboratory session on Day 3 113
Figure 3.3. Mean ± SEM of interstitial glucose concentrations over 24-hr period for A) day 1 intervention, B) day 2 intervention and C) mean of the 2-day intervention across the three conditions using continuous glucose monitoring system devices (iPro2). 128
Figure 3.4. Mean ± SEM of serum insulin concentrations and individual responses measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions 130
Figure 3.5. Mean ± SEM of the incremental area under curve of serum insulin concentrations and individual responses measured on the assessment day (Day 3) during the 3-hour postprandial period across the three conditions 131
Figure 3.6. Mean ± SEM of blood glucose concentrations and individual responses measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions 132
Figure 3.7. Mean ± SEM of serum TG concentrations and individual responses measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions....134
Figure 3.8. Mean ± SEM of plasma FFA concentrations and individual responses measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions....135
Figure 3.9. Mean ± SEM of heart rate and individual responses measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three condition 138
Figure 3.10. Mean ± SEM of the calf blood flow (BF) on the assessment day (Day 3) measured for 2 minutes using the venous occlusion plethysmography at baseline (fasting) and every 30 minutes during the 3-hour postprandial period across the three conditions....140
Figure 3.11. Mean ± SEM of interstitial glucose concentrations over a 24-hr period for A) day 1 intervention, B) day 2 intervention and C) mean of the 2-day intervention using two continuous glucose monitoring systems (iPro2 versus Abbott)....147
Figure 3.12. Mean ± SEM of glucose concentrations on the assessment day (Day 3) measured at baseline (fasting) and the 3-hour postprandial period using the iPro2, Abbott and YSI at baseline and every 10 minutes during the 3-hr postprandial period 148

Figure 3.13. Presents two Bland–Altman plots comparing interstitial glucose concentrations recorded by iPro2 sensors and Abbott sensors against the reference method YSI; A) iPro2 vs YSI and B) Abbott vs YSI. 152
Figure 4.1. Flow chart summarising participant recruitment and randomisation. 170
Figure 4.2. Schematic diagram of the 5-day study design in the Control trial and laboratory measurements on Day 1 and Day 6 172
Figure 4.3. Schematic diagram of the 5-day study design in the Inactivity trial and laboratory measurements on Day 1 and Day 6 172
Figure 4.4. Duplex-Doppler ultrasound (Toshiba Aplio 300 Diagnostic Ultrasound System). 176
Figure 4.5. Brachial artery FMD measurement technique. 176
Figure 4.6. Digital hand dynamometer. 178
Figure 4.7. Mean ± SEM of physical activity intensity data recorded by the GENEActiv devices during the 5-day intervention period of each trial. 187
Figure 4.8. Mean ± SEM of postural type data recorded by the GENEActiv devices during the 5-day intervention period of each trial.. 187

List of Tables

Table 1.1. Key studies on physical inactivity consequences with and without overfeeding following bed rest 39
Table 1.2. Key studies on physical inactivity consequences with and without overfeeding following prolonged sitting or limb immobilisation 44
Table 1.3. Key studies on physical inactivity consequences with and without overfeeding following reduced steps count or detraining 53
Table 2.1. Reliability data obtained for insulin, glucose, triglyceride (TG) and free fatty acids (FFA) 95
Table 3.1. Key studies comparing the performance of factory calibrated CGMs against well-established CGMs and reference methods 104
Table 3.2. Participant characteristics. 122
Table 3.3. Body weight, energy intake, number of steps and time spent on each physical activity intensity during the intervention period of each main trial 124
Table 3.4. Descriptive statistics of the 2-day intervention period measured by continuous glucose monitoring system (iPro2) compared between the three conditions 125
Table 3.5. HOMA-IR and Matsuda Indices during each condition on the assessment day (Day 3) during the 3-hour postprandial period across the three conditions 133
Table 3.6. Serum TG concentrations, plasma FFA concentrations and area under the curve on the assessment day (Day 3) measured at baseline (fasting) and during the 3-hour postprandial period (PP) across the three conditions. 136
Table 3.7. Summary of the cardiovascular parameters measured continuously for three hours on the assessment day (Day 3) during baseline (fasting) 3-hour postprandial using the Finometer across the three conditions 139
Table 3.8. Energy metabolism on the assessment day (Day 3) measured by indirect calorimetry at baseline (fasting) and the 3-hour postprandial period across the three conditions 143
Table 3.9. Head-to-head comparison between iPro2 and Abbott systems against the YSI on the assessment day (Day 3) of the Control trial 150
Table 4.1. Participant characteristics. 183
Table 4.2. Body weight, blood pressure and heart rate measurements at baseline and the end of each trial 184
Table 4.3. 24-hr step counts during the 5-day intervention period of each trial 185
Table 4.4. Brachial artery vascular measures during FMD at baseline and the end of each trial. 189
Table 4.5. Brachial artery %FMD at baseline and the end of each trial. 189
Table 4.6. Maximal handgrip strength (kg) of the non-dominant hand measured at baseline and the end of each trial 190

List of abbreviations

AD	Absolute Differences
AEE	Activity energy expenditure
ARD	Absolute Relative Differences
BMR	Basal Metabolic Rate
CGM	Continuous Glucose Monitoring Systems
DBP	Diastolic Blood Pressure
DIT	Diet-Induced Thermogenesis
eNOS	endothelial Nitric Oxide Synthase
FFA	Free Fatty Acids
FMD	Flow-Mediated Vasodilatation
GLUT-4	Glucose Transporter-4
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
iAUC	incremental Area Under the Curve
MAD	Mean Absolute Differences
MAP	Mean Arterial Pressure
MARD	Mean Relative Absolute Differences
MET	Metabolic Equivalent of Task
MVPA	Moderate to Vigorous Physical Activity
NO	Nitric Oxide
OGTT	Oral Glucose Tolerance Test
PAL	Physical Activity Level

PAR	Physical Activity Ratio
REE	Resting Energy Expenditure
RER	Respiratory Exchange Ratio
RMR	Resting Metabolic Rate
SBP	Systolic Blood Pressure
SEM	Standard Error of the Mean
TEE	Total Energy Expenditure
TG	Triglycerides
TPR	Total Peripheral Resistance
VOP	Venous Occlusion Plethysmography

Chapter 1: Introduction

1.1. Introduction

The question of how much of the obesity epidemic is owing to excessive calorie intake and how much is owing to decreased physical activity has long been a topic of argument (Swinburn, Sacks and Ravussin, 2009; Scarborough et al., 2011). The growth in obesity in the United States since the 1970s has been almost entirely caused by increased calorie consumption, while changes in physical activity appear to have played a minor influence. Further, physical activity levels have not reduced, and in some cases have grown, over the time when obesity prevalence has been rising (Swinburn, Sacks and Ravussin, 2009; Vandevijvere et al., 2015). Previous investigators used their data (from 1970–2000) to estimate how much weight Americans would have acquired throughout the period of thirty years had food intake been the only factor. The assumption was that if the actual weight gain was the same as expected, food intake would have been almost completely to blame. If it was not, this would have meant that changes in physical activity had a role to play, whereas if the actual weight gain was greater than projected, a decrease in physical activity could have played a role (Swinburn, Sacks and Ravussin, 2009). They expected to be 10.8 kilograms heavier, but the findings showed they were only 8.6 kilograms heavier. The excess calorie consumption still accounts for weight gain, but the increases in physical activity may have mitigated what would otherwise have been a greater weight gain. Similarly, other researchers studied the implications of increases in calorie intake and declines in physical activity levels for body weight gain in the UK population between 1980 and 2000 (Scarborough et al., 2011). The findings revealed that males and females

have different causes for increasing their body weight. Only a portion of the increase in body weight in males can be attributed to increased energy intake, implying that physical activity levels have also played a role. Increases in calorie consumption account for the complete rise in body weight in females, implying that this was the primary cause of weight gain.

Although it is vital to promote a balanced diet and increased physical activity to prevent weight gain and enhance other health outcomes, it is also necessary to determine if one side or the other of the energy balance equation is the primary cause of the rise in obesity. This would help prevention efforts to be better targeted at addressing the epidemic's underlying causes. It also has clinical significance for determining and preventing individual weight gain. In the subsequent sections, the researcher will discuss the definition, prevalence and adverse effects of physical inactivity and overfeeding, followed by an overview of human experimental studies on the cardiometabolic effects of physical inactivity with or without overfeeding using different methodological approaches.

1.2. Behavioural components

1.2.1. Definition

Many terms have been used to describe individuals, including trained, physically active, physically inactive and sedentary. Physical activity is referred to as any body movement that can increase energy expenditure. The amount of energy expended depends on the body's structure and content. Physical activity can be performed for recreation, leisure,

occupation, housework or athletic purposes at different frequencies, intensities, modalities and durations (Plasqui and Westerterp, 2007; Bussmann and van den Berg-Emons, 2013; Thivel et al., 2018). The quantity of energy needed to complete an activity is measured in kilojoules (kJ) or kilocalories (kcal); 4.184kJ is roughly equal to 1kcal. The kJ is favoured scientifically because it is a measure of energy expenditure; however, traditionally, the kcal, which is a measure of heat, has been used (Caspersen, Powell and Christenson, 1985). Exercise and physical activity are often used interchangeably, and they share a number of characteristics. For instance, both comprise any energy-expending movement produced by skeletal muscles and are positively associated with physical fitness as the intensity, length and frequency of movements increase (Caspersen, Powell and Christenson, 1985). However, exercise is a subtype of physical activity that is organised, structured and repeated with the goal of maintaining or improving fitness levels (Thivel et al., 2018). Physical inactivity can be defined as not meeting the guidelines of the recommended activity level of at least 150 minutes per week. This could be thirty minutes per day for five days in the form of moderate physical activities or twenty minutes per day for three days per week for more vigorous exercise types (Pate et al., 1995; Hamilton, Hamilton and Zderic, 2007; Hamilton et al., 2008; Lee et al., 2012; Same et al., 2016; van der Ploeg and Hillsdon, 2017; Dempsey and Thyfault, 2018; Panahi and Tremblay, 2018). Others have defined physical inactivity as reduced activity levels in the form of prolonged sitting time rather than not meeting guidelines. This assumes that metabolic alterations caused by prolonged sitting might play an equal or greater role than the absence of exercise (Hamilton et al., 2008; Owen et al., 2010; Dempsey and Thyfault, 2018). The term ‘sedentary’ is derived from the Latin word

‘sedentarius’ or ‘sedere’ and interpreted as ‘sitting in one position’ with an energy expenditure of ≤1.5 metabolic equivalents of task, which implies a different set of behaviours than lack of moderate-to-vigorous physical activities (MVPA) (Bussmann and van den Berg-Emons, 2013; Gibbs et al., 2015; Young et al., 2016; van der Ploeg and Hillsdon, 2017; Panahi and Tremblay, 2018; Thivel et al., 2018; Homer, Owen and Dunstan, 2019). Sedentary behaviour and physical activity are not mutually contradictory. Individuals are deemed active when they meet the physical activity guidelines for their age, but this does not exclude them from engaging in sedentary habits for a large portion of their time (Thivel et al., 2018). Researchers investigating exercise, physical inactivity and sedentary behaviour are currently considering those terms as distinct entities with specific determinants and health effects (Young et al., 2016). In the present thesis, both physical inactivity (avoiding MVPA) and sedentary behaviours (increased sitting time) were applied to reduce physical activity levels during interventions, which was hypothesised to be a better reflection of current lifestyles in most countries.

1.2.2. Prevalence

Nowadays, a sedentary lifestyle has encouraged unhealthily low levels of physical activity together with increased incidence of overweight, obesity and obesity-related disorders (Hu, 2003; Dunstan et al., 2007; Healy et al., 2008; Chaput and Tremblay, 2009; Homer, Owen and Dunstan, 2019). It has been reported that people are inactive during 50–60 percent of their daily time and approximately only one in three adults are meeting guidelines worldwide (Wilmot et al., 2012; Homer, Owen and Dunstan, 2019). Moreover, one quarter of the population of the United Kingdom does not get even thirty minutes of

moderate exercise per week, and 90 percent of Americans do not meet physical activity guidelines (Bowden Davies et al., 2019). Based on a national survey, 95 percent of total waking time is spent on sedentary and light-intensity physical activities, while 5 percent is spent on moderate to vigorous physical activities (Dempsey and Thyfault, 2018).

1.2.3. Adverse effects of physical inactivity

Physical inactivity has lately been implicated as a primary cause of death in Western nations, and it contributes to a variety of medical disorders. Cardiovascular (e.g., coronary heart disease) and metabolic illnesses (e.g., type 2 diabetes mellitus, lipid metabolism changes and increased oxidative stress) are among the chronic diseases exacerbated by physical inactivity (Hamilton, Hamilton and Zderic, 2007; Hamilton et al., 2008; Lee et al., 2012; Wilmot et al., 2012; Gaetano, 2016; Same et al., 2016; Booth et al., 2017; Dempsey and Thyfault, 2018; Winn et al., 2019). In addition, physical inactivity can promote muscle mass reduction, glucose homeostasis impairment, exercise intolerance, energy metabolism alterations, decreased fat oxidation, intramuscular lipid (IMCL) accumulation, muscle protein metabolism imbalance and increased production of reactive oxygen species (ROS) in the mitochondria (Lakka et al., 2003; Biolo et al., 2005; Bergouignan et al., 2006; Cree et al., 2010; Wall, Dirks and van Loon, 2013; Gram et al., 2015; Dirks et al., 2018; Narici et al., 2021). The necessity of being physically active for maintaining health is widely recognised, and the current scenario of significantly reduced physical activity to levels substantially below the daily requirement of 7,500–10,000 steps per day will increase health problems associated with physical inactivity (Booth et al., 2017). The metabolic alterations in adipose tissue and skeletal muscle caused by physical inactivity

are illustrated in Figure 1.1. Five thousand steps per day has been linked to poor cardiometabolic risk. When the change is abrupt, such as from >10,000 to fewer than 5,000 or as low as 1,500 daily step counts, severe health impacts can appear rapidly (within 3–14 days) (Tudor-Locke et al., 2013). In the following subsections, the researcher will review the consequences of physical inactivity for the cardiovascular system, endothelial function, insulin sensitivity and energy metabolism.

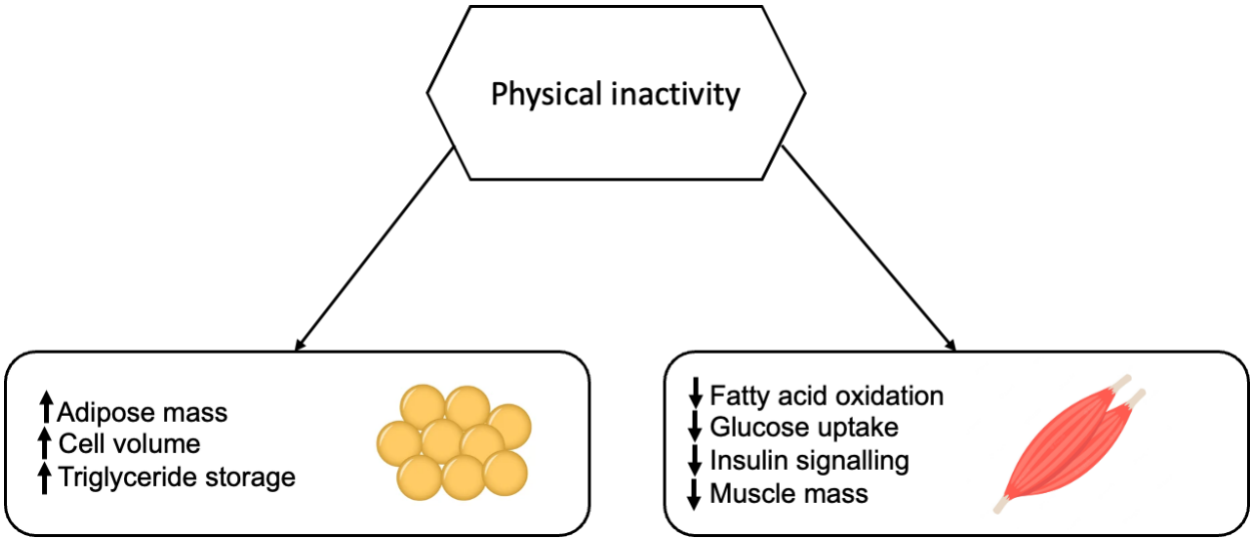


Figure 1.1. Illustration of the metabolic alterations in adipose tissue and skeletal muscle caused by physical inactivity

1.2.3.1. Cardiovascular system and endothelial function

Recent research has shown that physical inactivity increases the risk of developing cardiovascular disease and has also established a clear link between a sedentary lifestyle and deaths related to cardiovascular disease. Factors that are thought to increase the risk are the development of insulin resistance, metabolic syndrome, lipoprotein metabolism irregularities (specifically lipoprotein lipase in the muscle) and vascular defects (Same et al., 2016). However, the exact mechanisms by which cardiovascular health is influenced by physical inactivity are not completely defined and have thus become a subject of recent studies (Vranish et al., 2018). Hyperinsulinemia has been linked to increased sympathetic activity and blood pressure (Landsberg, 1994). Resistance to insulin-mediated glucose clearance can trigger the release of more insulin to maintain glycaemic control. Decompensation of glucose homeostasis can be avoided if this compensatory effort is effective, but the resulting hyperinsulinemia stimulates the sympathetic nervous system (SNS), resulting in a hyperdynamic state and a higher risk of developing hypertension (Landsberg, 1994; Facchini, Stoohs and Reaven, 1996). Furthermore, a strong relationship between heart rate and insulin resistance has been documented (Facchini, Stoohs and Reaven, 1996). This association is thought to be mediated by sympathetic stimulation, which explains why both insulin resistance and an increase in heart rate have been linked to the development of hypertension (Stern et al., 1992; Moan et al., 1994; Facchini, Stoohs and Reaven, 1996). In addition, insulin can stimulate sodium reabsorption, leading to extracellular fluid volume expansion, which may predispose the individual to hypertension (Vierhapper, 1985). Figure 1.2 illustrates

the postulated relationship among insulin resistance, heart rate and blood pressure via the sympathetic system.

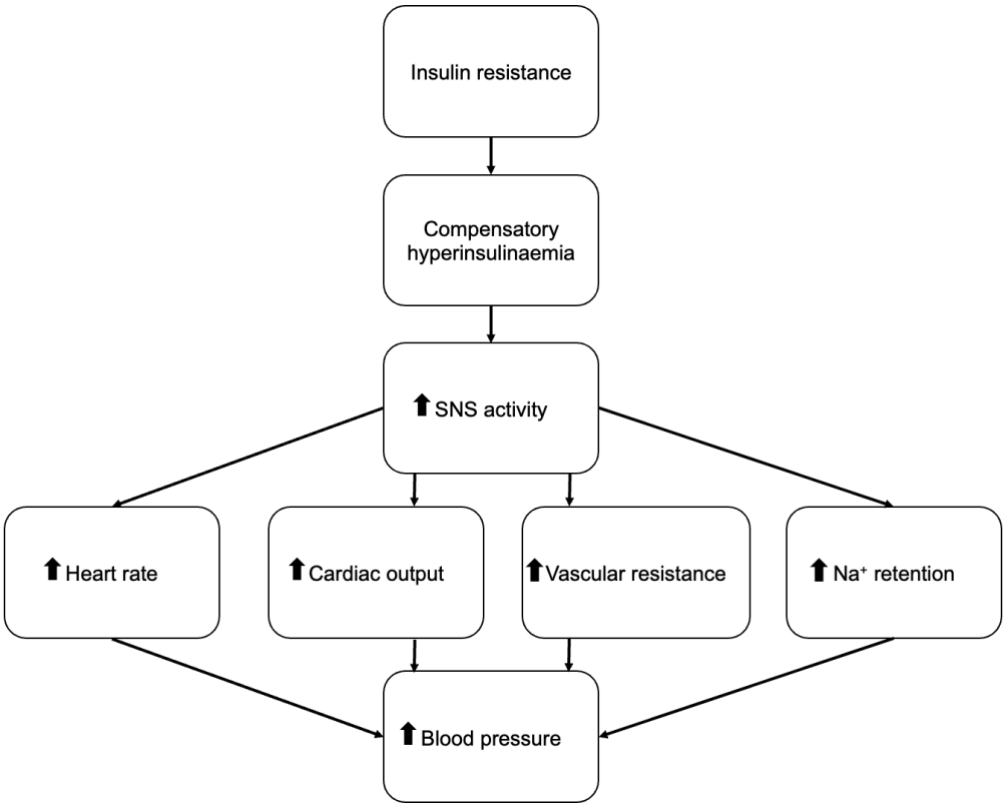


Figure 1.2. Hypothesised causation between insulin resistance and hypertension mediated by sympathetic nervous system (SNS) activation.

The vascular endothelium (intima) plays both structural and functional roles in the human body. It appears small in histological sections, consisting of a thin layer of cells 1–2 microns thick resting on top of a layer of smooth muscle cells (media) and a dense layer of connective tissues (adventitia). It is responsible for regulating vascular tone, preventing thrombogenicity (antithrombosis) and inhibiting platelet and leukocytes aggregation (anticoagulation) and adhesion. Thus, any disturbance in the vascular endothelium can lead to cardiovascular disorders such as hypertension, atherosclerosis and cardiac failure by promoting vasoconstriction and thrombosis (Raitakari and Celermajer, 2000; Corretti et al., 2002; Wheatcroft et al., 2003; Moens et al., 2005; Jansson, 2007; Potenza, Addabbo and Montagnani, 2009; Harris et al., 2010; Tomiyama and Yamashina, 2010; Chiu and Chien, 2011; Flammer et al., 2012; Alley et al., 2014). A major physical inactivity-related risk factor is atherosclerosis. Atherosclerosis is a significant contributor to the occurrence of about 90 percent of cardiovascular disorders and linked to other systemic risk factors such as diabetes mellitus, hypertension and dyslipidaemia (Malek, 1999; Ratcliffe et al., 2017). Defects in endothelial function are thought to be an early step in the development of atherosclerosis, leading to an increase in arterial stiffness and subsequent cardiovascular disorders (Wheatcroft et al., 2003; Flammer et al., 2012). Haemodynamic conditions within the blood vessels result in superficial stress near the walls of the vessel, which can be categorised into two types: circumferential stress and shear stress. The circumferential stress is secondary to variation in pulse pressure within the vessel. It acts perpendicular to the vessel wall and applies stress on all layers (intima, media and adventitia), whereas shear stress acts at a tangent to the vessel wall to generate frictional force mainly at the intima layer and is primarily determined by the blood

flow. Poiseuille's law is used to estimate the extent of the shear stress in the majority of the vessels, which states that shear stress is proportional to the viscosity of the blood flow and inversely proportional to the third power of the inner radius (Malek, 1999; Chiu and Chien, 2011). Shear stress level within the venous system ranges from 1–6 dyne/cm^2, while in the arterial network it ranges between 10 and 70 dyne/cm^2 (Malek, 1999; Chiu and Chien, 2011). The endothelium controls – that is, increases or decreases – the production of vasodilators depending on the changes in shear stress force (Kelm, 2002; Moens et al., 2005; Stoner and Sabatier, 2012). Thus, blood vessels' ability to respond to local environmental changes and increased shear stress in the lumen indicates good self-regulatory tone and blood flow distribution adjustment of the affected vessels (Corretti et al., 2002). Furthermore, it has been documented that an arterial shear stress level of more than 15 dyne/cm^2 induces an endothelial atheroprotective profile, while arterial shear stress level of less than 4 dyne/cm^2 stimulates an atherogenic state (Malek, 1999; Papaioannou and Stefanadis, 2005; Papaioannou et al., 2006). The vascular endothelium is composed of non-voltage calcium-activated potassium channels, which are activated by the increase in blood flow (i.e., shear stress), leading to calcium release that activates an enzyme known as endothelial nitric oxide synthase (eNOS). Subsequently, eNOS activates L-arginine conversion to nitric oxide (NO), which is an unstable molecule with a rapid tendency to convert into a stable by-product. NO will then infiltrate into the smooth muscle cells, resulting in vascular dilatation, and prevent platelet adhesion. This phenomenon is referred to as flow-mediated vasodilatation (FMD) (Corretti et al., 2002; Wheatcroft et al., 2003; Moens et al., 2005; Harris et al., 2010; Alley et al., 2014). There are several other vasodilators (e.g., prostaglandins, Adenosine triphosphate (ATP) and

endothelium-derived hyperpolarising factor), but owing to its primary role in FMD response, NO has been intensively investigated since its discovery in 1980 (Kelm, 2002; Muniyappa et al., 2007; Harris et al., 2010; Tomiyama and Yamashina, 2010). Vasodilation, which results in greater blood flow, is a primary physiological effect of insulin-stimulated NO release in the endothelium. There are two steps to insulin-mediated dilatation in skeletal muscle. First, dilatation of terminal arterioles causes an increase in the number of perfused capillaries within minutes without affecting total limb blood flow. Second, relaxation of larger resistance arteries enhances overall limb blood flow within thirty minutes, reaching maximum after two hours (Kim et al., 2006). The harmful consequences of low NO bioactivity for the endothelium, including the increase in vascular tone and the high risk of developing thrombosis, can be caused by eNOS malfunction/insufficiency or increased NO antagonists release (Potenza, Addabbo and Montagnani, 2009). It has been documented that different mechanisms control NO production in response to shear stress changes. In the highly acute changes, the intracellular calcium released by the endothelial cells is thought to be primarily responsible for NO release. During longer periods (i.e., within minutes), a continuous influx of NO is attributed to the activation of eNOS through serine-/threonine-specific protein kinase and protein kinase B (PKB). However, when the duration is increased to several minutes or hours with high shear stress levels, NO production is believed to be maintained by the enhancement of eNOS transcription (Corretti et al., 2002; Moens et al., 2005). Various factors can acutely influence FMD response such as stress, smoking, high fat intake, and changes in glucose and sex hormones (oestrogen and progesterone) levels (Kelm, 2002). Several hormonal and metabolic activities are tightly regulated in the

postprandial state. Previous researchers have linked endothelial disease to changes in the postprandial state, which is the period following meal intake (Lefèbvre and Scheen, 1998). The postprandial state lasts two to three hours after a high-carbohydrate meal, three to five hours after a mixed meal and up to ten hours after a high-fat meal (Lefèbvre and Scheen, 1998). The formation of ROS may be one mechanism by which high postprandial glucose levels promote endothelial dysfunction (Giugliano, Ceriello and Paolisso, 1996; Lefèbvre and Scheen, 1998). FMD response can help in predicting the risk of developing cardiovascular disorders in the long term (Thijssen, Green and Hopman, 2011). Endothelial dysfunction has been linked to the development of arterial hypertension and coagulation stimulation (Ceriello, 1993; Lefèbvre and Scheen, 1998). In the lower limbs, endothelial dysfunction is of clinical interest given evidence that the leg vasculature is particularly susceptible to atherosclerosis compared to disease-resistant vessels such as the brachial artery (Morishima et al., 2016).

1.2.3.2. Insulin sensitivity

Insulin resistance is a major factor triggering the development of type 2 diabetes mellitus, metabolic syndrome and atherosclerotic disorders (Abel, O'Shea and Ramasamy, 2012). Stressful events, infections and reduced levels of physical activity are examples of factors that can acutely and temporarily lead to insulin resistance, whereas high caloric intake, obesity and diabetes mellitus cause chronic and persistent insulin resistance (Trout, Homko and Tkacs, 2007). Insulin sensitivity refers to the ability of insulin to maintain a normal blood glucose level through enhancing glucose disposal in the muscle and adipose tissues, promoting glucose storage in the liver as glycogen and inhibiting glucose

release from the liver (Trout, Homko and Tkacs, 2007). Insulin also plays a key role in the inhibition of adipose tissue lipolysis (Sears and Perry, 2015). Normally, when insulin binds to its receptor on the cell surface, it phosphorylates both the Src-homology-collagen-like protein (SHC) and the insulin receptor substrate (IRS), which subsequently will activate a series of proteins pathways. On the one hand, the SHC protein will activate the mitogen-activated protein kinase (MAPK). The activation of MAPK promotes vascular constriction through endothelin-1 (ET-1) induction, which is a potent vasoconstrictor present in the vascular endothelial cells. On the other hand, the IRS activates phosphatidylinositol 3-kinase (PI3K) and subsequently phosphoinositide-dependent protein kinase (PDK), which activates Akt (protein kinase B) and atypical protein kinase C (aPKC). Akt will promote (a) vascular relaxation by increasing NO production, which is a potent vasodilator, and (b) glucose uptake in the skeletal muscle and adipose tissues. aPKC will also increase glucose uptake through GLUT-4 (Muniyappa et al., 2007). Figure 1.3 illustrates insulin signalling pathways and interacting mechanisms under normal states. There is a natural balance between the actions of the two signalling pathways, which promotes metabolic and cardiovascular homeostasis under healthy environments (Pessin and Saltiel, 2000; Schinner et al., 2005; Kim et al., 2006; Abel, O'Shea and Ramasamy, 2012). Insulin controls the use of substrates in various tissues, including muscular, adipose and liver tissues.

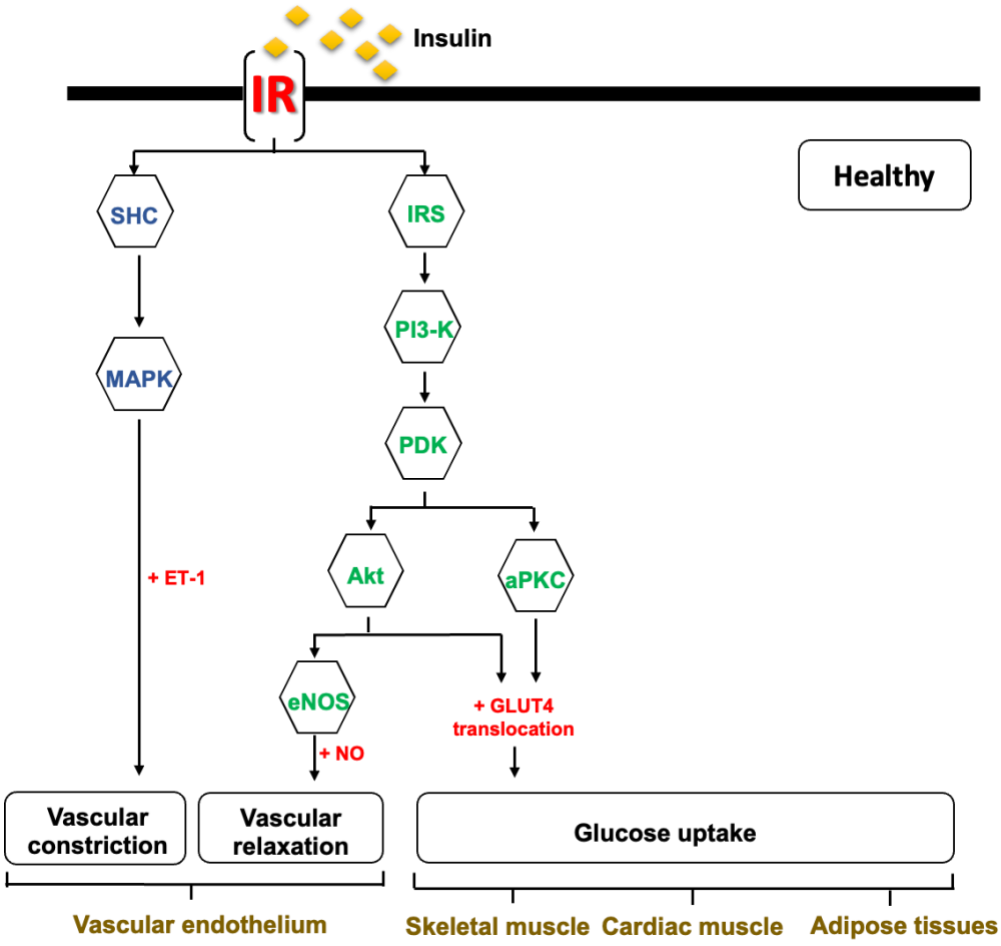


Figure 1.3. General features of insulin signalling pathways and interacting mechanisms of glucose uptake and vascular tone under healthy conditions. Abbreviations: insulin receptor (IR); Src-homology-collagen-like protein (SHC); mitogen-activated protein kinase (MAPK); endothelin-1 (ET-1); insulin receptor substrate (IRS); phosphatidylinositol 3-kinase (PI3K); phosphoinositide-dependent protein kinase (PDK); atypical protein kinase C (aPKC); endothelial nitric oxide synthase (eNOS); nitric oxide (NO); glucose transporter-4 (GLUT-4).

Insulin resistance is a state characterised by the reduced responsiveness of tissues to insulin actions at normal blood insulin concentration. Under those conditions, a greater insulin level is required to sustain normal glucose level in the circulation (Trout, Homko and Tkacs, 2007). Thus, insulin is unable to maintain the metabolism of glucose and free fatty acids (FFA) (Schinner et al., 2005; Potenza, Addabbo and Montagnani, 2009; Turner et al., 2014). Previous studies have shown that physical inactivity can increase post-absorptive glucose and insulin levels, while decreasing glucose disposal during oral glucose tolerance test (OGTT) and hyperinsulinemic clamp (Mikines et al., 1991; Sonne et al., 2010). In addition, insulin resistance associated with physical inactivity can lead to dyslipidaemia and hypertension (Ginsberg, 2000). It can also cause a decrease in skeletal muscle lipoprotein lipase function, leading to a reduction in plasma triglyceride (TG) clearance and glucose disposal (Balkau et al., 2008; Owen et al., 2010; Healy et al., 2011). One of the most serious health consequences of impaired insulin sensitivity is decreased blood disposal of dietary carbohydrates, which leads to a higher frequency and severity of hyperglycaemic excursions during the day (van Dijk and van Loon, 2015) and is associated with the onset of metabolic inflexibility (Kelley and Mandarino, 2000). Metabolic inflexibility limits the body's ability to transition between fat and carbohydrate utilisation as needed depending on the prandial state of the body (Dirks et al., 2018). Skeletal muscle is a major organ for glucose and FFA clearance from the plasma (Turner et al., 2014). When skeletal muscle is consistently contracted, the demand for ATP increases, requiring more glucose to be actively delivered into muscle cells. As a result, enhanced insulin sensitivity is promoted by increased contractions/exercise and depletion of glycogen stores. When skeletal muscles are passive, ATP demand is low, and

glycogen levels are high, glucose demand is low and insulin sensitivity diminishes (Booth et al., 2017). It has been reported that 30–40 percent of glucose is disposed into the skeletal muscle after a meal and about 15 percent is stored as muscle glycogen (Kelley et al., 1988), whereas during hyperinsulinemic clamps, more than 70 percent of glucose is oxidised in the skeletal muscles (Klip and Paquet, 1990). Furthermore, the effect of insulin and muscle contractions on glucose uptake is additive, and both ultimately result in GLUT-4 translocation (Rose and Richter, 2005). There is a high risk of developing insulin resistance when any disruption to the insulin-induced glucose disposal process occurs (Richards, Raines and Attie, 2010; Wasserman et al., 2011). Skeletal muscle glucose transportation is a rate-limiting phase in blood glucose clearance under physiological settings, and it is considered to occur as a result of attenuated insulin signalling and/or GLUT-4 translocation to the plasma membrane (Garvey et al., 1998). For its role in GLUT4 translocation, maintaining insulin signalling through the IRS/Akt pathway appears to be critical for insulin-mediated muscle glucose utilisation (Kim et al., 1999; Morino, 2005). Intramyocellular lipid deposition is another possibility for physical inactivity-induced impairment of muscle glucose clearance. A positive link occurs between plasma FFA levels and insulin resistance, while a greater link exists between intramyocellular lipids accumulation and insulin resistance (Krssak et al., 1999). Further, increased FFA levels cause endothelial dysfunction by directly damaging the vessel wall (Kim et al., 2006). Decreased insulin sensitivity can occur as a consequence of elevated ROS, which impairs insulin-mediated GLUT-4 translocation, and glucose metabolism reduces NO bioavailability and impairs vasodilation (Kim et al., 2006). Physical inactivity can induce insulin resistance leading to secondary hyperinsulinemia, which triggers

endothelial proliferation causing the formation of atherosclerotic plaques, thus aggravating the development of cardiovascular disorders (Biolo et al., 2005). It has been documented that glucotoxicity, lipotoxicity and inflammation are all factors that contribute to insulin resistance and vascular dysfunction (Kim et al., 2006), which could play a role in the reciprocal relationship between metabolic and cardiovascular disorders (Figure 1.4).

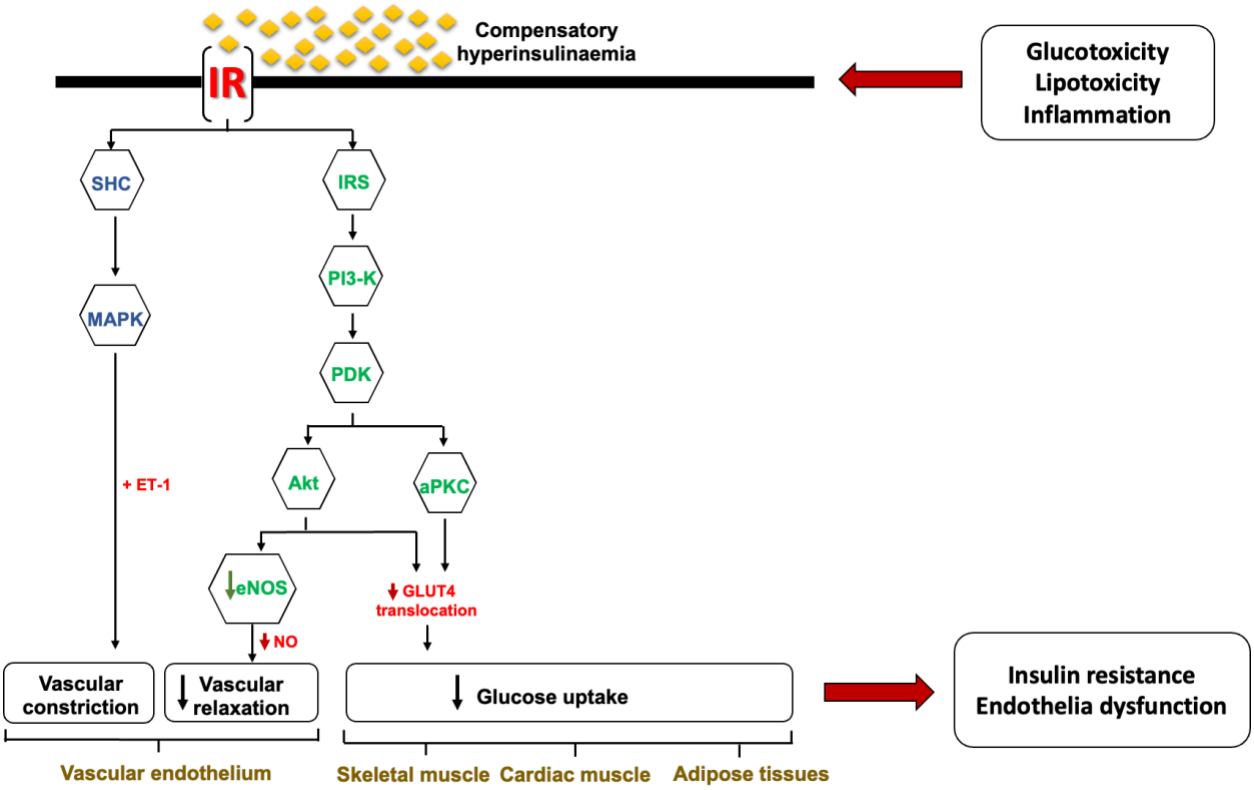


Figure 1.4. Under pathological settings of glucotoxicity, lipotoxicity and inflammation, parallel degradation in PI3K-dependent insulin-signalling pathways contributes to a reciprocal interaction of insulin resistance and endothelial dysfunction. Abbreviations: insulin receptor (IR); Src-homology-collagen-like protein (SHC); mitogen-activated protein kinase (MAPK); endothelin-1 (ET-1); insulin receptor substrate (IRS); phosphatidylinositol 3-kinase (PI3K); phosphoinositide-dependent protein kinase (PDK); atypical protein kinase C (aPKC); endothelial nitric oxide synthase (eNOS); nitric oxide (NO); glucose transporter-4 (GLUT-4).

In endothelial cells, hyperglycaemia stimulates aPKC. Induction of aPKC appears to increase the production of adhesion molecules, which injure the endothelium and reduce NO levels. Hyperglycaemia also promotes the release of vasoconstrictive substances such as endothelin by the endothelium. The role of the vasculature is dramatically altered to vascular constriction via this dual process (Haller, 1997; Lefèbvre and Scheen, 1998). Insulin's vasodilator effects are important in maintaining haemodynamic and metabolic equilibrium in healthy people.

1.2.3.3. Energy metabolism and appetite

Numerous factors, such as nutrition, gender, age and adiposity, influence whole-body energy metabolism. Physical inactivity, meanwhile, is an underestimated component that influences energy metabolism and substrate utilisation (Booth et al., 2017). The body reaches energy balance when the energy expended is compensated by an equal amount of energy intake, which helps to maintain body mass and energy stores (Landsberg, 2006; Hall et al., 2012). The energy balance formula [energy storage = energy intake – energy expenditure] expresses the energy conservation concept (Hall et al., 2012; Lam and Ravussin, 2016). Energy intake reflects the amount of energy contained in the macronutrients of the diet consumed, mainly carbohydrates, fat and protein. It is essential to maintain the function of the body's multiple internal and external functions, for example, for respiration, core temperature preservation, circulation, tissue growth and performing physical activities (Brooks et al., 2004; Hall et al., 2012). Carbohydrate energy density is 4 kcal/g, protein energy density is 4 kcal/g and fat energy density is 9 kcal/g. These are population averages for metabolisable energy, which is the amount of fuel really available

to cells for undertaking biological mechanisms (Hall et al., 2012). In vivo, macronutrients are converted to substrates that can either be oxidised or stored. Food consumption may not be matched by the reduction in energy requirement associated with inactivity in sedentary individuals. Mental leisure or work activities may increase appetite, potentially owing to changes in hormones and glucometabolic imbalance (Panahi and Tremblay, 2018; Narici et al., 2021).

The rate of change in the body's macronutrient reserves is referred to as energy storage. Carbohydrate is mostly stored in skeletal muscle and the liver as intracellular glycogen. Glucose is a limited supply substrate with only 4 g in circulation and is essential for brain metabolism, whereas the amount of glycogen is about 300–500 g in the liver and muscle, and its turnover is extremely fast (Booth et al., 2017). Glycogen levels are highest in the postprandial state. The main source of stored energy in most individuals is lipids, specifically TG. Lipids are predominantly shuttled to energy storage or kept in stores if their consumption is restricted owing to reduced energy expenditure or consistent intake (Booth et al., 2017). Depots of glucose stored in muscle and liver glycogen, as well as lipids stored in adipose tissue, muscle and liver, can be rapidly released to power muscular contraction and other critical functions as physical activity and energy demands rise (Booth et al., 2017). Any disproportion in these macronutrients' consumption and use will result in a change in body composition content (Hall et al., 2012). Inactivity has been associated with increases in triglyceride levels and a decrease in fat oxidation, which has been correlated to a decrease in metabolic rate (Blanc et al., 2000; Crossland et al., 2019; Narici et al., 2021). The decrease in lipid oxidation is accompanied by the emergence of insulin resistance (Blanc et al., 2000).

The total energy expenditure (TEE) produced daily is the result of three main components (Hall et al., 2012; Hill, Wyatt and Peters, 2012). First is the resting metabolic rate (RMR), which contributes a major part of TEE at about two thirds (~70–80 percent) in inactive people. It reflects the energy needed to maintain baseline cellular and organs functions and is affected by body size, body content (fat-free mass contributes about 70 percent of the RMR variance) and recent energy imbalances (Hall et al., 2012; Lam and Ravussin, 2016). The second major component of TEE is activity energy expenditure (AEE). There are two types of activity thermogenesis: exercise-related activity thermogenesis and non-exercise activity thermogenesis (NEAT) (Hall et al., 2012). Exercise-related activity thermogenesis is the energy generated when performing daily activities and exercises. Its contribution to TEE is around 10–15 percent, but it varies depending on the type, intensity and duration of the activity, for example, reaching up to 50 percent in highly active individuals (Lam and Ravussin, 2016). NEAT, which is the energy expended on any activity aside from sleeping, eating or sports-like exercise is the most variable component of energy expenditure (Levine, 2004). The third major component of TEE is diet-induced thermogenesis (DIT), which is the energy cost of meal consumption and metabolism. In a Western diet, DIT represents about 10–15 percent of the total energy intake with variation between and within individuals (Brooks et al., 2004; Levine, 2004; Landsberg, 2006; van Baak, 2008; Melzer, 2011; Hall et al., 2012; Hill, Wyatt and Peters, 2012; Lam and Ravussin, 2016; Westerterp, 2017). Food consumption is primarily regulated by a feedback system that reacts to both physiological and environmental signals (e.g. smell, taste, amount of food available, stress and social habits) (Lam and Ravussin, 2016). These signals affect dietary behaviour and everyday energy intake.

Interactions between peripheral organs and tissues, including adipose tissue, the gastrointestinal system, the pancreas and the nervous system, regulate body weight via signals that inform brain centres about nutritional and physiological status (Wilson and Enriori, 2015).

Appetite is a psychological desire for food intake. Hunger is a subjective and urging feeling that indicates the need for food intake. Satiety refers to the act of terminating a meal to avoid overeating. The difference between the terms ‘satiation’ and ‘satiety’ is that satiation refers to the feeling of fullness during the meal ingestion, while satiety refers to the feeling of fullness between meals (Hall et al., 2012; Amin and Mercer, 2016). Satiety and satiation are influenced by the composition of food with protein having a higher impact than carbohydrate, which in turn has a greater impact than fat (Delzenne et al., 2010; Hall et al., 2012). Appetite is controlled by a variety of metabolic signals, which are categorised as short-acting factors and long-acting adiposity signals. Figure 1.5 illustrates short-term and long-term appetite regulatory pathways in the human body.

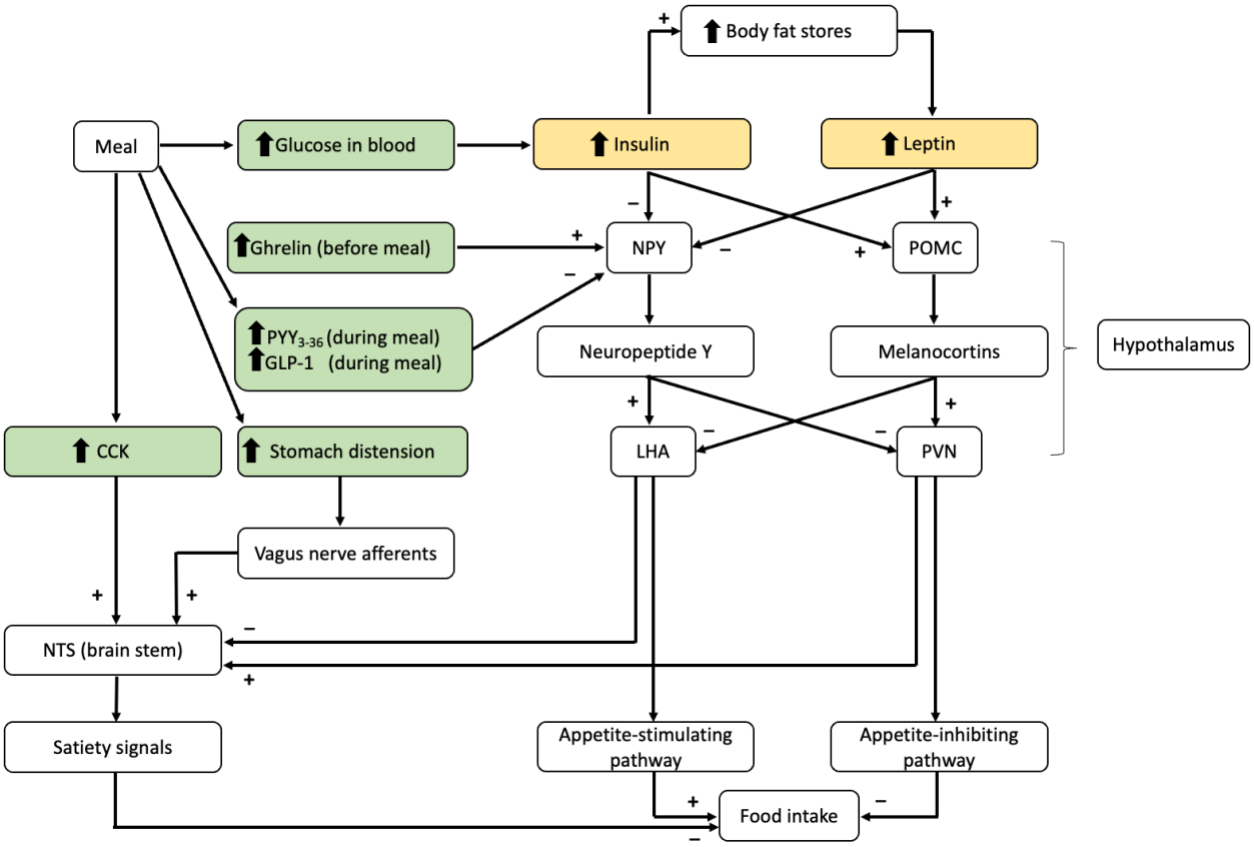


Figure 1.5. Overview of peripheral hormonal and centrally mediated neuronal appetite regulation. Green colour indicates short-term control, and yellow colour represents long-term control. Abbreviations: peptide YY (PYY); glucagon-like peptide 1 (GLP-1); cholecystokinin (CCK); nucleus tractus solitarius (NTS); neuropeptide Y (NPY); pro-opiomelanocortin (POMC); lateral hypothalamic area (LHA); paraventricular nucleus (PVN).

