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THE MODULATORY EFFECTS OF
SEDENTARY BEHAVIOUR AND
PHYSICAL ACTIVITY ON OLDER
ADULTS'
CARDIOVASCULAR/METABOLIC
PROFILES

DECLAN JOHN RYAN

A thesis submitted in partial fulfilment of
the requirements of the Manchester
Metropolitan University for the degree of
Doctor of Philosophy

Department of Exercise and Sport
Science

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Publications

Peer Reviewed Journal Articles

Ryan, D. J., Stebbings, G. K. and Onambele, G. L. (2015) 'The emergence of sedentary behaviour physiology and its effects on the cardiometabolic profile in young and older adults.' *Age*, 37(5) pp. 89-100.

- Chapter 01

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- Chapter 03 Part 1

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Ryan, D. J., Wullems, J. A., Stebbings, G. K., Morse, C. I., Stewart, C. E. and Onambele, G. L. (2018) 'Segregating the Distinct Effects of Sedentary Behavior and Physical Activity on Older Adults' Cardiovascular Structure and Function: Part 2— Isotemporal Substitution Approach.' *Journal of Physical Activity and Health*, [In Press].

- Chapter 03 Part 2

Conference Proceedings

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Ryan, D. J., Morse, C. I., Stebbings, G. K., Stewart, C. E. and Onambebe-Pearson, G. L. (2017) *Modelling any impact on cardiovascular health of sedentary behaviour and physical activity lifestyle substitution in older adults*. The 9th MMU Postgraduate Research Conference 2017, (Manchester, UK).

List of Abbreviations

₁₀MVPA	10-minute Moderate-Vigorous Physical Activity
ACP	Active Couch Potato
AL	Anterior Longitudinal
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BMI	Body Mass Index
B-Mode	Brightness Mode
BP	Blood Pressure
CAS	The Cheshire Algorithm for Sedentarism
CFM	Colour Flow Mode
CI	Confidence Interval
CoDA	Compositional Data Analysis
CV	Coefficient of Variation
CVD	Cardiovascular Disease
DNLL1	Dynein Light Chain 1
ECG	Electrocardiogram
eNOS	Endothelial Nitric Oxide Synthase
FMD	Flow Mediated Dilation
GENEA	GENEActiv
GLUT-4	Glucose Transporter Type-4
HbA1c	Glycated Haemoglobin
HDL-C	High Density Lipids
iAUC	Incremental Area Under The Curve
IL-6	Interleukin-6
IMT	Intima-Media Thickness
IPAQ	International Physical Activity Questionnaire
IR	Interquartile Range
ISM	Isotemporal Substitution Model
LDL-C	Low Density Lipids
LIPA	Light Intensity Physical Activity
LOG	Logarithmic
LPL	Lipoprotein Lipase
METs	Metabolic Equivalent Tasks
MVPA	Moderate-Vigorous Physical Activity
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
NO	Nitric Oxide
PA	Physical Activity
PB	Physical Behaviour
PIIINP	Procollagen III N-Terminal Propeptide
PL	Posterior Longitudinal
RI	Resistance Index
RMR	Resting Metabolic Rate
ROI	Region of Interest
SB	Sedentary Behaviour
SD	Standard Deviation
sMVPA	Sporadic Moderate-Vigorous Physical Activity
TV	Television
UK	United Kingdom
USB	Universal Serial Bus
Yrs	Years