In the short term, distension of the gastrointestinal tract and the production of a number of gut peptides are two pathways for satiation (Amin and Mercer, 2016). The hypothalamus, which regulates the body's basic functions including reproduction, temperature and hormone balances, is also in charge of energy balance (Wilson and Enriori, 2015). Energy control is linked to several hypothalamic regions, including the arcuate nucleus, comprising two interlinked neural circuits (pro-opiomelanocortin [POMC] and neuropeptide Y [NPY]) that vary in the expression of specific neuropeptides and hormones in response to changes in energy status, leading in adjustments in energy intake and expenditure. These neurons also connect to the brainstem and spinal cord (Austin and Marks, 2009; Wilson and Enriori, 2015). During a negative energy balance, NPY increases and POMC decreases. In contrast, when there is a surplus of energy, NPY decreases and POMC increases (Wilson and Enriori, 2015). The hormone ghrelin is the only peptide that is orexigenic, and its production arises from the fundus of the stomach with the time since the last meal to enhance food consumption (Delzenne et al., 2010; Wilson and Enriori, 2015). Once produced, it activates the hypothalamus through the NPY, which is responsible for the appetite-enhancing pathway, and the vagus nerve to stimulate food intake (Wilson and Enriori, 2015; Lam and Ravussin, 2016). It has been shown that the intensity and extent of postprandial ghrelin inhibition are dose-dependently related to the amount of caloric intake, that is, ghrelin is suppressed more by large meals (Callahan et al., 2004; Delzenne et al., 2010). At the same caloric load, lipids appear to reduce ghrelin levels less efficiently than carbohydrates or proteins (Delzenne et al., 2010). Gastric distension activates the vagal mechanosensitive fibres in the vagus nerve. The nucleus of the solitary tract in the brainstem is a significant receiver of vagal afferent

signals from the gut that transmits information to higher brain areas involved in appetite control, such as the hypothalamic centres. Individual meal sizes are regulated by the integration of all these afferent signals associated with food availability in the gut (Berthoud, 2012; Wilson and Enriori, 2015). Direct interaction of nutrients with the gastrointestinal system also induces the release of satiation hormones such as cholecystokinin (CCK) from I cells of the duodenum, and peptide YY (PYY_{3-36}) and glucagon-like peptide 1 from the L cells of the ilium and colon (GLP-1). The pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus, which are involved in the appetite-suppressing pathway, are activated by these hormones (Delzenne et al., 2010; Lam and Ravussin, 2016). CCK is produced shortly after a meal begins, while PYY levels rise one to two hours after a meal. High-fat and high-protein meals have been proven to enhance CCK and PYY production to a greater extent than a carbohydrate-rich diet at a similar calories level (Delzenne et al., 2010). Food intake increases circulation levels of GLP-1. GLP-1 is an incretin hormone that binds to specific receptors in pancreatic β-cells and stimulates insulin synthesis/release and reduces liver glycogen release (Girard, 2008; Delzenne et al., 2010; Lam and Ravussin, 2016). This is a crucial mechanism for enhancing the glycaemic signal, and it is thought to account for up to 70 percent of postprandial insulin production (Girard, 2008). Long-acting signals govern body weight and the amount of energy retained as fat over time by reflecting the amounts of fat storage (Wilson and Enriori, 2015). Insulin and leptin are hormones that provide information about overall nutritional status and serve as signals to influence longer-term dietary behaviour (Lam and Ravussin, 2016). Following a meal, the pancreas is acutely stimulated in response to the rise in circulating glucose levels to release insulin.

POMC transcription takes place in the hypothalamus arcuate nucleus when the insulin-signalling cascade is activated. Glucose-sensing neurons are also found in the hypothalamus, and they play a role in meal-related eating regulation (Lam and Ravussin, 2016). Leptin is a well-known adiposity signal to the brain because its circulating content is positively associated with TG stores (Wilson and Enriori, 2015; Pan and Myers, 2018). It has been documented that leptin's primary role is to signal insufficient energy stores or balance instead of an excess of fat stores (Rosenbaum and Leibel, 2014). It works in the same way that insulin does by binding to a receptor to initiate downstream signalling pathways that converge at the level of PI3K, causing the POMC neurons to enhance the sense of fullness (Wilson and Enriori, 2015; Lam and Ravussin, 2016). Insulin resistance is thought to impede leptin action and the body's energy homeostasis (Pan and Myers, 2018). Overall, the state of energy storage in adipose tissue highlights the need for longer-term food intake regulation to attain energy balance. Leptin and insulin suppress appetite, enhance energy expenditure, lower weight gain and optimise glucose homeostasis (Figure 1.6) (Pan and Myers, 2018).

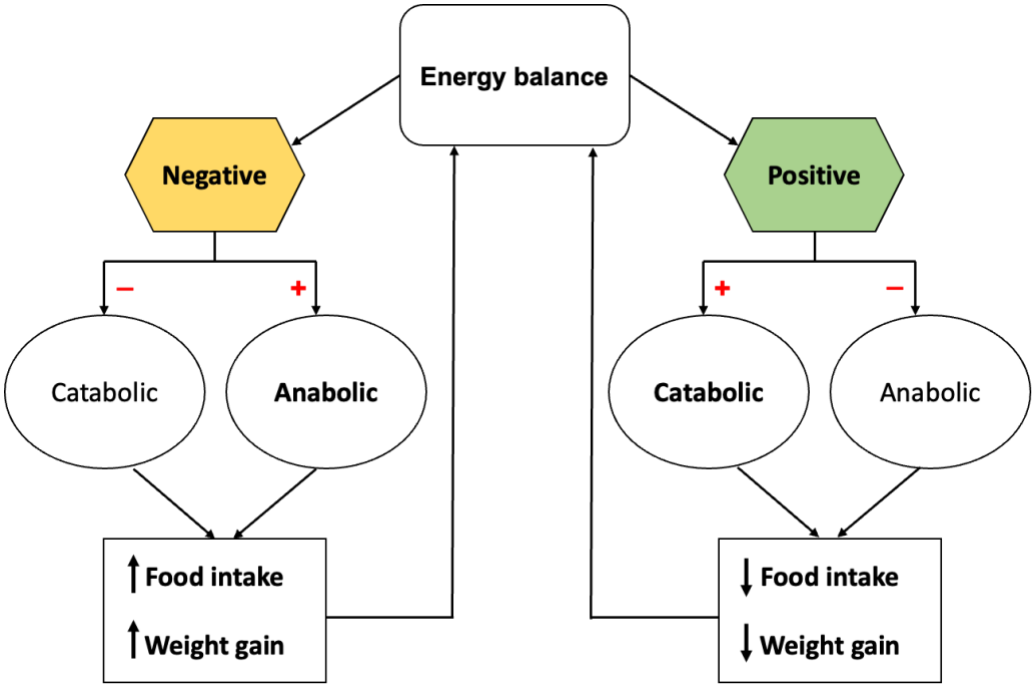


Figure 1.6. Insulin and leptin actions in response to energy imbalances.

1.3. Overfeeding

Overfeeding causes weight increase and fat accumulation in healthy people, as well as dyslipidaemia and changes in glucose metabolism and, in the long run, obesity and all of its well-known comorbidities (Jéquier and Tappy, 1999; Tappy, 2004). Human experimental studies of the adverse effects of overfeeding began to appear in the 1950s. Body weight and adiposity changes are often small during short-term overfeeding; that is, a duration of a few days is particularly interesting for investigating the impact of additional calories or excessive ingestion of specific nutrients on the metabolism. Overfeeding assessments of up to one month are likely to produce inconsistent findings because their measurements would be affected by the excess caloric intake, the dietary content and the body weight and adiposity changes occurring during the overfeeding time frame. Longer overfeeding experiments, lasting one month or longer, are especially valuable for assessing the combined effects of increased calories intake, different nutritional overload, weight gain and body composition changes (Bray and Bouchard, 2020).

1.3.1. Definition

Overfeeding occurs when the amount of dietary energy consumed or administered exceeds the amount of energy required, resulting in serious medical problems (Tappy, 2004).

1.3.2. Prevalence

It has been reported that population excess weight is mostly caused by increases in food availability and intake, and that this increase was more than enough to explain the growth in obesity in the United States beginning in the 1970s, as well as the majority of the weight gain in the United Kingdom since the 1980s (Swinburn et al., 2011). Obesity prevalence is expected to reach over 45 percent of the U.S. population by 2030, and 48 percent in the United Kingdom (Lean and Malkova, 2016).

1.3.3. Adverse effects of overfeeding

Overeating affects energy storage and adipose tissue, as well as hormonal and physiological responses. Metabolic disturbances, such as impaired glucose and lipid regulations, are linked to overeating (Tappy, 2004; Cornier, Bergman and Bessesen, 2006). Moreover, when increased energy intake is associated with macronutrient composition manipulation, it can lead to deleterious effects. For instance, acute high-fat overfeeding can elevate fasting glucose levels secondary to increased glucose output from the liver. It is also believed that it can trigger insulin production to compensate for the reduction in hepatic insulin sensitivity (Tappy, 2004; Brøns et al., 2009). During immobilisation, increased FFA supply owing to positive calorie intake and/or reduced physical activity could cause an increase in IMCL content (Crossland et al., 2019). Overfeeding's effects on the cardiovascular system, endothelial function, insulin sensitivity and energy metabolism will be discussed in the following sections.

1.3.3.1. Cardiovascular system and endothelial function

Increased calorie consumption, diet composition and obesity status can all result in changes in cardiovascular parameters (e.g., heart rate and blood pressure) and endothelial function. Increased heart rate was noted with overfeeding (van der Meer et al., 2008). Though, this change was not observed by others (Gentile et al., 2007). In overfeeding experiments, blood pressure remained stable (van der Meer et al., 2008), although diastolic blood pressure (DBP) and systolic blood pressure (SBP) increased (O'Dea et al., 1982). Cardiorespiratory fitness is a key indicator of future cardiovascular disorders (Lee, Blair and Jackson, 1999). In response to overfeeding, cardiorespiratory fitness showed an inverse relationship with the increase in resting SBP and DBP (Gentile et al., 2007). Overfeeding has an effect on endothelial function as measured by FMD of the brachial artery. Excessive intake reduced FMD from 9.1 to 7.8 percent. The decrease in FMD and the gain in visceral fat had a significant correlation (Romero-Corral et al., 2010).

1.3.3.2. Insulin sensitivity

Excessive intake usually raises insulin levels, although glucose levels are frequently unchanged. This is a result of regulatory mechanisms aimed at keeping glucose levels within a tight range. The amount of energy ingested, the period of overconsumption, the macronutrient content of the diet and the altered body composition are all factors that may influence glucose and insulin responses to overfeeding. During high-caloric intake, insulin resistance has been measured using a variety of methods, including the homeostatic assessment of insulin resistance index (HOMA-IR), glucose clearance during a

hyperinsulinaemic clamp, suppression of FFA and changes in liver glucose metabolism. It has been found that short-term overeating can increase insulin and glucose levels, increase liver glucose output, lower FFA and increase IMCL (Ravussin et al., 1985; Clore, Helm and Blackard, 1995; Magkos et al., 2014; Parry et al., 2017).

Insulin secretion was found to be increased in response to overfeeding to accommodate hepatic insulin resistance, which occurs before peripheral insulin resistance, implying that insulin itself may play a role in this regard (Brøns et al., 2009). The response is also influenced by the individual's metabolic state before overfeeding. This is especially true if the person has central obesity and is insulin resistant. A study by McLaughlin et al. (2016), individuals who were insulin sensitive developed unfavourable alterations in adipocytes and fat distribution, resulting in a 45 percent reduction in insulin-mediated glucose disposal after gaining weight. However, authors found that insulin-resistant individuals did not show changes in lipid metabolism or insulin activities, but energy surplus led to an 8 percent reduction in insulin-mediated glucose utilisation. The link connecting insulin resistance and alterations in fat cells and distribution suggests that these characteristics could be drivers of the overeating adverse response.

1.3.3.3. Energy metabolism and appetite

An overfeeding model has been used in research to determine if energy metabolism varies after overeating. Following excessive energy intake, body size increases, resulting in an increase in energy expenditure that is attributable to several passive factors

including increased RMR owing to increases in lean mass, increased AEE attributed to higher cost of moving a greater body mass and increased DIT accounting for the increase in calorie intake. Furthermore, tissue deposit and higher protein changeover result in an additional energy expense (Hall et al., 2012). There is no consistent evidence that excess calories generate an increase in RMR during short-term overfeeding. Some trials showed significant increases in RMR, whereas others did not (Ravussin et al., 1985; Dirlewanger et al., 2000; Joosen, Bakker and Westerterp, 2005; Thearle et al., 2013). Significant increases in RMR were observed after exposure to long durations of overfeeding lasting more than one month (Alligier et al., 2012). The amount of carbohydrate or fat in the overfeeding treatment had no effect on RMR fluctuations (Lammert et al., 2000). Nevertheless, the response of RMR to protein intake could differ, given Bray et al. (2015) found that only the normal and high-protein regimens exhibited a substantial rise in RMR.

When there is a positive energy balance, RMR increases with weight gain and when there is a negative energy balance, RMR decreases as body mass decreases. Presuming that calorie intake remains constant, energy expenditure should subsequently equal energy intake, and body weight will be maintained at a new set point. Alterations in energy expenditure are no longer predictable on the basis of metabolic mass when there is prolonged continuous deviation from energy balance (Lam and Ravussin, 2016). Calorie restriction reduced energy expenditure more than expected after accounting for changes in body composition, whereas overfeeding has the reverse effect (Leibel, Rosenbaum and Hirsch, 1995). These findings point to a disproportionate change in energy expenditure during and after the dynamic phase of weight loss or gain, a phenomenon

known as adaptive thermogenesis (Tappy, 2004; Lam and Ravussin, 2016). When there is a long-term energy excess, the enlarged fat mass releases more leptin, which is meant to exert negative feedback on energy intake to restore energy balance. However, insensitivity to leptin appears to undermine this protective process (Lam and Ravussin, 2016). In rats, and probably in humans as well, the SNS is activated when hypothalamic leptin receptors are stimulated, which increases energy expenditure in brown adipose tissue and suppresses appetite. The SNS is the sole source of innervation for brown adipose tissue. It has the potential to play a significant role in adaptive thermogenic responses to environmental stressors. Adaptive thermogenesis is believed to occur through increasing the expression and activity of mitochondrial uncoupling protein 1 (UCP-1) to uncouple oxidative phosphorylation and generate heat (Tappy, 2004; Wilson and Enriori, 2015). The question of whether there are adaptive changes in energy expenditure (i.e., NEAT may increase to partly counterbalance the effect of overfeeding) has been researched. Previous studies showed that NEAT increases as a result of overfeeding (Levine, 2004; Tappy, 2004). DIT was not affected by short-term overfeeding (Goran et al., 1994). However, carbohydrate overfeeding for a few days has been demonstrated to raise DIT by up to 39 percent (Weststrate and Hautvast, 1990). TEE was increased during short-term (within three days) overfeeding (Wijers, Saris and van Marken Lichtenbelt, 2007; Schmidt et al., 2013). In addition, TEE has been proven to be increased to a greater extent by carbohydrate than by fat overfeeding (Dirlewanger et al., 2000). Overfeeding with low protein causes TEE to increase less than when overfeeding with high protein (Bray et al., 2012). TEE did not increase in certain studies running from one to three weeks (Roberts et al., 1990) but did increase in others (Jebb et al., 1996).

TEE increased following prolonged overfeeding treatments lasting more than one month (Leibel, Rosenbaum and Hirsch, 1995). Overfeeding over a short period of time increases carbohydrate oxidation while decreasing fat oxidation (Weststrate and Hautvast, 1990; Schmidt et al., 2013). Respiratory quotient (RQ) often increases over longer periods of overeating, up to three weeks, indicating a growing dependence on glucose oxidation that is particularly noticeable after carbohydrate overfeeding (Welle and Campbell, 1983). Landsberg (1994) anticipated the role of energy surplus in developing insulin resistance, cardiovascular dysfunction and obesity in the long run. Figure 1.7 illustrates this hypothetical relationship.

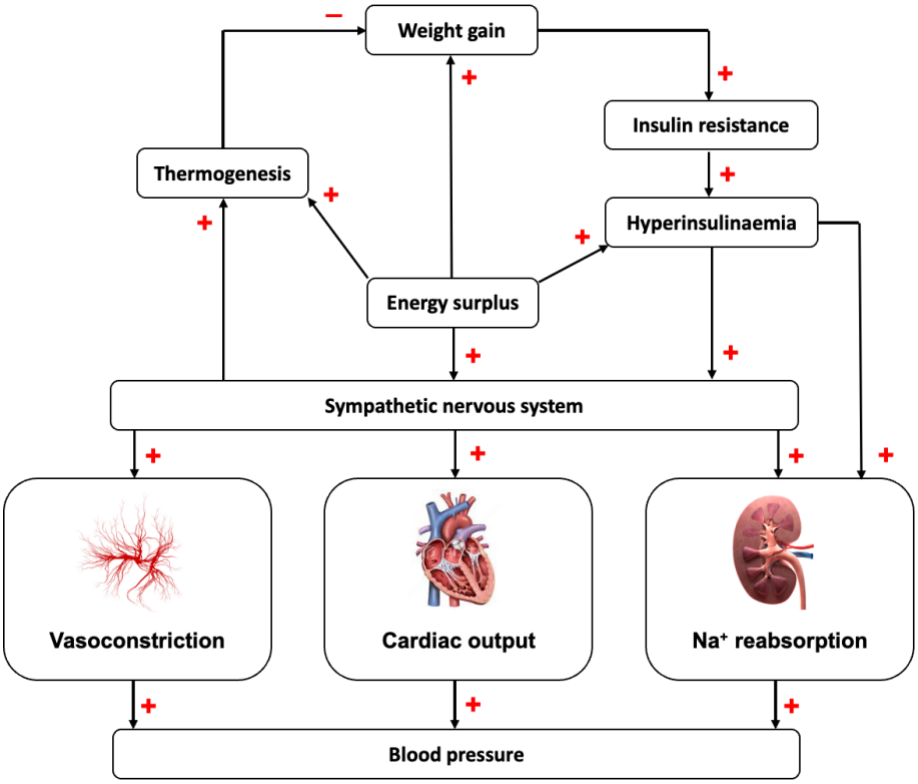


Figure 1.7. Schematic diagram explaining the potential metabolic–cardiovascular relationship associated with increased food intake.

Surplus caloric intakes, that is, energy intake – energy expended as heat, must be stored in the body with overeating. Because the human body's glycogen storage capacity is limited, surplus energy is ultimately stored as fat. Under normal circumstances, dietary lipid deposition into adipose tissue is regarded as the primary source of fat deposition. This mechanism, in which blood TG are metabolised by adipose tissue lipoprotein lipase and deposited as TG in adipocytes, is highly energy efficient, with very little heat dissipation. However, it is known that de novo lipogenesis can occur with a high carbohydrate consumption (Tappy, 2004). Lipogenesis (fat synthesis) can be measured

in various ways. The first approach is to assess RQ, and the other is to use isotopes to track fat formation. Carbohydrate is converted to lipid when the RQ is greater than one (Acheson et al., 1984; Aarsland, Chinkes and Wolfe, 1997). This process consumes more energy because it loses roughly 30 percent of energy as heat (Tappy, 2004). Indirect calorimetry can be used to calculate net de novo lipogenesis (Tappy, 2004). Aarsland, Chinkes and Wolfe (1997) believed that during overfeeding, de novo lipogenesis occurs not just in the liver but also in peripheral tissue. Carbohydrate storage as glycogen was also observed during overfeeding (Tappy, 2004). Following carbohydrate overeating, overexpression of lipogenic adipose tissue enzymes revealed that adipose tissue plays a significant role in de novo lipogenesis. As a consequence of the greater energy expenditure caused by lipid synthesis, this augmentation of de novo lipogenesis following carbohydrate overfeeding was significant (Tappy, 2004).

Overfeeding's impacts on hepatic fats were highly dependent on the diet's content as well as the amount of overfeeding. In the study by McDevitt et al. (2000), intrahepatic TG level increased in response to a short-term overfeeding treatment, but adipocytes gained the majority of the lipid. The basic metabolic actions of adipose tissue are controlled by hormones and the SNS. Adipose tissue is critical in the modulation of energy and metabolism. It also functions as an organ that produces and secretes adipokines, which are proteins that belong to the cytokine family (Rutkowski, Stern and Scherer, 2015). Adipokines have an impact on a variety of physiological processes, such as energy balance, immunity and inflammation. Leptin is an example of an adipokine that assists with energy metabolism and hormone sensitivity. Short- and long-term overfeeding investigations have shown an increase in serum leptin concentrations (Kolaczynski et al.,

1996; Wijers, Saris and van Marken Lichtenbelt, 2007). The breakdown of TG into FFAs occurs in adipose tissue under the coordinated action of three lipases. First, adipose triglyceride lipase converts TG to diglycerides. Second, hormone-sensitive lipase converts diglycerides to monoglycerides. Third, monoglyceride lipase cleaves monoglycerides into three FFAs and glycerol (Nielsen et al., 2014). The effects of gastrointestinal hormones have been studied, although the results are inconsistent. Overall, the majority of studies show that overeating has little effect on gastrointestinal hormones (Bray and Bouchard, 2020).

1.4. Previous human experimental studies

The online database PubMed was used to identify the publications reviewed. Original published papers on the effects of physical inactivity in humans with and without an energy surplus in young and healthy humans were searched for. The thirty-nine papers examined had a variety of study designs, experimental durations (three hours–three weeks) and sample sizes (five to forty-five participants per trial) with at least one of the main outcomes (metabolic responses, cardiovascular responses, energy metabolism and

endothelial function) being reported on. For reporting purposes, studies were grouped into three categories based on their methodological approach:

(1) Studies on physical inactivity consequences with and without overfeeding following bed rest (Table 1.1).

(2) Studies on physical inactivity consequences with and without overfeeding following prolonged sitting or limb immobilisation (Table 1.2).

(3) Studies on physical inactivity consequences with and without overfeeding following reduced steps count or detraining (Table 1.3).

Table 1.1. Key studies on physical inactivity consequences with and without overfeeding following bed rest

						Outcomes			
Author (year)	**Sample size**	**Participants' characteristics**	**Intervention**	**Diet**	**Duration**	**Metabolic**	**Cardiovascular**	**Energy metabolism**	**FMD**
Stuart *et al.* (1988)	**n=** 6 males	Age: 23 ± 2 yr BMI: N/A Healthy Activity level: active	Lab-based, repeated measures design **Measurements:** Insulin and glucose during OGTT at BL and at the end of intervention Whole body glucose utilisation during E-H clamp at BL and at the end of intervention	Liquid diet 30 kcal/kg/day (55% CHO, 31% fat, 14% protein)	7 days	Fasting glucose ➔ no difference vs BL (OGTT) Glucose AUC 16% > vs BL (OGTT) Fasting insulin 44% > vs BL (OGTT) Insulin AUC 44% > vs BL (OGTT) Glucose utilisation < vs BL (clamp)			
Mikines *et al.* (1991)	**n=** 6 males	Age: 25 ± 1 yr BMI: 22 ± 2.8 kg/m^2 Healthy Activity level: N/A	Lab-based, repeated measures design **Measurements:** Insulin, glucose, leg BF, whole body and leg glucose uptake rate during sequential E-H clamp at BL and at the end of intervention RER and substrate oxidation during sequential E-H using IC at BL and at the end of intervention	Reduced dietary intake mainly from fat (10–15%)	7 days	Insulin and glucose ➔ no differences vs BL Whole body glucose uptake rate 24% (37 µU/ml) < vs BL Leg glucose uptake rate 33% (10 µU/ml), 47% (18 µU/ml), 53% (37 µU/ml), ~20% (360 µU/ml) < vs BL	Leg BF ~32% < vs BL HR, SBP and DBP ➔ no difference vs BL	RER and substrate oxidation ➔ no difference vs BL	

			HR, SBP and DBP at BL and at the end of intervention						
Blanc *et al.* (2000)	**n=** 16 (8 males / 8 females)	Age: Males: 32.4 ± 1.9 yr Females: 27.9 ± 0.9 yr BMI: Males: 23.9 ± 0.7 kg/m^2 Females: 20.9 ± 0.6 kg/m^2 Healthy Activity level: N/A	Lab-based, repeated measures design **Measurements:** Daily fasting insulin and glucose Insulin, glucose and FFA during OGTT at BL and at the end of intervention Glucose turnover using stable isotope tracers REE, DIT and RER during OGTT using IC at BL and at the end of intervention	Conventional foods (146 kJ/kg.day of initial body mass; males 51% CHO, 31% fat, 19% protein; females 52% CHO, 32% fat, 16% protein)	7 days	**Males:** Fasting insulin 34% > vs BL Fasting glucose ➔ no difference vs BL Insulin AUC 48% > vs BL Glucose AUC ➔ no difference vs BL FFA AUC 41% < vs BL Endogenous glucose production 28% < vs BL Exogenous glucose oxidation 39% > vs BL **Females:** Fasting insulin 26% > vs BL Fasting glucose 7% < vs BL Insulin AUC 63% > vs BL Glucose AUC ➔ no difference vs BL		**Males:** REE ➔ no difference vs BL DIT ➔ no difference vs BL RER (0.906) > vs BL (1.004) CHO oxidation 25% > vs BL Fat oxidation 84% < vs BL Lipogenesis 304% > vs BL **Females:** REE ➔ no difference vs BL DIT 85% > vs BL RER (0.969) > vs BL (1.101) CHO oxidation ➔ no difference vs BL	

						FFA AUC 23% < vs BL Endogenous glucose production ➔ no difference vs BL Exogenous glucose oxidation ➔ no difference vs BL		Fat oxidation 80%< vs BL Lipogenesis 74% > vs BL	
Hamburg *et al.* (2007)	**n=** 20 (4 males / 8 females)	Age: 29 ± 7 yr BMI: N/A (weight: 70.4 ± 11.4 kg) Healthy Activity level: N/A	Lab-based, repeated measures design **Measurements:** Insulin sensitivity during OGTT at BL and at the end of intervention TG at BL and at the end of intervention Brachial artery, FMD, SBP, DBP and HR at BL, during days 3 and 5 of intervention	Maintain habitual dietary intake	5 days	Fasting insulin 47% > vs BL Fasting glucose 4% > vs BL Fasting TG 35% > vs BL Insulin AUC 67% > vs BL Glucose AUC 6% > vs BL HOMA-IR 50% > vs BL ISI 13% < vs BL	HR and DBP ➔ no difference vs BL SBP 3 mmHg (day 3) and 7 mmHg (day 5) > vs BL		No difference in FMD vs BL Reactive hyperaemia 16% (day 5) < vs BL
Sonne *et al.* (2010)	**n=** 20 males	Age: 25 ± 0.2 yr BMI: 24.1 ± 0.5 kg/m^2 Healthy Activity level: N/A	Lab-based, repeated measures design **Measurements:** Whole-body insulin sensitivity and insulin-stimulated FBF using VOP during E-H clamp at BL and at the end of intervention	Isocaloric diet (55% CHO, 30% fat, 15% protein)	10 days	M-value 34% < BL Insulin-stimulated forearm glucose clearance 75% < BL Insulin-stimulated FBF 53% < BL			

Nosova *et al.* (2014)	**n=** 5 (4 males / 1 female)	Age: 22 ± 2 yr BMI: 24 ± 3 kg/m^2 Healthy Activity level: N/A	Lab-based, repeated measures design **Measurements:** Fasting insulin, TG and glucose at BL and days 1, 3, 5 of intervention Brachial FMD, SBP, DBP and HR at BL and days 1, 3, 5 of intervention	Isocaloric meals	5 days	Fasting insulin and glucose ➔ no difference vs BL TG ➔ no difference vs BL	SBP and HR ➔ no difference vs BL DBP 4 mmHg > vs BL		Brachial %FMD 2% < vs BL
Dirks *et al.* (2016)	**n=** 10 males	Age: 23 ± 1 yr BMI: 23 ± 0.9 kg/m^2 Healthy Activity level: N/A	Lab-based, repeated measures design **Measurements:** Fasting insulin and glucose at BL and during intervention Insulin and glucose AUC during MTT at BL and on day 6 of intervention RMR using IC at BL and at the end of intervention GIR and insulin sensitivity during E-H clamp at BL and at the end of intervention Fasting/insulin-stimulated EE, RER and substrate oxidation during E-H clamp at BL and at the end of intervention $Vo_{2\,peak}$ at BL and at the end of intervention	Standardised meals (RMR X 1.35)	1 week	Fasting glucose ➔ no difference vs BL Fasting insulin 64% > vs BL (time effect) Insulin AUC 40% > vs BL (MTT) Glucose AUC ➔ no difference vs BL (MTT) HOMA-IR 63% > vs BL GIR 29% < vs BL	$Vo_{2\,peak}$ 6.4% vs BL	RMR 4% < vs BL Insulin-stimulated EE > vs fasting (clamp) in BL and post- intervention Insulin-stimulated RER (0.84) > vs fasting (0.93) in BL and post-intervention Insulin-stimulated CHO oxidation 85% > vs fasting (clamp) in BL and post-intervention BL insulin-stimulated fat oxidation 80% < vs fasting (clamp) Post-intervention insulin-stimulated fat oxidation 70% < vs fasting (clamp)	

Dirks *et al.* (2018)	**n=** 10 males	Age: 25 ± 3 yr BMI: 25.3 ± 2.5 kg/m^2 Healthy Activity level: habitually active	Lab-based, crossover design **Control:** habitually active + energy intake matched energy expended (energy balance) **BR-M:** bed rest + energy intake matched control (positive energy balance) **BR-B:** bed rest + energy intake reduced to match low energy expenditure (energy balance) **Measurements:** Glycaemic control using CGM (iPro2) during intervention Fasting insulin and glucose at BL and at the end of intervention Insulin, glucose and insulin sensitivity during OGTT at BL and at the end of intervention Substrate utilisation using IC at BL and at the end of intervention	Control/BR-M: 3409 kcal/day (56% CHO, 32% fat, 12% protein) BR-B: 2558 kcal/day (56% CHO, 32% fat, 12% protein)	24 hr	Glycaemic control (CGM) ➔ no difference between trials Fasting / PP insulin and glucose ➔ no difference between trials Insulin sensitivity ➔ no difference between trials		RER, EE and substrate oxidation ➔ no difference between trials RER, EE and CHO oxidation > vs BL in all trials	

Abbreviations: **BMI** body mass index; **BL** baseline; **FMD** flow-mediated vasodilatation; **CHO** carbohydrates; **SBP** systolic blood pressure; **DBP** diastolic blood pressure; **HR** heart rate; **OGTT** oral glucose tolerance test; **$Vo_{2\ peak}$** peak oxygen consumption; **HOMA-IR** homeostatic model assessment for insulin resistance; **AUC** area under the curve; **CGM** continuous glucose monitoring system; **RMR** resting metabolic rate; **DIT** diet-induced thermogenesis; **TG** triglycerides; **FFA** free fatty acids; **ISI** insulin sensitivity index; **E-H clamp** euglycaemic hyperinsulinaemic clamp; **BF** blood flow; **RER** respiratory exchange ratio; **IC** indirect calorimetry; **REE** resting energy expenditure; **VOP** venous occlusion plethysmography; **MTT** meal tolerance test; **GIR** glucose infusion rate; **EE** energy expenditure; **PP** postprandial.

Table 1.2. Key studies on physical inactivity consequences with and without overfeeding following prolonged sitting or limb immobilisation

						Outcomes			
Author (year)	**Sample size**	**Participants' characteristics**	**Intervention**	**Diet**	**Duration**	**Metabolic**	**Cardiovascular**	**Energy metabolism**	**FMD**
Stephens *et al.* (2011)	**n=** 14 males	Age: 26 ± 5 yr BMI: 24 ± 3 kg/m^2 Healthy Activity level: recreationally active (2.8 hr/wk of moderate - vigorous activities)	Lab-based, crossover design **NO-SIT:** active + energy intake matched energy expended (energy balance) **SIT:** sitting + energy surplus (positive energy balance) **SIT-BAL:** sitting + energy intake reduced to match low energy expenditure (energy balance) **Measurements:** Insulin during 1-hr continuous infusion of [6,6-^{2}H]-glucose at the end of intervention	Standardised meals (43% CHO, 39% fat, 18% protein)	24 hr	Insulin in SIT 41% > vs NO-SIT Insulin in SIT 18% > vs SIT-BAL Whole-body insulin action in SIT 39% < vs NO-SIT			
Birk *et al.* (2013)	**n=** 13 males	Age: 22 ± 1 yr BMI: 23 ± 2 kg/m^2 Healthy Activity level: recreationally active (2–5 hr of physical activity per wk)	Free-living, repeated measures design **Control:** active dominant arm **Intervention:** inactive non-dominant arm (cast) **Measurements:**	N/A	8 days				Brachial %FMD ➔ no difference vs BL Peak blood flow (37%) < vs BL

			Brachial FMD at BL and during days 4 and 8 of intervention						
Kim *et al.* (2014)	**n=** 9 males	Age: 24 ± 4 yr BMI: N/A (< 30 kg/m^2) Healthy Activity level: recreationally active	Lab-based, crossover design **Control:** sitting **MOD:** sitting + moderate exercise (1-hr running at 65% $Vo_{2\ max}$) **LOW:** sitting + low exercise (intermittent isoenergetic walking at 25% $Vo_{2\ max}$) **Measurements:** Insulin, glucose, TG and FFA during 6-hr high-fat tolerance test at the end of intervention Energy expenditure and substrate utilisation using IC at the end of intervention	**Control:** energy balanced (2521 kcal; 50.9% CHO, 30% fat,19.2% protein) **MOD:** same energy intake as in control (i.e., creating negative energy balance) **LOW:** same energy intake as in control (i.e., creating negative energy balance)	1 day	Insulin ➔ no difference between trials MOD and LOW glucose response < vs control (treatment effect) MOD postprandial TG AUC 24% < vs control LOW postprandial TG AUC 17% < vs control MOD and LOW FFA response > vs control (treatment effect)		MOD postprandial CHO oxidation 25% < vs control MOD postprandial fat oxidation 34% > vs control LOW postprandial fat oxidation 17% > vs control MOD postprandial CHO oxidation 34% > vs control TEE ➔ no difference between trials	
Thosar *et al.* (2014)	**n=** 12 males	Age: 24.2 ± 4 yr BMI: 23.7 ± 3.3 kg/m^2 Healthy Activity level: inactive	Lab-based, crossover design Prolonged sitting **Measurements:** SFA and brachial artery %FMD and shear rate at BL, 1 hr, 2 hr and 3 hr of sitting	Maintain habitual dietary intake	3 hr				**SFA:** 1st hr %FMD 4.5% < vs BL 2nd hr %FMD 3.5% < vs BL 3rd hr %FMD 3% < vs BL 1st hr shear rate 46% < vs BL

									2nd hr shear rate 39% < vs BL **Brachial artery:** %FMD and shear rate ➔ no difference vs BL
Restaino *et al.* (2015)	**n=** 11 males	Age: 27 ± 1 yr BMI: 25 ± 0.4 kg/m^2 Healthy Activity level: recreationally active	Lab-based, repeated measures design Prolonged sitting **Measurements:** Brachial and popliteal FMD at BL and at the end of intervention	Two standardised meals (29g CHO, 7g fat, 12g protein). Each 230 calories. First meal given 2 hrs prior to intervention and second meal at 4th hr during intervention	6 hr				Brachial %FMD ➔ no difference vs BL Brachial resting mean shear rate 46% < vs BL Brachial reactive hyperaemia 31% < vs BL Popliteal %FMD ~4% < vs BL Popliteal resting mean shear rate 53% < vs BL Popliteal reactive hyperaemia 43% < vs BL
Thosar *et al.* (2015)	**n=** 12 males	Age: 24.2 ± 4 yr BMI: 23.7 ± 3.4 kg/m^2 Healthy Activity level: inactive	Lab-based, crossover design **SIT:** prolonged sitting **ACT:** prolonged sitting + 5 min treadmill exercise at 30 min. 1 hr 30 min and 2 hr 30 min during sitting **Measurements:**	Maintain habitual dietary intake	3 hr				**SIT:** 1st hr %FMD < 89% vs BL and 90% vs ACT 2nd hr %FMD < 65% vs BL and 69% vs ACT

			SFA %FMD at BL, 1 hr, 2 hr and 3 hr of sitting						3rd hr %FMD < 53% vs BL and 68% vs ACT 1st hr shear 47% < vs 2nd hr shear rate 38% < vs BL **ACT:** %FMD and shear rate ➔ no difference vs BL
Morishima *et al.* (2016)	**n=** 11 (7males / 4 females)	Age: 26 ± 1 yr BMI: 25.0 ± 1.1 kg/m^2 Healthy Activity level: recreationally active	Lab-based, parallel design Prolonged sitting (one leg control, one leg fidgeting 1 min on: 4 min off) **Measurements:** Bilateral popliteal artery shear rate, BF and %FMD at BL and at 3 hr of sitting MAP at BL and at 3 hr of sitting	Light meal 2 hr prior to measurements	3 hr		MAP 2 mmHg > vs BL		**Control leg:** BF 63% < vs BL Sear rate 72% < vs BL %FMD 2.9 % < vs BL **Fidgeting leg:** BF 49% < vs BL BF 44% > vs control leg Sear rate 52% < vs BL

									Shear rate 73% > control leg %FMD > 2.9% vs BL and 5% vs control leg
Restaino *et al.* (2016)	**n=** 10 males	Age: 26 ± 1 yr BMI: 26.8 ± 1.3 kg/m^2 Healthy Activity level: N/A	Lab-based, parallel design Prolonged sitting (one leg dry, one leg heated) **Measurements:** Bilateral popliteal artery shear rate, BF and %FMD at BL and at the end of intervention	N/A	3 hr				**Dry leg:** Shear rate 45% < vs BL BF < 48% vs BL and 45% vs heated leg %FMD < 4.3% vs BL and 8.1% heated leg **Heated leg:** Shear rate ➔ no difference vs BL %FMD ➔ no difference vs BL

Ballard *et al*. (2017)	**n=** 11 males	Age: 21.2 ± 1.9 yr BMI: 24.7 ± 1.0 kg/m^2 Healthy Activity level: recreationally active (≤ 3 days/week of moderate-intensity physical activity)	Lab-based, crossover design **REST:** prolonged sitting with a prior 45 min rest **EX:** prolonged sitting with a pre-treadmill exercise at 65% Vo_{2max} **Measurements:** SFA %FMD at baseline and every 1 hr during sitting MAP at baseline and every 1 hr during sitting	Maintain habitual dietary intake	3 hr		REST MAP ➔ no difference vs EX		**REST**: 1st hr %FMD 1.52% < vs BL 2nd hr %FMD < 2.12% vs BL and 1.82% vs EX 3rd hr %FMD 2.27% < vs BL **EX:** %FMD ➔ no difference vs BL
Morishima *et al.* (2017)	**n=** 15 (10 males / 5 females)	Age: 26.7 ± 0.5 yr BMI: 25.6 ± 0.5 kg/m^2 Healthy Activity level: recreationally active (moderate exercise 2–3 days/wk)	Lab-based, crossover design **SIT:** prolonged sitting with a pre 45 min supine resting **Ex:** prolonged sitting with a pre 45 min leg cycling **Measurements:** Popliteal artery %FMD and BF at BL and 3 hr of sitting MAP at BL and 3 hr of sitting	Light meal 2 hr prior to measurements	3 hr		SIT MAP 8 mmHg > vs BL Ex MAP 6 mmHg > vs BL SIT MAP ➔ no difference vs Ex		SIT BF 39% < vs BL Ex BF 45% < vs BL SIT %FMD 2.8% < vs BL Ex %FMD ➔ no difference vs BL

Vranish *et al.* (2017)	**n=** 20 (8 males / 12 females)	Age: Males: 22 ± 1 yr Females: 20 ± 0 yr BMI: Males: 25.7 ± 0.9 kg/m^2 Females: 24.0 ± 0.8 kg/m^2 Healthy Activity level: recreationally active (exercise 3.5–4.4 hr/wk)	Lab-based, parallel design Prolonged sitting **Measurements:** Popliteal artery %FMD, shear rate and BF at BL and at the end of intervention HR and MAP at BL and at the end of intervention	N/A	3 hr		Males MAP ➔ no difference vs BL and females Males HR ➔ no difference vs BL and females		Males BF 50% < vs BL Females BF 49% < vs BL Males %FMD 3.9% < vs BL Females %FMD ➔ no difference vs BL Males and females shear rate < vs BL
Carter *et al.* (2019)	**n=** 15 (10 males / 5 females)	Age: 35.8 ± 10.2 yr BMI: 25.5 ± 3.2 kg/m^2 Healthy Activity level: high-moderately active	Lab-based, crossover design **SIT:** prolonged sitting **2-WALK:** prolonged sitting + 2 min light walk every 30 min **8-WALK:** prolonged sitting + 8 min light walk every 2 hr **Measurements:** SFA %FMD and BF at baseline and 4 hr of sitting	Low calorie, low fat and low glycaemic index snacks at BL, 2nd hr and 4th hr of sitting	4 hr				SIT %FMD ➔ no difference vs BL, 2-WALK and 8-WALK BF reduction in SIT (17%) > vs 8-Walk (2%)
O'Brien *et al.* (2019)	**n=** 20 Group 1 = 10 males Group 2 = 10 females	Age: Males: 24 ± 2 yr Females: 23 ± 2 yr BMI: Males: 26.2 ± 2.0 kg/m^2	Lab-based, parallel design Prolonged sitting **Measurements:** Popliteal artery %FMD and BF at BL and 3 hr of sitting	N/A	3 hr		**Males:** HR 6% > vs BL HR 11% < vs females		**Males:** %FMD 2.5% < vs BL %FMD ➔ no difference vs females

		Females: 24.2 ± 3.2 kg/m^2 Healthy Activity level: habitually active (~10,000 steps/day)	HR, SV, SBP, DBP and MAP at BL and 3 hr of sitting				SV 5% < vs BL SV ➔ no difference vs females SBP 7 mmHg > vs BL BL SBP 6 mmHg > vs females DBP 4 mmHg > vs BL DBP ➔ no difference vs females MAP 5 mmHg > vs BL MAP ➔ no difference vs females TVC 6% < vs BL TVC ➔ no difference vs females **Females** HR 7% > vs BL SV 4% < vs BL SBP 10 mmHg > vs BL		BF 77% < vs BL BL BF 44% > vs Females 3rd hr BF 50% > vs Females **Females:** %FMD 2.8% < vs BL BF 78% < vs BL

							DBP 5 mmHg > vs BL MAP 6 mmHg > vs BL TVC 8% < vs BL		
Burns *et al.* (2021)	Study 1: **n=** 10 males Study 2: **n=** 9 males	Age: Study 1: 24.9 ± 0.6 yr Study 2: 22.4 ± 1.3 yr BMI: Study 1: 23.1 ± 0.6 kg/m^2 Study 2: 22.6 ± 0.8 kg/m^2 Healthy Activity level: Study 1: N/A Study 2: N/A	Free-living, repeated measures design **Control:** active dominant arm **Intervention:** inactive non-dominant arm (cast) **Measurements:** **Study 1:** Glucose, insulin, FFA, TG and brachial BF during OGTT at BL and at the end of Day 1, 2 and 3 of intervention **Study 2:** Glucose, insulin, FFA, TG and brachial BF during high-fat mixed meal challenge (43% CHO, 47% fat, 10% protein) at BL and at the end of Day 1, 2 and 3 of intervention	N/A	3 days	**Study 1:** FGU 38% (day 1) and 57% (day 2) < vs BL FGU 63% (day 3) < vs control Insulin AUC ➔ no difference vs BL and control **Study 2:** FGU 46% (day 1) and 51% (day 2) < vs BL Insulin AUC ➔ no difference vs BL and control FFA uptake and TG disposal ➔ no difference vs BL and control	**Study 1:** Brachial BF ➔ no difference vs BL and control **Study 2:** Brachial BF➔ no difference vs BL and control		

Abbreviations: **BMI** body mass index; **BL** baseline; **MAP** mean arterial pressure; **SBP** systolic blood pressure; **DBP** diastolic blood pressure; **HR** heart rate; **SV** stroke volume: **TVC** total vascular conductance; **FMD** flow-mediated vasodilatation; **CHO** carbohydrates; **OGTT** oral glucose tolerance test; **$Vo_{2\ max}$** maximal oxygen consumption; **BF** blood flow; **TEE** total energy expenditure; **TG** triglycerides; **FFA** free fatty acids; **IC** indirect calorimetry; **FGU** forearm glucose uptake; **AUC** area under the curve; **SFA** superficial femoral artery; **PTA** posterior tibial artery

Table 1.3. Key studies on physical inactivity consequences with and without overfeeding following reduced steps count or detraining

						Outcomes			
Author (year)	**Sample size**	**Participants' characteristics**	**Intervention**	**Diet**	**Duration**	**Metabolic**	**Cardiovascular**	**Energy metabolism**	**FMD**
Vukovich *et al.* (1996)	**n=** 7 (5 male / 2 female)	Age: 33 ± 7 yr BMI: 22 ± 3 kg/m^2 Healthy Activity level: endurance runners (at least 5–7 days/wk for a minimum of 45 min/day)	Free-living, crossover design **Active:** one training bout **Inactive:** no training **Measurements:** Fasting plasma glucose and insulin 18 hr post-active and at the end of Inactive Insulin and glucose during E-H clamp at low (15 mU.m^{-2}.min^{-1}) and high (40 mU.m^{-2}.min^{-1}) insulin infusion 18 hrs post-active and at the end of inactive GLUT-4 content before each clamp	Maintain habitual dietary intake	6 days	Inactive fasting glucose ➔ no difference vs active Inactive fasting insulin 75% > vs active Inactive GLUT-4 content 17% > vs active **Clamp at low insulin infusion:** Inactive insulin 22% > vs active Inactive GDR 30% < vs active Inactive insulin clearance 20% < vs active **Clamp at high insulin infusion:** Inactive insulin 10% > vs active Inactive GDR 14% < vs active			

						Inactive insulin clearance 8% < vs active			
Arciero *et al*. (1998)	**n=** 8 males Age: 21 ± 1 yr BMI: 21 ± 1 kg/m^2 Healthy Activity level: endurance training athletes (at least 5–7 days/wk for a minimum of 45 min/day)	Free-living, crossover design **Active:** one training bout **Inactive:** no training **Measurements:** Glucose, insulin and FFA during fasting and OGTT at 24 hrs post-active and at the end of Inactive RMR, DIT, EE and RER during fasting and OGTT using IC at 24 hr post-active and at the end of inactive CBF and FBF using VOP at 24 hrs post-active and at the end of inactive	Maintain habitual dietary intake	7–10 days	Inactive fasting glucose 4.4% > vs active Inactive fasting insulin → no difference vs active Inactive glucose AUC 65% > vs active Inactive insulin AUC 73% > vs active Inactive FFA → no difference vs active	Active CBF 22% > vs BL Active FBF → no difference vs BL Inactive CBF and FBF → no difference vs BL and active	Inactive RMR 4% < vs active Inactive DIT 5% < vs active at 15–30 min Inactive RER (0.84) 3.6% > vs active (0.81)	
Stubbs *et al*. (2004)	**n=** 6 males Age: 23 ± 3 yr BMI: 22 ± 2 kg/m^2 Healthy Activity level: moderately active (PAL1.7–1.8)	Lab-based, crossover design **Inactive:** RMR X PAL1.4 **Active:** RMR X PAL1.8 **Measurements:**	Ad libitum (40% CHO, 47% fat, 13% protein)	7 days			Active EE 32% > vs inactive Daily EB was positive throughout the 7 days in both trials Inactive cumulative FB > active	

			Energy metabolism using whole-body IC throughout the 7 days					CB and EI ➔ no difference between trials	
Hagobian and Braun (2006)	**n=** 9 (6 males / 3 females)	Age: males 30 ± 8 yr / females 23 ± 2 yr BMI: N/A (weight: 73 ± 14.5 kg) Healthy Activity level: habitually active (PAL 1.5–1.8)	Free-living, repeated measures design **OF** (day 1–3): inactive (no exercise) + OF (+25%) **OF+Ex** (day 4): single bout of exercise + OF (+50%) **Measurements:** Fasting insulin, glucose and FFA at BL, post OF and post OF+Ex Insulin AUC, glucose AUC and insulin sensitivity during OGTT at BL, post OF and post OF+Ex	Increase dietary intake (56% CHO, 28% fat, 15% protein)	3 days	Fasting FFA in OF 32% < vs BL Insulin AUC in OF 38% > vs BL ISI in OF 30% < vs BL Fasting insulin in OF+Ex 43% > vs BL Insulin AUC in OF+Ex 20% < vs OF			
Olsen *et al.* (2008)	Study 1: **n=** 8 males Study 2: **n=** 10 males	Age: Study 1: 27 ± 5.7 yr Study 2: 24 ± 4.6 yr BMI: Study 1: 23 ± 4 kg/m^2 Study 2: 22 ± 2.1 kg/m^2 Healthy	Free-living, repeated measures design **Study 1:** reduce daily steps count by 78% **Study 2:** reduce daily steps count by 87% (study 2) **Measurements:** Insulin AUC during OGTT at BL and wk 1, 2, 3 (study 1) and at BL and wk 2 (study 2)	Maintain habitual dietary intake	**Study 1:** 3 wk **Study 2:** 2 wk	**Study 1:** Insulin AUC 53% (wk 1), 61% (wk 2) and 79% (wk 3) > vs BL **Study 2:** Insulin AUC 57% (wk 2) > vs BL (OGTT) Insulin AUC 50% (wk 2) > vs BL (OFTT)			

		Activity level: Study 1: ~600 steps/day Study 2: ~10,000 steps/day	TG AUC during OFTT at BL and wk 2 (study 2)			TG AUC 21% (wk 2) > vs BL (OFTT)			
Krogh-Madsen *et al.* (2010)	**n=** 10 males	Age: 24 ± 2 yr BMI: 22 ± 0.7 kg/m^2 Healthy Activity level: recreationally active (> 10,000 steps/day)	Free-living, repeated measures design Reduce daily steps count (~1,500) **Measurements:** Insulin sensitivity during E-H clamp at BL and at the end of intervention Fasting TG and FFA at BL and at the end of intervention Daily EE via Actiheart during intervention Vo_{2max} at BL and at end of intervention	Maintain habitual dietary intake	14 days	GIR 17% < vs BL Peripheral insulin sensitivity (R_d) < vs BL Fasting TG and FFA ➔ no difference vs BL	Vo_{2max} 7% < vs BL	Daily EE 40% < during intervention	
Knudsen *et al.* (2012)	**n=** 9 males	Age: 24 ± 3 yr BMI: 22 ± 3 kg/m^2 Healthy Activity level: active (10,000 steps/day)	Free-living, repeated measures design Reduce daily steps count (~1,500) + OF (+50%) **Measurements:** Insulin sensitivity during OGTT and E-H clamp at BL, days 3, 7 and immediately after intervention	Increase dietary intake (+50%)	14 days	Insulin AUC 34% (day 3), 68% (day 7), 37% (day 14) > vs BL Matsuda index 37% (day 3) and 43% (day 7) < vs BL GIR ~44% (day 14) < vs BL	$Vo_{2\,max}$ 3.8% < vs BL		

			$Vo_{2\ max}$ at BL and at end of intervention						
Mikus *et al.* (2012)	**n=** 12 (8 male / 4 female)	Age: 29 ± 1 yr BMI: 24 ± 0.9 kg/m^2 Healthy Activity level: active (≥10,000 steps/day)	Free-living, crossover design **Active:** maintain habitual steps count (≥10,000 steps/day) **Inactive:** reduce steps count (< 5,000 steps/day) **Measurements:** Glycaemic control using CGM (iPro) during intervention Fasting insulin, glucose and insulin sensitivity at BL and during OGTT at end of intervention	Maintain habitual dietary intake	3 days	Inactive postprandial glucose > active (CGM) Inactive fasting insulin 47% > vs active Inactive insulin > active Inactive Matsuda index 31% < vs active Inactive HOMA-IR 49% > vs active			
Boyle *et al.* (2013)	**n=** 11 males	Age: 25 ± 2 yr BMI: 25 ± 1 kg/m^2 Healthy Activity level: recreationally active (> 10,000 steps/day)	Free-living, repeated measures design Reduce daily steps count (< 5,000) **Measurements:** Brachial and popliteal FMD at BL and during days 1, 3 and 5 of intervention	Standardised meals (57% CHO, 28% fat, 15% protein)	5 days				Brachial %FMD → no difference vs BL Popliteal %FMD ~3% (day 5) < vs BL
Dixon *et al.* (2013)	Lean group: **n=** 9 males Overweight group: **n=** 9 males	Age: Lean (52 ± 2 yr) Overweight (49 ± 1 yr) BMI:	Free-living, parallel design Reduce daily steps count (<4,000) **Measurements:**	Maintain habitual dietary intake	7 days	Glucose AUC > vs BL in both groups (OGTT) Insulin AUC > vs BL in both groups			

		Lean (24 ± 0.7 kg/m^2) Overweight (29 ±1 kg/m^2) Healthy Activity level: active (> 10,000 steps/day)	Insulin and glucose during OGTT at BL and at end of intervention Fasting TG and FFA at BL and at end of intervention			Overweight glucose AUC > vs lean Overweight insulin AUC > vs lean Fasting TG > vs BL in both groups Fasting FFA➔ no difference vs BL in both groups Overweight fasting TG and FFA > vs lean			
Walhin *et al*. (2013)	SUR: **n=** 14 males SUR+Ex: **n=** 12 males	Age: 25 ± 7 yr BMI: 23.8 ± 2.5 kg/m^2 Healthy Activity level: habitually active (PAL 1.9 ± 0.4)	Free-living, parallel design **SUR:** reduce steps count (≤4,000) + OF (+50%) **SUR+Ex:** reduce steps count (≤4,000) + OF (+75%) + Ex (45 min/day) **Measurements:** RMR and RER using IC at BL and at end of intervention Insulin, glucose and insulin sensitivity during OGTT at BL and at end of intervention	Increase dietary intake	7 days	Insulin AUC in SUR (2-fold) > vs BL and SUR+Ex HOMA-IR in SUR > vs BL and SUR+Ex Matsuda index in SUR < vs BL and SUR+Ex		RMR in SUR 11% > vs BL RER in SUR (0.98) > vs BL (0.83) RER in SUR+Ex (0.90) > vs BL (0.80)	
Lyden *et al*. (2015)	**n=** 10 (4 male / 6 female)	Age: 25 ± 6 yr BMI: 25 ± 4 kg/m^2 Healthy Activity level: recreationally active (>150 min/wk of moderate physical activity)	Free-living, repeated measures design Reduce daily steps count (< 5,000) **Measurements:**	Maintain habitual dietary intake	7 days	Insulin 82% > vs BL Insulin AUC 32% > vs BL Glucose ➔ no difference vs BL			

			Insulin, glucose and ISI during OGTT at BL and at end of intervention Fasting TG at BL and at end of intervention			ISI 17% < BL Fasting TG ➔ no difference vs BL			
Reynolds *et al.* (2015)	**n=** 14 males	Age: 24 ± 1 yr BMI: 24 ± 0.8 kg/m^2 Healthy Activity level: recreationally active (> 10,000 steps/day)	Free-living, repeated measures design Reduce daily steps count (< 5,000) **Measurements:** Glycaemic control using CGM (iPro) during intervention Insulin sensitivity during OGTT at BL and at end of intervention Brachial and femoral blood flow during OGTT at BL and at end of intervention	Maintain habitual dietary intake	5 days	Peak PP glucose 9% > vs BL (CGM) Matsuda index 29% < vs BL	Brachial and femoral blood flow ➔ no difference vs BL		
Bowden Davies *et al.* (2018)	**n=** 45 (17 male / 28 female)	Age: 36 ± 14 yr BMI: 2.5 ± 4 kg/m^2 Healthy Activity level: habitually active (>10,000 steps/day)	Free-living, repeated measures design Reduce daily steps count by 81% (~1,500) **Measurements:** Insulin, glucose and insulin sensitivity during OGTT at BL and at end of intervention $Vo_{2\ peak}$ at BL and at end of intervention SBP and DBP at BL and at end of intervention	Maintain habitual dietary intake (56% CHO, 20% fat, 24% protein)	14 days	Insulin AUC > vs BL Glucose AUC > vs BL Matsuda index < vs BL	SBP 4 mmHg > vs BL $Vo_{2\ peak}$ 2.2 ml/min/kg < vs BL		

Credeur *et al.* (2018)	**n=** 10 males	Age: 25 ± 4 yr BMI: 25 ± 2 kg/m^2 Healthy Activity level: recreationally active (> 10,000 steps/day)	Free-living, repeated measures design Reduce daily steps count (< 5,000) **Measurements:** Insulin sensitivity in response to OGTT at BL and at end of intervention Compliance and stiffness of brachial and femoral arteries during OGTT at BL and at end of intervention	Standardised meals (57% CHO, 28% fat, 15% protein)	5 days	HOMA-IR 88% > vs BL Matsuda index 33% < vs BL	Brachial compliance and stiffness ➔ no difference vs BL Femoral artery compliance 40% < vs BL Femoral artery stiffness 76% > vs BL		
Winn *et al.* (2019)	Group A: **n=** 10 (6 male / 4 female) Group B: **n=** 5 males	Age: Group A: 24 ± 1 yr Group B: 26 ± 1 yr BMI: Group A: 25.2 ± 1.2 kg/m^2 Group B: 26.6 ± 1.3 kg/m^2 Healthy Activity level: active (> 10,000 steps/day)	Free-living, parallel design **Group A:** Inactive (daily steps count < 5,000) + energy intake reduced to match low energy expenditure (energy balance) **Group B:** Inactive (daily steps count < 5,000) + energy surplus (positive energy balance) **Measurements:** Fasting insulin, glucose, TG and FFA at BL and at end of intervention	**Group A:** decrease dietary intake (15–20%) **Group B:** increase dietary intake (35–40%)	10 days	**Group A:** Fasting / PP insulin ➔ no difference vs BL Fasting / PP glucose ➔ no difference vs BL Fasting / PP TG and FFA ➔ no difference vs BL **Group B:** Fasting insulin 49% > vs BL			**Group A:** Brachial FMD ➔ no difference vs BL **Group B:** Brachial FMD ➔ no difference vs BL

			Insulin, glucose, FFA , TG and insulin sensitivity in response to OGTT at BL and at end of intervention Brachial FMD at BL and at end of intervention			Fasting glucose 4% > vs BL PP insulin > vs BL PP glucose > vs BL HOMA-IR > vs BL Fasting / PP TG and FFA ➔ no difference vs BL			
Bowden Davies *et al.* (2021)	**n=** 28 (10 male / 18 female)	Age: 32 ± 11 yr BMI: 24.3 ± 2.5 kg/m^2 Healthy Activity level: habitually active (≥10,000 steps/day)	Free-living, repeated measures design Reduce daily steps count (~1500) **Measurements:** Insulin sensitivity during OGTT at BL and at end of intervention Brachial FMD at BL and at end of intervention	Maintain habitual dietary intake (56% CHO, 20% fat, 24% protein)	14 days	Matsuda index < vs BL			FMD 1.8% < vs BL

Abbreviations: **BMI** body mass index; **BL** baseline; **FMD** flow-mediated vasodilatation; **CHO** carbohydrates; **SBP** systolic blood pressure; **OGTT** oral glucose tolerance test; **$Vo_{2\ max}$** maximal oxygen consumption; **$Vo_{2\ peak}$** peak oxygen consumption; **HOMA-IR** homeostatic model assessment for insulin resistance; **AUC** area under the curve; **CGM** continuous glucose monitoring system; **RMR** resting metabolic rate; **PAL** physical activity level; **TG** triglycerides; **FFA** free fatty acids; **E-H clamp** euglycaemic hyperinsulinaemic clamp; **ISI** insulin sensitivity index; **RER** respiratory exchange ratio; **IC** indirect calorimetry; **VOP** venous occlusion plethysmography; **GIR** glucose infusion rate; **OFTT** oral fat tolerance test; **OF** overfeeding; **GLUT-4** glucose transporter type 4; **EE** energy expenditure; **Ex** exercise; **PP** postprandial; **GDR** glucose disposal rate; **CBF** calf blood flow; **FBF** forearm blood flow; **EB** energy balance; **FB f**at balance; **CB** carbohydrate balance; **DIT** diet-induced thermogenesis

In recent years, there has been a growing interest in determining the role of physical inactivity in the development of metabolic and cardiovascular disorders (Table 1.1., 1.2, 1.3). The traditional 'bed rest' model has been used to investigate the cardiometabolic effects (Stuart et al.,1988; Mikines et al., 1991; Blanc et al., 2000; Hamburg et al., 2007; Sonne et al., 2010; Nosova et al., 2014; Dirks et al., 2016; Dirks et al., 2018). Experimental investigations using a prolonged sitting or limb immobilisation strategy may have clinical implications (Stephens et al., 2011; Birk et al., 2013; Kim et al., 2014; Restaino et al., 2015; Burns et al., 2021), whereas lowering ambulatory activity via step reduction is thought to be a more relevant paradigm for simulating physical inactivity. Daily steps are frequently lowered from >10,000 to 1,500–5,000 over many days to weeks (Vukovich et al., 1996; Arciero et al., 1998; Stubbs et al., 2004; Hagobian and Braun, 2006; Olsen et al., 2008; Krogh-Madsen et al., 2010; Knudsen et al., 2012; Mikus et al., 2012; Boyle et al., 2013; Dixon et al., 2013; Walhin et al., 2013; Lyden et al., 2015; Reynolds et al., 2015; Bowden Davies et al., 2018; Credeur et al., 2018; Winn et al., 2019; Bowden Davies et al., 2021). Overall, findings from previous studies suggest that reducing physical activity level for a short period (within days) can contribute to the deleterious effects on cardiovascular function, insulin sensitivity and energy metabolism. In healthy men, 7–14 days of reduced activity lowers insulin sensitivity and increases visceral adiposity, which is possibly associated with positive energy balance (Olsen et al., 2008; Knudsen et al., 2012). In addition, increased visceral adiposity is coupled with insulin resistance and a higher risk of cardiovascular disorders (Tchernof and Després, 2013). Physical inactivity with a positive energy balance has a significant impact on metabolic control, body composition and cardiovascular risk profile. Low-grade inflammatory response and increased oxidative stress are triggered by excessive food intake, inactivity and

fat deposition (Narici et al., 2021). Limiting calorie intake during periods of physical inactivity may help prevent metabolic impairment linked to reduced ambulation. Stephens et al. (2011) discovered that preserving energy balance through reduced calorie intake throughout one day of inactivity did not totally eliminate decreases in insulin sensitivity. However, it is worth noting that this team used a model of prolonged sitting, which could cause a substantially higher metabolic impairment than step decrease in free-living situations.

Previous researchers encountered several methodical limitations that may affect their findings' reliability. For instance, studies on physical inactivity's adverse effects using complete inactivity protocols via bed rest, prolonged sitting or limb immobilisation do not resemble the normal 'free-living' environment, which is characterised by long periods of inactivity interspersed with bouts of light to moderate physical activity. Findings from these experiments are more relevant to hospitalised patients and immobile individuals and thus cannot be generalised to most of the population. Further, measurements could be influenced by glucose and insulin distribution volumes, which are likely reduced during bed rest, as seen by a significant reduction of blood volume (Mikines et al., 1991). Some authors did not report the habitual physical activity levels of participants (Mikines et al., 1991; Blanc et al., 2000; Sonne et al., 2010; Dirks et al., 2016). These values are important to rule out any additive inactivity behaviour before the intervention. Other investigators, though, did not account for caloric intake, that is, ad libitum (Mikus et al., 2012; Birk et al., 2013; Burns et al., 2021). Reporting dietary intake during intervention is essential to know the status of energy balance before the intervention and properly interpret the results, whether it is solely owing to the imposed reduction in physical activity or to an additive effect from

an energy imbalance. For these downsides, the present researcher applied steps reduction and prolonged sitting time protocol to replicate the habitual daily activity pattern in most healthy people. Physical activity level was assessed before and during each trial. In addition, energy requirement and energy intake were measured and prescribed for each participant to control for their energy balance. Assessing glycaemic variability during a free-living setting can add valuable information about the glycaemic pattern during the day. However, only two research teams used continuous glucose monitoring systems (CGM) to measure glycaemic control for three days (Mikus et al., 2012) and five days (Reynolds et al., 2015) of reduced steps count, which showed a significant increase in glucose levels during the reduced activity intervention. Cardiovascular parameters including SBP and DBP and heart rate were measured using an indirect sphygmomanometer at single time points (Nosova et al., 2014). Continuous beat-to-beat monitoring using a Finometer would give a comprehensive physiological picture of these variables' responses to applied intervention. Other parameters including mean arterial pressure, cardiac output, stroke volume and total peripheral resistance have so far not been thoroughly investigated. Furthermore, researchers used daily steps reduction to assess vascular function at the brachial artery (Boyle et al., 2013; Reynolds et al., 2015; Credeur et al., 2018). Reducing step count only may not yield a sufficient decrease in upper limb movements, which can affect the quality of findings. A drawback in Restaino et al.'s (2015) study is that participants were in the postprandial state when FMD measurements were taken. According to current guidelines (Alley et al., 2014; Rodriguez-Miguelez, Seigler and Harris, 2016), FMD responses should be measured in the fasting state to establish a steady haemodynamic state. Thus, results cannot be solely attributed to the effect of inactivity, that is, baseline FMD values may have been overestimated by the increased

blood flow secondary to meal ingestion. Novelties in the current thesis include: continuous cardiovascular parameters measurement in response to inactivity using a Finometer (Study 1, Chapter 3); applying a control trial to properly compare cardiometabolic and energy metabolism changes during intervention (Study 1, Chapter 3); the duration of only two days of steps reduction intervention with CGM application (Study 1, Chapter 3); steps count reduction in combination with arm immobilisation to create a whole-body inactivity model (Study 2, Chapter 4); reducing energy intake to create an energy balance to match whole-body inactivity (Study 2, Chapter 4).

Research on the implications of physical inactivity and overfeeding will reveal new insights into the physiological changes that drive our current obesity pandemic. It is an effective method for properly understanding how systems are regulated, which may serve to improve future understanding of the mechanisms related to human obesity and metabolism, thereby leading to recognition of possible targets for body weight control and metabolic health. It is anticipated in the current thesis that restricting caloric intake to prevent weight gain caused by physical inactivity maintains glycaemic control in young healthy individuals. Because physical inactivity is commonly accompanied by excessive energy consumption, it is hypothesised that glycaemic dysfunction is more prevalent when inactivity is combined with overfeeding. Thus, the aim is to evaluate the short-term effects of reduced physical activity and energy balance on cardiovascular responses, insulin sensitivity and energy metabolism under free-living and controlled laboratory environments, taking into consideration methodological limitations in the literature.

1.5. Objectives

The first primary objective is to assess the effects of a two-day free-living period of reduced physical activity and increased sitting time, with and without a proportional decrease in energy intake (creating neutral or positive energy balance, respectively), on cardiovascular responses, insulin sensitivity and energy metabolism (Study 1, Chapter 3). In addition, two secondary objectives were investigated, to explore the gender differences and to compare the performance of two CGMs (Medtronic iPro2 and FreeStyle Libre Abbott) in the control trial. The second primary objective is to measure brachial artery endothelial function using the FMD technique before and after a 5-day reduced physical activity and increased sitting time with a proportional decrease in energy intake to create a neutral energy balance (Study 2, Chapter 4).

Chapter 2: General materials and methods

To prevent duplication, this chapter describes general methodological components from study 1 (Chapter 3) and study 2 (Chapter 4). The related chapters explain methods unique to individual studies.

2.1. Ethical approval and recruitment

All experimental studies were performed in the David Greenfield Human Physiology Unit, School of Life Sciences, Queen's Medical Centre, University of Nottingham and approved by the University of Nottingham Medical School Ethics Committee.

- Study 1: The effects of two days of physical inactivity on cardiovascular responses, insulin sensitivity and energy metabolism in healthy humans (Ethics Reference No: I200317; ClinicalTrials.gov ID: NCT04172090).
- Study 2: The effects of five days of physical inactivity on endothelial function in healthy humans (Ethics Reference No:120-1810; ClinicalTrials.gov ID: NCT04199949).

All participants' visits took place within the unit. Participants were recruited from staff and students at the University of Nottingham and Queen's Medical Centre via poster advertisements.

2.2. Medical screening

On the screening day, each participant was greeted and seated in a screening room where the door closed for confidentiality and only the investigators were present. The visit started with a detailed explanation of the study protocol and all participants' questions and concerns were addressed. Participants were informed that they are free to withdraw from the study at any stage without the need to explain. Once they have indicated that they would like to participate, they were asked to sign two written consent forms (Appendix 1), one copy was given to the participant and the other was kept by the researchers. Then they completed a general health questionnaire (Appendix 2), the short form of the International Physical Activity Questionnaires (IPAQ) (Appendix 3), the Beck Depression Inventory (BDI) questionnaire (Appendix 4) and the Eating Attitude Test-26 (EAT-26) (Appendix 5). The general health questionnaire is composed of multiple-choice questions, yes or no questions and a check list. These questions sought information about common disorders, allergies, smoking and alcohol consumption and supplementation or medications. The BDI is a self-rated scale aimed at evaluating the severity of depressive symptoms (Beck et al., 1961). It is a commonly used tool to evaluate depression worldwide. It is composed of 21 questions each scaled from 0 (symptom absent) to 3 (severe symptoms) and the final score is calculated from the sum of all answers. The EAT-26 is a self-reporting questionnaire to assess eating behaviour and disorders (Garner et al., 1982). It consists of 26 questions with a choice of six answers: always, usually, often, sometimes, rarely and never. Each answer has a specific number of points allocated: for questions 1 to 25: always (3), usually (2), often (1), sometimes (0), rarely (0) and never (0) and for question 26: always (0), usually (0), often (0), sometimes (1), rarely (2) and never (3). The final EAT-26 score is calculated by summing the points of all

answers. The IPAQ is a reliable self-administered short survey of the last seven days and one of the most used physical activity questionnaires to estimate the physical activity level (PAL) (Craig et al., 2003; van Poppel et al., 2010). IPAQ evaluates physical activity levels by reporting three different intensities of exercise which are vigorous, moderate and walking. The final score for physical activity is the sum of frequency (days) and length (minutes) for each activity form and classified into (low = non-active, moderate = moderately active, high = very active). This was followed by measurements of the height using a stadiometer (Scaleways Ltd., Leicester, UK) and the body weight using an electronic weight scale (Seca Ltd., UK). Before measuring body weight, participants wore light clothes without shoes and removed any items in their pockets. The values of body weight and height were then used to calculate the body mass index (BMI) based on the standard equation: BMI (kg/m^2) = Body weight (kg) / Height (m^2) (Keys et al., 1972). In addition, three heart rate and blood pressure measurements were recorded using a Dinamap Pro 1000 vital signs monitor while sitting, and after one- and five-minutes standing. A 12-lead electrocardiogram (ECG) (Schiller At-10) was also performed to rule out any cardiac abnormalities and two 8 ml blood samples were obtained by an experienced research nurse and allocated to a serum separator tube for urea and electrolytes analysis and an ethylenediaminetetraacetic acid (EDTA) tube for full blood count analysis to confirm participants were healthy. Blood samples were analysed by the Clinical Chemistry and Haematology Laboratories in the Queen's Medical Centre, University of Nottingham. ECG and blood sample results were checked and approved by the medical staff in our Unit. Body fat content was assessed through skinfold measurements using a Skinfold Caliper (Harpenden Skinfold Caliper, Baty International Ltd, UK) on four body areas (biceps, triceps, subscapular and suprailiac), and waist circumference, both are non-

invasive and simple methods, which are routinely used in our laboratory. For all subjects, skinfold measures were taken from the right side of the body and by the same person to assure consistency in the results. Body fat percentage was calculated using the following linear regression equation and Siri's equation (Durnin and Womersley, 1974), where C and M are constants varied according to age and gender:

The linear regression equation:

$$\text{Body Density (BD)} = C - [\, M \times (\log_{10} \text{Sum of all skinfolds})]$$

The Siri equation:

$$\text{Fat \%} = [(4.95 / BD) - 4.5] \times 100$$

Waist circumference is a marker to identify the risk of the development of obesity and obesity-related pathologies (WHO, 2008). It was measured by placing the tape measure at the midway between the edge of the last palpable rib and the top of the iliac crest (normal values are ≤94 cm for men and ≤80 cm for women) (WHO, 2008). Before leaving the lab, participants were given training in the completion of a 3-day food diary (two weekdays and one weekend day) to be filled based on household measures including the type, quantity and time of food and drinks intake and a detailed description of the contents (Appendix 6). They were requested to return the diary by post or bring it back on the next preliminary visit. The diary was then reviewed and analysed. The online nutrient analysis software package NUTRITICS (Nutrition analysis software, v4.3 Academic Edition) was used to analyse food diaries and to assess participant's habitual energy intake, macronutrient percentages and type of preferred food. NUTRITICS integrates UK-based nutrient data capable of editing and inserting additional non-database products. The nutrient content of goods was

obtained from published values or food labels. Nutritional results from the software were then used to design meal plans for participants based on their habitual food intake and food preferences to follow during the intervention periods of each main study (study 1 in Chapter 3 and study 2 in Chapter 4).

2.3. Inclusion and exclusion criteria

The inclusion criteria for all research studies described in this thesis included healthy, young (age ranges between 18 – 35 years), males and/or females with normal BMI between 18 and 27 kg/m^2 and moderate physical activity status. While the exclusion criteria were well-trained individuals, smokers, high alcohol consumption (>14 units/week for men and women) (Department of Health, 2016), presence of health problems: metabolic (e.g. diabetes), endocrine (e.g. hyperthyroidism or hypothyroidism) or cardiovascular (e.g. heart or blood) abnormalities including hypertension or heart failure, or clinically significant abnormalities on screening including ECG abnormalities, clinical symptoms of depression (BDI score >20) (Jackson-Koku, 2016) or eating disorder (EAT-26 score >20) (Garfinkel and Newman, 2001). Participants on routine medication that may alter cardiovascular function and blood flow (e.g., blood pressure-lowering drugs or drugs that cause hypertension) or on an energy-restricted diet or seeking to lose weight (body weight should be stable during the last 3 months) were also excluded. Other exclusion criteria included a history of food allergy, intolerance or strong dislike of foods or drinks offered as part of the test breakfast or during the intervention period; females who were pregnant, lactating or have irregular menstrual cycles.

2.4. Blinding and randomisation

Blinding is a method used in experimental research to keep study participants, researchers and data analysers unaware of their group assignment (control vs intervention). The goal of blinding is to limit the risk of bias produced by knowing who is in which group. Thus, outcomes can be then credited to the intervention rather than being impacted by behaviour or appraisal of outcomes that can arise solely from knowing the group assignment. In experimental research, there are three primary types of blinding: 1) unblinded research, where all parties are informed of the participant's treatment. 2) single blinded research, in which participants are the only one who do not know which treatment they are receiving. This is made to prevent any possible behavioural changes that could occur if participants knew whether they were in the control or treatment group. 3) double blinded research, where participants and researchers are both uninformed of the treatment the subject is receiving. It is used to reduce biases that may arise because of disparities in researcher behaviour. Therefore, researchers may unintentionally approach those in the treatment group different than those in the control group if they are known of which participants are receiving the treatment. Performance bias is defined as systematic variations between the treatment and control group. It may occur as a consequence of care provided or exposure to factors other than the intervention. If blinding is not used, participants may respond better if they are aware that they are receiving a promising new treatment. Participants who are aware that they are not receiving an active treatment, on the other hand, may be less likely to follow the study protocol (Forbes, 2013). In some research, blinding may not be achievable because the intervention is visible to participants and/or those who deliver it. Then, alternative approaches could be taken to minimize the risk of bias in such research. For example, providing participants with

a precise protocol to lower the risk of discriminatory behaviour by the researchers and blinding the assessors of the results/statistical analysis which can be accomplished by using non-identifying codes to label the data of the participants.

The experiments in the present thesis were unblinded. This is because the interventions were visible to both, the researcher (i.e., to calculate the level of activity and amount of food intake during each intervention) and the participant (i.e., the number of daily steps and amount of food intake were provided prior to each intervention for proper intervention application). To lower the risk of research bias, a well-controlled design was applied to reduce the discriminatory behaviour by the researcher and participant. Also, data of the participants were labelled with non-identifying codes to blind the assessor of the results/statistical analysis (e.g., for study 1, first participant was coded PI01 and the second participant was coded PI02 etc., and for study 2, the first participant was coded F01 and the second participant was coded F02 etc.).

Randomisation is important in research because it reduces the risk of selection bias and helps to distribute known and unknown prognostic factors equally across treatment group. In experimental research, there are several types of randomisations, but the common randomisation techniques are (Suresh, 2011): 1) simple randomisation (easy to implement but in research with small sample size it can result in unequal number of participants among groups). 2) block randomisation (it can establish balance in sample size but may result in groups that are hardly similar in terms of some covariates). 3) stratified randomisation (controls and balances the

influence of covariates, but it only functions when every subject has been identified prior to group assignment). 4) covariate adaptive randomisation (utilizes the minimization method by determining the sample size disparity between several covariates).

For both main studies in the present research, randomisation was done by the researcher. The randomisation process was made prior to the start of study using an online random number generator for research (www.randomization.com). The software allows the researcher to specify the number of participants, the number of treatments (up to 20 treatments) and the number of blocks (1 for simple randomisation and >1 for block randomisation). The generator starts by sorting treatments internally and then allocate participants randomly to treatments. For the current research, block randomisation was used to allocate participants to treatments in a balanced cross-over fashion. Other types of randomisations were not used because they were not suitable for small sample size i.e., simple randomisation or suitable for studies that aim to randomise their participants based on certain covariates, e.g., age, gender, health status etc i.e., stratified randomisation. Block randomisation may result in imbalance of covariates and can influence the interpretation of research results, but this limitation was strengthened by the inclusion and exclusion criteria in the present studies. These criteria aimed to control for certain covariates like age, health status and activity level to reduce the effect of prognostic factors on the conclusions of the research.

2.5. Physical activity monitoring

In the current research, physical activity was assessed using pedometers, GENEActiv watches and Actihearts. Pedometers were used before intervention to measure habitual steps count and during the intervention periods to verify prescribed daily steps count for both studies (study 1 in Chapter 3 and study 2 in Chapter 4). Actiheart devices were used for habitual physical activity level assessment before intervention (study 1 and study 2) and GENEActiv watches were applied for continuous activity monitoring during intervention periods (study 2). General information about these devices were discussed below. Details about how they were used and how their data was analysed were described in the relevant chapter.

2.5.1. Accelerometers

Accelerometers are time and cost-effective devices that objectively monitor body movements over a certain period and provide accurate and real-time information. The majority of accelerometers used in physical activity testing rely on piezoelectricity, which is produced by microscopic crystalline structures. These crystals undergo compression and create an electrical charge proportional to the amount of the acceleration force. The electrical charge formed is filtered and transformed into multiple samples. According to the user's preference, these samples can be saved in internal memory and summed over a specific duration and frequency (e.g., every one second, 15 seconds or 60 seconds) which is called an epoch. Accelerometers are programmed to exclude any force outside the normal range of movement in the human being. To avoid data distortion, the sampling frequency should be at least twice the highest frequency of movement (CHEN and BASSETT, 2005; Cliff, Reilly and Okely, 2009). However, their recording can be limited to the body part attached to it. For

instance, when applied to the hip, movements in the upper limbs or any added activities such as weight-lifting may not be accurately measured or could be missed. Alternatively, when placed on a wrist, some lower limb activities may not be captured. In addition, some accelerometers cannot differentiate between the different intensities of physical activity levels such as light, moderate, or vigorous and sedentary behaviours (Cliff, Reilly and Okely, 2009). The advantage of using accelerometry-based devices is their ability to evaluate how daily or weekly physical activity varies. This can provide a better understanding of different physical behaviour profiles.

A. GENEActiv

GENEActiv is developed by Unilever Discover (Colworth, United Kingdom) and manufactured and distributed by ActivInsights Ltd. (www.geneactiv.co.uk; Kimbolton, Cambridgeshire, United Kingdom). GENEActiv is a wrist-based device that contains a triaxial accelerometer sensor that measures body acceleration within a range of forces (± 8g) and sampling range of between 10 to 100 Hz and is housed in a small, light-weight and waterproofed covering (Zhang et al., 2012). GENEActiv saves raw data for analysis offline which allows a better data organisation, processing algorithms and outliers cleaning. Data exported provide detailed information including activity intensity, time spent on the activity, number and duration of breaks during activity, time of day when activity occurs and how often an individual is exercising over a certain time duration. Pavey et al. (2016) examined the validity of GENEActiv to measure sedentary time during waking hours when compared to the activPAL during free-living environments among adults. The activPAL is a thigh-based accelerometer and it is the most used technique for measuring free-living sedentary behaviour. Results showed a significant correlation and agreement between the two accelerometers with

Pearson's coefficient r = 0.81. This suggests that using GENEActiv is a valid method for measuring sedentary time.

B. Actiheart:

The Actiheart is a one-piece device that consists of a built-in heart rate monitor and an accelerometer for body movement recording. The underlying principle of Actiheart and its validity to measure heart rate and movement has been described by Brage et al. (2005). Data recorded consists of heart rate, heart rate variability and physical activity scores. Plasqui and Westerterp (2007) compared methods of energy expenditure measurement and accelerometers against the gold standard technique the Doubly labelled water (DLW). DLW measures energy expenditure under free-living conditions. DLW is an isotope-based technique where two types of elements are used, oxygen (^{18}O) and hydrogen ($2H_2$). These two elements have similar chemical and physical properties of the natural existing forms in the human body. The underlying principle of the DLW is that when the isotopes are administered to the subject, the behaviour of ^{18}O and $2H_2$ can be traced. ^{18}O turnover accounts for the flow of water within the body and the amount of oxygen (O_2) inspired and carbon dioxide (CO_2) expired. While $2H_2$ turnover accounts for only the flow of water within the body. Since it is flushed out of the body by water and CO_2, the oxygen isotope leaves the body faster than the hydrogen isotope. The amount of CO_2 produced is used to measure the energy expended (Speakman, 1998). In contrast to DLW, measuring energy expenditure using an accelerometer built-in device can have more beneficial outcomes. Data recorded can be viewed and analysed for each day separately, whereas DLW can only show the end results for the whole measurement period. Also, the accelerometer is less expensive than the DLW. The accuracy of Actiheart to

measure energy expenditure has been evaluated by Crouter, Churilla and Bassett (2008) who compared the performance of Actiheart against the standard reference indirect calorimetry. Their findings showed that Actiheart devices are capable of estimating energy expenditure during a wide range of physical activities in a similar way to indirect calorimetry. Nevertheless, Brage et al., (2004) documented the disadvantages of using heart rate monitors and accelerometers separately to assess the energy expended during physical activity. Heart rate monitors readings can be altered by several biological factors such as training status, age, gender, amount of lean body mass involved, hydration and stroke volume. However, accelerometers are sensitive to biomechanical variability, for example, walking, running, cycling or weight-lifting activities. These limitations can be overcome by combining heart rate monitors and accelerometers in one device and calibrate it using branched model equations to accurately measure the energy expenditure of different physical activities (Brage et al., 2004).

2.6. Blood Glucose measurements

For study 1 in Chapter 3, continuous glucose monitoring systems (CGM) were used to assess daily free-living glycaemic control during the intervention days and Meal Tolerance Test (MTT) was applied to measure glycaemic control and insulin sensitivity in response to food ingestion during the measurement day. General information about these methods was discussed below. Details about how they were used and how their data was analysed were described in the relevant chapter.

2.6.1. Continuous glucose monitoring systems (CGM)

In comparison to the commonly used finger-prick blood glucose measurements, CGMs are devices that measure glucose levels through a sensor inserted subcutaneously into the interstitial fluid (ISF), which is the fluid located between the blood vessels and the cells. The sensor contains glucose oxidase which reacts with the glucose in the ISF resulting in electronic signal generation (Rossetti et al., 2010). These signals are converted to estimated mean values of glycaemia by the sensor using calibration capillary glucose values (Melki et al., 2006). To reach the cell after leaving the blood vessel, glucose must pass through the ISF first. Thus, the glucose level recorded from the ISF briefly lags the actual values of glucose in the blood (Melki et al., 2006). For instance, the average time delay between one type of the CGM (FreeStyle Libre, Abbott) versus the gold standard Yellow Spring Instrument (YSI) glucose reading was found to be 4.5 ± 4.8 minutes (Bailey et al., 2015). The advantages of the CGM over the finger-prick glucose measurements are that CGMs are less invasive, produce real-time continuous frequent glucose readings, are associated with better glucose and HbA_{1c} control and insulin dose adjustment in diabetic patients and are more sensitive to sense extreme conditions of low and high levels of glucose (Vashist, 2013). However, CGMs are more expensive, do not directly measure blood glucose, some types require repeated calibration using finger-pricks and some sensors can cause discomfort and skin irritation and must be changed regularly (Melki et al., 2006; Vashist, 2013). CGMs are of great value to deliver real-time data of glucose variability and their ability to link how daily activities and stress exposure can generate these fluctuations (Suh & Kim, 2015). These monitors can detect glucose levels between 2.2 and 22.2 mmol/L, any values that go above or below this range will not be shown in the data recorded. In the current research, two types of CGMs were used, the

Medtronic iPro2 and the FreeStyle Libre Abbott for study 1 in Chapter 3. The Medtronic iPro2 sensor was applied subcutaneously to the anterior abdominal wall and it was covered securely with an adhesive pad. iPro2 sensor sampled signals every five minutes and required at least 4 capillary glucose readings per day for calibration taken by the participants. After removing the sensor, the data were uploaded through the CareLink iPro™ Therapy Management Software for Diabetes using a special Dock and a USB connection (Medtronic iPro2 User Guide, 2016). The FreeStyle Libre Abbott is a factory-calibrated CGM, which implies that the calibration process is part of the sensor's production procedure and is carried out under controlled research laboratory environments (Hoss and Budiman, 2017). The FreeStyle Libre Abbott was composed of a wireless transmitter (sensor) and a handheld reader. The sensor was inserted subcutaneously using a special sensor applicator into the back of the upper arm. The reader was used to scan the sensor, collects and visualise glucose readings. The FreeStyle Libre Abbott can display the pattern and trend of glucose levels and receive glucose readings from up to a distance of three meters (Vashist, 2013; Bailey et al., 2015). The key benefits of factory-calibrated over user calibrated CGMs are (a) more convenient for the user because no finger-picks are needed to calibrate, and (b) they minimize sensor errors related to improper finger-prick calibration, e.g., washing hand before the test, transcription error and delayed values entry (Hoss and Budiman, 2017). A comparison study between the performance of the Medtronic iPro2 and the FreeStyle Libre Abbott CGMs was conducted in study 1.

2.6.2. Yellow spring instrument (YSI)

The YSI 2300 STAT PLUS was used to measure fasting and postprandial blood glucose for study 1 in Chapter 3. It is a glucose analyser machine that consists of an inner reaction chamber with a specific quantity of buffer and catalase reagent at 37°C. In addition, the chamber encompasses a hydrogen peroxidase-sensitive electrode and a glucose oxidase membrane. To support the reaction, the chamber contents are stirred by an air-driven diaphragm made from silicon that also ensures the right amount of O_2 is delivered. Aqueous standards are used instead of serum- or plasma-based standards in the YSI 2300 STAT PLUS. Thus, once the test sample is injected into the YSI chamber, the subsequent reactions occur (Chua and Tan, 1978):

1) D-Glucose + oxygen $\xrightarrow{\text{Glucose oxidase}}$ gluconolactone + hydrogen peroxide (H_2O_2)
2) The H_2O_2 sensitive electrode oxidises a constant portion of the H_2O_2:

   $H_2O_2 \quad 2H^+ + 2e^- + O_2$

3) Oxygen is reduced to water: $4H + O_2 \rightarrow 2H_2O + 4e^-$

Glucose concentration is directly proportional to the electrical current generated by the reactions in the chamber. The analyser measurements occur within a fixed rate and duration and provide a direct digital readout in mmol/L (Chua and Tan, 1978). The YSI was shown to have acceptable within-batch and between-batch precision. Within-batch coefficient variation of ≤ 1.2% for glucose concentrations between 5.2 and 22.1 mmol/L and between-batch coefficient variation of ≤ 5.8% for glucose concentrations between 1.6 and 16 mmol/L. In addition, the YSI illustrated good agreement with the Beckman glucose analyser (r = 0.997) (Chua and Tan, 1978) and the HemoCue glucose analyser (r = 0.979) (Stork et al., 2005).

2.6.3. Meal Tolerance Test (MTT)

The MTT was applied for study 1 on Chapter 3 to assess glycaemic control and insulin sensitivity in response to meal ingestion. The meal was in the form of a drink that contained a mixture of macronutrients (carbohydrates, protein and fat). It has been documented that performing MTT with a mixed meal is physiologically more relevant to the real-life setting than the Oral Glucose Tolerance Test (OGTT) (Patarrao, Lautt and Macedo, 2014).

2.7. Blood analysis

Serum insulin, serum triglycerides (TG) and plasma FFA were collected for study 1 in Chapter 3. Serum separator tubes were used for serum insulin and TG samples analysis, and lithium heparin tubes with added 15 µl of chelating agent ethylene glycol tetraacetic acid (EGTA) were used for plasma FFA samples analysis. The EGTA was added to prevent the degradation of FFA during storage. After that, tubes were gently mixed well by inverting the tube and then centrifuged (eppendorf, Centrifuge 5702 R). The centrifuging was immediately for the plasma FFA and after a least 15 minutes for serum insulin and TG to allow the sample to clot at room temperature. The tubes were centrifuged at 3000g for 10 minutes at a temperature of 4°C. Then, the supernatant part was transmitted using a Pasteur pipette into appropriately labelled microtubes (each 1.5 ml) and stored in the freezer at a temperature of -80°C. To prevent FFA degradation, a 10 µl Tetrahydrolipstatin (THL) was added to the microtube before to the supernatant transformation. The THL is a lipase inhibitor that prevents lipolysis and subsequently false increase in FFA readings. Thus, adding THL provides a more accurate determination of FFA levels. All samples collected were then analysed by a

single expert research technician to assure consistency and to eliminate individual variability.

2.7.1. Radioimmunoassay (RIA)

Serum insulin samples (each sample is 1 ml) were analysed using a radioimmunoassay. Millipore's Human Insulin-14K kit, USA, was used (intra-assay 2.2 – 4.4% and inter-assay 2.9 – 6.0%). The general principle of the radioimmunoassay is based on competitive binding, i.e., unlabelled antigen competes with a fixed concentration of labelled tracer antigen for binding to an antibody. Insulin samples were incubated with radiolabelled antigens and a constant dilution of insulin antiserum to limit the binding sites on the antibody. The concentration of radiolabelled antigens bound to the antibody decrease as the number of insulin antigens increase. After washing the free radiolabelled antigens, the number of insulin antigens in the sample was quantified using a Gamma Counter. The test was run according to the manufacturer's manual.

2.7.2. Enzymatic colour test

Both serum TG samples (each sample 1 ml) and plasma FFA samples (each sample 2 ml) were analysed using the enzymatic colorimetric method. ABX Pentra Triglycerides CP Kit, France, was used for TG samples (intra-assay 1.00 – 2.83, inter-assay 1.37 – 1.91). Wako NEFA C test kit, Germany, was used for FFA samples (intra-assay ≤ 2.7 %) . The general principle of the test is to determine the amount of a chemical element or compound in a sample with the assistance of a colour reagent. TG and FFA levels were determined following multiple enzymatic reactions ended by

adding hydrogen peroxide to the samples to oxidise a combination of phenol and 4-aminoantipyrine to produce quinoneimine dye. The intensity of the pigmentation was proportional to TG and FFA concentrations in the samples. Both tests were run according to the manufacturer's manual.

2.8. Insulin sensitivity

Several methods and indices have been developed to assess insulin sensitivity when the glucose clamp technique, which is the gold standard method, cannot be performed (Patarrão, Wayne Lautt and Paula Macedo, 2014). These include the Matsuda index and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). Because the glucose clamp technique was not feasible, both Matsuda index and HOMA-IR were used for study 1 in Chapter 3 to measure insulin sensitivity.

2.8.1. Matsuda index

The Matsuda index was first developed by Matsuda and DeFronzo (1999). It is used to assess whole-body insulin sensitivity, i.e., hepatic and peripheral tissues, using plasma insulin and blood glucose concentrations while fasted and during an OGTT as shown below:

$$\text{Matsuda index} = 10{,}000 / \sqrt{[G_0\ (\text{mg/dl}) \times I_0\ (\text{mIU/l}) \times G_{mean}\ (\text{mg/dl}) \times I_{mean}\ (\text{mIU/l})]}$$

Where is G_0 is fasting blood glucose concentrations, I_0 is fasting plasma insulin concentrations, G_{mean} is mean blood glucose concentration during OGTT and I_{mean} is mean blood insulin concentration during OGTT. The 10,000 is a simplifying constant

to get values ranging between 0 to 12 and the square root is used for correcting for non-linear distribution (Matsuda and DeFronzo, 1999; Patarrão, Wayne Lautt and Paula Macedo, 2014).

2.8.2. HOMA-IR

The HOMA-IR model was first documented by Matthews et al. (1985). The model assesses glucose-insulin homoeostasis by predicting the steady-state of fasting glucose and insulin levels using a set of non-linear equations. It assumes the presence of a feedback loop between pancreatic β-cells and the liver in which blood glucose levels are controlled by insulin-dependent hepatic glucose production, whereas insulin concentrations are regulated by pancreatic β-cells responsiveness to changes in blood glucose levels. Therefore, the absence of insulin response to changes in blood glucose levels reflects pancreatic β-cells dysfunction, and the lack of insulin inhibitory function on hepatic glucose production reflects insulin resistance. The HOMA-IR index is defined by the product of fasting insulin and fasting glucose divided by a constant as shown below:

$$\text{HOMA-IR} = \text{Fasting insulin } (\mu\text{IU/ml}) \times \text{Fasting glucose (mmol/L)} / 22.5$$

The constant 22.5 is a normalisation factor based on the notion that the normal fasting plasma insulin concentration is 5 µIU/ml and normal fasting plasma glucose concentration is 4.5 mmol/L (based on values acquired from healthy individuals). When HOMA-IR equals 1, it indicates a normal insulin sensitivity response (Matthews et al., 1985; Patarrão, Wayne Lautt and Paula Macedo, 2014).

2.9. Cardiovascular Function

For study 1 in Chapter 3, cardiovascular parameters including, mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, cardiac output, stroke volume, and total peripheral resistance (TPR) were continuously measured using Finometer and calf blood flow was assessed using Venous Occlusion Plethysmography (VOP). General information about these devices was discussed below. Details about how they were used and how their data was analysed were described in the relevant chapter.

2.9.1. Finometer

The Finometer is a non-invasive instrument that can continuously measure a number of cardiovascular parameters including the MAP, cardiac output, stroke volume and TPR in a beat-to-beat fashion. Finapres Medical System, Amsterdam, The Netherlands, was used. The device consists of two main parts, an inflatable finger cuff placed on the finger (the middle finger is commonly used) and a wrist box. The finger cuff is made of an infrared plethysmograph to detect any changes in the finger arterial volume. The wrist-box contains a fast servo-controlled pressurising system to keep the diameter of finger arteries constant through changes in finger cuff pressure. The central unit composes of three parts: a computer, an air pump and electronics (Imholz et al., 1998; Maestri et al., 2005). The Finometer is fitted with newly developed waveform filtering and level correction software, designed to minimize differences between finger and proximal arterial waveforms. Furthermore, it performs a return-to-flow test to calibrate the level of blood pressure measured in the finger concerning brachial blood pressure (Parati et al., 2003). Waveform filtering and correction of blood pressure levels have been established because pulse waves are physiologically

distorted from the aorta to the finger arteries, where they are wider and sharper, and because mean blood pressure rates are gradually decreased from arteries located more proximal to more peripheral. Thus, a mathematical filter function to correct the distortion of the pulse wave and a correction of the blood pressure level equation has been applied to allow an approximate reconstruction of the finger pulse wave shape which should make it identical to that found at the level of the brachial artery. These corrections have shown to decrease the standard deviation discrepancy between the finger and brachial average arterial blood pressure within the guidelines of the American Association for Medical Instrumentation (AAMI) (Parati et al., 2003). The Finometer incorporates the patented Modelflow method which uses a model to obtain continuous cardiac output from finger pressure. Modelflow is a three-element model and algorithm for estimating the aortic flow waveform from the waveform of the arterial finger pressure. These elements are the non-linear aortic characteristic impedance and Windkessel compliance which both depend on arterial pressure and peripheral resistance. The Finometer computes stroke volume by measuring the area under the curve of the flow pulse in systole and consequently cardiac output by multiplying stroke volume by the instantaneous heart rate. The total systemic peripheral resistance is the sum of aortic characteristic impedance and peripheral resistance values (Bogert and van Lieshout, 2005; Finometer^{TM} User's Guide, 2002; Parati et al., 2003; Wesseling et al., 1993). These reconstruction procedures run entirely automatically as the Finometer's default settings, but they may be de-selected (Finometer^{TM} User's Guide, 2002). The Finometer has shown to be a reliable method to assess blood pressure (Imholz et al., 1998; Schutte et al., 2004; Silke and McAuley, 1998). It has been found that the differences between the mercury sphygmomanometer and the Finometer in measuring systolic and diastolic blood pressure were -1.83 ± 6.8 mmHg and 0.88 ±

7.5 mmHg, respectively. These values fulfilled the criteria of the Association for the Advancement of Medical Instrumentation, which requires mean differences smaller than 5 mmHg and standard deviation less than 8 mmHg (Schutte et al., 2004).

2.9.2. Venous occlusion plethysmography (VOP)

In the late 1800s, the principles of plethysmography were first appreciated. VOP has been used in the research field to measure limb blood flow in normal and pathological conditions since the early 1900s (Joyner, Dietz and Shepherd, 2001). The principle of VOP is based on the concept that blood flow can be measured by obstructing the venous drainage temporarily in a limb without altering the arterial blood inflow by inflating a cuff to sub-diastolic pressure around the mid-thigh of the lower limb. This will lead to a gradual blood accumulation in the obstructed limb. A strain gauge is positioned around the widest area of the calf to measure any changes in leg volume. The linear increase in blood volume is proportional to arterial blood inflow (Greenfield, Whitney and Mowbray, 1963; Joyner, Dietz and Shepherd, 2001). To avoid non-linear blood flow results, it is a standard practice to exclude the foot. This can be achieved by inflating a cuff to supra-systolic pressure around the ankle. The reasons for this exclusion are that the feet are high in arterio-venous shunts, and they are very sensitive to temperature changes (Green et al., 2011; Wilkinson and Webb, 2001). The VOP instrument used for study 1 in Chapter 3 composed of a Hokanson EC6 plethysmograph, a Hokanson E20 rapid cuff inflator and a Hokanson AG101 cuff inflator air source. The direct relation between the volume and girth changes measured by the VOP is based on the presumption that the limb is a cylinder and that in the presence of unaltered limb length and cross-section, the percentage change in volume is equal to twice the percent change in its circumference (Greenfield, Whitney and

Mowbray, 1963; Joyner, Dietz and Shepherd, 2001; Whitney, 1953). It has been published that VOP is a reliable technique to measure limb blood flow. A high correlation between the VOP and Doppler ultrasound measurements (r^2= 0.87 - 0.98) was found across a wide range of flows in healthy individuals (Tschakovsky, Shoemaker and Hughson, 1995).