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Abstract

Cardiovascular disease (CVD) is the greatest cause of mortality, after cancer, in older adults (>60 years). CVD risk can be modulated by sedentary behaviour (SB) and physical activity (PA) however, research examining the effect of SB and PA on older adults' cardiovascular/metabolic profile is lacking. This thesis aimed to address this gap in the literature. Ninety-three independently living older adults wore a thigh-mounted triaxial accelerometer for 7 consecutive days to assess habitual SB and PA engagement and patterns. Fasting blood samples to assess seven cardiometabolic marker concentrations and ultrasound to assess vascular structure and function were conducted. Engagement in light intensity PA (LIPA) decreased popliteal intima-media thickness (IMT) and further ageing of popliteal IMT. Replacing one hour of SB with LIPA decreased carotid artery diameter. Replacing an hour of any SB or PA with moderate to vigorous PA (MVPA, ≥ 10 mins bouts) reduced triglyceride concentration. Those with a 'low' triglyceride and 'high' lipoprotein lipase concentration engaged in 48% and 11% more MVPA (≥ 10 mins bouts) than the entire sample population, respectively. Patterns of SB, specifically W50% was associated with an increase in popliteal IMT and resting heart rate. Furthermore, participants with a 'high' procollagen 3 N-terminal peptide concentration had a larger W50% than the 'low' group. For SB, patterns of engagement appeared to be better predictors of older adults' cardiovascular/metabolic status than total engagement time. LIPA is suggested to be a useful replacer of SB time due to their high co-dependence. MVPA (≥ 10 min bouts) engagement, which is already recommended in government PA policies, was a strong mediator of cardiometabolic markers. Overall, this thesis suggested that government PA policies should also include objective recommendations for SB and LIPA, as the entire intensity spectrum of SB and PA affected older adults' cardiovascular/metabolic profile.

Chapter 01:

Literature Review

‘The emergence of sedentary behaviour physiology and its effects on the cardio-metabolic profile in young and older adults.’

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The emergence of sedentary behaviour physiology and its effects on the cardio-metabolic profile in young and older adults.

Introduction

The 2008 Health Survey for England found 39% of males and 29% of females aged 16.0 years (yrs) and over met the recommended physical activity (PA) guidelines (Craig et al., 2009). However, the average self-reported time spent performing sedentary behaviour (SB) was still 2.8 ± 0.03 hrs·day⁻¹ (Craig et al., 2009). Whilst these surveys provide valuable information, they have their limitations, as they are highly reliant on the participant's recall ability. Indeed, it is now thought that the prevalence of SB and physical inactivity (not meeting PA recommendations) is even higher than previously reported, with accelerometer-derived survey data proposing that adults engage in SB for 9.91 ± 0.04 hrs·day⁻¹ and less than 10.0% meeting the recommended PA guidelines (Craig et al., 2009). SB is associated with an increased risk of developing factors associated with cardiovascular disease (CVD) (Healy et al., 2008a), such as type 2 diabetes mellitus (Oggioni et al., 2014) and atherosclerosis (Laufs et al., 2005).

Government health interventions have focused on improving physical health status by increasing bouts of moderate to vigorous physical activity (MVPA). The National Health Service (NHS) recommends 19.0 - 64.0+ yrs old adults should engage in 150 minutes (mins) of MVPA per week, which is commonly divided into five, 30.0 mins bouts a week (National Health Service, 2013a). However, individuals who meet the PA guidelines still display cardiometabolic risk factors associated with CVD and this is thought to be linked to their amount of SB, irrespective of the amount of MVPA undertaken (Healy et al., 2008a). Therefore, the research community has shifted focus towards PA and SB levels (collectively known as physical behaviour [PB] (Bussmann and van den Berg-Emons, 2013)) during the remaining 15.5 hours (hrs) of daily waking time (Hamilton et al., 2008). As the independent physiological characteristics of SB and MVPA have become apparent, a new PA population known as 'active couch potatoes' (ACP) has emerged (Gennuso et al., 2013; Healy

et al., 2008a; Peddie et al., 2013). The ACP population meet the PA guidelines for MVPA yet still spend the majority of their waking hours performing SB.

Ageing is associated with an increase in SB (Harvey et al., 2013) and with life expectancy increasing, populations aged 60.0 yrs and older are projected to account for 25.0% of the UK population by the year 2035 (Office for National Statistics, 2012a). Therefore, studies examining the associations between SB and CVD risk are warranted as the prevalence of CVD cases in the UK is greatest in populations aged 65.0 yrs and over (British Heart Foundation, 2014).