2.9.3. Duplex-Doppler Ultrasound

The endothelial function in the peripheral vessels can be examined in response to infusions (e.g., acetylcholine or endothelium-derived vasoactive substances) using angiography and strain gauge plethysmography which both are standard diagnostic methods to examine endothelium-dependent vasodilation. Such invasive approaches might not be suitable for healthy or asymptomatic individuals. Thus, non-invasive methods to investigate cardiovascular risk factors were introduced, including local vasodilation by flow-mediated vasodilatation (FMD), laser Doppler flowmetry and augmentation index (AI). Measuring FMD response by ultrasound is the most widely used method to assess endothelial function (Alley et al., 2014; Faulx, Wright, & Hoit, 2003; Flammer et al., 2012; Harris et al., 2010; Lekakis et al., 2011; Moens et al., 2005; Raitakari and Celermajer, 2000; Rodriguez-Miguelez, Seigler and Harris, 2016; Šejda et al., 2005; Stoner and Sabatier, 2012; Tomiyama and Yamashina, 2010). The ultrasound machine was first introduced in the 90's to assess conduit artery endothelial function (Celermajer et al., 1992; Harris et al., 2010). There are three main aspects to evaluate the endothelial function. First, by assessing the properties of the arterial wall, for example, the intimal layer thickness, compliance and remodelling changes. Second, measuring the endothelial anticoagulant factors such as plasminogen activator, thrombomodulin and von Willebrand factor. Third, assessing local vascular

tone by measuring the FMD response (Moens et al., 2005). The measurement of FMD has a great role in the research and clinical fields as a method to assess the effect of different diagnostic or therapeutic interventions, or as a prognostic and preventive tool to evaluate the short- and long-term risks of developing cardiovascular dysfunction (Black et al., 2008; Moens et al., 2005). In comparison to the VOP technique, using the high-resolution duplex-Doppler ultrasound in the assessment of FMD response yields a better temporal resolution and allows for the direct visualisation and measurement of the arterial wall diameters (Thijssen et al., 2010). The FMD test is a physiological examination and thus many factors must be considered to carry out an objective evaluation of endothelial function in humans. For study 2 in Chapter 4, participants were well prepared before the brachial artery FMD measurements. Participant preparation aimed to eliminate any factors that may interfere with FMD response including age, gender, pre-existing metabolic or cardiovascular disorder (Moens et al., 2005). In addition, participants were fasted for at least eight to 12 hours before the measurements and avoided aerobic or resistance exercise, vasoactive medicines, substances or supplements that could affect FMD such as vitamin C, alcohol or caffeinated drinks, tobacco or high-fat content food. The study room temperature was controlled and quiet (Alley et al., 2014; Amato et al., 2013; Corretti et al., 2002; Faulx, Wright, & Hoit, 2003; Harris et al., 2010; Hijmering et al., 2002; Hijmering et al., 2007; Johnson et al., 2013; Maruhashi et al., 2017; Papamichael et al., 2005; Peretz et al., 2007; Rodriguez-Miguelez, Seigler and Harris, 2016; Stoner and Sabatier, 2012; Thijssen et al., 2011; Widlansky et al., 2007). The timing of measurements was consistent to avoid diurnal variation (Harris et al., 2010; Kelm, 2002). Before measurements, participants laid down in a supine position for the upper limb for at least 45 minutes. An electrocardiogram (ECG) was applied for continuous

monitoring of the cardiac cycle. The imaged arm was at the level of the heart and a sphygmomanometric cuff was placed around the mid-forearm. A high frequency (short wavelength) ultrasound probe was used to provide optimal image resolution (Stoner and Sabatier, 2012). Before inflating the cuff, baseline arterial diameter and velocity at an insonation angle of ≤ 60° were recorded. The cuff was then inflated to 200 mmHg to block the arterial blood flow. This resulted in ischaemia in the conduit vessel and dilatation in the small resistance vessels. After five minutes (standardised occlusion time), the cuff was deflated leading to a high-blood flow state called reactive hyperaemia and dilatation of the artery. Post-ischaemic blood velocity was recorded directly after the deflation and not later than 15 seconds. Post-occlusion arterial diameters were measured at 50-, 60-, 70- and 80-seconds. To roll out the variability in measurement, one trained sonographer (research nurse and a senior technician in our laboratory) collected all images. The sonographer had full knowledge of arterial anatomy and basic scanning techniques. After collecting the data, images were analysed using a brachial analyser for research software. With the guide of ECG signals, the arterial diameter was captured in each cardiac cycle at the end of diastole (R wave) to minimize any inter-individual differences (Black et al., 2008; Bots et al., 2005; Corretti et al., 2002; Faulx, Wright, & Hoit, 2003; Harris et al., 2010; Hwang, 2017; Peretz et al., 2007; Raitakari and Celermajer, 2000; Ratcliffe et al., 2017; Rodriguez-Miguelez, Seigler and Harris, 2016; Thijssen et al., 2011). It was reported that arterial width sizes between 2.5 and 5 mm are easily scanned. Arterial diameter lower than 2.5 mm is hard to image and absolute diameter value is less accurate and reproducible because small changes can lead to a greater percentage change. Also, arterial diameters higher than 5 mm tend not to significantly dilate even with intact endothelial function. This is attributed to the inverse relation between arterial diameter

size and FMD response (Raitakari and Celermajer, 2000). Different researchers prefer different locations of cuff occlusion, above or below the antecubital fossa. Although both are acceptable ways to measure FMD, it has been reported that placing the cuff on the upper mid-arm can result in a greater FMD response. This can be explained by the anatomical direct occlusion of the brachial artery and the larger number of resistance vessels. Collapsed brachial artery and subsequently shifting of the surrounding tissues may affect the quality of images (Berry, Skyrme-Jones and Meredith, 2000; Corretti et al., 2002; Faulx, Wright, & Hoit, 2003; Harris et al., 2010; Peretz et al., 2007; Rodriguez-Miguelez, Seigler and Harris, 2016). Baseline diameter values can easily affect between-group data comparison. For example, larger baseline diameters lead to smaller percent changes in FMD (Corretti et al., 2002). Endothelial integrity, blood velocity and blood viscosity can affect the magnitude of shear stress and subsequently the FMD response (Rodriguez-Miguelez, Seigler and Harris, 2016). FMD response was calculated as the change in arterial diameter following the release of cuff occlusion as a percent of the baseline diameter measured at rest (Rodriguez-Miguelez, Seigler and Harris, 2016). The FMD response was determined using the following formula:

FMD (%) = [(Peak diameter – Baseline diameter) / Baseline diameter] X 100

2.10. Energy metabolism

Indirect calorimetry is a method that is usually used to calculate energy expenditure from the respiratory gas exchange, i.e., oxygen uptake (VO_2) and carbon dioxide production (VCO_2) (Ferrannini, 1988). In addition, it is used to assess whole-body substrate oxidation by indirectly measuring the respiratory exchange ratio (RER),

which is the ratio between CO_2 production to O_2 consumption (Turner et al., 2014). Indirect calorimetry was used for study 1 in Chapter 3 to measure energy metabolism at rest. It was applied using a metabolic cart (COSMED Cardiopulmonary Diagnostic Suite, OMNIA software, UK) that collects gases through a Perspex hood which is known to be the most convenient way to measure energy expenditure at rest in human studies (Fellows and Macdonald, 1985). The system is composed of a hood with a flow-through open-circuit and a mass flow meter connected to mixing chambers and a vacuum pump that draws room air at a rate of 50 to 60 l/min over the participant's face (Fellows and Macdonald, 1985). It measures VO_2 and VCO_2 in ml/min. During measurements, it was made sure that the hood was properly air-tight to provide constant O_2 flow. The fraction of expired air that was CO_2 ($FeCO_2$) was kept at 0.5% to ensure safe levels of CO_2 inside the hood. Any increase or decrease in FeCo2 percentage can lead to hyper- or hypo-ventilation and metabolic disturbance. The principle of measurements and applications of indirect calorimetry has been previously reported by da Rocha, Alves and da Fonseca (2006). The standard operating procedure (SOP) followed to run the COSMED is shown in Appendix 7.

Before the use of COSMED, calibrations of the system were performed. The COSMED unit was connected to two cylinders of pressurised gas composed of 16% O_2 and 5% CO_2. The pressure was kept at 5 bar and the machine was turned on to warm up for at least 30 minutes. Room air calibration was performed by the system to ensure that the gas composition that passes through the canopy is stable and known. This was followed by flowmeter and gas calibrations. During the flowmeter calibration, a three litres syringe and a flowmeter turbine were used. To ensure a proper flow calibration, six strokes were performed by pulling the syringe handle in and out while keeping the

flow curve within the range of -1 and +1 L/seconds and 1 and 4 % of error. After that, the gas calibration was automatically run by the system. COSMED was calibrated multiple times before and during each experimental trial to correct any drift in the sensitivity of the analyser. To ensure that COSMED was working correctly and that accurate values were obtained, monthly repeated series of alcohol calibrations were made using a known amount of alcohol (ethanol) burnt for 20 minutes under a special canopy. The alcohol respiratory exchange ratio was around 0.67 and the recovery percentage was above 95%. The SOP followed to calibrate the COSMED is shown in Appendix 7.

2.11. Maximal handgrip strength test

The acute maximum voluntary force that can be produced by skeletal muscles is called muscle strength. Muscle strength test helps in assessing a person's overall strength, mobility limitation, nutrition status and predicting the risk of health deterioration. However, muscle strength can differ by age, gender, an individual's physical activity level, hand length, forearm circumference, hand dominance, body height and weight (Bohannon, 2015; Lopes et al., 2018). Maximal handgrip strength test is a common method used in research and clinical fields. It is non-invasive, cost-effective, simple and quick to perform. For study 2 in Chapter 4, a maximal handgrip strength test was performed on the non-dominant hand using a digital hand dynamometer (Digital Analyser, MIE Medical Research Ltd, Leeds, UK).

2.12. Reliability for outcomes measures

Coefficient of variation (CV) and Pearson corelation coefficient (*r*) were calculated using two blood samples for eight participants taken five minutes apart at baseline during control phase for serum insulin, blood glucose, serum TG and plasma FFA (Table 2.1).

Table 2.1. Reliability data obtained for insulin, glucose, triglyceride (TG) and free fatty acids (FFA)

Variable	*r*	CV %
Serum insulin	0.700	16.50
Blood glucose	0.935	2.68
Serum TG	0.985	3.93
Plasma FFA	0.942	3.88

The Finometer (Finapres Medical System) reliability was assessed by Deltsidou *et al*. (2017). Authors documented inter-rater reliability (intraclass correlation = 0.794-0.993) and internal consistency (Cronbach's coefficient alpha 0.794-0.993). Quark CPET COSMED was shown to be reliable (CV ≤ 3%) and accurate (within 2% of actual value) in measuring respiratory exchange ratio, oxygen percent and carbon dioxide recovery (Kaviani *et al.*, 2018). The MIE digital grip analyser reliability was assessed by Wardand Adams (2007). It was found to be reliable (CV of 30.77%) for the dominant hand and (CV of 31.46%) for the non-dominant hand.

2.13. Statistical analysis

For study 1 in Chapter 3 and for study 2 in Chapter 4, Data cleaning was done using Microsoft Excel 365 and data analysis and graphing was made through IBM SPSS version 28 and GraphPad Prism software (version 9 for Windows). Descriptive statistics were obtained for every outcome and expressed as a mean ± standard error (SD). All variables were checked for normality were Shapiro-Wilk test was used in SPSS and D'Agostino-Pearson Omnibus normality test was used in Prism. Shapiro-Wilk test was adopted to check for normality because the sample size was between 3 to 50 (D'Agostino, 1971; Shapiro and Wilk, 1965). Normal distribution was assumed when Shapiro-Wilk test was not significant ($p > .05$). When raw data normality was detected, parametric analysis was applied. Sphericity was examined to check for covariance equality using Mauchly's test and sphericity was assumed when p value was not significant ($p > .05$). Results were expressed as a mean ± standard error of the mean (SEM). $P < .05$ was set as the value of statistical significance. Data were analysed using paired t-test and repeated measures ANOVAs when appropriate. If statistical differences were reached, Bonferroni post-hoc testing was used to correct for family-wise error. For each outcome, effect size was calculated and reported as Cohen's d values (d) for t tests and as partial eta-squared values (η^2_p) for ANOVA and the interpretation was according to Cohen's classification (Lakens, 2013); large effect = 0.14, medium effect = 0.06 and small effect = 0.01.

The current research follows a crossover design that can be susceptible to carry-over effects. The term "carry-over effect" refers to how a previous treatment's effects might change the outcome of a subsequent treatment. Thus, the possibility of carry-over effects during the present studies (study 1 in Chapter 3 and study 2 in Chapter 4) were

examined by comparing the primary variables in terms of visit regardless of the treatment applied. The presence of carry-over effect between treatments was not assumed when there was no significant difference between visits, i.e., the washout period between treatments was sufficient to prevent any carry-over effects.

Chapter 3: The effects of two days of physical inactivity on cardiovascular responses, insulin sensitivity and energy metabolism in healthy humans

3.1. Introduction

In the epidemiological literature, evidence pointing to adverse health consequences on cardiovascular health linked to occupational sitting was identified as early as the 1950s (Morris and Crawford, 1958). Recent evidence indicates a positive association between reduced physical activity and cardiometabolic risks (Dunstan et al., 2007; Healy et al., 2008; Homer, Owen and Dunstan, 2019). Current lifestyle changes, such as increased intake of high-calorie foods in combination with physical inactivity are considered to play an important role in the drastic rise in obesity worldwide. In addition, reduced physical activity can lead to an impaired insulin function (Credeur et al., 2018; Mikus et al., 2012; Stephens et al., 2011). It has been documented that the elimination of physical inactivity would remove between 6% to 10% of the major non-communicable diseases and increase life expectancy (Lee et al. 2012; Hu 2003). Factors that may contribute to health adverse effects following periods of low muscle activity are not well understood. Investigating the acute effects of physical inactivity will shed light on the early processes involved in the development of these cardiovascular and metabolic diseases. Recent research examined the short-term adverse effects of physical inactivity with energy surplus on cardiometabolic risk factors. It has been reported that only five days of bed rest yield cardiovascular detrimental effects manifested as increases in systolic and diastolic blood pressures, and decreases in arterial reactive hyperaemia (Hamburg et al., 2007; Nosova et al.,

2014). In addition, inactivity for less than two weeks can increase arterial stiffness, decrease arterial compliance and reduce cardiovascular fitness indicated by the low maximal oxygen consumption (VO_{2max}) levels (Credeur et al., 2018; Krogh-Madsen et al., 2010). Furthermore, inactivity has been shown to acutely affects insulin sensitivity and glycaemic control. These harmful effects were expressed as changes in blood insulin and glucose concentrations in response to the oral glucose tolerance test or insulin sensitivity measured by hyperinsulinaemic/hyperglycaemic clamp (Credeur et al. 2018; Dixon et al. 2013; Hamburg et al., 2007; Knudsen et al. 2012; Krogh-Madsen et al. 2010; Lyden et al. 2015; Mikus et al. 2012; Reynolds et al. 2015). Moreover, inactivity for one to two weeks can decrease energy expenditure and increases fasting triglycerides (TG) (Dixon et al. 2013; Krogh-Madsen et al. 2010; Stubbs et al., 2004). Previous literature attributed these adverse cardiometabolic effects to low muscle activity, i.e., physical inactivity *per se*. Whether these unfavourable effects were partly mediated by the concurrent energy surplus has not been thoroughly tested. Stephens et al. (2011) conducted a study to examine the effect of 24 hours of physical inactivity on insulin action with or without adjusting energy intake to either maintain energy balance or achieve positive energy balance. Insulin sensitivity was measured on the following day by a one hour continuous infusion of [6,6-2H]-glucose. Findings showed that one day of reduced physical activity with and without energy surplus decreased insulin sensitivity, but this decrease in insulin sensitivity was greater when associated with positive energy intake. This suggests that energy surplus associated with inactivity may be a contributing factor to the adverse effects of physical inactivity. Thus, the current study aimed to assess the effects of a 2-day free-living period of reduced physical activity and prolonged sitting time, with and without a proportional decrease in energy intake (creating neutral or positive energy balance, respectively), on

cardiovascular responses, insulin sensitivity and energy metabolism in response to food ingestion. The rationale for choosing a two-day intervention period is to investigate the acute effects (less than three days) of inactivity would yield greater adverse effects. Because to our knowledge, the shortest intervention duration applied in previous studies using a daily steps reduction model to measure the adverse effects of inactivity lasted for three days (Mikus et al., 2012) with significant insulin and glucose changes. Moreover, investigating the effects of inactivity with and without energy surplus was assessed by Stephens et al. (2011) using prolonged sitting (laboratory-based) approach for one day. Prolonged sitting model do not reflect the normal habitual activity level which may limit the generalisation of finding to most of population. Thus, a similar intervention design of Stephens et al. study using a daily steps reduction (free-living) approach with one day longer period was applied in the present experiment to investigate whether these findings in the latter study persists to the following day or not and to measure the cardiovascular and energy balance responses alongside the metabolic responses which were not measured together previously. Participants recruited were young and healthy. The rationale for choosing such population is of importance because it can helps understanding how cardiometabolic illness develops and can give a mechanistic explanation for the negative effects of physical inactivity with and without overfeeding.

In addition to the primary objective of the present study, two secondary objectives were investigated: 1) an exploratory analysis for gender differences and 2) assessing the reliability of the newly established continuous glucose monitoring system (CGM), the Freestyle Libre Abbott, during the Control trial. CGMs were designed to monitor glucose levels for three consecutive days to enhance knowledge of glucose

fluctuations (hypoglycaemic and hyperglycaemic episodes) that could not be identified using self-monitoring blood glucose measurements. More than 15 years ago, the first CGM were developed. Since then, CGMs have been continually improved for better metabolic control. These technological improvements included increasing the lifetime of the sensor, setting up auditory and visual warnings for hypoglycaemic and hyperglycaemic occurrence, visualising real-time glucose levels, minimising the hardware size and facilitate access to the data remotely by direct connection to smartphones (Facchinetti, 2016; Vashist and Luong, 2017). Current CGMs are wearable devices that enable the glucose concentration to be calculated and visualized continuously for several days in real-time and provide hypo- and hyper-glycaemic warnings, and information on glucose trends. They are commonly consisting of three main parts including (1) a needle-based sensor implanted subcutaneously in the upper arm or anterior wall of the abdomen which measures electrical signals proportional to the interstitial glucose level; (2) a transmitter that facilitates the transmission of the signals received and processed by the sensor; and (3) a portable device to receive, record and visualise the signals. Though CGMs are called continuous, the interstitial fluid glucose is typically sampled intermittently with frequency varying between measurements. Glucose values are averaged every few minutes to a quarter of an hour and presented as a single reading (Facchinetti, 2016; Vashist and Luong, 2017; Villena Gonzales, Mobashsher and Abbosh, 2019). CGMs are classified into professional (retrospective) devices and personal (real-time) devices. The professional CGMs are operated by health care providers and are commonly used for the collection of masked data. Patients are unaware of the effects of testing until the health care provider downloads and analyse them. This allows for an objective evaluation of glycaemic control. A major limitation of the professional

CGMs is the absence of warning alarms for hypoglycaemic or hyperglycaemic episodes. On the contrary, personal CGMs patients and values of glucose are constantly visible. This enables instant treatment modifications in the case of hypoglycaemic or hyperglycaemic events (Ida et al., 2018; Vashist and Luong, 2017). The FreeStyle Libre Abbott systems are disposable and water-resistant personal CGMs. They can be worn on the back of the upper arm for up to 14 days (336 hours). Through a thin filament that is placed just beneath the skin, the sensor continuously detects glucose levels in the interstitial fluid every few minutes. The sensor can collect up to 1,340 readings during the period. No calibration by finger-prick is needed because the sensor is factory calibrated. With just a painless one-second scan, glucose readings can be taken as many times per day as required. The results are transmitted via wireless radio frequency technology to the receiver. To make the testing more convenient, scanning can be done while the sensor is under clothing. Each scan shows a glucose result in real-time, and the reader can keep data for up to 90 days. In addition, these systems have been shown to be unaffected by subject characteristics, user-friendly, cost-effective, light-weighted with a better technique sensor attachment (Bailey et al., 2015; Fokkert et al., 2017; Kumagai et al., 2019; Ida et al., 2018; Rodbard, 2017; Sato et al., 2019; Vashist and Luong, 2017). The Medtronic iPro2 systems are professional CGMs that can record interstitial glucose concentration up to 7 days (168 hours). The sensor is commonly inserted on the anterior abdominal wall and must be calibrated at least four times per day using finger-prick glucose values. It can display glucose readings every 5 minutes (Akintola et al., 2015; Ida et al., 2018; Kumagai et al., 2019; Sato et al., 2019). To date, several studies have investigated the accuracy of factory calibrated CGMs to measure blood glucose

against the reference methods which include capillary or/and venous blood glucose and commonly used CGMs (Table 3.1).

Table 3.1. Key studies comparing the performance of factory calibrated CGMs against well-established CGMs and reference methods

Author (year)	Sample size	Participant's characteristics	Comparison	Duration	Outcomes
Bailey et al. (2015)	**n=** 72	Age: 46 ± 15 yr BMI: 28 ± 5 kg/m^2 Diabetic (type 1 and 2)	FreeStyle Libre Abbott vs capillary and venous blood [glucose]	14 days	Abbott MARD 11.4% vs capillary Abbott MARD 12% vs venous **Linear regression:** Good correlation Abbott vs capillary (r =0.95)
Bonora et al. (2016)	**n=** 8 (7 male / 1 female)	Age: 43 ± 8 yr BMI: 27 ± 3 kg/m^2 Diabetic (type 1)	FreeStyle Libre Abbott vs Dexcom G4 Platinum and capillary blood [glucose]	14 days	Abbott MARD 18.1% vs Dexcom Abbott MARD 16.6% vs capillary Dexcom MARD 19% vs capillary **Pearson's correlation:** Good correlation Abbott vs Dexcom (r^2 = 0.76)
Aberer et al. (2017)	**n=** 12 (7 male / 5 female)	Age: 33 ± 11 yr BMI: 23 ± 2 kg/m^2 Diabetic (type 1)	FreeStyle libre Abbott, Dexcom G4 Platinum and Medtronic MiniMed 640G vs venous blood [glucose] during simulated real-life environments	12 hr	Abbott MARD 13.2% vs venous Dexcom MARD of 16.8% vs venous Medtronic MARD 21.4% vs venous **Bland–Altman analysis:** Abbott 7.6% < vs venous Dexcom 7.2% > vs venous Medtronic 5.1% > vs venous **95% limits of agreement:** Abbott (35.5%), Dexcom (35%) and Medtronic (50%)
Fokkert et al. (2017)	**n=** 20 (14 male / 6 female)	Age: 24-74 yr BMI: 21-31 kg/m^2	FreeStyle Libre Abbott vs Medtronic iPro2 and capillary blood [glucose] in response to OGTT	7-14 days	Abbott glucose < vs iPro2 and capillary Abbott MARD 8.3% vs capillary

		Diabetic (type 1 and 2)			iPro2 comparable to capillary
Ji et al. (2017)	**n=** 40 (24 male / 16 female)	Age: 53.1 ± 12.5 yr BMI: 26.5 ± 3.9 kg/m^2 Diabetic (type 1 and 2)	FreeStyle Libre Abbott vs capillary and venous blood [glucose]	14 days	Abbott MARD 10% vs capillary Abbott MARD 10.7% vs venous **Linear regression:** Good correlation Abbott vs capillary (r= 0.94) Good correlation Abbott vs venous (r= 0.95)
Ólafsdóttir et al. (2017)	**n=** 50	Age: 47.2 ± 15.7 yr BMI: 25.5 ± 4.2 kg/m2 Diabetic (type 1)	FreeStyle Libre Abbott vs capillary blood [glucose]	14 days	Abbott MARD 13.2 % vs capillary **Pearson's correlation:** Good correlation Abbott vs capillary (r= 0.96)
Ida et al. (2018)	**n=** 15 (10 male / 5 female)	Age: 66 ± 12 yr BMI: 27 ± 5 kg/m^2 Diabetic (type 2)	FreeStyle Libre Pro Abbott vs Medtronic iPro2	7 days	Abbott mean glucose 16% < vs iPro2 Abbott MARD 17.4% vs iPro2 **Linear regression:** Good correlation Abbott vs iPro2 (r = 0.91)
Kumagai et al. (2019)	**n=** 10 (7 male / 3 female)	Age: 60 ± 18 yr BMI: 25 ± 4 kg/m^2 Diabetic (type 1 and 2)	FreeStyle Libre Pro Abbott vs Medtronic iPro2 and capillary blood [glucose]	7 days	Abbott glucose 2.6% < vs capillary Abbott glucose 65.3% < vs iPro2 No differences iPro2 vs capillary Abbott median ARD 8.1% vs capillary Abbott median ARD 6.5% vs iPro2 iPro2 median ARD 5% vs capillary **Linear regression:** Good correlation Abbott vs iPo2 (q = 0.96, p < 0.01).

Sato et al. (2019)	**n=** 5 (3 male / 2 female) Age: 53 ± 10 yr BMI: 25 ± 5 kg/m^2 Diabetic (type 2)		FreeStyle Libre Pro Abbott and the Medtronic iPro2 vs venous and capillary blood [glucose]	5-7 days	iPro2 MARD of 9.2% vs venous Abbott MARD 8.2% vs venous **Linear regression:** Good correlation iPro2 vs venous (r^2= 0.86) Good correlation Abbott vs venous (r^2= 0.90) **Bland-Altman analysis:** Abbott 8.7% < vs iPro2 95% limits of agreement: Abbott vs iPro2 (33.5%)

Abbreviations: **MARD** = mean absolute relative differences; **CHO** = carbohydrate; **OGTT** = oral glucose tolerance test; **ARD** = absolute relative differences; **AD** = absolute differences; **SD** = standard deviation.

CGMs were designed to detect high fluctuations of blood glucose levels in diabetic patients. Table 3.1 showed that most of the published literature studied CGMs performance among diabetic middle-aged or elderly individuals. Their suitability for healthy normo-glycaemic populations that exhibit smaller daily excursions has not been studied extensively. Acknowledging the performance of these devices during a healthy state would give an insight into how these sensors can work at baseline levels to compare the results against the diabetic state. The Medtronic iPro2 devices are well-established CGMs and they have been used as references alongside the capillary and/or venous blood glucose to examine the accuracy of new CGMs (Fokkert et al., 2017; Ida et al., 2018; Kumagai et al., 2019; Sato et al., 2019). In the present research, the FreeStyle Libre Abbott performance was compared to the Medtronic iPro2 during the free-living period rather than venous/capillary blood glucose because of the practical limitations of frequent blood collection. Previous research has shown different views about the performance of factory calibrated CGMs. Some found good agreement with reference methods (Bailey et al., 2015; Bonora et al., 2016; Ida et al., 2018; Sato et al., 2019), while others observed underestimated blood glucose levels (Aberer et al., 2017; Fokkert et al., 2017; Kumagai et al., 2019). No prior research has compared the CGMs against arterialised blood glucose measurements under standardised laboratory environments among young normo-glycaemic subjects. Thus, a performance comparison was included in the present study to explore the agreement of a newly established factory-calibrated sensor (FreeStyle Libre Abbott) against the Medtronic iPro2 and arterialised blood glucose measurements in young and normo-glycaemic individuals under free-living conditions and standardised laboratory settings.

3.2. Materials and Methods

Eight young, healthy, moderately active males and females with normal body mass index were recruited. Each volunteer completed a total of 8 laboratory visits. Before the main visits, two separate visits (medical screening and preliminary assessment) were conducted to ensure that volunteers met the inclusion criteria for the study and to familiarize them with the equipment. The medical screening visit and the inclusion and exclusion criteria were as described in Chapter 2 (section 2.3). The level of activity of each participant was assessed using the short version of the International Physical Activity Questionnaires (IPAQ) during the medical screening visit (Appendix 3). A 3-day food diary was given for each participant to record food and drinks consumed during three consecutive days (two weekdays and one weekend) for habitual food intake and preferences assessment (Appendix 6).

Providing the individual's medical screening results were satisfactory, participants were asked to attend a physical assessment session after an overnight fast and complete 3-day food diaries which were collected on this visit. Food diaries were analysed using the online dietary analysis package NUTRITICS (Nutrition analysis software, v4.3 Academic Edition). Results from the software were used to design meal plans for participants based on their habitual food intake and food preferences to follow during the 2-day intervention period. During the assessment visit, body weight was first measured and then the participant was asked to lay in a semi-supine position for 30 minutes on a hospital bed at a controlled room temperature (22°C - 25°C) (Compher et al., 2006). Thereafter, resting energy expenditure (REE) was measured for 20 minutes using the metabolic cart (COSMED Cardiopulmonary Diagnostic Suite, OMNIA software, UK) with a ventilated hood placed over the head linked to the gas

analysis system. A wireless heart rate monitor (COSMED) was placed on the participant's chest via specialized straps. Heart rate was recorded throughout the test, which helped in estimating the energy cost at rest. During the recording, the participant was instructed to remain silent and immobile with close monitoring and reassurance to keep the participant awake and comfortable. At the end of the visit, the heart rate monitor was detached, and lunch was provided. After that, a pedometer (OMRON Walking Style One 2.1, Step counter HJ-321-E, OMRON Healthcare UK Ltd.) was fitted on the participant's waist and an Actiheart (CamNtech Ltd. UK) on the participant's chest to measure their habitual step counts and energy expenditure, respectively, over three days. The amount of physical activity that free-living individuals engage in each week may be estimated using the data from a pedometer for at least three days (Tudorlock et al., 2005).

Each participant was then randomly assigned to complete three experimental trials. Each trial lasted for three days, including two days of intervention and one day of measurements. The washout period between each trial was a minimum of two weeks and a maximum of three months. A flow chart summarising participant recruitment and randomisation is shown in Figure 3.1. For female participants, trials were conducted during the follicular phase of their menstrual cycle (approximately 3 weeks washout period) to control for the sex hormone effects on substrate oxidation and vascular function (Williams et al. 2001; Zderic, Coggan and Ruby, 2001).

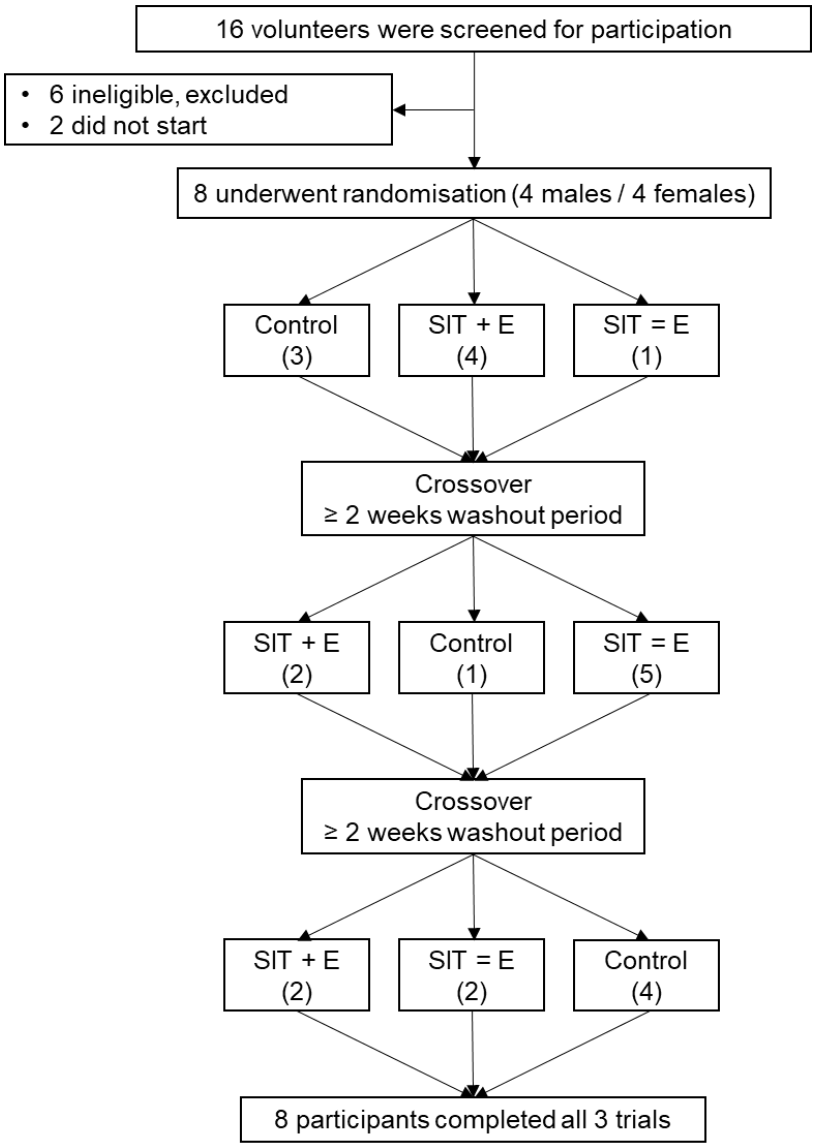


Figure 3.1. Flow chart summarising participant recruitment and randomisation.

The level of physical activity and energy intake during the 2-day intervention of each trial were varied. In the Control trial, participants were asked to continue with their habitual daily physical activities at a physical activity level (PAL) of ~1.85 and consume an isoenergetic intake. In the SIT+E trial, physical activities were reduced to PAL of ~1.40 but maintaining the same energy intake as that consumed in the Control trial to achieve a positive energy balance. In the SIT=E trial, both physical activity and energy intake were reduced to a PAL of ~1.40 to reach an energy balance. In SIT+E and SIT=E trials, participants were instructed to reduce their normal daily step count by 80% on both days, which was verified using a pedometer.

Participants were requested to come to the lab for a short pre-visit (approximately 60 minutes) two days before each main experimental visit. During this pre-visit, two subcutaneous continuous glucose monitoring (CGM) devices were inserted. The first CGM sensor was placed on the back of the arm (FreeStyle Libre Flash Glucose Monitoring System, Abbott 2016, UK) and the other sensor on the anterior abdominal wall (iPro2, Medtronic, USA). These CGM devices provide 24 hours continuous interstitial glucose level records. The two CGM devices were placed throughout the control trial only for direct comparison between the two and only one (iPro2) was placed during the SIT+E and SIT=E trials and removed at the end of Day 3 for all trials. The reason why CGMs were inserted two days before the trial is to allow enough time for sensor equilibration and to resolve any micro-hematoma caused by the insertion. Previous studies found that the sensor performance is worst on the first 24-hour of insertion while maintaining good performance during the remaining period of assigned time of use (Aberer et al., 2017; Akintola et al., 2015). For iPro2 sensors, calibrations with blood glucose taken by finger-pricks were required at least 4 times per day.

Participants were instructed and familiarised to perform the finger-prick on themselves by the researchers using special needles (Unistik 3 Comfort Safety Lancets, 28G X 1.8mm, Owen Mumford), strips (ACCU-CHEK Inform II TEST STRIPS, Roche) and blood glucose reader (Accu-Chek Aviva). Each participant documented finger-prick blood glucose before breakfast, lunch, dinner and sleep (Akintola et al., 2015). A pedometer was supplied and an Actiheart was applied around the chest to provide easily accessible feedback of each participant's daily step count and physical activity corresponding to the prescribed physical activity level. Activity templates for day 1 and day 2 of the intervention designed to cover the range of likely total energy expenditure (TEE) values required by different participants to achieve the proposed PAL values are shown in Appendix 8. The physical activity ratio (PAR) for each activity was taken based on published values by the Department of Health in 1992 and the Food and Agriculture Organization (FAO) in 2004. The PAR value is the energy cost for different physical activities calculated as multiples of resting metabolic rate (RMR) per minute, while the PAL value represents a multiple of RMR per 24 hours (Scientific Advisory Committee on Nutrition, 2011). PAL was estimated from the average 24-hour TEE and RMR measured by the indirect calorimetry (PAL = TEE / RMR). Before each trial phase, volunteers were asked to maintain their normal habitual physical activities and food intake, avoid alcohol intake for two days, and to refrain from caffeine consumption throughout the trial. These factors were controlled to prevent additive or false effect on the study measurements. For instance, it has been documented that drinking caffeine may enhances thermogenesis and lowers energy intake (Westerterp-Plantenga et al. 2006) and that alcohol consumption can negatively affect lipid metabolism (Jeon and Carr, 2020). Figure 3.2 shows a schematic diagram of the study design and sequence of measurements made during each trial.

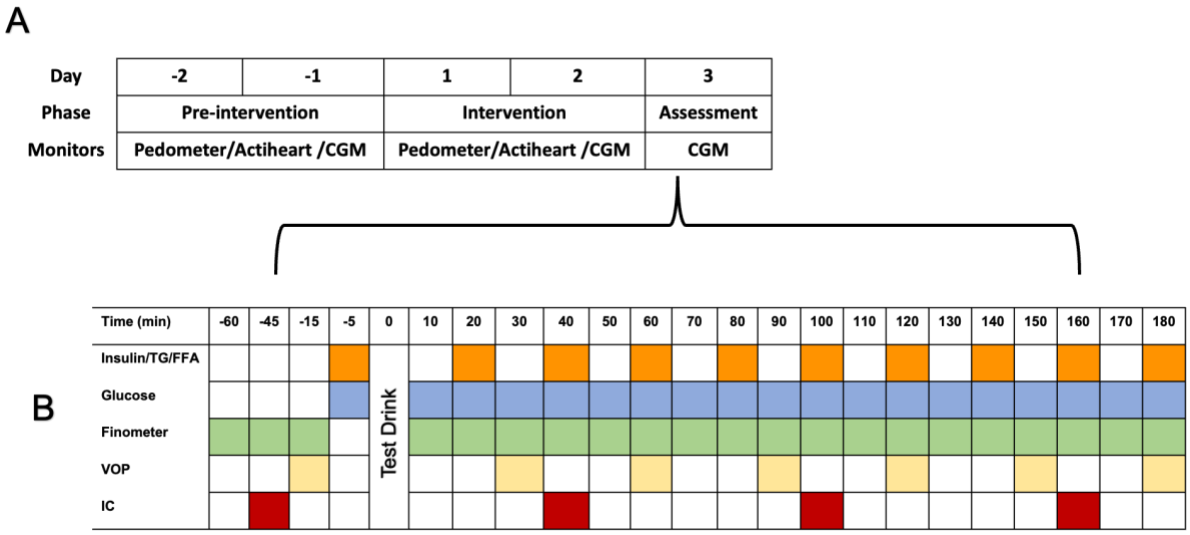


Figure 3.2. Schematic diagram of the 2-day study design (A) and the sampling intervals for laboratory session on Day 3 (B) Abbreviations: CGM = continuous glucose monitoring system; TG = triglycerides; FFA = free fatty acids; VOP = venous occlusion plethysmograph.

Meal templates were designed based on foods that are commonly consumed by the UK population. On days 1 and 2, all food (three main meals and three snacks) and food weighing scales (SALTERS, ARC ELECTRONIC SCALE with add and weigh) were supplied to take home. Main meals and snacks provided ~50% carbohydrates, ~35% fat and ~15% protein of total energy intake over the day. The energy derived from the prescribed macronutrients was calculated through multiplying the amount of intake in grams by the associating Atwater general factor (4 kcal/g for carbohydrates, 9 kcal/g for fat and 4 kcal/g for protein). An example of meal templates designed for the intervention days is presented in Appendix 9. Participants were guided to weigh food themselves to ensure amount consistency and to achieve the required energy intake. Participants were asked to record any food not consumed, or if they consumed any non-prescribed foods. They were encouraged to consume all foods as prescribed to reduce the variability between participants.

On the measurement visit (day 3) of each experimental trial, participants came to the lab after an overnight fast of at least 10 to 12 hours. Cardiovascular and metabolic assessments were made in response to a standardised test drink given approximately at 9:30 a.m. to assess the relative effects of the previous two intervention days. A data sheet was designed to ensure detailed participant information and measurements were recorded. Upon arrival, the participant's body weight was measured followed by a rest on a semi-supine position on a hospital bed for 30 minutes at a controlled room temperature (22°C - 25°C) (Compher et al., 2006). While the participant was rested, a retrograde cannula (Venflon IV cannula 20g pink) was inserted by medical staff into a dorsal hand vein under local anaesthesia (1% w/v Lidocaine Injection 50 mg / 5 ml, B. Braun) for subsequent blood sampling. The participant's hand was then placed in a

hot, air-warmed, ventilated perspex box kept at about 55°C for at least 10 min to arterialise the venous blood. It has been documented that arterialized venous blood provides a reasonable estimation of the actual arterial value (Liu et al., 1992). The cannula was kept patent by dripping of normal saline solution with 0.9% Sodium Chloride (Baxter Healthcare Ltd. Thetford, UK) at a fixed speed rate and it was replaced once finished and the total amount used was documented for each participant. A three-way tap was used to draw blood samples and the first 2 mL of each sample (diluted blood with the saline) was discarded. The temperature of the hand and room were continuously monitored. After completing baseline measurements, standardise test drink was served as a breakfast in an open glass consumed via a straw at room temperature over two minutes. The test drink was a vanilla-flavour milkshake (Ensure compact, Abbott). It is a complete and balanced nutritional drink. Each bottle was 125 ml and provided 2.4 kcal/ml. The volume was based on the participant's body weight recorded on arrival (an average of 10 $kcal \cdot kg^{-1}$ body weight) and total energy intake comprised approximately 50% from carbohydrate, 35% from fat and 15% from protein. A liquid mixed meal was used for a better standardisation between trials and allows faster physiological response measurements than a solid meal which can take a longer time to be absorbed by the gut (Achour, Méance and Briend, 2001). REE and whole-body substrate oxidation assessment for 20 minutes using COSMED were done at baseline and hourly during the 3 hours post-test drink. Furthermore, cardiovascular parameters including mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, cardiac output, stroke volume, and total peripheral resistance (TPR) were recorded continuously throughout the measurement session using the Finometer. Calf blood flow responses were measured for two minutes by venous

occlusion plethysmography (VOP) at baseline and every 30 minutes during the three hours postprandial. Moreover, blood samples were taken every 10 minutes for blood glucose and every 20 minutes for serum insulin, serum TG and plasma free fatty acids (FFA) for three hours after the standardized test drink. Blood glucose measurements were made immediately using the Yellow Spring Instrument (YSI 2300 STAT PLUS) directly from the syringe. Upon the completion of the experiment, the cannula and CGM device were removed before the participant could leave the laboratory. All data collected were saved in a private and secured place.

3.2.1. Power calculation

A *priori* power analyses using G*Power (version 3.1.9) was used to estimate the sample size for this experiment. Pervious study by Lyden *et al*. (2015) reported a 32% (effect size = 0.73) increase in insulin AUC compared to baseline following seven days of inactivity among 10 participants. If a similar difference was expected in the present study following two days of physical inactivity, we should have been able to detect this effect in 6 participants with a power of 80% at a 5% significance level. Recruitment continued until the required sample size was reached. To preserve statistical power in situations of withdrawal, more volunteers were recruited.

3.2.2. Data handling

A. CGM

Data recorded by the iPro2 Medtronic sensor were uploaded through the official website (https://carelink.minimed.eu/ipro/hcp/login.jsf?bhcp=1). Before uploading the data, finger pricks blood glucose readings were manually inserted for retrospective

analysis of stored electronic signals that were then transformed to concentrations of glucose. After that, an excel file composing raw data of interstitial glucose levels, with five minutes recording interval, was generated and downloaded for the analysis. The FreeStyle Libre Abbott glucose measurements were uploaded through the FreeStyle Libre Software (version 1.0) via a USB connection to a laptop. The raw data of the interstitial blood glucose were then exported into an Excel file with 15 minutes intervals. Before the analysis, all raw data was cleaned and organised. Before the analysis, all raw data was cleaned and organised. For the 2-day intervention, measures of glycemia included 24-hour mean glucose, day-time mean glucose (from 7:30 a.m. until 12 a.m.) and night-time mean glucose (from 12 a.m. until 7:30 am). The day and night mean glucose assessments were done to explore detailed CGMs performance during the day (participant is awake, eating and taking finger-pricks for calibration) and during the night (participant is sleeping and fasted with no calibration). The iPro2 glucose values were averaged every 15 minutes to allow direct comparison between the two CGMs. On the assessment day (day 3), the Abbott sensor was scanned every 10 minutes in accordance with blood sampling and the iPro2 data were averaged every 10 minutes to evaluate the agreement between the Abbott, iPro2 and arterialised blood glucose measurements.

B. Insulin sensitivity

Fasting and postprandial insulin sensitivity were calculated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Matsuda Index using the following equations documented by Gutch et al. (2015) and Patarrao, Lautt and Macedo (2014):

$$\text{HOMA-IR} = \text{Fasting insulin } (\mu\text{IU/ml}) \times \text{Fasting glucose (mmol/L)} / 22.5$$

$$\text{Matsuda index} = 10{,}000 / \sqrt{G0\ (\text{mg/dl}) \times I0\ (\text{mIU/l}) \times G_{mean}\ (\text{mg/dl}) \times I_{mean}\ (\text{mIU/l})}$$

Normal insulin sensitivity was indicated when HOMA-IR value was less than 2.5 and Matsuda Index more than 4.3 (Gutch et al., 2015).

C. Finometer and VOP

Before usage, Finometer and VOP were calibrated according to our laboratory standard operating procedure (Appendix 10 and Appendix 11). Both machines were connected to a laptop with LabChart software. Values of cardiovascular responses were inputted and displayed as line graphs in LabChart, directly from the VOP for calf blood flow and through 4 main channels from the Finometer for MAP, stroke volume, TPR and cardiac output. At each blood flow measuring time point, the rapid cuff inflation system of the VOP was set to inflate for 10 seconds and deflate for 5 seconds until eight curves have been recorded which took about two minutes. The linear part of each curve (about four squares on the LabChart) was selected. The average of these values represented the blood flow at that time point. The Finometer run continuously throughout the trial and displayed MAP, stroke volume, TPR and cardiac output recordings on the LabChart with 10 seconds intervals. Finometer calculates MAP and TPR based on the following equations:

$$\text{MAP (mmHg)} = 1/3\ \text{SBP} + 2/3\ \text{DBP}$$

$$\text{TPR (mmHg.L/min)} = \text{MAP} / \text{cardiac output}$$

Total vascular conductance (TVC), which reflects how smooth the blood flows (Lautt 1989), was calculated using the measurements of cardiac output and MAP based on the equation:

$$TVC\ (mL.min^{-1}.mmHg^{-1}) = cardiac\ output / MAP$$

D. Indirect calorimetry

To ensure that the participant was in a steady state and for reliable measurements, the first and last 2.5 minutes of the 20 minutes COSMED recordings were discarded, and only the middle 15 minutes values were taken for analysis. The REE obtained was used to calculate the estimated energy requirement (EER) for each arm of the study according to the following equation: EER = REE X PAL, where PAL was selected based on the experimental trial, i.e., PAL = 1.85 for the active state and PAL = 1.40 for the inactive state (Brooks et al., 2004). The Oxygen uptake (VO_2) and Carbon Dioxide production (VCO_2) values measured were used to calculate both carbohydrate (CHO) and fat utilisation following the Frayn (1983) equations:

$$CHO\ (g/min) = (4.55 \times VCO_2) - (3.21 \times VO_2)$$

$$Fat\ (g/min) = (1.67 \times VO_2) - (1.67 \times VCO_2).$$

Energy units obtained from the indirect calorimetry were in kilocalories. The units were converted to kilojoules by multiplying the energy by 4.184. Since measurements were taken under resting state, i.e., absence of activity-induced energy expenditure, DIT was measured as the increase in postprandial energy expenditure above fasting levels during the three hours post-test drink intake. DIT has shown to be affected by circadian

variations (morning versus evening) where lower levels were found during the evening compared to the morning (Romon et al., 1993). In addition, Morris et al. (2015) reported no effect of behavioural variations (sleep/awake and fasting/feeding) on DIT measurements. Thus, DIT measurements timing was made consistent in the present experiment, i.e., in the morning for each visit, to avoid circadian variations.

3.2.3. Statistical analysis

Using the Shapiro-Wilk test in SPSS, the normality of all variables was examined. In Prism, the D'Agostino-Pearson Omnibus normality test was used when the data set included more than 99 time points. When the Shapiro-Wilk test had a non-significant result (p >.05), normal distribution was assumed. Parametric analysis was used once the normality of the raw data was confirmed. Levene's test of equality of error variances were applied to examine homogeneity when in between-subject analysis was used. Homogeneity was assumed when p value was not significant (p > .05). Descriptive statistics including mean, standard deviation, maximum, minimum and percent coefficient of variation of the 24-hour interstitial glucose concentration were obtained. Differences in of the interstitial glucose during the 2-day intervention where analyse using repeated measure mixed-model two-way ANOVA (Treatment x Time). Differences in body weight, daily steps count, energy intake, daily energy expenditure, fasting serum insulin, fasting blood glucose, fasting serum triglycerides (TG), fasting plasma free fatty acids (FFA), fasting cardiovascular parameters, fasting calf blood flow responses and diet-induced thermogenesis (DIT) to each main trial were analysed using repeated measure mixed-model one-way ANOVA. Differences in postprandial serum insulin, blood glucose, serum TG, plasma FFA, cardiovascular parameters, calf blood flow, oxygen uptake (VO_2), carbon dioxide production VCO_2, resting energy

expenditure (REE), substrate utilisation and respiratory quotient (RQ) responses were analysed using repeated measure mixed-model two-way ANOVA (Treatment x Time). Differences in insulin incremental area under the curve (iAUC), blood glucose (iAUC), TG iAUC, FFA decremental area under the curve (dAUC) were analysed using repeated measures one-way ANOVA. Insulin sensitivity indices were analysed using repeated measure mixed-model one-way ANOVA. Gender differences were analysed for each main outcome. Differences in time spent on each physical activity type measured by the Actiheart were assessed using three-way (Treatment x Activity type x Day).

Differences between the iPro2 and the Abbott devices during the 24-hour intervention period and the differences between the iPro2, Abbott and Yellow Spring Instrument (YSI) were analysed using two-way (Treatment x Time) repeated measures ANOVA with Bonferroni post-hoc tests to correct for family-wise error when statistical significance was reached. Coefficient of determination (r^2), mean differences, mean absolute differences (MAD), mean absolute relative differences (MARD) were computed for each participant and for pooled data from all participants. CGM performance was analysed using the Bland–Altman analysis. Differences between MADs and MARD's were assessed using paired t-test. Results were expressed as a mean ± standard error of the mean (SEM). $P < .05$ was set as the value of statistical significance. If statistical differences were reached, Bonferroni post-hoc testing was used to correct for family-wise error. Carry-over effect was assessed by comparing measurement time (fasting and postprandial) for each primary outcome between visits 1, 2 and 3 using repeated measures 2-way ANOVA (3 visits X 2 time). No carry-over

effect was assumed in the presence of non-significant (p > .05) difference between visits.

3.3. Results

Eight young and healthy male and female participants completed all three main trials. Detailed anthropometric measurements are shown in Table 3.2.

Table 3.2. Participant characteristics.[1]

Variable	Men	Women
n	4	4
Age (yrs)	23 ± 3	23 ± 2
Height (m)	1.78 ± 0.07	1.61 ± 0.07
Body weight (kg)	70.50 ± 5.53	56.5 ± 10.72
BMI (kg/m^2)	22.40 ± 1.94	21.7 ± 2.70
WC (cm)	77.00 ± 3.92	67.8 ± 7.93
Fat (%)	17.38 ± 4.37	25.80 ± 6.06

[1]All values are means ± standard deviation (SD). Abbreviations: BMI = body mass index; WC = waist circumference.

3.3.1. Day 1 and Day 2 of the intervention

A. Body weight, energy intake, steps count and physical activities

No difference was found in body weight measurement across study visits [F(2,12) = 0.06, p = .944] (Table 3.3). Steps counts showed significant differences between Control (23842 ± 1143 steps/day) versus SIT+E (5282 ± 561 steps/day) and SIT=E (4726 ± 298 steps/day) trials [F(2,12) = 264.92, p < .001] with mean differences of 18560 and 19116 steps, respectively (see Table 3.3). No significant differences were

found between SIT+E and SIT=E (mean differences of 556 steps). Energy intake was prescribed for each participant according to their baseline RMR multiplied by the specific PAL for each main trial, Control (RMR X 1.85), SIT+E (RMR X 1.85) and SIT=E (RMR X 1.40). Participants consumed all food with no leftover or non-prescribed food intake were reported. Thus, an exact compliance was assumed since prescribed meals were carefully calculated in terms of energy content, macronutrient composition and weight of food. There was a main treatment effect on energy intake [$F(2,12) = 1235.61$, $p < .001$ with large effect size of $\eta^2_p = 1.00$]. Compared to SIT=E (8503.83 ± 241.93 kJ/day), energy intake in Control (11237.19 ± 319.69 kJ/day) and SIT+E (11237.19 ± 319.69 kJ/day) was significantly higher with a mean difference of 2733.36 kJ (Table 3.3). Physical activity time measured by the Actiheart are shown in Table 3.3. Because of circumstances beyond our control, the physical activity data were measured in only four participants (3 female / 1 male). There was a main effect of physical activity type between conditions. Moderate activity time were higher during the Control (236 ± 52 minutes/day) compared to SIT=E (57 ± 17 minutes/day) with a mean difference of 179 minutes/day [$F(1.57,9.41) = 8.38$, $p = .011$]. Vigorous activity time was significantly higher during Control (17 ± 6 minutes/day) compared to SIT+E (3 ± 1 minutes/day) and SIT=E (1 ± 1 minutes/day) with mean differences of 14 and 16 minutes/day, respectively, [$F(1.10,6.60) = 9.67$, p .017].

Table 3.3. Body weight, energy intake, number of steps and time spent on each physical activity intensity during the intervention period of each main trial.[1]

	Control	SIT+E	SIT=E
Body weight (kg)	63.6 ± 3.2	63.5 ± 3.1	63.5 ± 3.0
Energy Intake (kJ/day)	11237.2 ± 319.7 #	11237.2 ± 319.6 #	8503.8 ± 241.9
Steps/day	23842 ± 1143 § #	5282 ± 56	4726 ± 298
Sedentary (min/day) [a]	939 ± 102	1000 ± 146	1191 ± 67
Light activity (min/day) [a]	249 ± 53	316 ± 102	191 ± 51
Moderate activity (min/day) [a]	236 ± 52	122 ± 45	57 ± 17*
Vigorous activity (min/day) [a]	17 ± 6	3 ± 1*	1 ± 1*

[1]All values are means ± SEM. * different from Control $p < .05$, § different from SIT+E $p < .05$, # different from SIT=E $p < .05$. [a] (n=4)

B. Interstitial glucose concentrations (iPro2)

A summary of the interstitial glucose levels recorded continuously by the iPro2 during the 2-day intervention period of each main trial are shown in Table 3.4, including the mean ± standard deviation, maximum, minimum and percent coefficient of variation (%CV) values of the 24 hours, day-time (07:30 to 00:00 hours) and night-time (00:00 to 07:30 hours) periods.

Table 3.4. Descriptive statistics of the 2-day intervention period measured by continuous glucose monitoring system (iPro2) compared between the three conditions (n=8).

	Control			SIT+E			SIT=E		
	Mean ± SD	**Range**	**%CV**	**Mean ± SD**	**Range**	**%CV**	**Mean ± SD**	**Range**	**%CV**
24 hours	5.08 ± 0.17	5.41 - 4.75	3.31	5.35 ± 0.18	5.65 – 4.91	3.42	5.04 ± 0.27	5.74 - 4.45	5.28
Day-time[a]	5.09 ± 0.26	5.28 – 4.91	5.14	5.40 ± 0.02	5.41 – 5.39	0.32	5.14 ± 0.04	5.17 – 5.11	0.81
Night-time[b]	4.96 ± 0.30	5.18 - 4.75	6.07	5.16 ± 0.11	5.24 – 5.08	2.11	4.76 ± 0.06	4.81 - 4.72	1.33

[a]Day hours were from 07:30 to 00:00. [b]Night hours were from 00:00 to 07:30. Abbreviations: SD = standard deviation; %CV = percent coefficient of variation.

Figure 3.3. presents the interstitial glucose concentrations measured by the continuous glucose monitoring system devices (iPro2) during the 24-hr period for day 1 intervention, day 2 intervention and the mean of the 2-day intervention across the three conditions. On Day 1 of intervention, interstitial glucose concentrations showed significant treatment main effect [$F(2,14) = 5.97$, $p = .013$ with large effect of $\eta^2_p = 0.46$]. Glucose levels in SIT+E (5.31 ± 0.01 mmol/L) were higher compared to Control (4.86 ± 0.01 mmol/L) and SIT=E (4.99 ± 0.02 mmol/L) with mean differences of 0.45 and 0.32 mmol/L, respectively. There was a significant treatment main effect during the day-time between the trials [$F(2,14) = 7.06$, $p = .008$ with large effect of $\eta^2_p = 0.50$] where glucose levels in SIT+E (5.41 ± 0.01 mmol/L) were higher compared to Control (4.91 ± 0.02 mmol/L) and SIT=E (5.11 ± 0.02 mmol/L) with mean differences of 0.50 and 0.30 mmol/L, respectively. However, during night-time no differences were found between the main trials [$F(180,1260) = 0.99$, $p = .512$]. On Day 2 intervention, no differences were found in interstitial glucose between the three conditions [$F(576,4032) = 0.76$, $p > .999$]. Moreover, day-time glucose concentrations were not different across the three conditions [$F(396,2772) = 0.72$, $p > .999$]. Whereas, night-time showed significant treatment main effect [$F(2,14) = 5.14$, $p = .021$ with large effect of $\eta^2_p = 0.42$]. Glucose levels were greater at night during Control (5.17 ± 0.01 mmol/L) and SIT+E (5.24 ± 0.01 mmol/L) than SIT=E (4.81 ± 0.02mmol/L) with mean differences of 0.36 and 0.43 mmol/L, respectively. The mean of the 2-day intervention period across the three trials showed a significant treatment main effect [$F(2,14) = 4.06$, $p = .041$ with large effect of $\eta^2_p = 0.22$] where SIT+E (5.35 ± 0.01 mmol/L) resulted in greater interstitial glucose concentrations than Control (5.08 ± 0.01 mmol/L) and SIT=E (5.04 ± 0.02 mmol/L) with mean differences of 0.27 and 0.31 mmol/L, respectively. The 2-day intervention day-time mean displayed a significant interaction

effect [$F(2,14) = 4.28$, $p = .035$ with large effect of $\eta^2_p = 0.38$]. SIT+E (5.40 ± 0.01 mmol/L) showed higher interstitial glucose levels than Control (5.09 ± 0.02 mmol/L) and SIT=E (5.14 ± 0.03 mmol/L) with mean differences of 0.31 and 0.26 mmol/L, respectively. While demonstrated a treatment main effect during night-time [$F(2,14) = 5.06$, $p = 0.02$ with large effect of $\eta^2_p = 0.42$]. Interstitial glucose levels in SIT+E (5.16 ± 0.08 mmol/L) were greater than in Control (4.96 ± 0.21 mmol/L) and SIT=E (4.76 ± 0.04 mmol/L) with mean differences of 0.20 and 0.40 mmol/L.

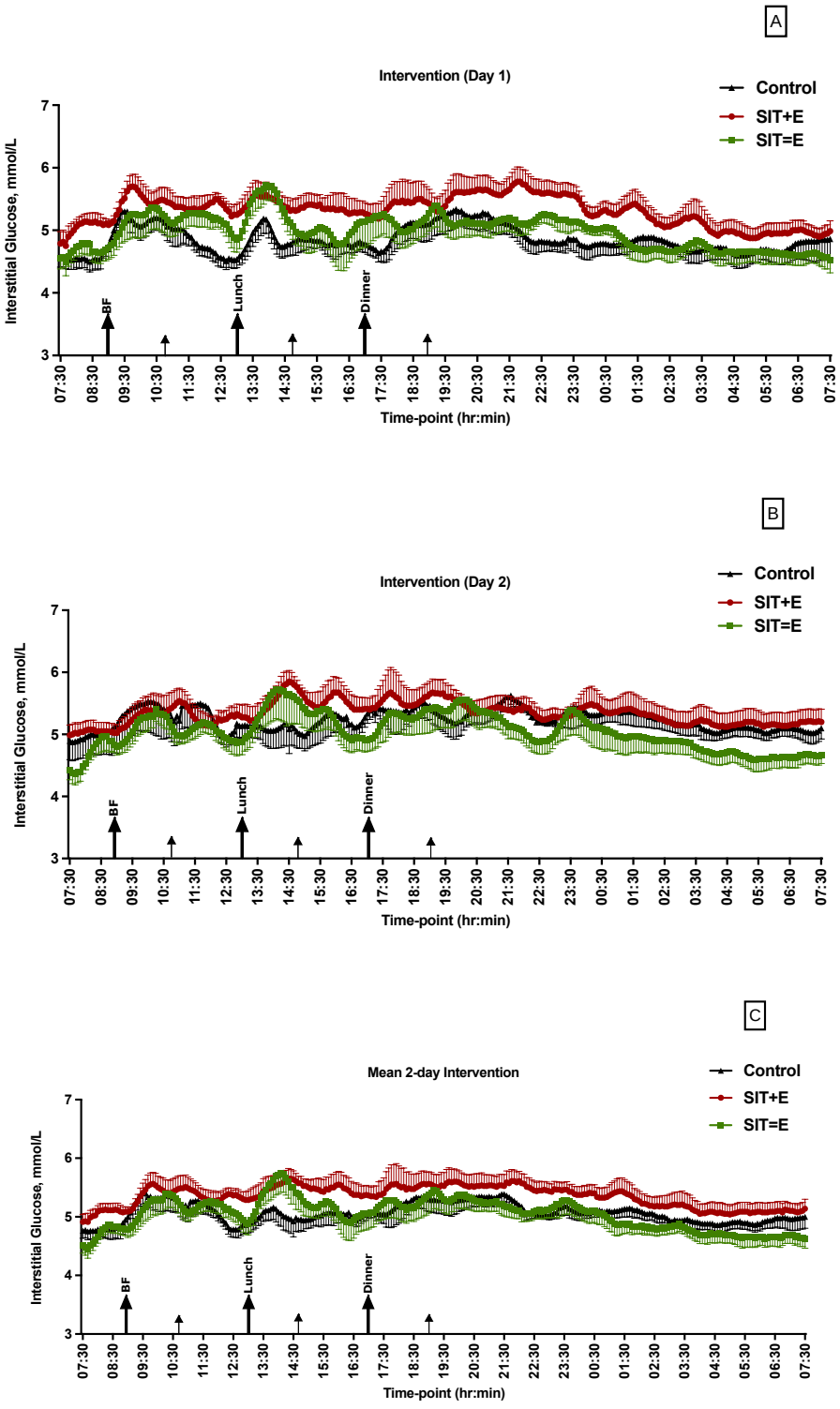


Figure 3.3. Mean ± SEM of interstitial glucose concentrations (n=8) over 24-hr period for A) day 1 intervention, B) day 2 intervention and C) mean of the 2-day intervention across the three conditions using continuous glucose monitoring system devices (iPro2).

3.3.2. Assessment day (Day 3)

3.3.2.1. Metabolic responses

No differences in fasting serum insulin levels were found $F(2,12) = 0.10$, $p = .909$ nor postprandial concentrations [$F(18,108) = 0.79$, $p = .708$] between the three conditions (Figure 3.4). Serum insulin iAUC showed close significant treatment main effect [$F(2,14) = 3.71$, $p = .051$ with large effect $\eta^2_p = 0.35$]. Higher values by 25% were observed in SIT+E (9817 ± 1108 mIU/L) compared to Control (7868 ± 718 mIU/L) with a mean difference of 1949 mIU/L (Figure 3.5).

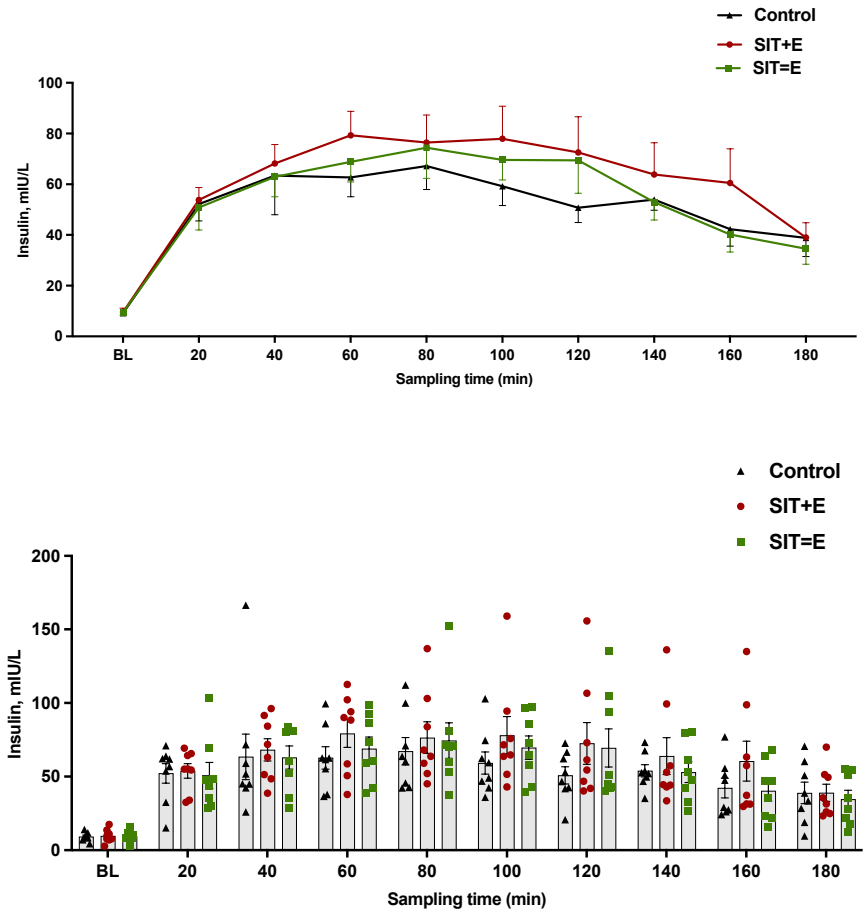


Figure 3.4. Mean ± SEM of serum insulin concentrations (above) and individual responses (below) measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions (n=8).

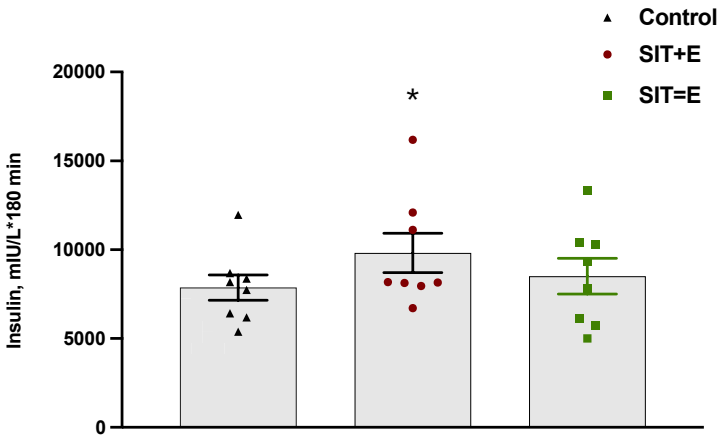


Figure 3.5. Mean ± SEM of the incremental area under curve of serum insulin concentrations and individual responses measured on the assessment day (Day 3) during the 3-hour postprandial period across the three conditions (n=8). * different from Control p = .051.

Mean blood glucose concentrations measured by the YSI showed no significant differences between all trials during fasting [$F(2,12) = 2.46$, $p = .127$] and during the three-hour postprandial period [$F(36,216) = 0.66$, $p = .930$] (see Figure 3.6). Furthermore, there were no differences between trials in insulin sensitivity indices including HOMA-IR at baseline [$F(2,12) = 0.43$, $p = .662$] and Matsuda index during the three-hour postprandial period [$F(2,12) = 0.27$, $p = .770$] (Table 3.5).

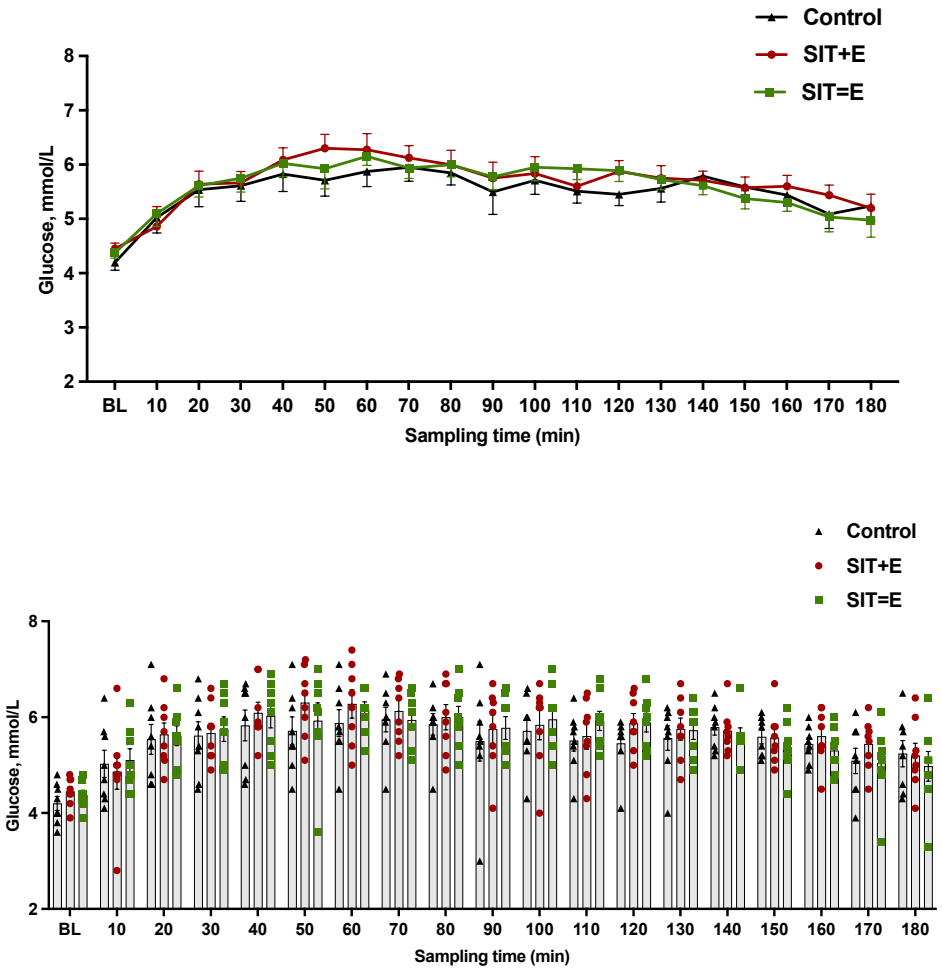


Figure 3.6. Mean ± SEM of blood glucose concentrations (above) and individual responses (below) measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions (n=8).

Table 3.5. HOMA-IR and Matsuda Indices during each condition on the assessment day (Day 3) during the 3-hour postprandial period across the three conditions.[1]

	Control	SIT+E	SIT=E
HOMA-IR	1.71 ± 0.24	1.93 ± 0.37	1.86 ± 0.26
Matsuda Index	5.52 ± 0.53	5.14 ± 0.83	5.23 ± 0.59

[1]All values are means ± SEM (n=8).

Mean fasting serum TG levels were not different between main trials [$F(2,12) = 0.68$, $p = .527$] and same wise in response to the test drink [$F(18,108) = 0.27$, $p = .999$] (Figure 3.7). There was a no significant differences in fasting [$F(2,12) = 0.64$, $p = .544$] and postprandial [$F(18,108) = 0.54$, $p = .934$] mean plasma FFA concentrations between trials (Figure 3.8). A summary of TG and FFA responses presented in Table 3.6. No differences were found in iAUC of TG [$F(2,14) = 0.06$, $p = .940$] and dAUC of FFA [$F(2,14) = 0.92$, $p = .420$].

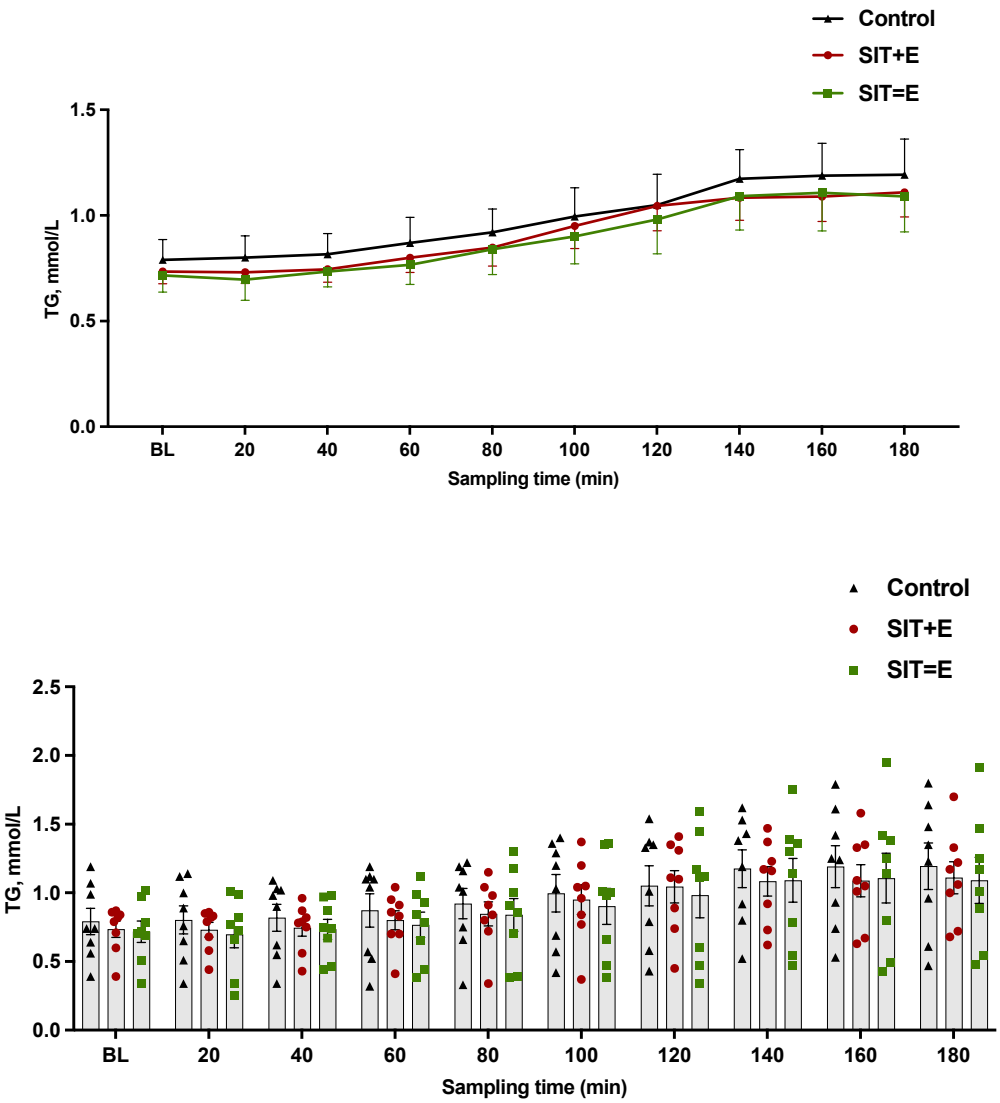


Figure 3.7. Mean ± SEM of serum TG concentrations (above) and individual responses (below) measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions (n=8).

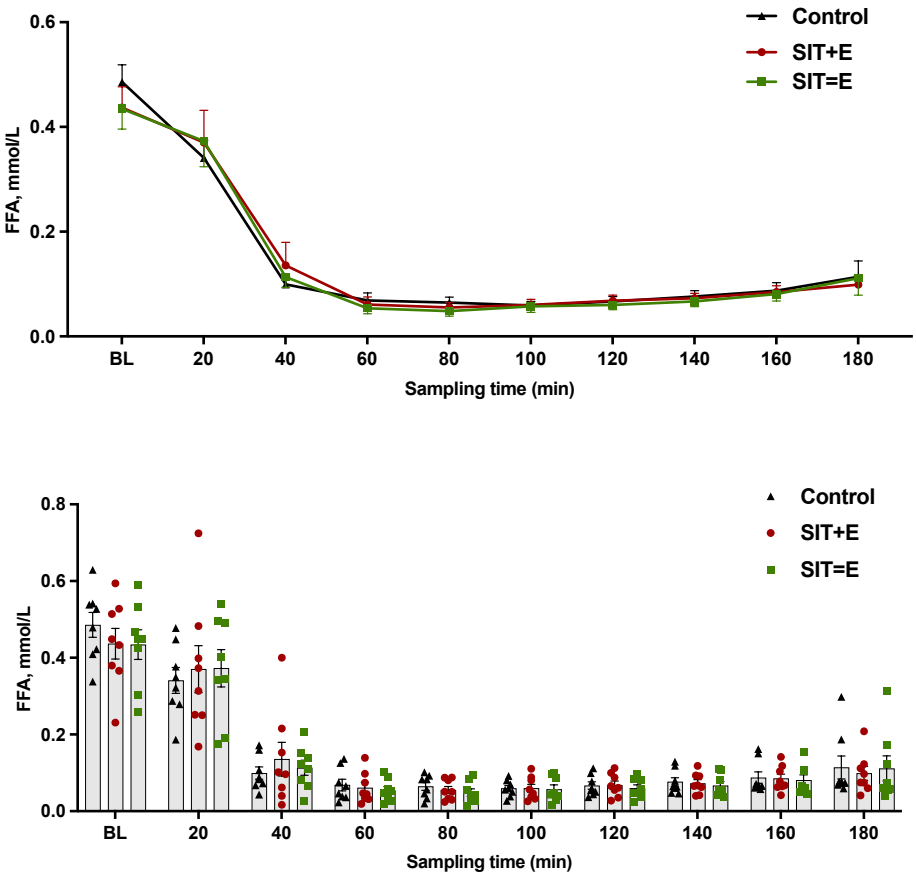


Figure 3.8. Mean ± SEM of plasma FFA concentrations (above) and individual responses (below) measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions (n=8).

Table 3.6. Serum TG concentrations, plasma FFA concentrations and area under the curve on the assessment day (Day 3) measured at baseline (fasting) and during the 3-hour postprandial period (PP) across the three conditions.[1]

	Control	SIT+E	SIT=E
TG (mmol/L)			
Fasting	0.79 ± 0.06	0.74 ± 0.06	0.72 ± 0.07
3h-PP	0.98 ± 0.07 $	0.91 ± 0.08 $	0.89 ± 0.08 $
iAUC	35.69 ± 0.00	32.69 ± 0.00	34.30 ± 0.00
FFA (mmol/L)			
Fasting	0.49 ± 0.03	0.44 ± 0.04	0.44 ± 0.04
3h-PP	0.15 ± 0.01 $	0.14 ± 0.02 $	0.14 ± 0.02 $
dAUC	-64.18 ± 0.00	-55.35 ± 0.00	-55.77 ± 0.00

[1]All values are means ± SEM (n=8). Abbreviations: PP = postprandial; TG = triglycerides; FFA = free fatty acids; iAUC = incremental area under curve; dAUC = decremental area under curve. $ different from fasting $p < .05$.

Metabolic responses carry-over effect

Analysis of variance showed no significant differences in baseline and postprandial metabolic responses between the measurement visits; insulin [$F(2,14) = 2.37$, $p = .130$], blood glucose [$F(2,14) = 2.18$, $p = .150$], TG [$F(2,14) = 0.71$, $p = .511$] and FFA [$F(2,14) = 0.90$, $p = .564$].

3.3.2.2. Cardiovascular responses

Cardiovascular parameters responses measured continuously by the Finometer at baseline (fasting) and during the 3-hour postprandial period. A summary of cardiovascular responses are presented in Table 3.7. No significant differences were found in baseline [$F(2,14) = 0.56$, $p = .585$] and postprandial [$F(180, 1260) = 0.80$, $p = .972$] levels of cardiac output between the three main trials. No significant differences were found in baseline [$F(2,14) = 0.20$, $p = .824$] and postprandial [$F(180, 1260) = 1.09$, $p = .223$] levels of stroke volume between the three main trials. No significant differences were found in baseline heart rate [$F(2,14) = 1.21$, $p = .328$]. However, there was a significant interaction difference in postprandial levels between the three main trials [$F(180, 1260) = 1.65$, $p < .001$] with large effect size of $\eta^2_p = 0.19$] illustrated in Figure 3.9. During the second hour, postprandial heart rate levels were greater in Control (80.28 ± 3.95 bpm) and SIT+E (80.39 ± 4.26 bpm) compared to SIT=E (74.47 ± 3.40 bpm) with mean differences of 5.81 and 5.93 bpm, respectively. During the third hour, postprandial heart rate levels were higher in SIT+E (82.06 ± 3.85 bpm) compared to Control (77.45 ± 2.90 bpm) and SIT=E (76.86 ± 3.07 bpm) with mean differences of 4.61 and 5.20 bpm, respectively. No significant differences were found in baseline [$F(2,14) = 0.19$, $p = .826$] and postprandial [$F(180, 1260) = 0.79$, $p = .978$] levels of MAP between the three main trials. No significant differences were found in baseline [$F(2,14) = 0.69$, $p = .516$] and postprandial [$F(180, 1260) = 0.68$, $p = .999$] levels of SBP between the three main trials. No significant differences were found in baseline [$F(2,14) = 0.02$, $p = .980$] and postprandial [$F(180, 1260) = 0.57$, $p > .999$] levels of DBP between the three main trials. No significant differences were found in baseline [$F(2,14) = 0.68$, $p = .525$] and postprandial [$F(180, 1260) = 0.67$, $p = .999$] levels of TPR between the three main trials. No significant differences were found in baseline

[*F*(2,14) = 0.57, *p* = .581] and postprandial [*F*(2,12) = 1.26, *p* = .319] levels of TVC between the three main trials.

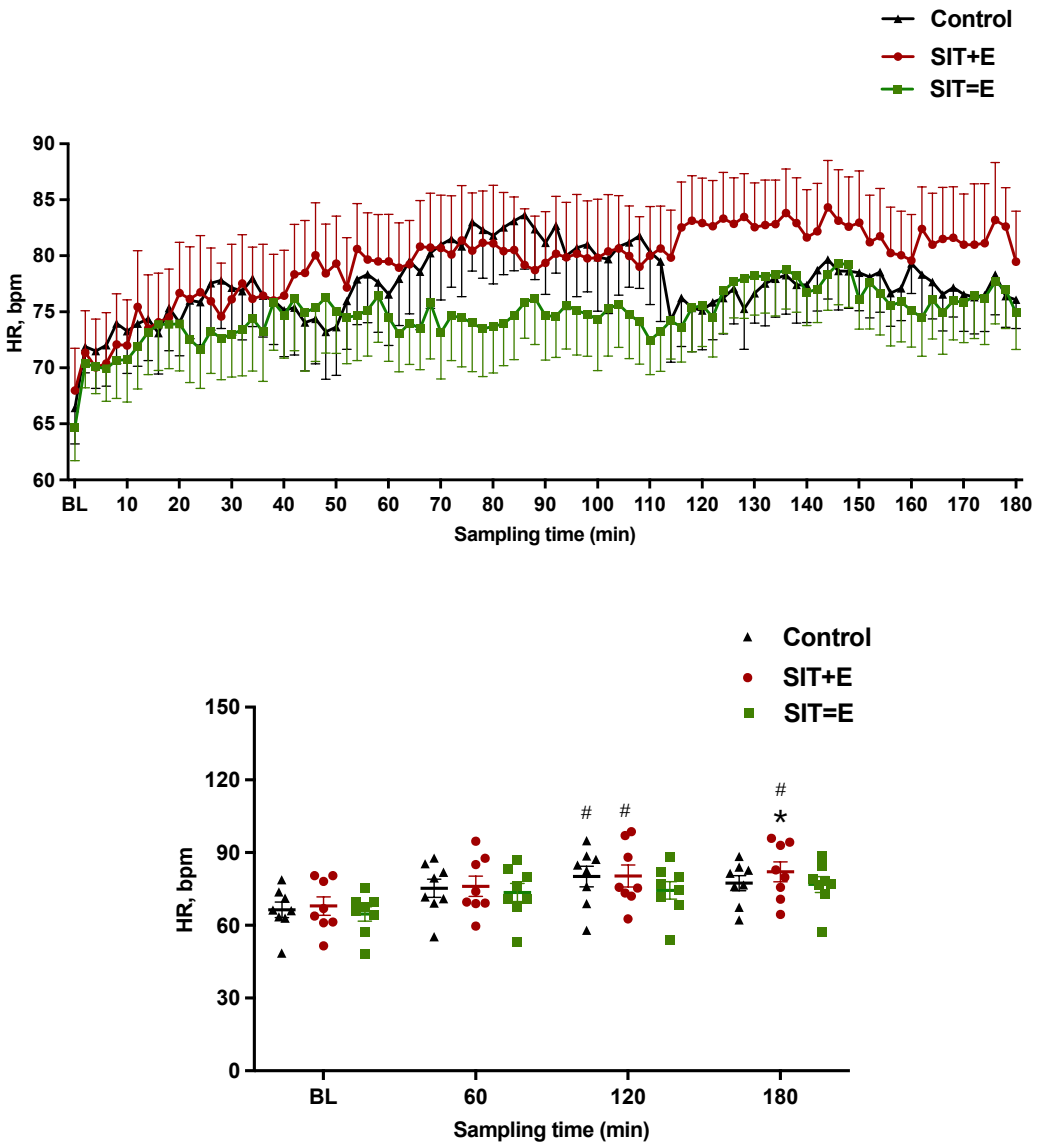


Figure 3.9. Mean ± SEM of heart rate (above) and individual responses (below) measured on the assessment day (Day 3) at baseline (fasting) and during the 3-hour postprandial period across the three conditions (n=8). * different from Control $p < .05$, # different from SIT=E $p < .05$

Table 3.7. Summary of the cardiovascular parameters measured continuously for three hours on the assessment day (Day 3) during baseline (fasting) 3-hour postprandial using the Finometer across the three conditions.[1]

	Control	SIT+E	SIT=E
CO (L/min)			
Fasting	5.02 ± 0.44	5.26 ± 0.31	5.12 ± 0.35
3-hr PP	6.40 ± 0.64	6.29 ± 0.38	6.58 ± 0.51
SV (ml/beat)			
Fasting	76.35 ± 5.20	78.18 ± 3.66	78.85 ± 5.18
3-hr PP	82.92 ± 7.27	79.09 ± 3.70	87.73 ± 5.64
HR (bpm)			
Fasting	66.42 ± 3.18	67.98 ± 3.77	64.72 ± 3.00
3-hr PP	77.66 ± 3.59 $	79.52 ± 4.21 $ *	74.96 ± 3.48 $ * §
MAP (mmHg)			
Fasting	84.08 ± 2.15	82.69 ± 2.46	84.45 ± 3.36
3-hr PP	85.31 ± 2.38	86.42 ± 2.69	88.70 ± 2.88
SBP (mmHg)			
Fasting	119.51 ± 4.41	114.19 ± 2.85	118.08 ± 4.25
3-hr PP	125.41 ± 5.34	123.66 ± 3.88 $	125.67 ± 4.20 $
DBP (mmHg)			
Fasting	69.98 ± 1.93	69.64 ± 2.51	70.17 ± 2.78
3-hr PP	71.30 ± 2.90	71.87 ± 2.46	72.37 ± 2.34
TPR (mmHg.L/min)			
Fasting	17.47 ± 1.56	15.96 ± 0.70	16.79 ± 1.30
3-hr PP	14.23 ± 1.10	14.20 ± 0.61	14.28 ± 0.98
TVC (mL.min^{-1}. mmHg ^{1})			
Fasting	59.71 ± 1.39	63.61 ± 0.87	60.63 ± 1.01
3-hr PP	75.02 ± 0.47	72.78 ± 0.45	74.18 ± 0.47

[1]All values are means ± SEM (n=8). Abbreviations: PP = postprandial; CO = cardiac output; SV= stroke volume; HR = heart rate; MAP = mean arterial pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; TPR = total peripheral resistance. $ different from fasting $p < .05$, * different from Control $p < .05$, § different from SIT+E $p < .05$

3.3.2.3. Calf blood flow

No differences in fasting calf blood flow across the three conditions [$F(2,12) = 0.11$, $p = .893$]. However, postprandial blood flow responses increased from baseline in all three trials, time main effect [$F(6,36) = 10.41$, $p < .001$ with large effect of $\eta^2_p = 0.63$], but no differences were found between trials [$F(12,72) = 0.86$, $p = .591$] (Figure 3.10).

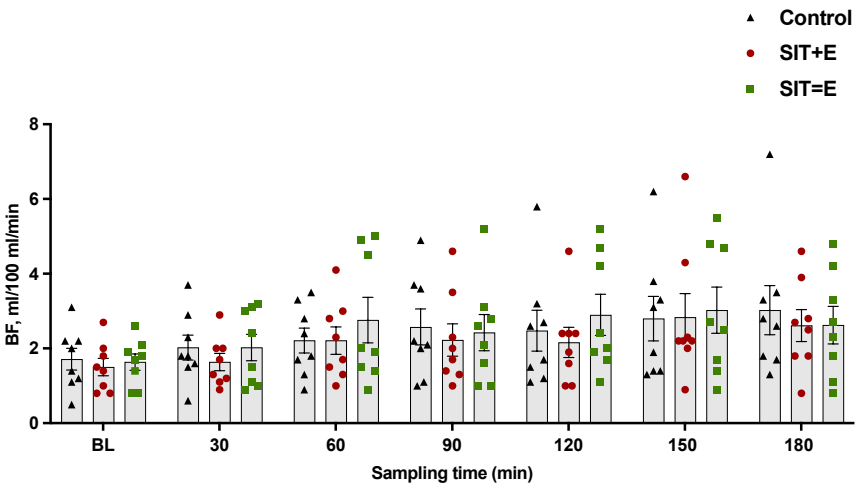


Figure 3.10. Mean ± SEM of the calf blood flow (BF) on the assessment day (Day 3) measured for 2 minutes using the venous occlusion plethysmography at baseline (fasting) and every 30 minutes during the 3-hour postprandial period across the three conditions (n=8).

Cardiovascular responses carry-over effect

Analysis of variance showed no significant differences in baseline and postprandial cardiovascular responses between the three measurement visits; CO [$F(2,14) = 0.16$, $p = .857$], SV [$F(2,14) = 1.20$, $p = .330$], HR [$F(2,14) = 1.71$, $p = .217$], MAP [$F(2,14) = 0.32$, $p = .728$], SBP [$F(2,14) = 0.33$, $p = .726$], DBP [$F(2,14) = 0.03$, $p = .971$], TPR [$F(2,14) = 0.36$, $p = .706$], TVC [$F(2,14) = 0.04$, $p = .959$] and calf blood flow [$F(2,14) = 0.13$, $p = .877$].

3.3.2.4. Energy metabolism

Mean energy expenditure and substrate oxidation measured at baseline (fasting) and during the 3-hour postprandial period across all three trials were presented in Table 3.8. Postprandial REE, VO2, VCO2, RQ and substrate utilisation were increased significantly compared to fasting levels ($p < .001$). No differences in fasting [$F(2,14) = 2.69$, $p = .103$] and postprandial [$F(6,36) = 0.41$, $p = .867$] VO_2 were found between the three trials. Fasting [$F(2,14) = 2.10$, $p = .165$] and postprandial [$F(6,30) = 0.56$, $p = .762$] VCO_2 were not different between across trials. REE was not different between trials during fasting [$F(2,12) = 2.71$, $p = .107$] and postprandial [$F(2,12) = 1.56$, $p = .250$]. No significant difference in DIT between the three trials [$F(2,12) = 0.03$, $p = .975$]. Fat utilisation during fasting [$F(2,12) = 2.17$, $p = .266$] and postprandial [$F(6,36) = 0.86$, $p = .537$] showed no significant difference. Fasting [$F(2,12) = 1.08$, $p = .371$] and postprandial [$F(6,36) = 0.79$, $p = .582$] CHO utilisation were not different between the three trials. Fasting [$F(2,12) = 1.65$, $p = .232$] and postprandial [$F(6,36) = 1.06$, $p = .406$] RQ were not different between the main trials.

Table 3.8. Energy metabolism on the assessment day (Day 3) measured by indirect calorimetry at baseline (fasting) and the 3-hour postprandial period across the three conditions.[1]

	Control	SIT+E	SIT=E
REE (kJ/min)			
Fasting	3.86 ± 0.19	3.92 ± 0.20	3.64 ± 0.29
3h-PP	4.58 ± 0.23 $	4.59 ± 0.22 $	4.34 ± 0.30 $
VO_2 (L/min)			
Fasting	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01
3h-PP	0.23 ± 0.01 $	0.23 ± 0.01 $	0.22 ± 0.02 $
VCO_2 (L/min)			
Fasting	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
3h-PP	0.19 ± 0.01 $	0.18 ± 0.01 $	0.18 ± 0.01 $
CHO (mg/kg/min)			
Fasting	1.53 ± 0.29	1.17 ± 0.23	1.62 ± 0.30
3h-PP	2.83 ± 0.45 $	2.45 ± 0.36 $	2.99 ± 0.42 $
CHO (%)			
Fasting	26.08 ± 1.53	18.62 ± 3.04	31.77 ± 3.70
3h-PP	40.23 ± 6.51 $	34.18 ± 5.33 $	46.83 ± 7.26 $
Fat (mg/kg/min)			
Fasting	1.66 ± 0.15	1.84 ± 0.08	1.49 ± 0.13
3h-PP	1.57 ± 0.21	1.73 ± 0.16	1.31 ± 0.27
Fat (%)			
Fasting	73.92 ± 1.66	81.38 ± 3.04	68.24 ± 3.70
3h-PP	59.77 ± 6.51 $	65.82 ± 5.33 $	53.18 ± 7.26 $
RQ			
Fasting	0.78 ± 0.02	0.76 ± 0.01	0.79 ± 0.01
3h-PP	0.83 ± 0.02 $	0.81 ± 0.02 $	0.84 ± 0.02 $
DIT (kJ/min)	0.72 ± 0.11	0.68 ± 0.12	0.71 ± 0.17
DIT (%)	18.65 ± 2.74	17.09 ± 3.35	19.23 ± 5.13

[1]All values are means ± SEM (n=8). Abbreviations: PP = postprandial; REE = resting energy expenditure; VO_2 = oxygen uptake; VCO_2 = carbon dioxide production; CHO = carbohydrate; RQ = respiratory quotient; DIT = diet-induced thermogenesis. $ different from fasting $p < .05$.

Energy metabolism carry-over effect

Analysis of variance showed no significant differences in baseline and postprandial energy metabolism between the measurement visits; VO2 [$F(2,14) = 2.70$, $p = .102$], VCO2 [$F(2,14) = 1.74$, $p = .211$]**,** REE [$F(2,14) = 2.21$ $p = .147$], CHO utilisation [$F(2,14) = 1.42$, $p = .275$], fat utilisation [$F(2,14) = 2.82$, $p = .094$] and RQ [$F(2,14) = 2.06$, $p = .165$].

3.3.3. Secondary analysis

3.3.3.1. Gender differences

No gender differences were found in serum insulin, blood glucose and plasma FFA. Males had higher levels of serum TG compared to females [$F(1,6) = 8.35$, $p = .028$ with large effect of $\eta^2_p = 0.58$] with mean differences of 0.55 mmol/L in Control, 0.17 mmol/L in SIT+E and 0.50 mmol/L in SIT=E. There were significant gender differences in stroke volume [$F(1,6) = 9.91$, $p = .020$, large effect of $\eta^2_p = 0.62$], cardiac output [$F(1,6) = 11.36$, $p = .015$, large effect of $\eta^2_p = 0.65$], TPR [$F(1,6) = 17.33$, $p = .006$, large effect of $\eta^2_p = 0.74$] and TVC [$F(1,6) = 11.75$, $p = .014$, large effect of $\eta^2_p = 0.66$]. Males (94.24 ± 4.94 ml/beat) had greater stroke volume response than females (72.26 ± 4.94 ml/beat) with a mean difference of 21.98 ml/beat. Cardiac output response was higher in men (7.46 ± 0.43L/min) compared to women (5.39 ± 0.43 L/min) with a mean difference of 2.07 L/min. Whereas, TPR responses in females (16.09 ± 0.63 mmHg.min/L) were greater than males (12.39 ± 0.63 mmHg.min/L) with a mean difference of 3.70 mmHg.min/L. TVC values were higher in males (75.90 ± 3.44 $mL.min^{-1}.mmHg^{-1}$) compared to females (59.22 ± 3.44 $mL.min^{-1}.mmHg^{-1}$) with a mean difference of $mL.min^{-1}.mmHg^{-1}$. There was a significant gender difference in VO_2 [$F(1,6) = 28.19$, $p = .002$ with large effect $\eta^2_p = 0.83$]. Males (0.24 ± 0.00 L/min) had a greater VO_2 levels compared to females (0.19 ± 0.00 L/min) with a mean difference of 0.06 L/min. There was a significant gender difference in VCO_2 [$F(1,5) = 23.19$, $p = .005$ with large effect $\eta^2_p = 0.82$]. Males (0.20 ± 0.00 L/min) had a greater VCO_2 levels compared to females (0.15 ± 0.00 L/min) with a mean difference of 0.05 L/min. There was a significant gender difference in REE [$F(1,6) = 51.29$, $p < .001$ with large effect $\eta^2_p = 0.93$]. Males (4.71 ± 0.11 kJ/min) had greater REE levels compared to females

(3.60 ± 0.11 kJ/min) with a mean difference of 1.12 kJ/min. There was no differences in substrate oxidation between genders.

3.3.3.2. FreeStyle Libre Abbott reliability analysis (Control trial)

Figure 3.11 present 24-hour interstitial glucose concentrations measured by the iPro2 and the Abbott sensors for Day 1 intervention, Day 2 intervention and mean of the 2-day intervention. Abbott's glucose readings showed different patterns over the 24-hour periods between the two devices; on both days the Abbott exhibited lower values during the night-time and higher spikes during the day-time when compared to the iPro2. On Day 1, there was a significant interaction effect [$F(96,672) = 1.80$, $p < .001$ with large effect of $\eta^2_p = 0.20$] between the two CGMs. In compared to iPro2 (4.86 ± 0.02 mmol/L), interstitial glucose recorded by the Abbott (4.59 ± 0.04 mmol/L) were lower with a mean difference of 0.27 mmol/L. Similarly on Day 2, there was a significant interaction effect [$F(96,672) = 1.94$, $p < .001$ with large effect of $\eta^2_p = 0.22$] between the two CGMs. iPro2 (5.24 ± 0.02 mmol/L), interstitial glucose concentrations were greater than Abbott (4.94 ± 0.05 mmol/L) with a mean difference of 0.30 mmol/L. The mean of the two days has shown a significant interaction effect [$F(96,672) = 3.52$, $p < .001$ with large effect of $\eta^2_p = 0.33$] were Abbott (4.79 ± 0.04 mmol/L) was lower than iPro2 (5.06 ± 0.02 mmol/L) with a mean difference of 0.27 mmol/L.

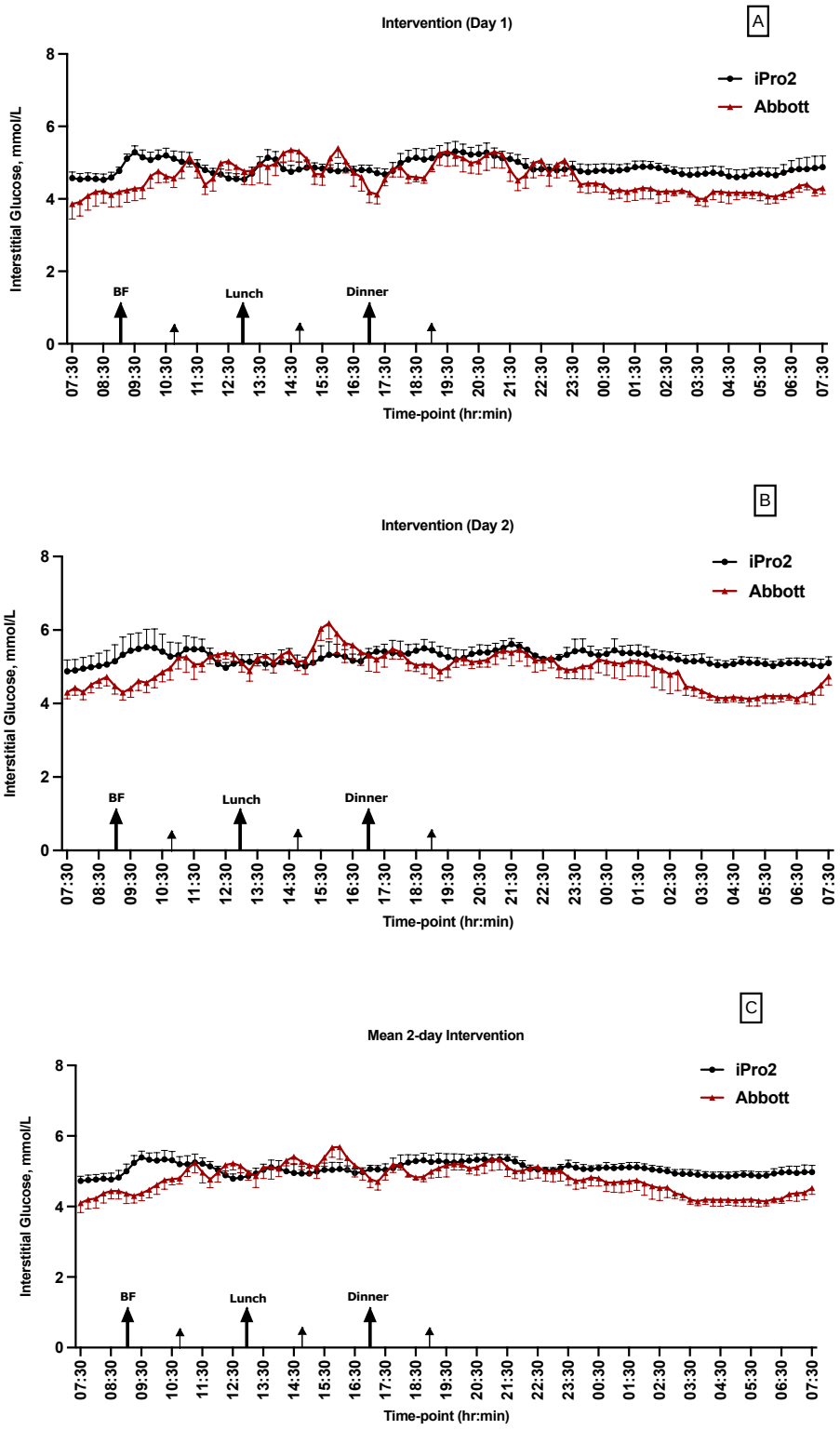


Figure 3.11. Mean ± SEM of interstitial glucose concentrations over a 24-hr period for A) day 1 intervention, B) day 2 intervention and C) mean of the 2-day intervention using two continuous glucose monitoring systems (iPro2 versus Abbott). Main meals (indicated by big arrows) were provided at 09:00, 13:00 and 17:00 hr. Snacks (indicated by small arrows) were given at 10:45, 14:45 and 19:00 hr. Participants were instructed to sleep from 23:30 to 07:30 hr (n=8). Abbreviations: BF=breakfast**.**

On day 3, under standardised laboratory conditions, glucose concentration measurements were compared between the YSI, iPro2 and Abbott at baseline (fasting) and during a 3-hour postprandial period (Figure 3.12). There was a treatment main effect [$F(1.77,12.36) = 9.23$, $p = .004$ with large effect of $\eta^2_p = 0.57$]. iPro2 (6.12 ± 0.10 mmol/L) showed higher glucose concentrations compared to the YSI (5.50 ± 0.09 mmol/L) and Abbott (5.53 ± 0.10 mmol/L) with mean differences of 0.62 and 0.59 mmol/L, respectively.

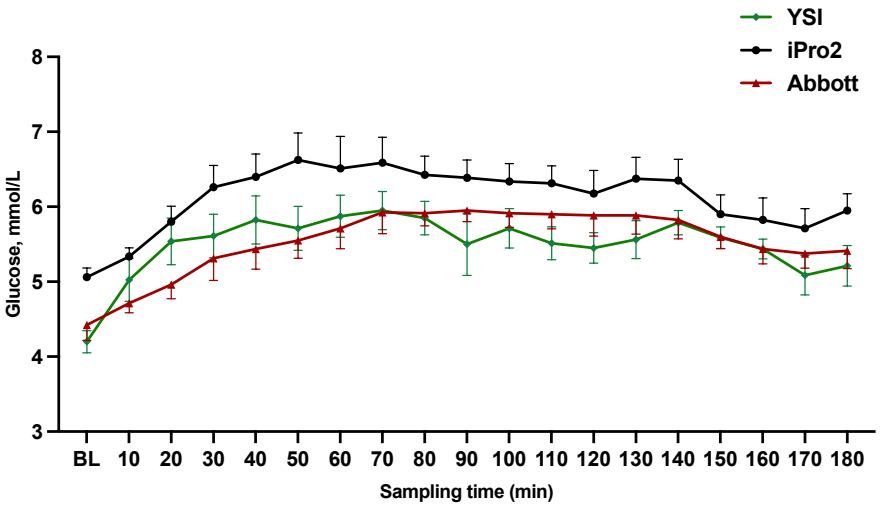


Figure 3.12. Mean ± SEM of glucose concentrations on the assessment day (Day 3) measured at baseline (fasting) and the 3-hour postprandial period using the iPro2, Abbott and YSI at baseline and every 10 minutes during the 3-hr postprandial period (n=8).

Table 3.9 shows the results of the comparison between the CGMs against the reference blood glucose measured by the YSI. The correlation between iPro2 versus YSI and Abbott versus YSI was assessed using the squared Person's coefficient r^2, ranged from very poor (participant 3, $r^2 = 0.017$) to very good (participant 6, $r^2 = 0.875$) in the case of iPro2 versus YSI, while ranged from very poor (participant 3, $r^2 = 0.008$) to very good (participant 6, $r^2 = 0.930$) in the case of Abbott versus YSI. As a result, the overall r^2 value for pooled data from all participants was equal to 0.131 for iPro2 versus YSI and 0.210 for Abbott versus YSI. The mean difference between iPro2 versus YSI (0.63 ± 0.53 mmol/L) was significantly greater than between the Abbott versus YSI (0.03 ± 0.45 mmol/L) with a mean difference of 0.60 mmol/L [7) = 4.40, p = .024, d = 1.22 (large effect)]. There was no significant difference between MARD's [t(7) = 0.87, p = .415] and [t(7) = 1.13, p = .297] MAD's of the two comparison.

Table 3.9. Head-to-head comparison between iPro2 and Abbott systems against the YSI on the assessment day (Day 3) of the Control trial

Participant no.	Pooled	P1	P2	P3	P4	P5	P6	P7	P8
iPro2 versus YSI									
r^2	0.131	0.700	0.236	0.017	0.335	0.748	0.875	0.149	0.044
Mean difference iPro2 - YSI (mmol/L)	0.63 ± 0.53	0.45 ± 0.46	0.02 ± 0.56	1.40 ± 0.72	1.04 ± 0.53	0.14 ± 0.28	0.86 ± 0.80	0.05 ± 0.63	1.06 ± 1.24
MAD (mmol/L)	0.82 ± 0.46	0.48 ± 0.42	0.47 ± 0.28	1.40 ± 0.72	1.06 ± 0.48	0.22 ± 0.21	1.02 ± 0.57	0.49 ± 0.38	1.41 ± 0.79
MARD, %	16.15 ± 10.41	9.53 ± 9.72	8.96 ± 5.50	33.79 ± 22.29	21.65 ± 11.94	4.09 ± 4.10	16.53 ± 7.97	8.02 ± 6.22	26.63 ± 16.71
Abbott versus YSI									
r^2	0.210	0.597	0.775	0.008	0.335	0.036	0.930	0.149	0.044
Mean difference YSI-Abbott (mmol/L)	0.03 ± 0.45	-0.28 ± 0.50	0.19 ± 0.47	0.65 ± 0.66	0.43 ± 0.53	-0.28 ± 1.16	-0.34 ± 0.40	-0.56 ± 0.63	0.45 ± 1.24
MAD (mmol/L)	0.68 ± 0.26	0.47 ± 0.32	0.43 ± 0.25	0.77 ± 0.51	0.54 ± 0.41	0.97 ± 0.65	0.43 ± 0.30	0.68 ± 0.50	1.11 ± 0.67
MARD, %	12.69 ± 5.28	8.35 ± 5.45	8.13 ± 4.67	18.82 ± 16.75	11.19 ± 9.59	16.60 ± 10.30	6.99 ± 4.24	10.72 ± 7.19	20.69 ± 13.36

All values are means ± SD (n=8). Abbreviations: YSI = Yellow Spring Instrument; MAD = mean absolute difference; MARD = mean absolute relative difference.

The agreement of each CGM with the YSI blood glucose measurement method was assessed via one sample t-test, Bland-Altman plot and linear regression analysis to detect if systematic differences exist between measurements. One sample t-test for the differences between iPro2 and YSI measurements showed significant difference [$t(7) = -3.28$, $p = .014$, d = 0.55 (large effect)] which indicates that both device were not similar. In contrast, one sample t-test for the differences between Abbott and YSI measurements showed non-significant difference [$t(7) = -0.31$, $p = .77$] which indicates that the two devices were similar. Because t-test was not statistically significant for the latter comparison, Bland-Altman plot can be generated to compare the performance of Abbott against the YSI (see Figure 3.13B). Although there was a statistically significant difference between the iPro2 and YSI, a Bland-Altman plot was generated to visually compare the performance of the two CGMs against the YSI measurements (see Figure 3.13A). Bland-Altman analysis showed a mean difference between the iPro2 and YSI methods (bias) of -0.64 mmol/L, with 95% limits of agreement (confidence interval; CI) between 0.44 mmol/L to -1.72 mmol/L. When the Abbott values were compared to YSI blood glucose values, the bias was -0.05 mmol/L, with 95% limits of agreement between 0.86 mmol/L to -0.96 mmol/L. Abbott against YSI showed lower bias value and less scattered measurements compared to iPro2 against YSI. Linear regression analysis was conducted to check if there was a proportional bias between the Abbott and YSI measurements. Results showed that there was no significant linear relationship between the difference and the mean of measurements [b = 1.02, $t = 2.43$, $p = .051$] which indicates the absence of proportional bias.

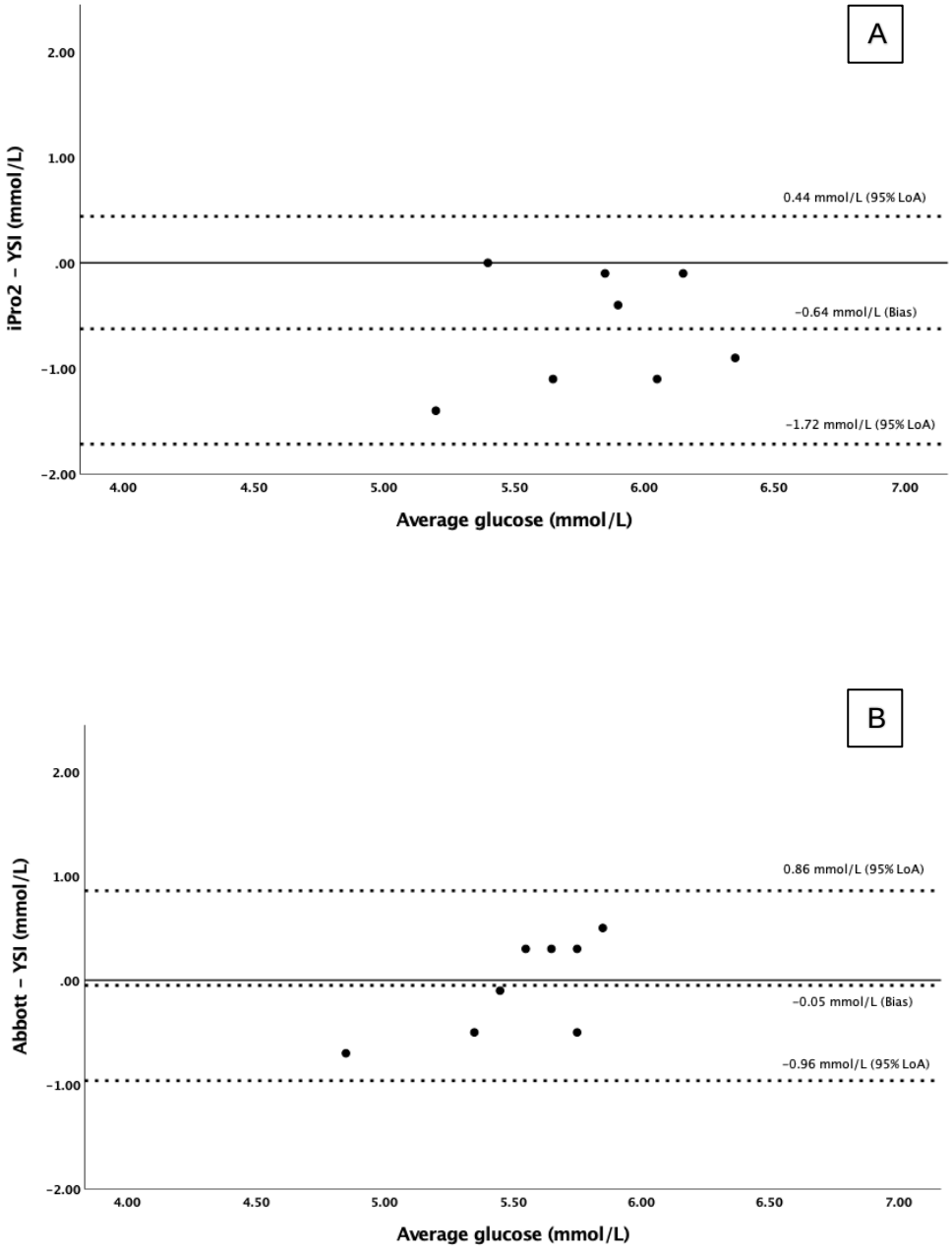


Figure 3.13. Presents two Bland–Altman plots comparing interstitial glucose concentrations recorded by iPro2 sensors and Abbott sensors against the reference method YSI; A) iPro2 vs YSI and B) Abbott vs YSI. Each plot displays the bias and 95% levels of agreement (95% LoA). The x-axis represents the average of the two measurements obtained by YSI and each CGM devise. The y-axis represents the difference between YSI and the corresponding CGM values (n=8).

3.4. Discussion

3.4.1. Cardiometabolic effects of physical inactivity

The present study aimed to investigate the acute cardiometabolic effects of two days of imposed reduced physical activity, with and without a proportional decrease in energy intake among healthy and moderately active men and women. It was speculated that low levels of activity, combined with increased energy intake, predispose people to deleterious effects, which can be ameliorated if energy intake is reduced to match the decrease in activity level. The protocol was designed as free-living to mimic current daily lifestyles, where results can be generalised to most of the population. The main findings suggest physical inactivity with an energy surplus led to a 6.1% increase in 24-hour interstitial glucose concentrations. This effect was not seen when physical inactivity was matched with reduced energy intake. Although no changes were observed in postprandial glucose concentrations and insulin sensitivity indices in the present study, postprandial serum insulin iAUC tended to show an increase after two days of physical inactivity in the presence of positive energy balance compared to the control group. When energy intake was reduced, this effect was ameliorated. No changes in cardiac output, stroke volume, MAP, SBP, DBP, TPR and TVC responses after inactivity were observed. However, postprandial heart rate levels increased following two days of inactivity with an energy surplus, compared to the active state but not during inactivity with isoenergetic food intake. In addition, inactivity with and without an energy surplus did not affect calf blood flow among the three conditions. The carry-over effect assessment supported that the washout period of at least two weeks between the main trials was sufficient to prevent any carry-over effects on the study outcomes.

It appears that positive energy balance, frequently associated with physical inactivity, rather than physical inactivity *per se*, might be responsible for these detrimental effects on 24-hour interstitial glucose concentrations. Two previous studies applied CGM sensors during the free-living intervention but for longer periods of inactivity, three days (Mikus et al., 2012) and five days (Reynolds et al., 2015). The findings from both studies showed that reduced daily steps from >10,000 to <5,000, with an energy surplus, significantly increased interstitial glucose levels. This supports the negative effect of inactivity with a positive energy balance on the interstitial glucose levels found in the present experiment.

Only two days of physical inactivity with a high energy intake could trigger alterations in insulin action or secretion. A potential mechanism to explain this effect on insulin action might be related to energy surplus, which inhibits key components of the insulin signalling pathway secondary to the overabundance of glucose, fatty acids and/or amino acids (Patti, 1999). In a similar methodological approach, two studies examined the short-term effects of inactivity with and without an energy surplus (Stephens et al., 2011; Winn et al., 2019). Stephens et al. (2011) detected a significant decrease in insulin action by 39% following one day of prolonged sitting with increased energy intake, and this was partially improved when energy intake was reduced to create an energy balance. Winn et al. (2019) observed significant increases in insulin and glucose concentrations and a decrease in insulin sensitivity after 10 days of reduced steps count, from 10,000 to < 5,000 steps/day, with increased energy intake by 35–40%. These changes were abolished when energy intake was decreased by 15–20% to match energy expended during inactivity. The findings from these studies support our results, in which positive energy balance has a major role in initiating the

deleterious effects on insulin sensitivity. The early changes in insulin action noted in the Stephens et al. (2011) study could be due to the nature of inactivity imposed. The authors conducted each trial in a restricted laboratory-based environment of prolonged sitting, while increasing energy intake by 4,184 kJ during the inactivity with the energy surplus trial — whereas ours, a free-living protocol, was applied with 24-hour continuous monitoring of glycaemic control, with increasing energy intake by 2,733 kJ during the inactivity with an energy surplus trial. In the former study, the participants were asked to refrain from any exercise before each main trial, which may lower their habitual physical level while participants in the present study maintained their habitual physical activities with no restrictions. Applying an extreme form of inactivity (i.e. prolonged sitting), preceded by a day with low activity, may enhance the earlier appearance of negative health outcomes. In contrast, Winn et al. (2019) had a longer intervention period (10 vs 2 days) and a greater amount of energy intake increase (35–40% vs 25%) compared to the present study protocol, which highlight the major role of energy intake manipulation and long periods of inactivity exposure on the human body. Several research studies have examined the acute effects of inactivity (ranging from 3 to 14 days) using the daily steps reduction approach (<5,000 steps per day) with an energy surplus on glycaemic control and insulin sensitivity (Bowden Davies et al., 2018, 2021; Credeur et al., 2018; Dixon et al., 2013; Knudsen et al., 2012; Krogh-Madsen et al., 2010; Lyden et al., 2015; Mikus et al., 2012; Olsen et al., 2008; Reynolds et al., 2015; Walhin et al., 2013). The findings from these studies showed a significant reduction in glycaemic control and insulin sensitivity, which indicates a few days of reduced physical activity is sufficient to trigger significant adverse changes in glucose and insulin responses. Increases in insulin AUC were similarly observed in previous research (Bowden Davies et al., 2018; Dixon et al., 2013; Knudsen et al.,

2012; Lyden et al., 2015; Olsen et al., 2008; Walhin et al., 2013). Following three days of inactivity with an energy surplus, the insulin AUC was increased by 34% from baseline in healthy young men. The findings from these studies are compatible with the present results, but detrimental changes of physical inactivity with positive energy intake emerged earlier during the first couple of days using free-living and standardised laboratory environments.

Postprandial heart rate levels increased during inactivity with an energy surplus. The heart rate responses to a standardised mixed meal in healthy young men were previously measured by Fagan et al. (1986). The findings from Fagan et al.'s study showed heart rate levels were significantly increased during the three-hr postprandial period, with maximal values occurring one hour after the meal. In the present experiment, the postprandial increase in heart rate persists till the third hour with no concomitant changes in cardiac output or stroke volume. However, the increase in heart rate was associated with an increase in insulin response, which is consistent with the possibility that increased heart rates were secondary to postprandial insulin-induced sympathetic nervous system activation through the β-adrenergic receptors in the heart (Facchini, Stoohs and Reaven, 1996; Moreira et al., 2015; Scherrer and Sartori, 1997). Similarly, O'Brien et al (2019) documented an increase in heart rate levels by 6% in men and 7% in women. In contrast to the present experiment, heart rate elevation was associated with decreases in stroke volume levels (5% in men and 4% in women) and TVC (6% in men and 8% in women) following three hours of uninterrupted sitting. Significant changes in heart rate, stroke volume and TVC within a few hours of inactivity could be attributed to the restricted protocol design applied and the larger sample size. Energy intake was not reported, which makes it difficult to

interpret the underlying factor in regard to whether these changes were induced by the inactivity *per se* or by inactivity with a positive energy balance.

The amount of force applied by the blood on the arterial wall is expressed by the peripheral blood pressure, which also highlights the tremendous pressure inflicted on major organs. Based on a systematic review and meta-analysis by Paterson et al. (2022), in response to an acute bout of prolonged uninterrupted sitting, MAP and SBP showed small but significant changes, with mean differences of 3.3 mmHg and 3.2 mmHg, respectively, whereas DBP showed a non-significant increase, with a mean difference of 0.1 mmHg. The elevated blood pooling that has been seen in the lower extremities during prolonged sitting could be the reason for the increase in MAP and SBP. As a result, there would be a fall in venous return, which has been shown to reduce renal perfusion pressure and activate the renin-angiotensin-aldosterone pathway, eventually raising blood pressure (Atlas, 2007; Shvartz et al., 1983). Hamburg et al. (2007) reported that bed rest increased SBP by 3 mmHg on day 3 and 7 mmHg by day 7 compared to the baseline, with no differences found in DBP, whereas Nosova et al. (2014) found a 4 mmHg increase in DBP after five days of bed rest compared to the baseline, with no differences in SBP. The increase in SBP and DBP in these studies was attributed to the increase in basal arterial tone and vascular resistance secondary to muscular inactivity, which manifested by increased arterial wall stiffness. Furthermore, extreme forms of inactivity models (i.e. bed rest and prolonged uninterrupted sitting) could be major contributors to the development of such pathological changes in blood pressure compared to free-living settings. In addition, most measurements in these studies were taken at single time points while fasting. In the present study, the Finometer was used to assess cardiovascular

responses in a beat-to-beat fashion, which is more reliable for detecting small changes that may be missed with single measurements. In addition, cardiovascular parameters were measured during fasting and postprandial states. Postprandial measurements of cardiovascular parameters integrate metabolic and cardiovascular responses, which can assist in proposing risk factors and underlying mechanisms (Cavalot et al., 2006; O'Keefe and Bell, 2007). Physiologically, insulin is released in response to the rise in blood glucose levels after meal consumption. Insulin promotes an increase in blood flow secondary to the activation of nitric oxide (NO)-induced vasodilatation, which is seen mostly during the first hour of the postprandial period (Muniyappa et al., 2007). The current study showed postprandial calf blood flow increased from baseline but not between trials. In contrast, Hamburg et al. (2007) found a significant decrease in the calf reactive hyperaemia after non-free living (bed rest) and a longer period (five days) of inactivity, as measured using the VOP. The authors attributed this effect to the reduced resting blood flow levels, which led to decreases in shear stress and endothelial nitric oxide synthase (eNOS) expression. The reduction in calf blood flow in their study was associated with increases in glucose AUC by 6% and insulin AUC by 67% in response to OGTT. Reduced leg blood flow and insulin sensitivity following inactivity were also observed in other studies (Arciero et al., 1998; Mikines et al., 1991). It is thought these changes occurred concurrently because insulin action and eNOS activation share a common signalling pathway through the phosphatidylinositol-3 kinase (PI3-K) and Akt system to enhance NO-induced vasodilatation and peripheral glucose uptake (see Chapter 1, Figure 1.3. The general features of insulin signalling pathways and interacting mechanisms of glucose uptake and vascular tone under healthy conditions), which support the idea that there exists a direct relationship between insulin resistance and vascular malfunction. For instance, decreased calf

blood flow can lead to insulin resistance by decreasing insulin-mediated glucose disposal.

Irrespective of energy balance, the data showed no differences in REE, DIT and substrate oxidation among the different conditions. Significant changes in energy expenditure can be observed when there is a weight gain, specifically, fat-free mass. When food intake changes, DIT, which makes up around 10% of the total energy consumed during a normal diet, also changes, which in turn affects energy expenditure. A rise in DIT results from an increase in intake, which raises energy expenditure (Westerterp, 2017). Participants' body weight did not significantly change in this study among the three conditions, which indicates no significant changes occurred in body mass that can be explained by the short intervention period applied, which lowers the chance of developing major changes in body mass. The data suggested postprandial increases in CHO contribution and decreases in fat utilisation from a fasted state following active and inactive conditions, which was evident by the increase in postprandial RQ values, indicating the tendency toward CHO as a source of fuel. The balance macronutrient composition of the test drink given during measurements and the stable body weight of participants could prevent the occurrence of significant alterations in substrate oxidation. Prior studies that examined the effect of physical inactivity on energy metabolism had opposite findings (Arciero et al., 1998; Blanc et al., 2000; Dirks et al., 2018; Dirks et al., 2016; Kim et al., 2014; Krogh-Madsen et al., 2010; Mikines et al., 1991; Stubbs et al., 2004; Walhin et al., 2013). On the one hand, some studies have documented significant changes in energy metabolism. For instance, Arciero et al. (1998) found significant reductions in REE by 4% and DIT by 5% following seven days of detraining. Walhin et al. (2013)

documented a significant 11% increase in REE after seven days of reduced step count with overfeeding. Following one week of bed rest, Dirks et al. (2016) observed a significant decrease in REE by 4%. Changes in REE and DIT were mostly found after one week of applied inactivity, which may indicate longer periods of inactivity are required to detect alterations. One day of prolonged sitting yielded a significant increase of 25% in carbohydrate oxidation and a decrease in fat oxidation compared with a similar period of moderate-intensity physical activity in response to a high fat tolerance test (Kim et al., 2014). On the other hand, some studies could not find significant changes (Dirks et al., 2018; Mikines et al., 1991). In support to our data, Dirks et al. (2018) measured energy expenditure and substrate oxidation in response to bed rest with and without a proportional decrease in energy intake of a balanced diet and found no differences in energy metabolism between the two conditions. Despite the similar restrictive inactivity model and intervention duration in the Kim et al. and Dirks et al. studies, their findings highlight the importance of standardising energy intake composition to eliminate the cofounding factors of macronutrient abundance, which could influence the outcomes. The energy intake during the intervention and lab measurements were well-controlled and balanced in the present study.

There were no changes in fasting and postprandial serum TG and plasma FFA. Although many researchers have not observed significant changes (Arciero et al., 1998; Burns et al., 2021; Krogh-Madsen et al., 2010; Lyden et al., 2015; Nosova et al., 2014; Winn et al., 2019), others have (Blanc et al., 2000; Dixon et al., 2013; Hagobian and Braun, 2006; Hamburg et al., 2007; Olsen et al., 2008). Dixon et al. (2013) and Lyden et al. (2015) measured TG levels after one week of reduced daily steps to less

than 5,000, with an energy surplus. Lyden et al. (2015) did not find changes in fasting plasma TG in men and women, whereas Dixon et al. (2013) were able to detect increases in fasting TG concentrations among healthy men. These observations may suggest longer periods of more than two days of inactivity are required to detect changes in lipids. Another possibility is that gender-related effects could play a role, where differences were evident when only men were investigated. It has been documented that there are distinct differences in lipid oxidation and storage between men and women (Blaak, 2001).

3.4.2. Head-to-head comparison between two continuous glucose monitoring systems

The current reliability analysis aimed at exploring the performance of the newly established factory-calibrated FreeStyle Libre Abbott sensor. To our knowledge, no previous studies have investigated the agreement of the FreeStyle Libre Abbott sensor against the Medtronic iPro2 sensor and arterialised blood glucose measurements obtained using YSI among young and healthy normo-glycaemic participants over two days of a free-living period and during a three-hour postprandial period under standardised laboratory conditions. Arterialised blood glucose measurements were used as a reference method during the postprandial period to reflect true arterial blood glucose levels. The findings from the present study showed that, during the two-day free-living period, the FreeStyle Libre Abbott produced lower mean interstitial glucose values by 5% compared to the iPro2. The reason behind this difference between the two CGMs remains unclear. One possibility is the recommended site of insertion, where the FreeStyle Libre Abbott sensors are to be inserted only in the upper arm and the iPrco2 sensors in the abdomen according to the manufacturer's user guides.

Another possible reason is the differences in the algorithms used in each system. Moreover, finger-prick blood glucose tests used to calibrate professional CGMs cannot be affected by contaminated skin with environmental sugar (i.e. not washing the skin after eating with one's hand). Recent research comparing the performance of the factory-calibrated CGM with iPro2 used different types of Abbott CGM (FreeStyle Libre Pro Abbott) during longer periods (seven days) and restricted conditions (Ida et al., 2018; Kumagai et al., 2019). These studies found a greater reduction in the interstitial glucose concentrations recorded by the FreeStyle Libre Pro Abbott compared to the iPro2, which may be attributed to the differences in the methodological approach. For example, they have used a different type of Abbott CGM called the FreeStyle Libre Pro Abbott**.** According to the manufacturer user guide, the FreeStyle Libre Pro Abbott is also a factory-calibrated sensor and functions the same as the FreeStyle Libre Abbott but differs in that it blindly records interstitial glucose, and readings must be uploaded by a professional care provider after sensor removal. Furthermore, the performance of the Abbott against the iPro2 was compared under the hospitalised setting and among elderly diabetic individuals. Diabetic patients are known to have greater glycaemic variability (Suh and Kim, 2015) than normo-glycaemic people, which could result in a larger difference between the CGM values in these studies. During the three-hour postprandial period under the standardised laboratory conditions, the FreeStyle Libre Abbott showed better consistency with arterialised blood glucose measurements obtained using the YSI than the iPro2. Although not statistically significant, this was evident by the lower MARD between the Abbott and the YSI (MARD 12.69%) than between the iPro2 and the YSI (MARD 16.15%). The MARD percentage against YSI measurements is within the range published in the literature.

Previous studies have assessed the performance of FreeStyle Libre Abbott against the capillary/venous blood glucose measurements among middle-aged diabetic patients during 14 days, with findings showing MARD percentages ranging from about 10% to 17% (Bailey et al., 2015; Bonora et al., 2016; Ji et al., 2017; Ólafsdóttir et al., 2017). Bland-Altman's analysis revealed the FreeStyle Libre Abbott illustrated a higher level of agreement against the YSI compared to the iPro2. A good correlation between the FreeStyle Libre Abbot and capillary blood glucose measurements has been previously documented, where the Pearson correlation coefficient (*r*) ranged from 0.94 to 0.96 (Bailey et al., 2015; Ji et al., 2017; Ólafsdóttir et al., 2017). Direct comparisons between studies can be difficult due to differences in the study protocol regarding duration of intervention, type of reference method, type of factory-calibrated CGM and participants' health status. In diabetic patients, the accuracy of CGMs has been demonstrated (see Table 3.1). CGM is increasingly being used in normo-glycaemic individuals for research purposes, but the validity of CGM in such individuals is uncertain (Akintola et al., 2015; Fabricatore et al., 2011; Wijsman et al., 2013). The current study results thus add to the literature regarding the performance of CGM among young healthy normo-glycaemic people. Factory-calibrated CGMs offer more important details about glucose trends, such as nocturnal hypoglycaemia and postprandial hyperglycaemia compared to traditional CGMs. It is therefore more valuable for care optimisation and patient education to examine not only glucose levels but also glucose patterns.

In the present research, the outcomes relate to young and healthy individuals and cannot be generalised to older people or patients. Nevertheless, because both men and women participated in this study, the implications can be beneficial for both genders. Most participants were full-time students, so the class schedule may have affected the consistency of the timing of meal and snack intake and increased the risk of individual errors — for example, the use of the devices and reporting of glucose results. Standardisation for the hydration status before trial was not applied, which may have had some influence on the outcomes, specifically the ones related to energy metabolism. Armstrong (2005), for example, reported that low levels of hydration can decrease body mass. In addition, measurements were made after the two-day intervention. Measuring cardiometabolic responses and energy metabolism also after day 1 may add valuable understanding to the current data (i.e., how soon these effects appear, are these effects greater or lower after day 1 compared to day 2 and whether significant but transient changes were present after day 1 that could not be detected after day 2. However, CGM sensors were applied during the two-day intervention, which is of great value to exploring the glycaemic control across the different conditions. Actiheart was used to monitor physical activity of participants. Due to unavailability of devices, it was measured for four out of the eight participants. A factory-calibrated CGM (FreeStyle Libre Abbott) was applied in a daily free-living setting, in which its comparison against the standard reference arterial blood glucose by frequent blood sampling was not applicable — although fasting and postprandial blood glucose measurements by the FreeStyle Libre Abbott were comparable against arterialised blood glucose measurements under standardised laboratory conditions. The present study showed several strengths. It was randomised and cross-over and conducted under free-living conditions and standardised laboratory settings. All blood

samples were withdrawn from arterialised blood to ensure a valid and reliable comparison of the outcomes. Cardiovascular parameters were measured continuously in a beat-to-beat fashion using the Finometer instead of other devices that give individual readings (i.e. the sphygmomanometer). The accurate evaluation of the cardiovascular and metabolic adverse effects of reduced physical activity is essential for a better understanding of the pathophysiology, prevention and treatment of related disorders.

In conclusion, this study showed that two days of reduced physical activity with a positive energy intake is associated with an increase in 24-hour interstitial glucose concentrations and increases in postprandial serum insulin and heart rate in response to mixed meals. These metabolic and cardiovascular adverse changes were ameliorated when energy intake was reduced to match expenditure during the two-day physical inactivity period. These negative health effects draw attention to the importance of maintaining an active and healthy lifestyle. Consideration to decrease energy intake when increasing physical activity is not feasible. FreeStyle Libre Abbott is a useful tool to assess daily glucose profile in healthy normo-glycaemic individuals. Further studies are recommended to explore the short-term effects of physical inactivity with and without energy surplus on the cardiovascular and metabolic systems using free-living and standardised laboratory protocols in health and disease. This will aid in assessing various lifestyle habits such as exercise and dietary modalities to mitigate the negative effects of a sedentary lifestyle on metabolism and vascular health. Studying the interactions of physical inactivity and high-fat diets on cardiometabolic responses is therefore a vital area of further research.

Chapter 4: The effects of five days of physical inactivity on endothelial function in healthy humans

4.1. Introduction

The vascular endothelium is of essential importance for the maintenance of vascular tone and regulation of blood flow in response to changes in tissue and organ perfusion requirements. It is composed of a single layer of cells that produces several vasoactive substances including nitric oxide (NO), endothelial cell growth factor, plasminogen inhibitors, prostacyclins and endothelins (Alley et al., 2014). Endothelial dysfunction characterized primarily by a disruption of the balance between vasodilators and vasoconstrictors is considered a key event in the development of atherosclerosis and subsequently cardiovascular diseases (Chiu and Chien, 2011). The degree of endothelium dysfunction has been correlated with the severity of many diseases including hypertension, atherosclerosis and diabetes mellitus (Kelm, 2002; Rodriguez-Miguelez, Seigler and Harris, 2016). Accordingly, a reliable assessment of endothelial function in humans is a valuable tool that may help to better understand the aetiology of common cardiovascular diseases with the overall aim of enhancing treatment efficiency and disease prevention. Several methods have been established over the past years to provide an evaluation of endothelial function in humans (Flammer et al., 2012; Tomiyama and Yamashina, 2010). Flow-mediated vasodilatation (FMD) is a non-invasive ultrasonic technique for the assessment of vascular endothelial function. The FMD test was first introduced in 1989 and combines an occlusion of the forearm and subsequent reactive hyperaemia that promotes vasodilation of the brachial artery in response to NO release (Rodriguez-Miguelez, Seigler and Harris, 2016). NO is synthesized from L- arginine by the endothelial nitric oxide synthase (eNOS). Arterial

wall shear stress leads to a greater eNOS activation, causing NO release and consequently smooth muscle relaxation. NO plays a vital anti-atherogenic role in fostering vasodilatation and preserving the integrity of the endothelium. It controls vessel tone and diameter by regulating the balance between oxygen supply to the tissues and their metabolic need. Thus, NO bioavailability reduction is commonly used as an indicator of endothelial dysfunction (Rodriguez-Miguelez, Seigler and Harris, 2016).

Being inactive can increase the risk of endothelial dysfunction. A metanalysis by Paterson et al (2020) explored the acute effect of prolonged sitting on the endothelial function. Findings showed that uninterrupted sitting led to significant decrease in %FMD with a mean difference of 2.12% in lower limbs, but no significant changes were found when FMD was assessed in the forearm. It has been reported that reduced FMD responses can increase the risk of cardiovascular abnormalities up to 13% in the long-term and that performing simple types of exercise could be an effective approach to maintain vascular function while exposed to extended time of sitting (Inaba, Chen and Bergmann, 2010; Paterson et al., 2020). Previous studies examined endothelial function following physical inactivity for duration ranged from three hours up to eight days in different arteries (femoral artery, brachial artery and popliteal artery), and have mainly used complete removal of physical activity via bed rest, prolonged sitting or limb immobilisation (Ballard et al., 2017; Birk et al.,2013; Carter et al., 2019; Climie et al., 2018; Hamburg et al., 2007; Kruse et al. 2018; Morishima et al., 2017; Morishima et al., 2016; Nosova et al., 2014; O'Brien et al., 2019; Restaino et al., 2015; Restaino et al., 2016; Thosar et al., 2015; Thosar et al., 2014; Vranish et al., 2017). These study models are more relevant to special circumstances (for

example, in the case of immobilisation due to injury or prolonged hospital stay) and cannot be generalised to the overall population. Furthermore, the state of energy balance during the imposed physical inactivity period in these studies not well controlled. Whether their findings are caused by the inactivity *per se* or/and energy surplus has not been previously challenged. Research in the short-term (ranging from 5 days to 14 days) effects of inactivity on brachial FMD response using a freely living model with daily steps reduction are limited (Bowden Davies et al., 2021; Boyle et al., 2013; Winn et al., 2019). The present study aimed to assess brachial artery endothelial function via a free-living reduced physical activity and increased sitting intervention among young and healthy individuals where results can be generalised to most of the population. In addition, the endothelial function was measured using a non-invasive FMD technique before and after five days of reduced physical activity via an arm sling and daily step counts reduction with a proportional decrease in energy intake to match energy expenditure in healthy participants. The rationale for choosing a five-days intervention period is to apply a similar duration of reduced activity conducted by Nosova et al. (2014). Nosova and colleagues measured brachial FMD response using the bed rest model which resulted in a significant reduction in FMD percentage. However, the novelty of the current experiment is the intention to create a free-living whole-body inactivity model by combining upper and lower limbs reduced activity via arm sling and step count reduction, respectively. In addition, considering a greater percentage of step count reduction (80%) compared to previous studies (Boyle et al., 2013) and matching food intake with energy expenditure to exclude the confounding effects of positive energy balance. In addition, the study was designed as a cross-over where the inactive trial was compared to a standardised active trial with an isoenergetic diet.

4.2. Materials and methods

Eight young, healthy, moderately active males with normal body mass index (BMI) were enrolled. Each participant was asked to attend the laboratory for two main experimental trials. The flow chart summarising participant recruitment and randomisation is shown in Figure 4.1. Physical activity level (PAL) was self-reported using the short version of the International Physical Activity Questionnaires (IPAQ) (Appendix 3) and pedometers were used to verify daily steps. Each participant was required to attend the laboratory for a total of 8 occasions. The first visit was the medical screening. The medical screening visit and the inclusion and exclusion criteria were as described in Chapter 2 (section 2.3).

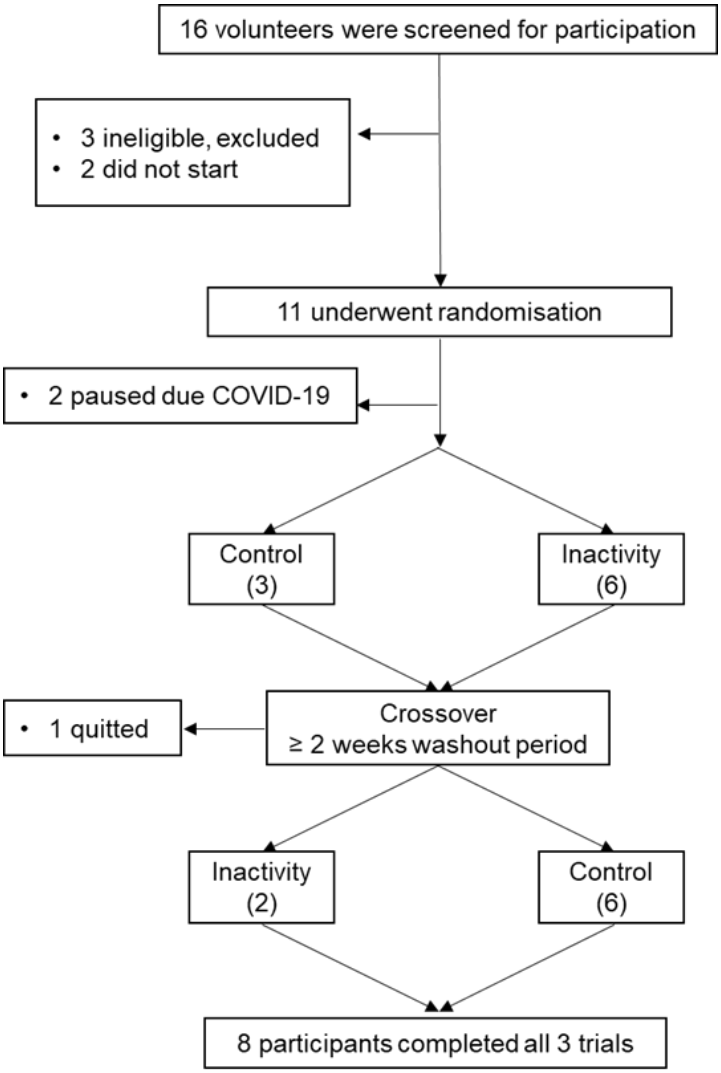


Figure 4.1. Flow chart summarising participant recruitment and randomisation.

When the medical screening was satisfactory, participants then attended the lab for a preliminary visit. In this visit, each participant was familiarised with procedures to reduce stress-induced sympathetic activity on the actual measurement day. In addition, participants were instructed to complete a 3-day food diary, which was used to evaluate their habitual food intake and food preferences. A pedometer (OMRON Walking Style One 2.1, Step counter HJ-321-E, OMRON Healthcare UK Ltd.) was fitted on the participant's waist and an Actiheart (CamNtech Ltd. UK) on the participant's chest to measure their habitual step counts and energy expenditure, respectively, over three days. The readings from a pedometer for at least three days can be used to estimate the quantity of physical activity that free-living persons perform in a week (Tudorlock et al., 2005).

After completion, participants returned the Actiheart, pedometer and food diary to the lab and randomly underwent two different treatments lasting six consecutive days each (five days of intervention plus one day of post-treatment measurements) and separated with a minimum washout period of two weeks. Figures 4.2 and 4.3 show schematic diagrams of the sequence of measurements made during each trial. Food diaries were analysed using the online dietary analysis package NUTRITICS (Nutrition analysis software, v4.3 Academic Edition). Results from the software were used to design meal plans for participants based on their habitual food intake and food preferences to follow during the 5-day intervention period.

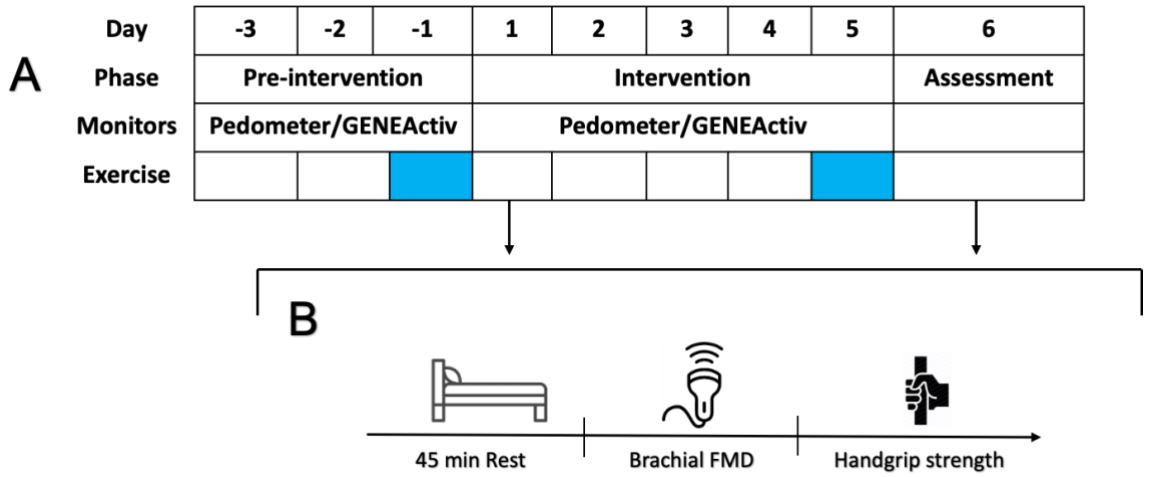


Figure 4.2. Schematic diagram of the 5-day study design in the Control trial (A) and laboratory measurements on Day 1 and Day 6 (B)**.**

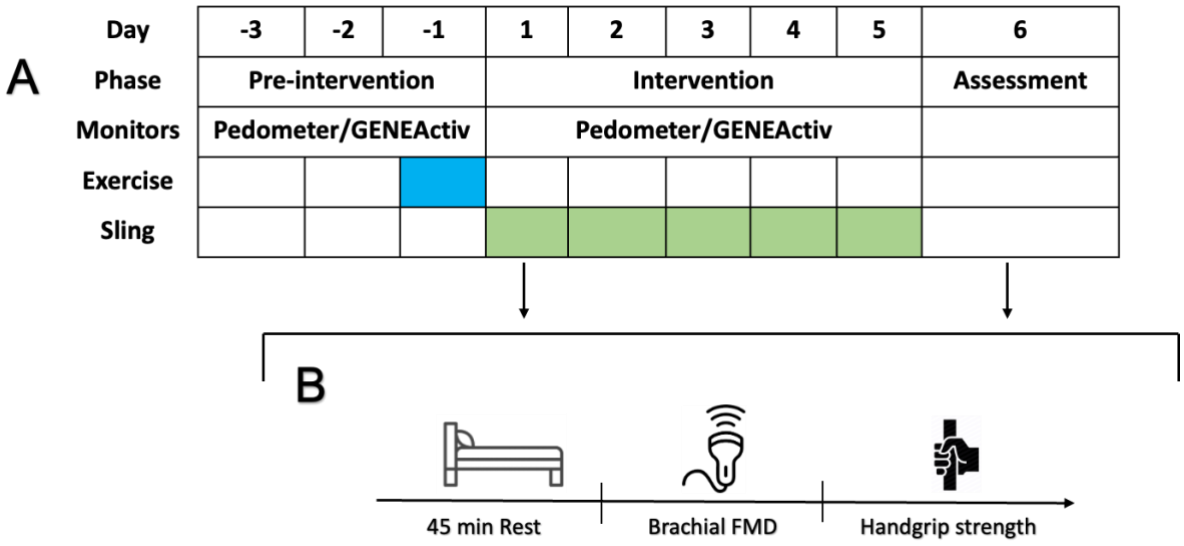


Figure 4.3. Schematic diagram of the 5-day study design in the Inactivity trial (A) and laboratory measurements on Day 1 and Day 6 (B).

Before each trial, participants were asked to continue with their normal daily physical activities and dietary intake, refrain from alcohol consumption for the previous two days and caffeine consumption for 24 hours before the day of the trial. Standardisation of these factors was made to minimise additional or false effect on the study outcomes. For example, previous studies have shown that caffeinated drinks can cause detrimental impacts on FMD response and that alcohol intake possesses dilatory effect on large arteries among healthy people (Papamichael et al., 2005; Vlachopoulos et al., 2003).

The level of physical activity and energy intake during the 5-day intervention of each trial were varied. The two trials were (a) Control trial: participants were asked to continue with their habitual daily physical activities at a PAL of ~1.85 and consume an isoenergetic intake and (b) Inactivity trial: participants were instructed to reduce their normal daily step count by 80% verified using a pedometer and placing their non-dominant arm in a sling. Both physical activity and energy intake were reduced to a PAL of ~1.40 to reach an energy balance.

Three days before each main trial, participants were asked to attend the laboratory for a quick visit (about 30 minutes) to attach continuous activity monitoring watches (GENEActiv, Activinsights Ltd. UK) on the participant's wrist and a pedometer on the waist. They were provided with a prescribed meal plan and daily steps count to follow during the 5-day intervention. Food weighing scales (Argos Home Digital Kitchen Scale) were also supplied for precise measurement of each meal. The day before each main trial, all participants performed one bout of moderate-intensity exercise. The

exercise is composed of brisk walking for 45 minutes anytime between 3 pm and 6 pm for a distance of about 3.75 kilometres. The intensity and duration were chosen based on a pilot study of a similar group. Participants were instructed to avoid any additional exercise after this exercise bout. The reasons for this exercise bout were to standardise the amount and time of the last structured physical activity before each trial to control for the exercise-induced hyperaemia and maintain the level of habitual physical activity patterns before baseline measurements (Boyle et al., 2013). In addition, participants were asked to exercise on day 5 of the Control trial only to allow meaningful comparison of baseline and post-intervention measurements in the latter trial.

On day 1 of each main trial, participants attended the laboratory after an overnight fast of at least 10 to 12 hours. A data sheet was designed to ensure detailed participant information and measurements were recorded. Participant's body weight using an electronic weight scale (Seca Ltd., UK), blood pressure and heart rate using a Dinamap Pro 1000 vital signs monitor were first measured. Because mental stress can affect FMD response, participants were asked to rest on a reclining bed for 45 minutes in a quiet and temperature-controlled room (Thijssen et al., 2019). Assessment timings were standardised to be undertaken during early morning to prevent diurnal fluctuation (Thijssen et al., 2019). After that, baseline brachial artery FMD of the non-dominant arm was measured according to guidelines reported by Corretti et al. (2002) and Thijssen et al., (2011). Measurements were done via duplex-Doppler ultrasound (Toshiba Aplio 300 Diagnostic Ultrasound System, TUS-A300) using a high-frequency ultrasound transducer (12 MHz) (Figure 4.4). An arm pneumatic pressure cuff (ERKA, range 29-40cm, Germany) was placed distal to the antecubital fossa (mid-forearm)

with measurements taken to ensure comparable cuff positioning on repeated visits (Figure 4.5). A 3-lead ECG was applied below the clavicle of the right and left shoulders, and below the pectoral muscle in the lateral base of the chest to ensure the arterial diameter is measured at the same point in the cardiac cycle for the pre-and post-occlusion measurements. During measurements, the participant's arm was extended laterally (approximately 80°) at the level of the heart and the distal forearm was supported by a pillow to maintain an accurate position throughout the measurement. Baseline diameter and velocity readings measured immediately before cuff inflation using the B mode and duplex mode imaging with an insonation angle corrected at 60° to maximise accuracy. Following cuff inflation to 200 mmHg for five minutes, blood velocity was measured at 15 seconds post occlusion, and post occlusion arterial diameters were measured at 50-, 60-, 70- and 80-seconds. These measurement timings were chosen because it was shown that 60 seconds after the cuff occlusion is released, the diameter increases to its maximum (Corretti et al., 2002).

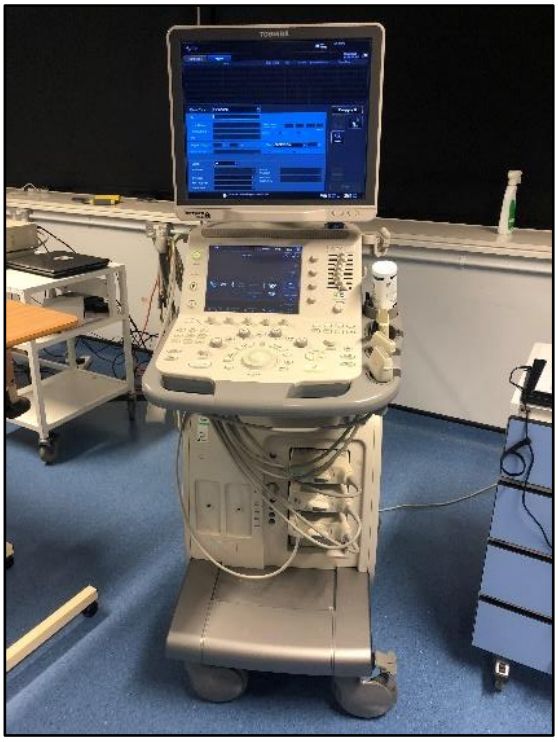


Figure 4.4. Duplex-Doppler ultrasound (Toshiba Aplio 300 Diagnostic Ultrasound System).



Figure 4.5. Brachial artery FMD measurement technique.

At the end of FMD measurements, the maximal voluntary handgrip strength test was done on the non-dominant hand using a digital hand dynamometer (Digital Analyser, MIE Medical Research Ltd, Leeds, UK) (Figure 4.6). The test was performed while the participant sitting upright on a chair with the upper arm adducted (the elbow at about 90°). To prevent using accessory muscles, the upper arm was kept away from the body. The forearm was supported by a pillow and the hand was placed comfortably around the dynameter handle. The handles provided different hand sizes to select an appropriate grip size for each participant and the analyser can display the force value in kilograms. After that, participants were given a count down and verbal encouragement to do three maximum handgrip muscle contractions separated with one-minute rest intervals. Before each measurement, the auto zeroing button was pressed for re-sitting. The average of the three readings was documented as the baseline for maximal handgrip strength. Before leaving the laboratory, a sling was applied on the non-dominant arm for the Inactivity trial only. The sling was adjusted by the researcher to ensure proper placement of the arm and to avoid swelling formation. The participants were familiarised with how to remove and re-apply the sling only before and after taking shower or when going to bed.



Figure 4.6. Digital hand dynamometer.

The meal plan of the 5-day intervention period was designed based on habitual food intake and estimated energy requirements. It was composed of three main standardised meals (breakfast, lunch, and dinner) with three snacks following each meal. Daily food intake was mixed in composition providing ~50% of total energy from carbohydrates, ~35% from fat, and ~15% from protein. Intake in grams was multiplied by the associated Atwater general factor, which is 4 kcal/g for carbohydrates, 9 kcal/g for fat, and 4 kcal/g for protein, to determine the energy generated from the recommended macronutrients. Any incidents of leftover or overfeeding were reported, but participants were encouraged to follow the prescribed meal plan. The estimated energy requirement (EER) for each arm of the study was calculated according to the following equation:

$$EER = BMR \times PAL$$

Where BMR is the basal metabolic rate estimated using the Harris-Benedict equation revised by Mifflin and St Jeor in 1990 for men (Mifflin et al., 1990):

$$BMR = [10 \times \text{weight (kg)}] + [6.25 \times \text{height (cm)}] - [5 \times \text{age (years)}] + 5$$

PAL was selected based on the experimental trial, i.e., PAL= 1.85 for the active state and PAL=1.40 for the inactive state (Brooks et al., 2004). In the Inactivity trial, both carbohydrates and fat content were reduced, but protein content was the same as the Control trial to avoid negative nitrogen balance and potential loss of muscle mass.

On Day 6 of each experimental trial, the participants were asked to attend the laboratory after an overnight fast of at least 10 to 12 hours and follow the same protocol applied for the baseline measurements described previously for Day 1. Upon the completion of the experiment, participants were given lunch before leaving the laboratory. All ultrasonic measurements and analysis were performed by a research nurse and a senior technician in our laboratory. Data collected were saved in a private and secure place.

4.2.1. Power calculation

To determine the sample size for this experiment, a *priori* power assessment using G*Power (version 3.1.9) was used. Nosova et al. (2014) demonstrated a significant 2.1 % (effect size = 0.73) decrease in brachial artery %FMD compared to baseline in response to five days of reduced physical activity in five participants. If a similar difference was expected in the present study following five days of physical inactivity, we should have been able to detect this effect in 8 participants with a power of 80% at a 5% significance level. Recruitment continued until the required sample size was reached. To preserve statistical power in situations of withdrawal, more volunteers were recruited.

4.2.2. Data handling

A. Duplex-Doppler ultrasound

Mean and peak blood velocity was obtained directly from the duplex-Doppler ultrasound recordings. The ultrasound recorded arterial diameters with a single decimal which can obscure the small changes in the vessel diameter. Thus, a brachial analyser for research software was used to measure and analyse arterial diameters with two decimal figures. The software allows to set the region of interest manually on the artery at the R wave (end of diastole) of the ECG recording. Four arterial diameters were measured and averaged in millimetres at each time point. The percent of post-occlusion diameter change from baseline diameter (%FMD) was calculated using the following equation (Boyle et al., 2013; Restaino et al., 2016):

$$\%FMD = [\text{ Peak diameter - Baseline diameter}] / \text{Baseline diameter X } 100$$

Brachial shear rate was determined using the equation (Boyle et al., 2013; Restaino et al., 2016):

$$\text{Shear rate } (s^{-1}) = [\text{ 8 X Mean blood velocity (cm/sec)}] / \text{Diameter (cm)}$$

Brachial blood flow was measured using the values of mean blood velocity (cm/sec) and cross-sectional area (cm^2) based on the equation below (Restaino et al., 2016):

$$\text{Blood flow (ml/min)} = 3.14 \text{ X } [\text{ Diameter (cm) / 2 }]^2 \text{ X Mean velocity (cm/min) X } 60$$

B. GENEActiv

The GENEActiv used in this study has the following features according to the manufacturer's user guide produced by Activinsights (2012): size (43 mm x 40 mm x 13 mm), weight (16g), sensor type (Microelectromechanical systems) with a range of +/- 8g and resolution of 12 bit (3.9 mg). The PC software version 3.2 (GENEActiv) was downloaded through the official website (https://www.activinsights.com/resources-support/geneactiv/downloads-software/). To set up the GENEActiv, the device was first placed on a cradle connected to the laptop through a USB cable. The software then recognised the device by displaying its unique serial code. After that, a configuration setup was selected and a sampling frequency of 100 Hz for 7 days period was chosen. Detailed subject information was inserted including subject code, body location, date of birth, age, gender, height, weight, BMI and handedness. Once all information was inputted, the erase and configure function was selected to start recording. Following configuration, the device was removed from the cradle and was applied to the participant's dominant hand. At the end of the trial, the GENEActiv was removed from the participant and attached to the cradle to download the data. To extract the data from the device, the data extractor function in the software was selected. The extraction process saved the data in the form of a (.bin) file. This was followed by selecting the data converter function to convert the (.bin) file into 15 seconds and 60 seconds epoch (.csv) files. The (.csv) files were then imported into two Excel macro files designed for GENEActiv data analysis. The first Excel macro file was used to analyse the 60 seconds epoch (.csv) files and generate summary graphs and tables of the different intensity of physical activities including sedentary activity, light activity, moderate activity and vigorous activity. Data of each physical activity type were reported as percentages and duration in minutes during each day. The second

Excel macro file was used to analyse the 15 seconds epoch (.csv) files and generate summary tables of the duration in minutes spent in different body postures i.e., sitting and standing. The mean of the duration (minutes) spent in each type of the physical activities and body postures during the 5-day intervention period was used for the statistical analysis.

4.2.3. Statistical analysis

Using the Shapiro-Wilk test in SPSS, all variables were examined for normality. When the Shapiro-Wilk test had a non-significant result (p >.05), normal distribution was assumed. Parametric analysis was used once the normality of the raw data was established. Differences in energy intake were analysed using paired-samples t test. Differences in daily steps count during the 5-day intervention were assessed using two-way (Treatment x Day) repeated measures ANOVA. Differences in physical activities and postures time during the 5-day intervention were assessed using three-way (Treatment x Activity type x Day) repeated measures ANOVA. %FMD differences were analysed using two-way (Treatment x Day) repeated measures ANOVA. Three-way (Treatment x Day x Time-point) repeated measures ANOVA was used for differences in mean blood velocity. Brachial diameter, shear rate and blood flow were analysed using three-way (Treatment x Day x Time-point) mixed-effects repeated measures ANOVA. Body weight, systolic blood pressure, diastolic blood pressure, heart rate and maximal handgrip strength differences were analysed using two-way (Treatment x Day) repeated measures ANOVA. Results were expressed as a mean ± standard error of the mean (SEM). P < .05 was set as the value of statistical significance. If statistical differences were reached, Bonferroni post-hoc testing was used to correct for family-wise error. Carry-over effect was explored via comparing measurement time (baseline on day 1 and post-intervention on Day 6) for each main outcome between visits 1 and 2 using repeated

measures 2-way ANOVA (2 visits X 2 time). No carry-over effect was assumed in the presence of non-significant ($p > .05$) difference between visits.

4.3. Results

Eight participants completed both trials. Detailed anthropometric measurements are shown in Table 4.1.

Table 4.1. Participant characteristics.[1]

Variable	M ± SD
n	8
Gender	Males
Age (yrs)	22 ± 5
Height (m)	1.76 ± 0.05
Body weight (kg)	75.8 ± 7.8
BMI (kg/m^2)	24.55 ± 2.77
WC (cm)	82.25 ± 4.50
Fat (%)	17.11 ± 3.64
Habitual steps	11129 ± 1085

[1]All values are means (M) ± Standard deviation (SD). Abbreviations: BMI = body mass index; WC = waist circumference.

No differences were found in body weight [$F(1,7) = 3.62$, $p = .099$], heart rate [$F(1,7) = 0.68$, $p = .802$], systolic blood pressure [$F(1,7) = 0.24$, $p = .638$]. There was a main effect of time on diastolic blood pressure between day 1 and day 6 of each trial [$F(1,7) = 11.07$, $p = .013$ with a large effect size of $\eta^2_p = 0.61$. Diastolic blood pressure was higher in day 1 (73.31 ± 2.64 mmHg) compared to day 6 (69.75 ± 3.32 mmHg) with a mean difference of 3.56 mmHg (see Table 4.2).

Table 4.2. Body weight, blood pressure and heart rate measurements at baseline and the end of each trial.[1]

	Control	Inactivity
Body weight (kg)		
Day 1	75.2 ± 3.0	75.5 ± 2.8
Day 6	75.4 ± 3.24	75.1 ± 2.9
SBP (mmHg)		
Day 1	127.25 ± 4.81	133.00 ± 2.89
Day 6	128.88 ± 5.89	132.75 ± 5.75
DBP (mmHg)		
Day 1	74.00 ± 3.07	72.63 ± 2.49
Day 6	69.25 ± 3.4 $	70.25 ± 3.40 $
HR (bpm)		
Day 1	71.75 ± 4.95	73.63 ± 4.10
Day 6	72.75 ± 4.10	76.00 ± 4.13

[1]All values are means ± SEM (n=8). Abbreviations: BW = body weight; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate. $ different from day 1 $p < .05$.

4.3.1. Energy intake and physical activity patterns during the 5-day intervention

Energy intake was significantly lower during the Inactivity trial (11365 ± 215 kJ/day) compared to Control (14207 ± 268 kJ/day) with a mean difference of 2841 kJ/day [t(7) = 52.91, $p < .001$, d = 18.71 (large effect)]. Daily steps count during the 5-day intervention period for both trials are shown in Table 4.3. There was a main effect of treatment on the daily steps count [$F(1,7) = 163.87$, $p < .001$ with large effect $\eta^2_p = 0.96$]. Daily steps were significantly reduced during the Inactivity trial (2496 ± 451 steps/day.

Table 4.3. 24-hr step counts during the 5-day intervention period of each trial.[1]

	Steps/day	
	Control	**Inactivity**
Day 1	12499 ± 1983	2794 ± 401 *
Day 2	11861 ± 909	2435 ± 484 *
Day 3	13676 ± 2155	2753 ± 796 *
Day 4	12071 ± 1215	2434 ± 542 *
Day 5	13502 ± 2075	2067 ± 219 *

[1]All values are means ± SEM (n=8). * different from Control $p < .05$.

Figure 4.7 presents the duration of different physical activity intensity type (sedentary, light, moderate and vigorous) and Figure 4.8 shows the postural type (sitting and standing) recorded in minutes by the GENEActiv devices during the 5-day intervention period. Significant treatment effect was found in moderate activity time [$F(1,7) = 7.98$, $p < .026$ with large effect size of $\eta^2_p = 0.53$] and vigorous activity time [$F(1,7) = 15.86$, $p < .005$ with large effect size of $\eta^2_p = 0.69$] between the Control and Inactivity trials. During Control, moderate activity time (203.30 ± 8.78 minutes/day) was greater than during Inactivity (123.10 ± 5.81 minutes/day) with a mean difference of 80.20 minutes/day. Similarly, vigorous activities were higher during Control (28.78 ± 3.62 minutes/day) compared to Inactivity (6.00 ± 0.90 minutes/day) with a mean difference of 22.78 minutes/day. There was a main effect of physical activity intensity type on the duration of physical activity in Control and Inactivity trials [$F(1.06,7.43) = 95.21$, $p < .001$ with large effect size of $\eta^2_p = 0.93$] during both trials. Sedentary time (929.60 ± 57.43 minutes/day) was significantly greater than light activity time (70.35 ± 7.61 minutes/day), moderate activity time (163.18 ± 8.01 minutes/day) and vigorous activity time (17.39 ± 4.40 minutes/day) with mean differences of 559.25, 466.43 and 612.21 minutes, respectively. Moderate activity was significantly higher than light and vigorous activities with mean differences of 92.83 and 155.79 minutes. Light activities were greater than vigorous with a mean difference of 52.96 minutes. There was a significant interaction effect in postural duration between the Control and Inactivity [$F(1, 7) = 6.79$, $p = .035$ with large effect of $\eta^2_p = 0.49$]. Sitting time was significantly greater during Inactivity (1126.90 ± 34.29 minutes/day) compared to Control (999.98 ± 29.97 minutes/day) with a mean difference of 126.92 minutes. In addition, standing time was significantly lower during Inactivity (313.30 ± 34.32minutes/day) compared to Control (440.18 ± 29.95 minutes/day) with a mean difference of 126.88 minutes.

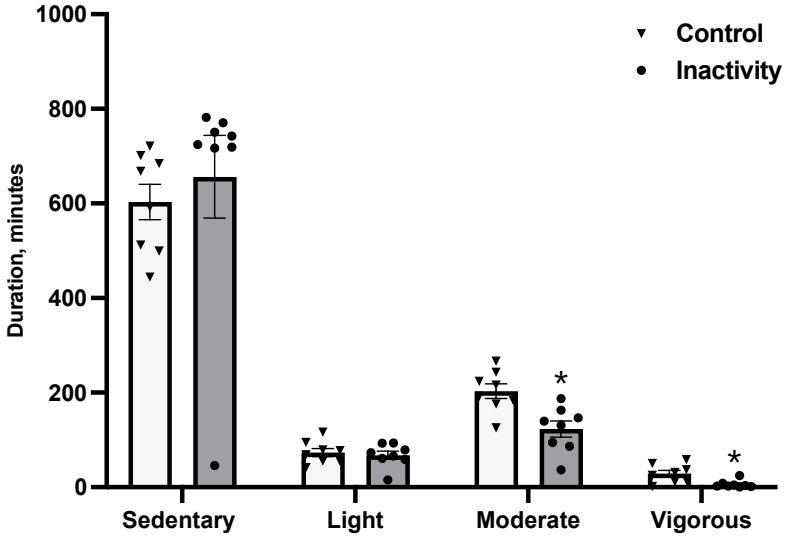


Figure 4.7. Mean ± SEM (n=8) of physical activity intensity data recorded by the GENEActiv devices during the 5-day intervention period of each trial. * different from control $p < .05$.

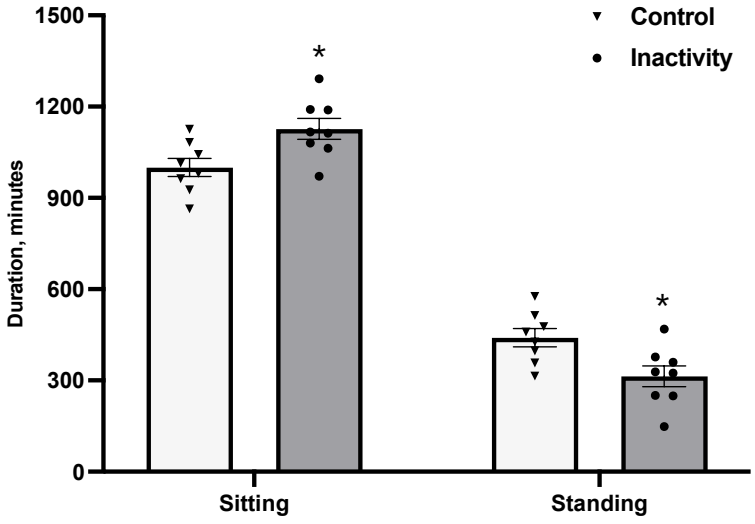


Figure 4.8. Mean ± SEM (n=8) of postural type data recorded by the GENEActiv devices during the 5-day intervention period of each trial. * different from control $p < .05$.

4.3.2. Brachial FMD measurements

Mean diameter, mean blood velocity, blood flow and Shear rate of the brachial artery were not different between Control and Inactivity. Though, there was a significant main effect of time-point, i.e., pre versus post occlusion measurements (see Table 4.4). Post-occlusion diameter (4.25 ± 0.15 mm) was significantly higher than pre-occlusion values (4.01 ± 0.14 mm) with a mean difference of 0.24 mm [$F(1,7) = 75.12$, $p < .001$ with large effect size of $\eta^2_p = 0.91$]. Post-occlusion blood velocity (53.08 ± 4.10 cm/sec) was significantly greater than pre-occlusion values (15.13 ± 1.46 cm/sec) with a mean difference of 37.94 cm/sec [$F(1,7) = 124.30$, $p < .001$ with large effect size of $\eta^2_p = 0.95$]. Post-occlusion shear rate (1026.57 ± 72.98 sec^{-1}) was higher than pre-occlusion values (302.38 ± 42.43 sec^{-1}) with a mean difference of 724.19 sec^{-1} [$F(1, 7) = 157.50$, $p < .001$ with large effect size of $\eta^2_p = 0.96$]. Post-occlusion blood flow (484.54 ± 66.52 ml/min) was significantly higher than pre-occlusion values (107.08 ± 23.05 ml/min) with a mean difference of 377.47 ml/min [$F(1,7) = 62.74$, $p < .001$ with large effect size of $\eta^2_p = 0.90$]. No significant interaction effect was observed in brachial artery %FMD between both trials [$F(1, 4) = 0.75$, $p = .435$] (see Table 4.5).

Table 4.4. Brachial artery vascular measures during FMD at baseline and the end of each trial.[1]

	Control		Inactivity	
	Day 1	**Day 6**	**Day 1**	**Day 6**
Diameter (mm)				
Pre-occlusion	4.15 ± 0.15	4.08 ± 0.13	3.94 ± 0.15	3.87 ± 0.13
Post-occlusion	4.42 ± 0.15 $	4.33 ± 0.15 $	4.16 ± 0.16 $	4.10 ± 0.15 $
MBV (cm/sec)				
Pre-occlusion	15.83 ± 1.96	15.44 ± 2.93	14.28 ± 1.94	15.00 ± 1.54
Post-occlusion	54.00 ± 4.44 $	50.15 ± 4.34 $	54.53 ± 4.75 $	53.63 ± 6.05 $
Shear rate (sec^{-1})				
Pre-occlusion	302.48 ± 49.37	309.89 ± 60.13	288.35 ± 33.14	308.79 ± 27.07
Post-occlusion	1104.23 ± 52.77 $	920.86 ± 67.23 $	1046.74 ± 75.63 $	1034.43 ± 96.27 $
Blood flow (ml/min)				
Pre-occlusion	91.60 ± 29.45	118.53 ± 24.59	108.98 ± 21.83	109.19 ± 16.35
Post-occlusion	527.64 ± 59.70 $	458.85 ± 60.97 $	460.45 ± 70.85 $	446.22 ± 74.56 $

[1]All values are means ± SEM (n=8). Abbreviations: MBV = mean blood velocity. $ different from pre-occlusion $p < .05$.

Table 4.5. Brachial artery %FMD at baseline and the end of each trial.[1]

	Control	Inactivity
Day 1	6.43 ± 0.48	6.14 ± 0.96
Day 6	5.59 ± 1.12	5.97 ± 1.27

[1]All values are means ± SEM (n=8).

Mean maximal handgrip strength showed near significant difference [$F(1,7) = 4.47$, $p = .072$ with large effect of $\eta^2_p = 0.39$] between Control and Inactivity. Although not significant, maximal handgrip strength decreased after the Inactivity trial by 8.5% compared to baseline and post-inactivity values were lower by 11% compared to post-Control (see Table 4.6).

Table 4.6. Maximal handgrip strength (kg) of the non-dominant hand measured at baseline and the end of each trial.[1]

	Control	Inactivity
Day 1	30.76 ± 1.61	30.70 ± 1.98
Day 6	31.65 ± 2.41	28.10 ± 1.91

[1]All values are means ± SEM (n=8).

FMD responses carry-over effect

Analysis of variance showed no significant differences in baseline and post-intervention FMD responses [$F(1,4) = 1.04$, $p = .688$] and maximal handgrip strength [$F(1,7) = 4.61$, $p = .069$] between the measurement visits.

4.4. Discussion

This study aimed to investigate changes in brachial artery endothelial function among healthy young men before and after five days of reduced physical activity with and without a proportional decrease in energy intake. Energy intake was reduced to match energy expended during inactivity to eliminate the confounding effect of positive energy balance. The novelty of the research is the non-traditional protocol design whereby physical inactivity was applied through a combined approach of daily step count reduction and arm sling application in a free-living setting.

The main findings of this study suggest that no changes in brachial vascular responses occurred during FMD measurement following inactivity with reduced energy intake to match reduced energy expenditure when compared to an active state with an isoenergetic diet. Handgrip strength showed a trend to decrease following reduced activity levels. Assessment of the carryover effects confirmed that the washout interval of at least two weeks between the main trials was adequate to eliminate any carryover effects on the study outcomes.

There is consistent evidence highlighting the negative consequences of increased sitting time on vascular function. Reductions in arterial FMD, shear rate and blood flow responses were the common reported deleterious effects of sitting (Morishima et al., 2016; Restaino et al., 2016). In the published literature, the effects of inactivity on brachial endothelial function are controversial. Some studies reported no significant differences in brachial artery %FMD (Boyle et al., 2013; Winn et al., 2019), while others did (Bowden Davies et al., 2021 Nosova et al., 2014). Nosova et al. (2014) found a

significant reduction by 2% of brachial artery %FMD associated with an increase in arterial stiffness after five days of bed rest among five young and healthy men and women. Though a similar period of inactivity to the present study was imposed, there were differences in the methodological approach. They applied restrictive sedentary protocol, which may have a greater influence on the development of brachial endothelial dysfunction compared to free-living inactivity protocols like decreasing daily steps (Boyle et al., 2013; Winn et al., 2019). Participants' habitual activity levels were not addressed to confirm that they were in the active state before starting the inactivity intervention. In the present research, habitual activity level was assessed prior to intervention, and physical activity patterns and body postures were monitored continuously during the intervention period using GENEActiv monitors. A significant decrease in brachial %FMD by 1.8% was found after 14 days of reducing daily steps from 10,000 to about 1,500 steps/day with an energy surplus among young and healthy males and females (Bowden Davies et al., 2021). The ability for the latter study to find significant changes could be attributed to the longer duration of intervention (14 vs. five days) and larger sample size (28 vs. eight participants) compared to the present research. In addition, the positive energy balance accompanying the inactivity could be responsible for the deterioration in endothelial function. Energy intake was reduced to match the low energy expended during inactivity in the current study, which may delay the appearance of any negative outcomes and preserve endothelial function. Using a similar approach, Winn et al. (2019) explored the effect of reducing activity (from 10,000 to < 5,000 steps/day) during 10 days with and without a reduction in energy intake to match the energy expended. In one group, 10 young men and women reduced their energy intake by 15–20% to match their energy expended during inactivity. Another group composed of five young men increased their energy intake

by 35–40% to create a positive energy balance. Both groups did not show significant changes in FMD response following intervention. Irrespective to the protocol applied, majority of studies have not found significant changes in brachial FMD (Birk et al.,2013; Boyle et al., 2013; Hamburg et al., 2007; Restaino et al., 2015).

FMD reflects the vasodilatory function of the macrovascular conduit arteries, i.e., brachial arteries. Reactive hyperaemia, which is the transient increase in blood flow following a brief period of ischemia, indicates the ability of the microvascular resistance arteries to dilate, which determines tissue perfusion. A significant decrease in brachial artery reactive hyperaemia by 16% was found after five days of bed rest (Hamburg et al., 2007) and by 31% after six hours of prolonged sitting (Restaino et al., 2015). This decrease in brachial artery reactive hyperaemia was not accompanied by changes in FMD, which suggest that downstream resistance arteries failed to vasodilate in response to the transient ischemia induced by cuff occlusion during the FMD and that changes in reactive hyperaemia might not be driven by changes in conduit artery diameter (Hamburg et al., 2007). Interestingly, findings from studies that investigated the effect of inactivity on brachial and popliteal arteries showed a significant decline in %FMD in the popliteal artery but not in the brachial artery (Boyle et al., 2013; Restaino et al., 2015). This may indicate that physical inactivity differentially impacts vascular function in a limb-specific manner and that the brachial artery could be more resilient to inactivity than the popliteal artery. A potential explanation could be that during inactivity, such as in prolonged sitting or reduced step count, lower limbs are exposed to a greater reduction in muscle activity and subsequently a greater decrease in shear stress and blood flow compared to upper limbs. Another potential mechanism is the increase in hydrostatic pressure, which is secondary to the low pumping action in

muscles and blood pooling in the lower limbs, leading to a further reduction in blood flow and shear stress (Padilla and Fadel, 2017). The partially preservative nature of the brachial artery against any vascular disturbance may be attributed to the fact that most studies do not control for the movement of upper limbs during inactivity (e.g., allowing desk-based activities).

Although not significant, maximal handgrip strength of the non-dominant hand decreased following five days of arm sling wear, reflecting some degree of deconditioning in the inactive forearm. Similarly, Burns et al. (2021) found a significant decrease by 10% in maximal handgrip strength following three days of forearm immobilisation using a cast compared to the contralateral non-immobilised forearms of 10 young and healthy males. Birk et al. (2013) found a significant reduction by 9.5% in maximal handgrip strength following eight days of unilateral non-dominant arm inactivity compared to baseline among 13 young men. The reduction in handgrip strength was not associated with brachial artery FMD changes. This suggests that a larger sample size, longer period of inactivity and/or more restrictive measures for limb immobilisation (i.e., using a cast rather than a sling to ensure a greater stabilisation effect on the arm) are required for detecting significant reductions in maximal handgrip strength.

The novelty of the study design can provide a useful resource for future studies to adopt. Moreover, it is a randomised crossover study that was conducted under free-living environments with standardised laboratory measurements. Participants were all men, and gender may have played a role in the results. However, a significant

difference between genders may not exist as reported in O'Brien et al.'s (2019) study, where both males and females exhibited similar popliteal FMD responses following three hours of sitting. Also, brachial FMD response showed no significant difference after 10 days of reduced daily steps with decreased energy intake to match energy expenditure among men and women (Winn et al., 2019). Applying physical activity monitors on both upper arms would have a better approach to monitor upper body movement and increase the reliability of measurements. There are different methodological approaches to the FMD test. FMD was measured at single time points (i.e., baseline and 60, 70 and 80 seconds post-occlusion), meaning the true peak vascular diameter post-occlusion could be missed. Measuring FMD continuously during post-occlusion using edge-detection software packages and stereotactic devices to fix the ultrasound transducer might increase the accuracy of the data (Harris et al., 2010). However, the absence of normal FMD cut-off values makes it challenging to define true abnormalities.

In conclusion, the present study could not demonstrate significant brachial FMD changes in response to short-term reductions in physical activity. Muscle strength could be the first to be negatively affected by low levels of physical activity. Further research with a larger sample size is required to confirm these observations. In addition, investigating the nature of possible effects of short-term physical inactivity on different limbs (e.g., comparing the vascular responses of brachial arteries compared to other conduit arteries) may improve our understanding of the limb-specific effects of reduced physical activity on endothelial function.

Chapter 5: General discussion

Inactivity is defined being not meeting recommended physical activity guidelines, while sedentary behaviours being any awake activity in which the energy expended is ≤1.5 metabolic equivalents when standing, sitting, or lying down. Both states of activities increase the risk of developing undesirable health outcomes (Admiraal et al., 2011; Bertoni et al., 2009; Bull et al., 2020; Tremblay et al., 2017; Tudor-Locke et al., 2013). It is possible that recurrent temporary deficits in vascular and metabolic function brought on by acute inactivity may be a factor in the well-documented long-term negative consequences on cardiovascular and metabolic health (Kim et al., 2018; Padilla and Fadel 2017). The fundamental processes, nevertheless, that connect the short-term impacts of inactivity and the long-term implications on human health are still poorly understood (Daniele, Lucas and Rendeiro, 2022). Detrimental health effects were observed during states of overfeeding (Bray and Bouchard, 2020). The growth in the incidence of obesity over the years has been linked to increased energy consumption and low levels of activity (Scarborough et al., 2011; Swinburn, Sacks and Ravussin, 2009). Human experimental studies have shown a growing interest to investigate the short-term effects of inactivity (Bates et al., 2021; Daniele, Lucas and Rendeiro, 2022; Narici et al., 2021; Paterson et al., 2022) and exploring the role of energy surplus during inactive conditions (Dirks et al., 2018; Knudsen et al., 2012; Stephens et al., 2011). Different inactivity models had been applied including bed rest, prolonged sitting on a chair and single limb immobilisation (Birks et al., 2013; Burns et al., 2021; O'Brien et al., 2019; Sonne et al., 2010; Varnish et al., 2017), but these models severely restricted body movement and do not reflect real-life scenarios where periods of ordinary inactivity are frequently interrupted in a typical workday. Assessing

inactivity during free-living environments would provide more relevant information of the consequences of low levels of physical activity to the population. Accordingly, several studies assessed inactivity in free-living settings (Bowden Boyle et al., 2013, Krogh-Madsen et al., 2010; Olsen et al., 2008). However, majority of these studies were limited in controlling the energy balance by maintaining habitual energy intake during the intervention (Dixon et al., 2013; Mikus et al., 2012). Studies which controlled for energy intake (Knudsen et al., 2012; Mikines, 1991; Stephens et al., 2011; Walhin et al, 2013) were either a lab-based experiments or the intervention lasted one to two weeks or did not have a control arm. To the authors' knowledge, no study has measured cardiovascular responses using a Finometer following free-living inactivity interventions. In addition, studies assessing endothelial function in a non-lab-based protocols are limited (Boyle et al., 2013, Bowden Davies et al., 2021; Winn et al., 2019). Taken into consideration these methodological and knowledge gaps in the literature, the aim of the current thesis is to explore the short-term effects of inactivity with and without energy surplus on metabolic responses, cardiovascular responses, endothelial function and energy metabolism under free-living and standardised laboratory measurements. Two studies were applied, the first study (Chapter 3) was intended to explore the cardiometabolic and energy metabolism responses to a 2-day period of reduced activity with and without proportional decrease in energy intake to create neutral energy balance or positive energy balance states, respectively, among young and healthy males and females. The second study (Chapter 4) was designed to assess endothelial function in response to a 5-day period of reduced activity with a concomitant decrease in energy intake to match energy expended in young and healthy males.

This research demonstrated that reduced physical activity with a positive energy balance was associated with an increase in 24-hour glucose concentration, and an increase in postprandial insulin incremental area under the curve (iAUC) and an increased in heart rate levels. These metabolic and cardiovascular adverse changes were attenuated when energy intake was reduced to match energy expended. According to these results, rather than physical inactivity itself being the cause of these negative consequences, a positive energy balance may be. In addition, reduced physical activity did not alter brachial artery endothelial function, but has shown a trend to reduce maximal handgrip strength.

Several short-term inactivity studies have shown significant increases in insulin concentration and decreases in insulin action (Knudsen et al., 2012; Krogh-Madsen et al., 2010; Stephens et al., 2011). In line with present findings, increases in insulin iAUC during low levels of physical activity with positive energy balance has been documented in other studies (Knudsen et al., 2012; Olsen et al., 2008; Walhin et al., 2013). Moreover, the beneficial role of reducing energy intake during inactivity was also observed (Stephens et al., 2011; Winn et al.,2019). Inactivity and energy surplus increases the risk of insulin resistance (Blanc et al., 1998; Stuart et al., 1988). Present study revealed that young and healthy individuals are prone to develop reduction in insulin sensitivity following short period of inactivity with energy imbalance. Dixon et al. (2013) documented that after one week of reduced activity and energy surplus, both lean and overweight showed similar increases in insulin and glucose area under the curve. This warrant attention on how serious and early metabolic alterations can occur and necessitate further exploration in higher risk groups that can develop insulin resistance, i.e., overweight and obese population. In addition, investigating whether

reducing energy intake in such population would yield beneficial outcomes is an interesting area of research. Increases in postprandial heart rate response detected is believed to be secondary to insulin-induced cardiac sympathetic nervous system activation (Facchini, Stoohs and Reaven, 1996; Moreira et al., 2015; Scherrer and Sartori, 1997; Siani et al., 1990). In the presence of high levels of insulin with no significant changes in blood glucose, insulin increases heart rate through the sympathetic nervous system enhancement among young and healthy participants (Siani et al.,1990). The sympathetic nervous system increases heart rate through the activation of β-adrenergic receptors in the heart (Mangmool et al., 2017; Salazar, Chen and Rockman, 2007). This reflects the integrated relationship between the metabolic and cardiovascular functions in the human body (Muniyappa et al., 2007). One of the essential roles of insulin is to maintain vascular function (Janus et al., 2016; Zheng and Liu, 2015). Any disturbance in insulin function can exhibit vascular dysfunction and consequently developing hypertension, atherosclerosis and endothelial dysfunction (Semenkovich, 2006; Steinberg et al., 1996; Tarray et al., 2014). Thus, the present research is of a great value since it showed that only two days of reducing activity can produce a combined alterations in metabolic and cardiovascular responses.

Peripheral vascular health has been shown to adversely affected by acute (within few hours) and short-term (within days or weeks) physical inactivity episodes (Daniele, Lucas and Rendeiro, 2022). FMD response is impacted by sedentary behaviour and has been shown to decrease with increased daily sitting time (Yamaji et al., 2022). The effects of prolonged sitting on endothelial function, one of the most researched models of inactivity, where endothelium-dependent dilation, blood flow, and shear rate

consistently diminish. In the current study, no significant changes in brachial artery endothelial function were detected in response to reduced physical activity. In support, brachial FMD response was not affected following sitting for three to six hours (Restaino et al., 2015; Thosar et al., 2014). However, when longer period of inactivity was applied (14 days) with no change in habitual energy intake, brachial artery FMD significantly reduced (Bowden Davies et al., 2021). It is difficult to interpret such findings where both inactivity and positive energy balance may contribute to the detrimental effects reported. Therefore, what strengthen the present study is that the confounding factor of energy surplus was eliminated by reducing energy intake to match energy expended during the intervention. Subsequently, findings can be attributed to inactivity *per se*. The effects of extended sitting time on the function of the endothelium in the upper and lower limbs have only been examined in a small number of studies (Daniele, Lucas and Rendeiro, 2022). Most studies have discovered that sitting mostly impacts endothelial function in the lower leg arteries among healthy and at-risk (overweight/obese) population (Kruse et al., 2018; Morishima et al., 2017; Vranish et al., 2017). There has been limited study comparing the effects of sitting on men and women, therefore it is unknown if vascular responses differ depending on gender (Daniele, Lucas and Rendeiro, 2022). Interestingly, sitting caused reductions in popliteal artery FMD in males, while vascular function was conserved in women (Vranish et al., 2017). This could be explained by the effect of estrogen, which protects the endothelial function (Maturana et al., 2007). This highlights the necessity for future research to assess the variations in vascular function during inactivity between genders. Studying the underlying processes relating to the lower limbs' selective vascular response to inactivity is also important.

It is generally known that sedentary lifestyle leads to a loss of skeletal muscle mass (Gianoudis et al., 2015). In addition, it increases the risk of developing sarcopenia later in life which is a disorder associated with aging that causes skeletal muscles to gradually lose strength and function (Cruz-Jentoft and Sayer 2019). It is unclear if vascular dysfunction brought on by inactivity is directly causing skeletal muscle tissue to lose some of its functional and structural properties (Daniele, Lucas and Rendeiro, 2022). Two potential factors that links inactivity with muscle function disturbance which are the inactivity-induced reduction in blood flow and increase in arterial stiffness that can affect muscle protein anabolism (Credeur et al., 2019; Mikines et al., 1991). Previous studies with different inactivity models and intervention duration (ranged from 5 days to 17 weeks) have shown that inactivity lowered muscle mass and function and decreased muscle protein synthesis rate (Burns et al., 2021; Birk et al., 2013; Dirks et al., 2014; Krogh-Madsen et al., 2010). Present findings showed that the handgrip strength tended to be reduced following inactivity. These findings greatly contribute to the exiting evidence in that inactivity *per se* under free-living environment and after short-term period increased the risk of developing muscle strength dysfunction among healthy population. Accordingly, further studies are necessary to detect early muscular changes following inactivity independent of energy imbalance among older population whom at a great risk of muscle function deterioration.

Taking together, findings from the current research demonstrated that few days of reduced physical activity has the potential to generate deleterious effects on health. Several preventive strategies have been proposed to counteract theses negative consequences (Daniele, Lucas and Rendeiro, 2022; Narici et al., 2021). One of the major interventions being used to mitigate the harmful consequences of inactivity is

physical exercise (Carter et al., 2019; Kim et al., 2014; Walhin et al., 2013). It has been shown that vigorous-intensity exercise has a tremendous impact on preventing the negative outcome of inactivity on insulin sensitivity even with a significant calorie excess (Walhin et al., 2013). There is promising evidence that various forms of physical exercise are successful in avoiding deficits in peripheral vascular function. Interrupting sitting time with frequent simple resistance exercises or performing aerobic exercises prior to prolong sitting have shown to prevent the inactivity-induced decreases in FMD responses (Ballard et al., 2017; Climie et al., 2018, Morishima et al., 2017). Interestingly, replacing sitting with standing can counteract the deterioration in cardiometabolic health (Morishima et al., 2017; Zeigler et al., 2016). However, specific recommendations on the frequency and length of standing that elicit the greatest benefit in conjunction with these findings is not yet have been made. Because eating and inactivity frequently go hand in hand, it has been documented that reducing daily energy intake of about 15 to 25 % can alleviate the detrimental consequences of inactivity (Narici et al., 2021). Findings from the present research highlighted the exacerbated effect of energy imbalance during inactivity and in support with this preventive dietary approach.

The detailed limitations for each main study can be found in the relative chapters, Chapter 3 for study 1 and Chapter 4 for study 2. Overall, the research was limited by the small sample size where findings may not be generalised to most of the population. However, the present thesis demonstrated several methodological strengths. Studies applied were designed in a randomised crossover fashion. Each participant served as his/her own control. In addition, control arms were applied to each main study to establish reliable comparison against well-controlled and standardised conditions.

Energy intake during intervention was well-controlled which facilitate the assessment of inactivity *per se*. Measurements were applied in free-living and standardised laboratory settings. Participants were young and healthy which are a low-risk population. This can help to understand the pathophysiological responses which can be transferred to at-risk population and utilised the knowledge for the prevention and management of disorders.

In conclusion, both excessive calorie consumption and physical inactivity harm the cardiovascular and metabolic systems. Such information ought to be addressed and utilised to direct lifestyle changes and to be incorporated to current physical activity guidelines. Recommendations to reduce energy intake, increase standing time and interrupting sitting time by frequent exercise sessions can have a major preventive role against deleterious health effects observed. Future studies applying similar study protocol among overweight/obese and older population is recommended. Gender difference in FMD responses under the same intervention would be an interesting area to explore.

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Appendices

Appendix 1: Consent form

University of Nottingham,
School of Life Sciences, The Medical School.

CONSENT FORM

Final version 1.1

Title of Study:

REC ref:

Name of Researchers:

Name of Participant:

Please initial box

1. I confirm that I have read and understand the information sheet version number 1.1 dated 08/12/15 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. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of data collected in the study may be looked at by responsible individuals from the University of Nottingham, the research group where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐

4. I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for 7 years after the results of this study have been published. ☐

5. I have not been a subject in any other research study in the last three months, which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg blood sample >50ml) or exposure to ionising radiation. ☐

6. **Optional:** I agree that the samples and data sets collected about me can be stored by the above research team at the University of Nottingham for possible use in future studies (approved by an Ethics Committee). ☐

7. I understand that some of these studies may be carried out by researchers other than the current Research team, who ran the first study. Any data used will be anonymised, and I will not be identified in any way. ☐

8. I understand that the tests or procedures are carried out for research only and not for clinical diagnostic purposes. However, if the study investigator should feel it necessary to inform my GP of my participation in the study, an adverse event or abnormal test result, I understand I am giving my consent to do so. ☐

9. I confirm that I have disclosed relevant medical information before the study. ☐

10. I voluntarily agree to take part in the above study. ☐

11. **Optional:** I would be interested in being contacted about further studies in the future. ☐

Name of Participant	**Date**	**Signature**

Name of Person taking consent (if different from Principal Investigator)	Date	Signature

Name of Principal Investigator	Date	Signature

Appendix 2: Health questionnaire

PERSONAL INFORMATION

Subject Number _____ Date ___/____/___

1. **Name** ……..

2. **Contact Address** …….

3. **Contact Telephone number** .....……...

4**.** **Date of Birth** ……./……./ 19…….

GENERAL HEALTH

---

1. In general would you say your health is;

Please tick one response

a.	**Excellent**	☐
b.	Very good	☐
c.	Good	☐
d	Fair	☐
e.	Poor	☐

2. Compared to one year ago, how would you rate your health in general now?
Please tick one response

a.	Much better than a year ago	☐
b. .	Somewhat better than a year ago	☐
c.	About the same	☐
d. .	Somewhat worse than a year ago	☐
e. .	Much worse than a year ago	☐

3. The following questions are concerned with any medical conditions you might have.

Please tick one box on each line

	No	*Yes*	*Don't Know*
Diabetes			
Epilepsy			
Depression			
Hayfever			
Asthma			
Eczema			
High blood pressure			
Heart problems			
Breathing problems *			
Indigestion / heartburn			
Irritable bowel syndrome			
Any other medical condition not listed above (please			

*** other than asthma**

4. **Do you have a tendency to faint in certain situations?**

☐ **Yes** ☐ **No** ☐ **Don't Know**

eg. on standing, in a warm room, after fasting

5. **Smoking** On average, how many cigarettes do you smoke a day?……..

(If a non – smoker, please put 'none')

Please detail any other medical condition/s

If you answered '*yes*' to any of the above, when did you first start having the problem and what medication (if any) do you use?

……
……
……

DIET AND HEALTH

1. The following questions relate to your diet and your health

Please tick one box on each line

a. Are you on a medically **prescribed diet?** ☐ ☐ ☐

eg diabetic, gluten-free etc **Yes** **No** **Don't Know**

If 'yes', what diet are you following? ……..……
……..……

b. Do you suffer from any **food allergies**? Eg nuts, wheat, milk ☐ ☐ ☐

Yes **No** **Don't Know**

If 'yes', which foods are you allergic to?

……..……
……..……

c. Do you regularly (at least once a week) take **vitamin** and / or **mineral** supplements ☐ ☐ ☐

Yes **No** **Don't Know**

If 'yes', which supplements do you take?

……..……

……..……

d. Do you regularly (at least once a week) take any other supplements for your health? **Yes** ☐ **No** ☐ **Don't Know** ☐

Eg. Creatine, Carnitine, Echinacea, St John's Wort, etc

If 'yes', which supplements do you take?

……..……

……..……..

2. **Alcohol** On average, how many units of alcohol do you drink in a week?

…….Units

(pint of beer/lager =3 units, pub measure of wine / spirits =1 unit)

PHYSIOLOGICAL MEASUREMENTS

1. **Height ……cm**

2. **Weight ……kg**

3. **BMI …….kg/m^2**

4. **Waist circumference ……cm**

5. **Skinfold test:**

   Biceps: ……mm /Triceps: ……mm /Suprailiac: ……mm /Subscapular: ……mm

6. **BP ……./……. Sitting ……./……. Standing (1min) ……./…….Standing (5 min)**

7. **HR: ……/……../……**

8. **12-lead ECG test** **Yes** ☐ **No** ☐

9. **Have you taken part, or do you intend to take part in** any other trials within the three months prior to or following this particular study? Yes ☐ No ☐

Appendix 3: International Physical Activity Questionnaires (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRES

IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health- related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken in 12 countries (14 sites) across 6 continents during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages. IPAQ is suitable for use in regional, national and international monitoring and surveillance systems and for use in research projects and public health program planning and evaluation. International collaboration on IPAQ is on-going and an international prevalence study is under development.

Using IPAQ

Worldwide use of the IPAQ instruments for monitoring and research purposes is encouraged.

It is strongly recommended, to ensure data quality and comparability and to facilitate the development of an international database on health-related physical activity, that

- no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments,
- if additional questions on physical activity are needed they should follow the IPAQ items,
- translations are undertaken using the prescribed back translation methods (see website)
- new translated versions of IPAQ be made available to others via the web site to avoid duplication of effort and different versions in the same language,
- a copy of IPAQ data from representative samples at national, state or regional level be provided to the IPAQ data storage center for future collaborative use (with permission) by those who contribute.

More Information

Two scientific publications presenting the methods and the pooled results from the IPAQ reliability and validity study are due out in 2002.

More detailed information on the IPAQ process, the research methods used in the development of the IPAQ instruments, the use of IPAQ, the published papers and abstracts and the on-going international collaboration is available on the IPAQ web-site.

www.ipaq.ki.se

INTERNATIONAL PHYSIC AL ACTIVITY QUESTIONNAIRE IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

NOTE: EXAMPLES OF ACTIVITIES MAY BE REPLACED BY CULTURALLY RELEVANT EXAMPLES WITH THE SAME METS VALUES (SEE AINSWORTH ET AL., 2000).

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. This is part of a large study being conducted in many countries around the world. Your answers will help us to understand how active we are compared with people in other countries.

The questions are about the time you spent being physically active in the last 7 days. They include questions about activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Your answers are important.

Please answer each question even if you do not consider yourself to be an active person.

THANK YOU FOR PARTICIPATING.

In answering the following questions,

- **vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder that normal.

- **moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder that normal.

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

Think about *only* those physical activities that you did for at least 10 minutes at a time.

_____days per week ⇨

or

☐ **none**

1b. How much time in total did you usually spend on one of those days doing vigorous physical activities*?*

_____hours_____minutes

2a. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____days per week ⇨

or

☐ **none**

2b. How much time in total did you usually spend on one of those days doing moderate physical activities*?*

_____hours_____minutes

3a. During the last 7 days, on how many days did you **walk** for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

_____days per week ⇨

or

☐ **none**

3b. How much time in total did you usually spend walking on one of those days*?*

_____hours_____minutes

The last question is about the time you spent sitting on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

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

____hours_____minutes

This is the end of questionnaire, thank you for participating.

Appendix 4: Beck Depression Inventory questionnaire

Beck Depression Inventory

Please read each group of statements carefully and pick out the one statement that best describes the way you have been feeling during the past two weeks, including today. Circle the number besides the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group.

A. Mood
- 0 I do not feel sad
- 1 I feel blue or sad
- 2a I am blue or sad all the time and I can't snap out of it
- 2b I am so sad or unhappy that it is very painful
- 3 I am so sad or unhappy that I can't stand it

B. Pessimism
- 0 I am not particularly pessimistic or discouraged about the future
- 1a I feel discouraged about the future
- 2a I feel I have nothing to look forward to
- 2b I feel that I won't ever get over my troubles
- 3 I feel that the future is hopeless and that things cannot improve

C. Sense of Failure
- 0 I do not feel like a failure
- 1 I feel I have failed more than the average person
- 2a I feel I have accomplished very little that is worthwhile or that means anything
- 2b As I look back on my life all I can see is a lot of failures
- 3 I feel I am a complete failure as a person (parent, husband, wife)

D. Lack of Satisfaction
- 0 I am not particularly dissatisfied
- la I feel bored most of the time
- lb I don't enjoy things the way I used to
- 2 I don't get satisfaction out of anything any more
- 3 I am dissatisfied with everything

E. Guilty Feeling
- 0 I don't feel particularly guilty
- 1 1 feel bad or unworthy a good part of the time
- 2a I feel quite guilty
- 2a I feel bad or unworthy practically all the time now
- 3 I feel as though I am very bad or worthless

F. Sense of Punishment
- 0 I don't feel I am being punished
- 1 I have a feeling that something bad may happen to me
- 2 I feel I am being punished or will be punished
- 3a I feel I deserve to be punished
- 3b I want to be punished

G. Self-Hate
- 0 I don't feel disappointed in myself
- 1a I am disappointed in myself
- 1b I don't like myself
- 2 1 am disgusted with myself
- 3 I hate myself

A. Self- Accusations

0 I don't feel I am any worse than anybody else
1 I am very critical of myself for weaknesses or mistakes
2a I blame myself for everything that goes wrong
2b I feel I have many bad faults

B. Self- Punitive Wishes

0 I don't have any thoughts of harming myself
1 I have thoughts of harming myself but I would not carry them out
2a I feel I would be better off dead
2b I have definite plans about committing suicide
2c I feel my family would be better off if 1 were dead
3 I would kill myself if I could

C. Crying Spells

0 I don't cry any more than usual
1 I cry more now than I used to
2 I cry all the time now. I can't stop it
3 I used to be able to cry but now I can't cry at all even though 1 want to

D. Irritability

0 I am no more irritated now than I ever am
1 I get annoyed or irritated more easily than I used to
2 I feel irritated all the time
3 I don't get irritated at all at the things that used to irritate me

E. Social Withdrawal

0 I have not lost interest in other people
1 I am less interested in other people now than I used to be
2 I have lost most of my interest in other people and have little feeling for them
3 I have lost all my interest in other people and don't care about them at all

F. Indecisiveness

0 I make decisions about as well as ever
1 I am less sure of myself now and try to put off making decisions
2 I can't make decisions any more without help
3 I can't make any decisions at all any more

G. Body Image

0 I don't feel I look any worse than I used to
1 I am worried that I am looking old or unattractive
2 I feel that there are permanent changes in my appearance and they make me look unattractive
3 I feel that I am ugly or repulsive looking

H. Work Inhibition

0 I can work about as well as before
1a It takes extra effort to get started at doing something
lb I don't work as well as I used to
2 I have to push myself very hard to do anything
3 I cannot do any work at all

I. Sleep Disturbance

0 I can sleep as well as usual
1 I wake up more tired in the morning than I used to
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
3 I wake up early every day and can't get more than 5 hours sleep

J. Fatigability

0 I don't get any more tired than usual
1 I get tired more easily than I used to
2 I get tired from doing anything
3 I get too tired to do anything

A. Loss of Appetite

0 My appetite is no worse than usual
1 My appetite is not as good as it used to be
2 My appetite is much worse now
3 I have no appetite at all any more

B. Weight Loss

0 I haven't lost much weight, if any, lately
1 I have lost more than 5 pounds
2 I have lost more than 10 pounds
3 I have lost more than 15 pounds

C. Somatic Preoccupation

0 I am no more concerned about my health than usual
1 I am concerned about aches and pains or upset stomach or constipation or other unpleasant feelings in my body
2 I am so concerned with how I feel or what I feel that it's hard to think of much else
3 I am completely absorbed in what I feel

D. Loss of Libido

0 I have not noticed any recent change in my interest in sex
1 I am less interested in sex than I used to be
2 I am much less interested in sex now
3 I have lost interest in sex completely

Thank you…

All information provided will be kept strictly confidential and used only for the purpose of this study

Appendix 5: Eating Attitude Test-26 Questionnaire

Please choose one response by marking a check to the right for each of the following statements:		Always	Very often	Often	Sometimes	Rarely	Never
1	Am terrified about being overweight						
2	Avoid eating when I am hungry						
3	Find myself preoccupied with food						
4	Have gone on eating binges where I feel that I may not be able to stop.						
5	Cut my food into small pieces.						
6	Aware of the calorie content of foods that I eat						
7	Particularly avoid food with a high carbohydrate content (i.e. bread, rice, potatoes, etc.)						
8	Feel that others would prefer if I ate more.						
9	Vomit after I have eaten.						
10	Feel extremely guilty after eating.						
11	Am occupied with a desire to be thinner						
12	Think about burning up calories when I exercise						
13	Other people think that I am too thin.						
14	Am preoccupied with the thought of having fat on my body						
15	Take longer than others to eat my meals						
16	Avoid foods with sugar in them.						
17	Eat diet foods.						
18	Feel that food controls my life						
19	Display self-control around food.						
20	Feel that others pressure me to eat						
21	Give too much time and thought to food						
22	Feel uncomfortable after eating sweets						
23	Engage in dieting behaviour.						
24	Like my stomach to be empty.						
25	Have the impulse to vomit after meals.						
26	Enjoy trying new rich foods.						

Appendix 6: Food diary

Subject ID -..... Day –No..... .....Date.....

Time	Type of food or drink with brand names and cooking method	Amount	Food or drink left over and amount	Office use only: code and weight

Appendix 7: Indirect calorimetry (COSMED) SOP



Standard Operating Procedure

Title: COSMED Quark RMR for resting energy expenditure

SOP ref: SOPL055

Version and Date: 1.1 5th January 2017

Effective from date:

5th January 2017

Review Cycle: 2 yrs after effective date

Superseded Version and Date:

Version 1.0, 25/03/2015

Author:

Name and position: J Mallinson Research technician

Authorised by:

Name and position: Prof Dilleep Lobo DGHPU Clinical Director

Document History:

1:

Modification to previous version:

1:

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1.BACKGROUND

Energy Expenditure can be measured indirectly with a metabolic cart. Gas exchange is analysed to determine the volume of air passing through the lungs, the uptake of oxygen (VO2) and the amount of Carbon Dioxide expelled to atmosphere (CO2 output – VCO2). These measurements allow the Resting Energy Expenditure (REE) and the Respiratory Quotient (RQ) to be calculated. The RQ represents the ratio of carbon dioxide exhaled to the amount of oxygen consumed by the individual. RQ assists in the interpretation of the REE results. The abbreviated Weir equation is a commonly used method to calculate the REE.

Abbreviated Weir equation:
REE = [3.9 (VO2) + 1.1 (VCO2)] 1.44

2. EQUIPMENT

For breath by breath mode:

1) Quark Unit
2) Turbine Flowmeter (small)
3) Sampling line
4) Resting Hood
5) 3L Calibration Syringe
6)

Equipment Examples:



3L Calibration Syringe



Resting Hood



Turbine Flowmeter(small)

4.0 PROCEDURE

a) Calibration

1. The volunteers should be given adequate explanation of the experimental protocol to ensure that an 'informed' consent to participate is given. A third party should be present during the explanation of the experiment/protocol and should witness the volunteer's signature on the consent form. This third party should have knowledge of the protocol and must be able to confirm that a full and correct description has been given, and also that the subject has a good understanding of what will take place.

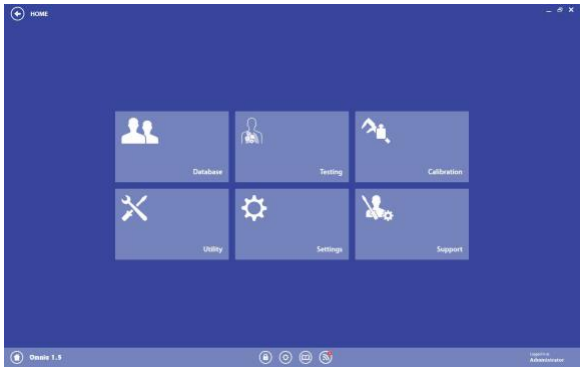
2. As with all 'human study' protocols, two experienced experimenters should be present throughout the study.

3. Prior to starting any exercise protocol, the investigator should perform a twelve lead ECG, together with BP and Heart Rate monitoring at rest, on all subjects. The ECG should be checked by a physician before the exercise test begins.

   Monitor heart rate throughout. If subject feels faint or unwell at any time, stop the test immediately.

4. Switch power on at supply, allow 10 mins for Quark to warm up.

5. Turn on computer and click on COSMED Omnia icon, enter password (on front of PC screen) and this screen will be shown.

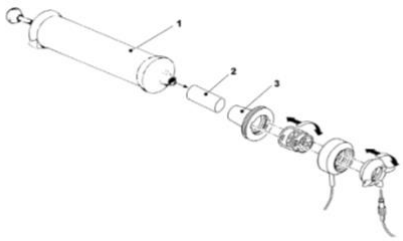


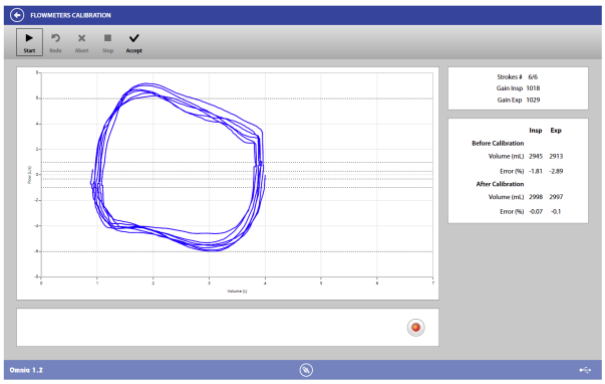
6. **Calibration**: Both the flowmeter and gas need to be calibrated before use.

a) Flowmeter: Perform a flowmeter calibration at the start of the study day (this doesn't need to be repeated throughout the day).
Select calibration, then flowmeter calibration. Attach turbine to 3 litre syringe and ensure cable and sampling line attached to the rear of the quark box. Ensure the syringe piston

is pushed all the way in. Press start and select device (25mm for exercise, 18mm for resting). Wait until red dot on right of screen turns green then move the piston in and out for 6 strokes, keeping line between a flow of -1 and 1 L/s. Once complete, if happy with values (1-4% error is ok), press accept.

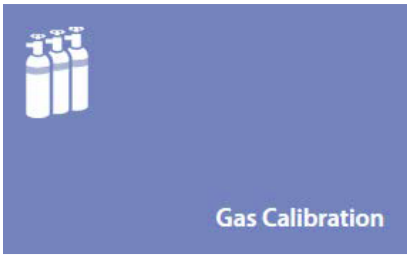


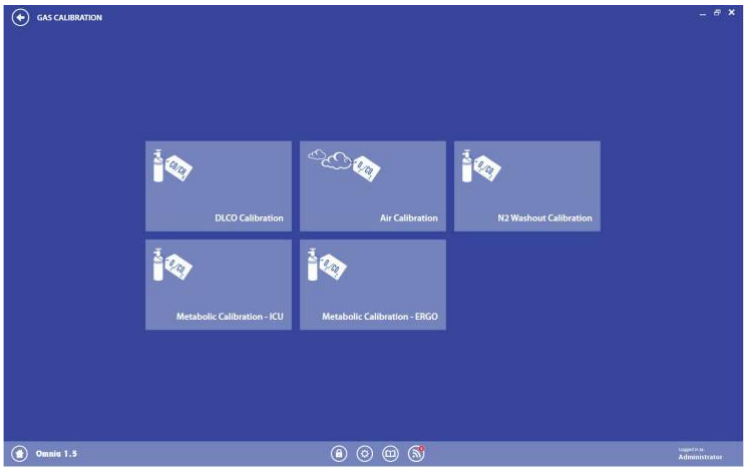




b) Gas calibration: this should be performed before each test. Ensure sampling line from the turbine is placed in the front of the quark analyser. Open the gas bottle and ensure pressure is 5 bar.

In the calibration panel select gas calibration then metabolic calibration – ERGO. Press start then check the warnings, the cylinder gas concentrations (O2 16%, CO2 5%) and the O2 span (22%). The system automatically has a 3min warm up before gas calibration, if the machine has been on for at least 10mins you can deselect this option. Press ok and the calibration will run.





After calibration the results are shown graphically (below). If any value is out of range it will be highlighted and calibration should be repeated. If calibration values remain unacceptable you should contact technical assistance. Otherwise, if values are in range press ok to finish the test.



b) Performing the test

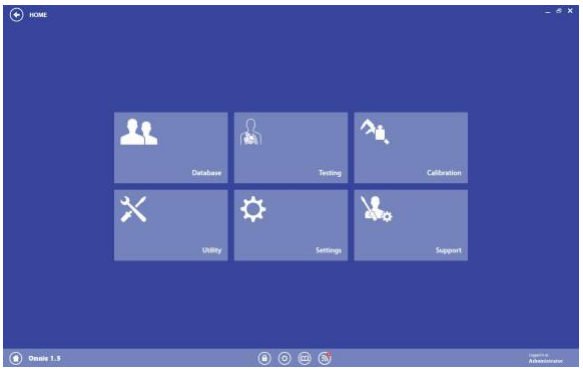
General Recommendations

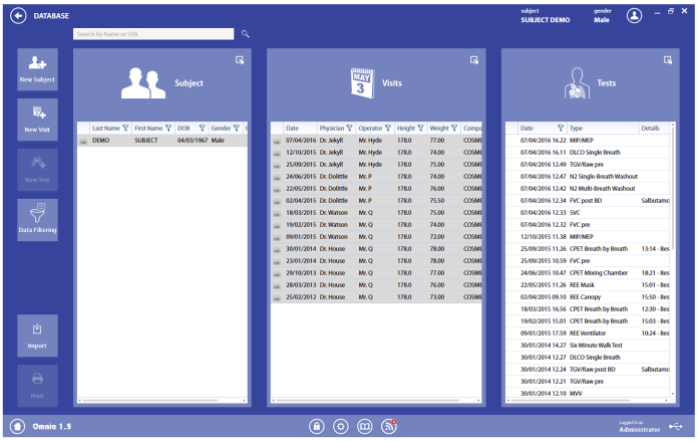
To allow for optimal conditions prior to, during and after the REE measurement:

• The subject is recommended not to eat for at least 12 hours prior to the measurement.
• Testing conditions should optimally remain quiet throughout the procedure, and a normal room temperature must be maintained.
• A steady state should be achieved during the test, which is defined as a 5 minute period when the average minute VO2 and VCO2 changes by less than 10% and the average RQ changes by less than 5%.
• The subject should refrain from moving during the test.
• A test of minimum 15-20 minutes is recommended to assure stable, interpretable measurements.
• The first 5 minutes of data acquisition should be discarded when performing the test.

1. The volunteer should be rested on a bed in a semi-supine position for ~15mins prior to testing.

2. **Entering a new subject:** From main panel select database, and new subject. Enter subject's details, mandatory fields are highlighted in red, press save. Please use a subject code rather than name. Subject details are now saved into the database. To start a test select new test.

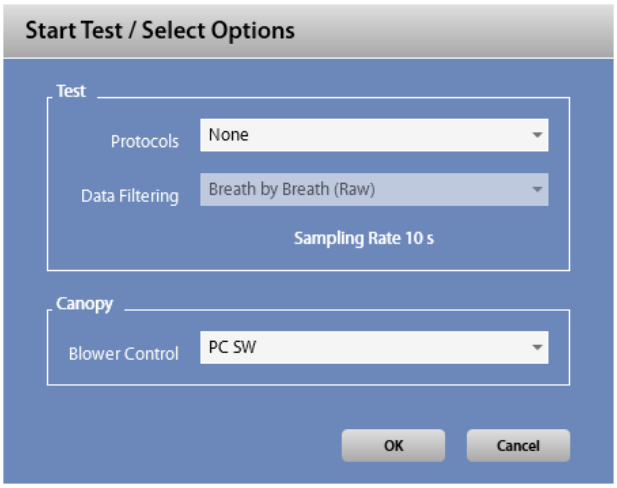




3. **Running a test:** Once subject details entered select new test. If subject returning for 2nd visit, select subject then click new visit. At this point the weight can be changed if it has

altered from the first visit. Next select metabolic then Resting Energy Expenditure, then REE - Canopy. You will then be prompted for test options, these should read:

Protocol: none, Blower control: PC SW



Ensure the turbine/flowmeter and tubing is placed into the back of the quark and the blower switch has been turned on (this will beep a couple of times). Place the canopy with tubing and bacterial filter attached over the volunteer. Do not place the canopy hood over a patient's head before the tube is properly connected and a continuous flow has been applied from the canopy blower.

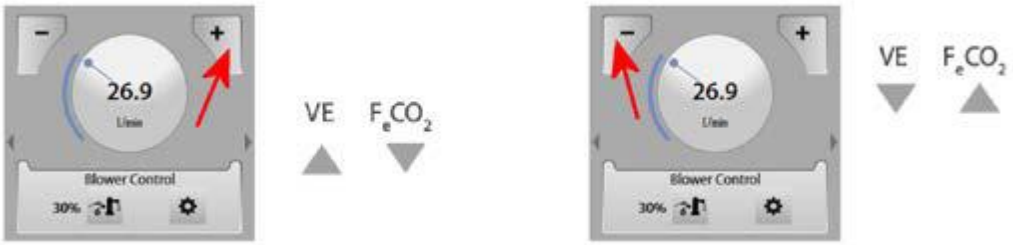


Once the volunteer is settled press ok and then press start. The machine will start up. Data is not stored until REC is selected or 2 mins has passed, after which the data automatically starts to be stored.

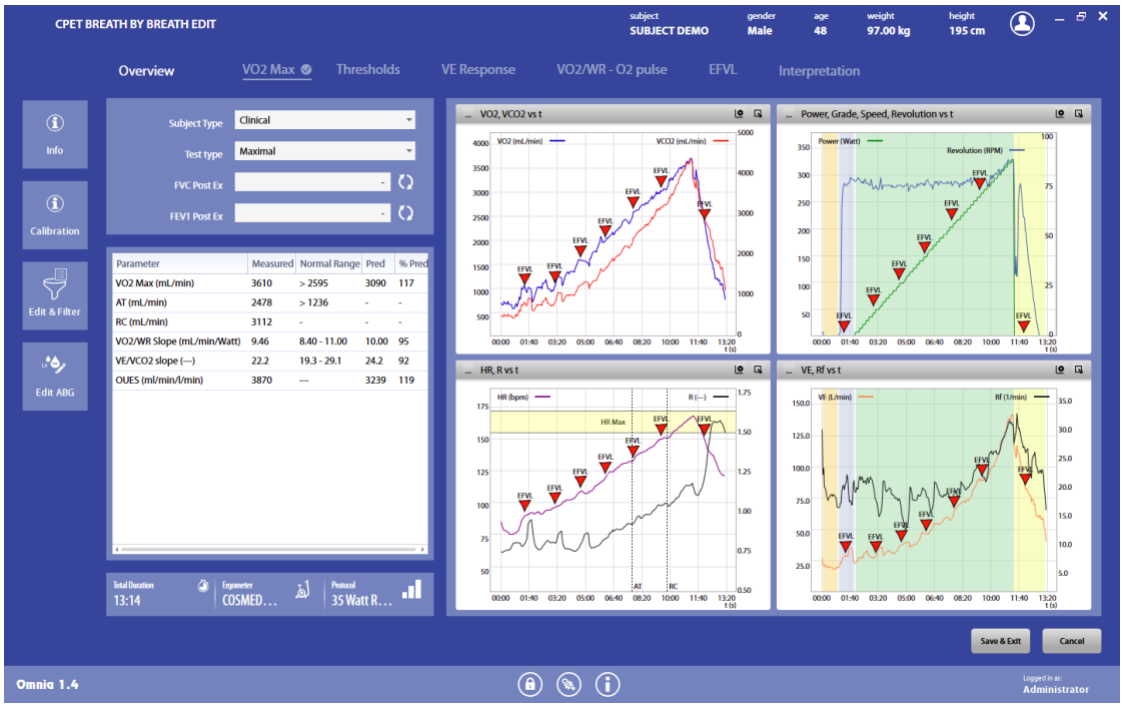
4. **Dashboard**: the dashboard can be configured to show data of interest. For resting measurements we show VO2/VCO2, EE/RQ, VP/FeCO2 and CHO%/FAT%. For any

other data to be displayed go to utility, customise views and select which parameter you would like to show.

5. **Running the test:** the main point during the test is to ensure the FeCO2 lies between 0.5 - 0.6%. This can be regulated by altering the air flow/ventilation through the hood using the blower control on screen. Increasing flow rate will decrease FeCO2 and vice versa. This should become stable after a few minutes into the test. Run the test for 15-20 mins and then press stop and remove the canopy from the volunteer.



6. **Data:** Once the test is completed you will be taken to the edit page. Here data can be filtered if needed, otherwise click & exit. The next page will then allow you to save data in excel format.



Medical/Staffing cover required

For the purposes of the instructions set out below ‘High risk’ individuals are those who:

- Are age >50 years
- Have a Body Mass Index (BMI) >30
- Have any medical condition that might affect or be affected by exercise

‘Low risk’ individuals are all other volunteers aged 18-49 years who are deemed otherwise fit and healthy.

Cover for ‘Low risk’ individuals:

- 2 people in the room responsible for running the test (at least one who has experience in running VO2 max tests)
- An ILS trained person immediately available on the unit

- Medical cover on phone/bleep

  Cover for ‘High risk’ individuals:

- 2 people in the room responsible for running the test, one of whom should be ILS trained and at least one who has experience of running VO2 max tests
- A medically qualified doctor on the unit immediately available

Hazards

- Ensure subject has had a health screening, given signed informed consent, and that medical cover is in place.

- Always monitor subject for signs of ill health, such as feeling unwell or faint, particularly if they have recently had an invasive procedure such as a muscle biopsy.

Any problems please contact Jo Mallinson or one of the technical staff on B Floor ex 19107

Measured parameters

Breath-by-Breath exercise testing Symbol	**UM**	**Parameter**
VO2	ml/min	Oxygen uptake
VCO2	ml/min	Carbon Dioxide production
Vt	l	Tidal Volume

FetO2	%	End Tidal O2
FetCO2	%	End Tidal CO2
R	---	Respiratory Quotient
VE	l/min	Ventilation
HR	1/min	Heart Rate
Qt	l	Cardiac Output
AT	---	Anaerobic Threshold
VE	l/min	Ventilation
SV	l/min	Stroke Volume
RF	1/min	Respiratory frequency
FeO2, FeCO2	%	Averaged expiratory concentration of O2 e CO2
VE/VO2	---	Ventilatory equivalent for O2
VE/VCO2	---	Ventilatory equivalent for CO2
VO2/HR	ml/beat	Oxygen pulse
VO2/Kg	ml/min/Kg	VO2 per Kg
Ti, Te, Ti/Ttot	sec	Time breaths
Vd/Vt	---	Vd/Vt ratio
PaCO2	mmHg	Arterial PCO2 (estimated)
P(a-et)CO2	mmHg	Delta PaCO2 – PetCO2

Appendix 8: Examples of activity templates for Study 1 and 2

(A) PAL 1.85 (B) PAL 1.40

A

Time	Activity	Duration (hr)	PAR	Duration*PAR	**RMR 1647 kcal/ day** = 68.6 kcal/ hr
23:30-07:30	Sleeping	8	1	8.0	548.8
07:30-08:00	Personal care	0.5	2.3	1.2	78.9
08:00-08:30	Breakfast	0.5	1.5	0.8	51.5
08:30-09:00	Walking	0.5	3.2	1.6	109.8
09:00-10:45	Sitting	1.75	1.45	2.5	174.1
10:45-11:00	Snack	0.25	1.5	0.4	25.7
11:00-13:00	Walking	2	3.2	6.4	439.0
13:00-13:30	Lunch	0.5	1.5	0.8	51.5
13:30-14:45	Sitting	1.25	1.45	1.8	124.3
14:45-15:00	Snack	0.25	1.5	0.4	25.7
15:00-16:30	Sitting	1.5	1.45	2.2	149.2
16:30-17:00	Walking	0.5	3.2	1.6	109.8
17:00-17:30	Dinner	0.5	1.5	0.8	51.5
17:30-19:00	Walking	1.5	3.2	4.8	329.3
19:00-19:15	Snack	0.25	1.5	0.4	25.7
19:15-19:30	Sitting	0.25	1.45	0.4	24.9
19:30-22:00	Walking	2.5	3.2	8.0	548.8
22:00-23:00	Sitting	1	1.45	1.5	99.5
23:00-23:30	Personal care	0.5	2.3	1.2	78.9
			TEE	44.4	**3046.7**
			PAL	**1.85**	**1.85**

B

Time	Activity	Duration (hr)	PAR	Duration*PAR	RMR 1647 kcal/ day = 68.6 kcal/ hr
23:30-07:30	Sleeping	8.00	1	8.0	548.8
07:30-08:00	Personal care	0.50	2.3	1.2	78.9
08:00-08:30	Breakfast	0.50	1.5	0.8	51.5
08:30-09:00	Public transportation	0.50	1.2	0.6	41.2
09:00-10:45	Sitting	1.75	1.45	2.5	174.1
10:45-11:00	Snack	0.25	1.5	0.4	25.7
11:00-13:00	Sitting	2.00	1.45	2.9	198.9
13:00-13:30	Lunch	0.50	1.5	0.8	51.5
13:30-14:45	Sitting	1.25	1.45	1.8	124.3
14:45-15:00	Snack	0.25	1.5	0.4	25.7
15:00-16:30	Sitting	1.50	1.45	2.2	149.2
16:30-17:00	Walking	0.50	3.2	1.6	109.8
17:00-17:30	Dinner	0.50	1.5	0.8	51.5
17:30-19:00	Sitting	1.50	1.45	2.2	149.2
19:00-19:15	Snack	0.25	1.5	0.4	25.7
19:15-19:30	Sitting	0.25	1.45	0.4	24.9
19:30-19:50	Walking	0.33	3.2	1.1	73.2
19:50-23:00	Sitting	3.17	1.45	4.6	314.9
23:00-23:30	Personal care	0.50	2.3	1.2	78.9
			TEE	33.5	**2297.7**
			PAL	**1.40**	**1.40**

Appendix 9: An example of meal template for Study 1 and 2

Meal time	Composition	Weight of food (g)
09:00-09:30	Sainsbury's British Semi Skimmed Milk	375
	Hovis Wholemeal Bread, Medium Sliced	120
	Sainsbury's English Butter, Salted	6
10:45-11:00	McVitie's Toasting Waffles	50
	Muller Corner Plain Yogurt with Strawberry Compote 150g	150
13:00-13:30	Tuna ans Mayo Sandwich	138
	McVitie's Digestive Biscuits (Original)	70
14:45-15:00	Sainsbury's Granny Smith Apples	260
	Walkers Ready Salted Crisps (1 pack 25g)	25
17:00-17:30	Sainsbury's Indian Chicken Tikka Masala with Pilau Rice	450
19:00-19:30	Muller Corner Plain Yogurt with Strawberry Compote 150g	150
	Walkers Ready Salted Crisps (1 pack 25g)	50

Appendix 10: Finometer SOP

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Standard Operating Procedure

Title: Finometer

SOP ref: SOPL058
Version and Date: 1.0 2nd May 2017

Effective from date:
2nd May 2017

Superseded Version and Date:

Review Cycle: 2 yrs after effective date

Author:
Name and position: Melanie Marshall Human Studies Technician
Sara Brown DGHPU Manager

Authorised by:
Name and position: Prof Dilleep Lobo DGHPU Clinical Director

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Document History:	Modification to previous version:
1:	1:

David Greenfield Human Physiology Unit, University of Nottingham, School of Life Sciences, QMC, Derby Road, Nottingham NG7 2UH. Tel: 01158230248

1. BACKGROUND

This SOP has been written to give general guidance to study personnel on how to correctly and safely use the Finapres Blood pressure machine within studies produce automated blood pressure readings.

The FINOMETER PRO is a non-invasive, FINGER TIP, stationary blood measurement and beat-to-beat hemodynamic monitoring system. It captures the continuous blood pressure waveform and automatically computes 15 beat-to-beat hemodynamic parameters including Cardiac Output (CO), Stroke Volume (SV), Total Peripheral Resistance (TPR), Pulse Rate Variability (PRV) and BaroReflex Sensitivity (BRS)

2. EQUIPMENT

1. Finapres Finometer
2. Various sized finger cuffs
3. Wrist Box
4. Arm Cuff (attached to the Finometer)

3. PROCEDURE

1. Explain to the subject what you are going to do. The volunteer should be given adequate explanation of the experimental protocol to ensure that an ‘informed’ consent to participate is given

2. The health and safety of the volunteer is of primary concern during the experiment. All studies, unless stated otherwise in the Ethics Committee submission, should have two observers, one being the investigator

3. Ask the Volunteer to lie supine on a couch/bed, to totally relax for approximately 30 minutes prior to data collection

4. Turn the Finometer on at the back of the machine, and then select the ‘Research’ option by pressing the rectangular button below this screen option. Press ‘Mark’ to confirm

5. Input ‘volunteer’ information, i.e. gender, age, weight etc. using the four arrow keys on the panel. Press the ‘Describe Subject’ button to confirm the data

6. Place the arm cuff firmly on the upper part of the arm

7. Place the wrist box firmly on the corresponding wrist, attach like a watch, i.e. facing outwards

8. Now place a finger cuff on the lower part of the ‘middle finger’, with the light sensors on the sides of the finger. The cuff should fit snugly, i.e. not too large (overlapping) or small (over stretched). See Fig. 1 and Fig. 2

Fig. 1

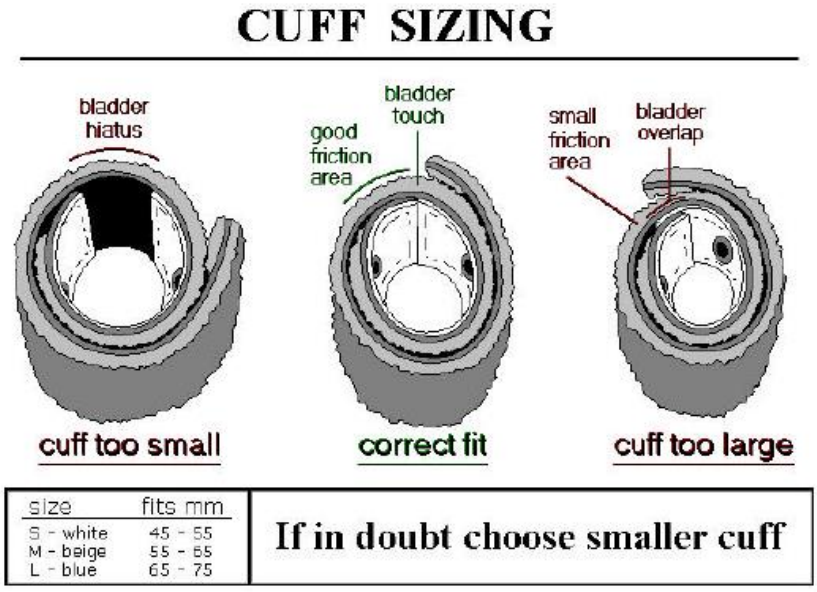
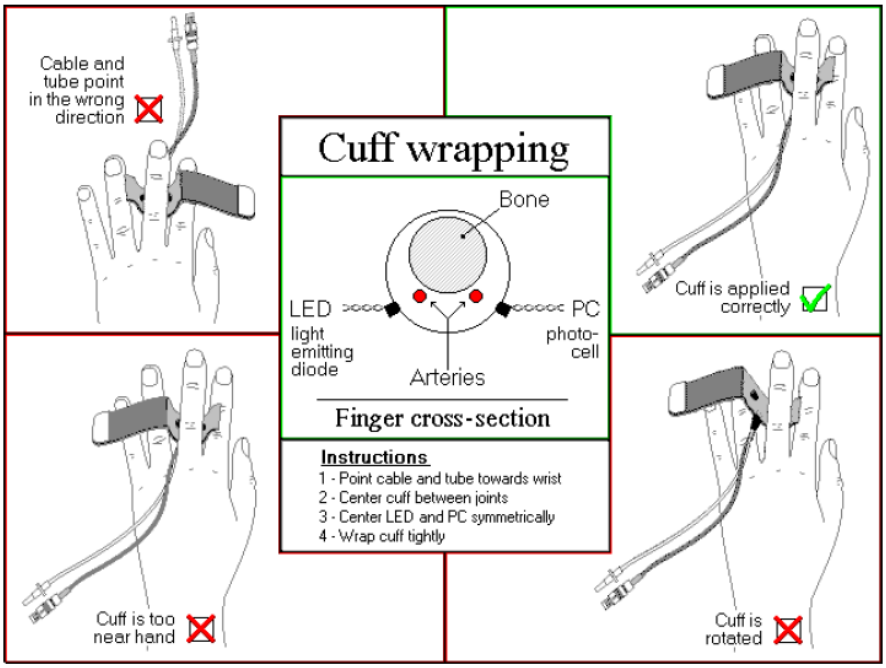


Fig 2.



9. Insert the sensor connections from the finger cuff into the wrist box. The metal end corresponding to the red dot on the box

10. Neutralise the 2 sensors by bringing them together and pressing the ‘Mark’ button on the Finometer. Now place the circular sensor on the arm cuff and the rectangular one on the top of the finger cuff

11. Now press the ‘Start’ button

12. Wait for at least 2 minutes until the ‘physiological measurements’ on the screen indicate ‘great’ or excellent

13. Use the arrow keys to tab along to the ‘RTF cal’ window. Arrow down to select ‘Ramp’ and press the ‘RTF cal button’. Wait for the ‘successful’ comment after approximately 2 minutes and then press ‘Mark’ to indicate the start of your ‘baseline’ readings

14. Always remember to make a note of the filename and date

15. Press ‘Mark’ and record the time on your study Proformata, at the various significant times of your study, i.e. when drinking/eating, making other physiological measurements

16. Upon completing your study and finishing, press ‘Mark’, then press the ‘Stop’ button once and then again to confirm

17. To turn the Finometer off press the << and >> buttons simultaneously

18. Remember to turn the Finometer off at the back and also at the mains supply

Note: Do not start the machine if the cuff is attached to the ‘wrist’ box but not around a finger. Do not take the cuff off the finger at any point unless it has been disconnected from the ‘wrist’ box or the machine is switched off

Downloading Data using ‘Fino Link’:

1. Using the wire and adaptor, connect the Finometer to a PC with the ‘Fino Link’ Download program

2. Turn the Finometer on

3. Select ‘Fino Link File Download’ from the desktop

4. Select ‘Choose Directory’ from the top left of the screen
5. Highlight the files required

6. Click on the < key to download

7. In the next window select the appropriate file and rename if necessary, putting the .fpf at the end

8. Click on ‘Start Download’ – this may take some time depending upon the size of the files

9. Now open ‘Beatscope Easy’ from the desktop

10. Open the file you have previously downloaded from ‘Fino Link’

11. Zoom out to see the entire file

12. Highlight all the data required, then select File/Export/Beats

13. Give the file a sensible name and put .txt at the end

14. The file can now be opened up in Excel as usual (- remember to select ‘semi colon’ option during the conversion process)

Appendix 11: Venous occlusion plethysmography SOP

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Standard Operating Procedure

Title: Limb Blood Flow using EC6 Hokanson Plethysmograph

SOP ref: SOPL029
Version and Date: 1.0 21st March 2017

Effective from date:
21st March 2017

Review Cycle: 2 yrs after effective date

Superseded Version and Date:

Author:

Name and position: Julie Littlewood Research Associate
Melanie Marshall Human Studies Technician
Sara Brown DGHPU Manager

Authorised by:

Name and position: Prof Dilleep Lobo DGHPU Clinical Director

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Document History:	Modification to previous version:
1:	1:

David Greenfield Human Physiology Unit, University of Nottingham, School of Life Sciences, QMC, Derby Road, Nottingham NG7 2UH. Tel: 01158230248

This SOP has been written to give general guidance to study personnel on how to correctly and safely use the EC6 Hokanson Plethysmograph to measure Limb Blood Flow in either the lower arm or calf.

1. BACKGROUND

Studies may require blood flow measurements to be taken. The EC6 Hokanson Plethysmograph and 'Labchart' software can be used to measure limb blood flow in either the lower arm or leg.

2. EQUIPMENT

1. Strain gauges of appropriate sizes



2. Tape Measure
3. Micropore or equivalent surgical tape
4. Automatic cuff inflator

5.  Hokkanson Plethysmograph



6. Limb cuffs appropriate to the measurement site

7. Limb  support and foam

8. Powerlab

9. Pc or Laptop with ‘Labchart’ software

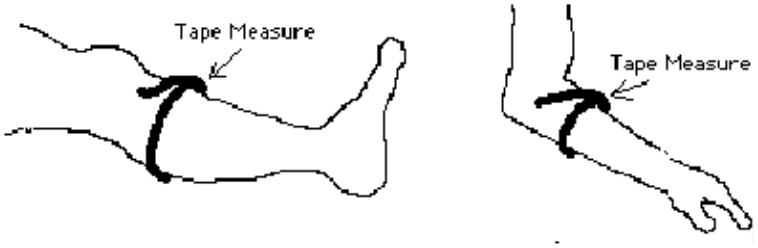
3. PROCEDURES

Blood Flow Measurements

1. The subject lies semi-supine on the bed. Ensure they are comfortable and explain the procedure to them.

The volunteer should be given adequate explanation of the experimental protocol to ensure that an ‘informed’ consent to participate is given. A third party should be present during the explanation of the experiment/procedure and should witness the volunteer’s signature on the consent form. This third party should have knowledge of the protocol, and must be able to confirm that a correct and full description has been given and that the subject has an understanding of what will take place.

2. Measure the limb around the widest part (see diagram) in cm. This figure represents the length of strain gauge required. Select this strain gauge.



3. Switch on the plethysmograph (red button on the front of the machine) and ensure that it is connected to the Powerlab via a jack plug which goes into the socket on the front of the plethysmograph marked ‘recorder’ and a BNC connection on the front of the Powerlab.

4. Insert the end of the strain gauge into the ‘gauge’ socket on the front of the plethysmograph. If necessary, an extension cable can be used to bridge the distance between equipment and volunteer.

5. Place the strain gauge sound the limb and secure the free loop around the black end of the gauge which has a small ridge to hold the loop in place.

6. Put a small amount of surgical tape (e.g. Micropore) over the black end of the strain gauge and attach to the skin to maintain the correct position on the limb.

Do not put the tape directly over the tubing (elastic bit of the strain gauge) as this can impede its function.

7. The strain gauge should fit snugly around the limb. If it is too loose or tight (causes indention in the skin), then select a smaller or larger strain gauge as appropriate

8. Place the low pressure (large) cuff around the top of the arm/leg, above the elbow/knee. Secure with a piece of tape to prevent the cuff from unravelling when inflated.

9. Place the high pressure (small) cuff around the wrist/ankle. Secure as before with a piece of tape.



10. Support the limb in an elevated position to allow venous drainage. See diagram below;

11. Turn Powerlab and computer on. Open Labchart software and set up recording fields using the ‘SET UP’ menus and drop down boxes located at the right hand side of the screen. Alternatively, open up ‘TEMPLATE’ already saved.

12. Save file with a unique name relevant to subject, visit number and study.

13. Ensuring that there is a cross over the recording icon at the bottom right hand side of the computer screen, click on the ‘START’ icon located to the right of the ‘RECORD’ icon. If there is no cross over the recording icon, click on it to display the cross before clicking on ‘START’.

The Labchart software will begin to display the output from the plethysmograph.

14. Looking at the display of the gauge output on the front of the plethysmograph press the auto balance switch (on the front of the machine) down. The needle should go into the middle of the display.

15. Looking at the computer screen, press the auto balance switch on the front of the plethysmograph down again. The trace should come into view on the chart display.

The position of the needle/trace can be altered using the ‘position’ dial on the plethysmograph if required.
Note: A recording of a calibration signal is needed at the beginning and end of the study. In addition, repeat calibration signal recordings will be required if any settings are changed during the study (including if the participant moves).
16. Push the ‘CALIBRATE’ switch (located on the front of the plethysmograph) to the right (1%). Look at the display on the front of the plethysmograph. The needle should move to the right, but not go all the way to ‘4’. If the needle deflection is too great adjust the range to a higher % (usually it is on 0.5% or 1%).

17. Push the ‘CALIBRATE’ switch to the right (1%) again. Look at the chart screen. The signal deflection should be at least half the width of the channel display. If it is too small adjust the chart settings (Drop down box to the right of the chart display) to a smaller voltage.

Repeat until you are happy with the signal.

<u>To make the calibration recording:</u>

18. Click the ‘RECORD’ icon on the computer screen (the cross is no longer displayed and so subsequent data will be recorded).

19. Push the calibrate switch to the right (as before), hold for a second, then release. A square wave should be displayed. Repeat 3 times.

20. Then record a 0.1% signal by pushing the calibrate switch to the left, hold for a second, then release. Repeat 3 times.

21. Click the ‘STOP’ button to stop the recording.
Note: Try to keep the settings the same throughout the experiment. Make a note of any changed that are made to the settings during the experiment.

22. If not already set up – go to the ‘WINDOW’ option on the main menu and select ‘DATA PAD’.

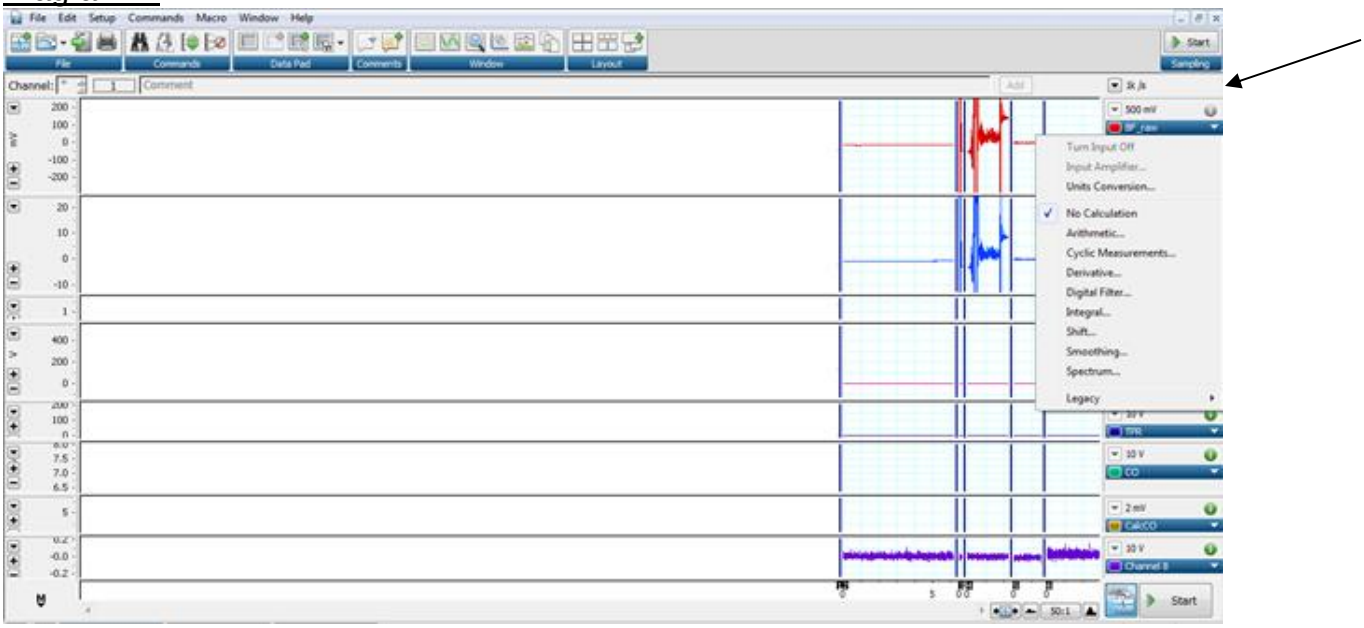
23. Select a new channel, click on the header and select ‘DATA FROM CHANNEL 1’. Make sure this is the raw data in mVs. Now select ‘STATISTICS’ and ‘MEAN’.

24. Go back to the main data collection screen and measure the ‘CALIBRATION SIGNAL HEIGHT’ in mV, by using the mouse to select a section of the baseline and adding to the data pad (ctrl + d). Then select a portion of the top of the calibration signal. Again record this value into the data pad. Repeat for all the calibration signals made at the start of the study.

25. From the data pad results, calculate the difference between the two values to determine the height of the signal. Calculate this for all 3 calibration signals and take a mean value.

26. Also calculate the mean average of the 3 ‘BASELINE’ measurements.
Note: If no changes to the setting were made, then a mean of these values can be used throughout. The 1% calibration signal is the mV equivalent to a 1% increase in forearm volume i.e. an increase of 1ml per 100ml of forearm. (0.1% signals should be x10 before comparison with 1% signals.)

27. In channel 1 on the main data collection screen, open the right hand drop down menu ▽ and select ‘UNITS CONVERSION’. See diagram 1:
Diagram 1.

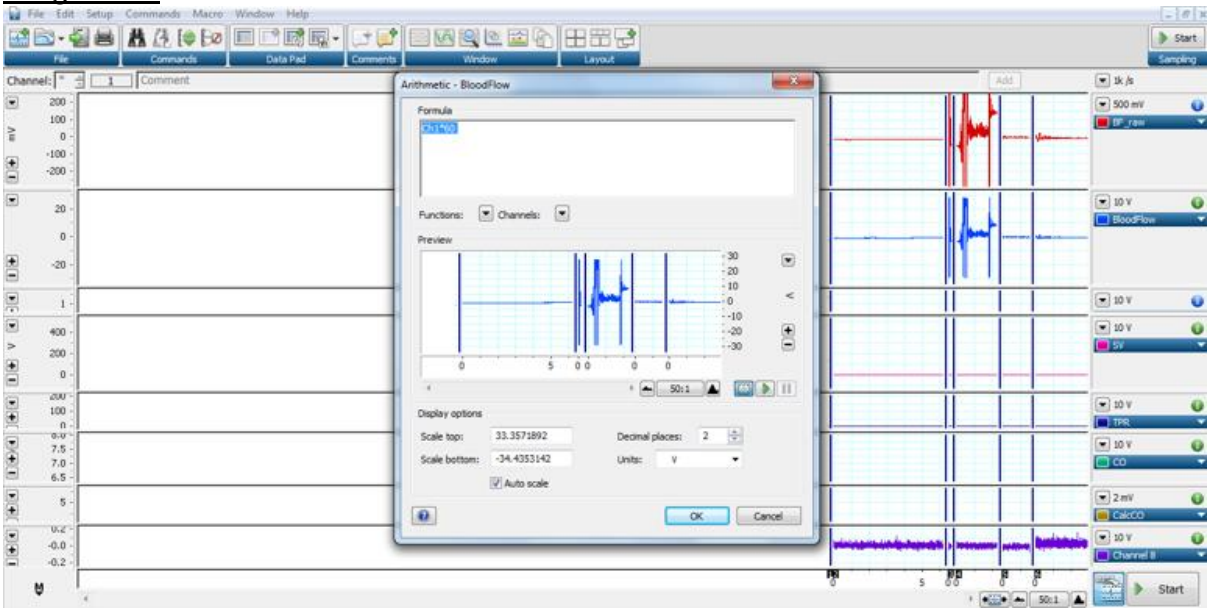


28. In this box make sure ‘POINT AND DIFFERENCE’ is selected and then input the ‘BASELINE’ mV average measurement into the top box (Point 1) and this should equal 0. Then put the average height measurement (∆) into the box below (Point 2) and this should equal 1. Select Units (top right) as % and make sure this is now turned ‘ON’ in the very top right hand corner and click ‘OK’. See Diagram 2:

Diagram 2.

29. To analyse the slopes from Channel 2 – If a ‘TEMPLATE FILE’ is not already set up:
a. In channel 2 on the main data collection screen, open the right hand drop down menu ▽ and select ‘ARITHMETIC’, (diagram 3), in here type ‘Ch1*60’ in the formula box and click ‘OK’ (this is multiplying the seconds by 60 to give values per minute).

Diagram 3.



b. Now still in channel 2 on the main data collection screen, open the right hand drop down menu ▽ again and select ‘UNITS CONVERSION’ (diagram 4.) Select ‘2 POINT CALIBRATION’: Point 1=1V → 1 and Point 2=0V → 0. ‘NO UNITS’ should be selected and this conversion should be turned ‘ON’ (top right) and click ‘OK’. (The raw data in the channel will now be represented in ml/100ml/min).

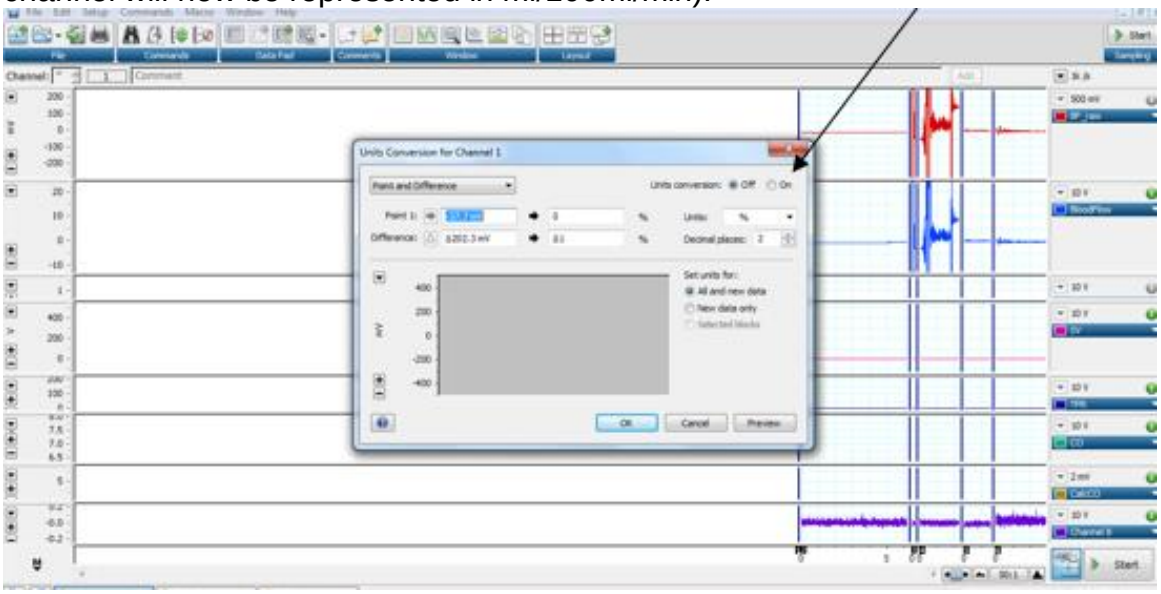
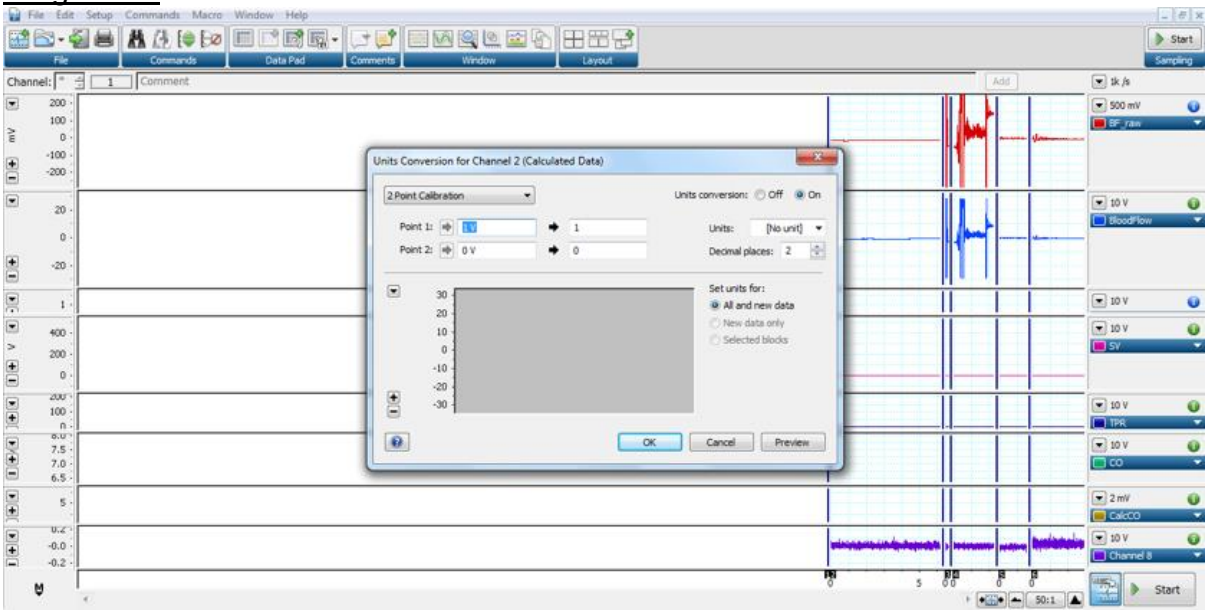


Diagram 4.



c. Go to the ‘WINDOW’ option on the main menu and select ‘DATA PAD’. Select ‘SLOPE CALCULATION’ by clicking once on a channel heading and select ‘SLOPE’, then ‘AVERAGE SLOPE’. Make sure this data is sampling data from Channel 2 (i.e. the corrected slope values in ml/100ml/min) and then click ‘OK’.

You can now go ahead and start collecting blood flow data

To check the cuffs

30. Switch on the inflator. This takes approximately 30 sec to reach adequate pressure.

31. Inflate the high and low pressure cuffs by pressing the ‘INFLATE’ button.

The high pressure cuff should be >180mmHg. Adjust the pressure using the regulator if required.

The low pressure cuff should be a little lower than diastolic blood pressure (usually around 40mmHg). This can be adjusted using the regulator if required.

During measurements, the high pressure cuff will remain on throughout and the low pressure cuff will inflate and deflate at timed intervals by an operator pushing the inflate switch.

32. Deflate all cuffs.

To record blood flow measurements

33. Instruct the volunteer to stay still and not to talk.

34. Inflate the high pressure cuff around the wrist or ankle to >180mmHg for 1 minute before recordings are required.

35. If the Lab Chart recording is not already running, click the ‘START’ icon on the computer screen.

36. Inflate the low pressure cuff for 10 secs. The trace displayed on the computer screen should gradually increase until a plateau is reached. Once the plateau is seen, deflate the low pressure cuff for 5 secs or until the trace falls back to baseline.

Turn low pressure cuff back on again and observe the trace as before.

37. Repeat for 2 minutes, i.e. until 7-8 curves have been recorded.

38. When recording has finished deflate both cuffs and click the ‘STOP’ icon, to stop the measurement – **UNLESS** you need to keep the recording running for other parameters being measured continuously.

The Hokanson inflator can be turned off if time to the next reading is >10 min.
Note: If the curves obtained are too small or too large (such that the trace goes out of range), adjust the Labchart settings to a lower or higher voltage as appropriate.
If the curves do not go back down to baseline when the low pressure cuff is deflated, check that the limb is at a sufficient angle to allow venous drainage and that venous drainage is not being impaired by the position of the low pressure cuff or limb supports.

39. If possible it is good practice to save recordings during the experiment. To do this, click ‘STOP’ to halt display, then click the ‘SAVE’ icon.

However: If you are recording BP, Cardiac Output and Stroke Volume data from the <u>Finometer</u>, keep the recording running continuously throughout the experiment.

40. At the end of the experiment, remove the strain gauge from the patient and plethysmograph, and carefully put back into its packer.

41. Remove all cuffs from the patient

42. Save the data on the computer to the unique patient file and switch of all equipment.

Slope Data Analysis

1. Go to the first blood flow recording and select the linear part of the slope for the calculation. Highlight the relevant section by using the mouse to place the cursor at the start of the slope, click, hold and drag the cursor to the end of the section. Release the cursor and the area of interest should be highlighted. Try and select 5 heartbeats or 3 or 4 squares on the grid for all slopes. Add to the data pad (ctrl + d).

2. Repeat for all slopes

3. Insert a blank row spacer in the data pad, before repeating slope analysis for further bouts of blood flow recordings
Note: If the subject or the strain gauge moves during the recording, turn the Units Conversion calculation ‘OFF’ in Channel 1, so you are recording raw data again and repeat the 3 x 1% calibration signals. Use the new calibration factors in the ‘UNIT CONVERSION’ box for any subsequent slope recordings.

Data Collected in the Data Pad can now be copied and pasted to an Excel spreadsheet for further analysis.