This literature review aims to discuss the emergence of the ACP lifestyle, and how the independent effects of SB and MVPA on cardio-metabolic markers, associated with CVD, develop with ageing.

Defining Sedentary Behaviour

The definition of SB has been inconsistent throughout the literature due to different SB and the methods used to determine these. Most studies classify SB as <1.50 Metabolic Equivalent Tasks (METs) (Tremblay et al., 2010; Pate et al., 2008; Rowlands et al., 2014; Owen et al., 2010; Ainsworth et al., 2000) however; certain light intensity physical activities (LIPA) (standing) can elicit similar MET values (Ainsworth et al., 2000). 'Sedentary' originates from 'sedere', Latin for 'to sit'. Therefore, the definition of SB should not only include the MET threshold (<1.50 METs) but also acknowledge postural positions (sitting, lying, TV viewing, driving) (Tremblay et al., 2010).

Tremblay et al. (2010) further suggested that, similar to the measurement of PA using the acronym 'FITT' (frequency, intensity, time, type), SB should be recorded by using 'SITT' (SB frequency, interruptions in SB, time duration of each SB, type of SB). 'SITT' is recommended as SB does not have large variations in intensity and the number of interruptions in SB appears to be an important determinant of health status (Chastin et al., 2015a).

The Effects of Sedentary Behaviour Can Be Measured Independent of Those of Moderate to Vigorous Physical Activity

Generally-speaking, PA may be considered as any activity of daily living that leads to the expenditure of energy, above the levels required for maintaining basal metabolic activities, with a threshold usually considered as anything at least 1.50 times greater than the resting metabolic rate (RMR) (Mansoubi et al., 2015). The misclassification of SB under the umbrella of PA stems from the data collection methods of early epidemiological research. The Harvard Alumni study (Paffenbarger Jr et al., 1986) used self-reports to gain insight into PA levels by recording the frequency of stair climbing, city blocks (0.13 km) walked, and sports played. Individuals who failed to expend more than 2000 kcal·week⁻¹ through these exercises were classed as sedentary even though there were no questions relating to SB. This assumption that too little MVPA equates to large amounts of SB may have come about through self-reports because it is easier for participants to recall more strenuous bouts of activity they have performed (Kriska, 2000), rather than how much time they have spent performing SB. Additionally, it can be difficult to quantify the frequency and length of SB when the focus of questions only relates to MVPA (e.g. “Ask only about activities that are at least the intensity of walking, but include walking.” - Stanford 7-day recall instructions (Sallis, 1985)). Therefore it is easy to understand how early PA research has influenced government health initiatives to focus on increasing MVPA to improve overall health, rather than aiming to reduce SB time.

Conversely, SB and MVPA cause quantifiably different cardio-metabolic responses which do not correspond to the PA spectrum. SB was found to decrease lipoprotein lipase (LPL) activity (relative to ambulatory controls) by 55.0%, in oxidative muscle fibres. Whereas, voluntary running (56.0 m·min⁻¹) caused no increase in relative LPL activity in oxidative muscle fibres (Bey and Hamilton, 2003). In the singular PA spectrum it would be expected that the decline in LPL from SB would be equally and oppositely matched by the benefits of MVPA. Therefore, in terms of physiological response, the PA spectrum should be bi-axial. The x axis should range from high SB to low SB and the y axis should range from insufficient MVPA to sufficient MVPA, subsequently, allowing for the acknowledgment of the ACP lifestyle.

In light of this independence between SB and MVPA, Healy et al. (2008a) found populations (47.3±13.1 yrs) that met the recommended PA guidelines still displayed associations between increased SB and CVD risk factors. This effect was more noticeable in their female sub-populations (triglyceride increase, from lowest to highest quartile of TV viewing hours: female 0.06 mmol·l⁻¹ [95% confidence interval [95%CI] 0.04, 0.09], *p*<0.001, male, -0.001 mmol·l⁻¹ [95%CI -0.03, 0.03], *p*=0.511). The greater association in female triglyceride concentration may be due to sex differences in fuel homeostasis, as a six day bed rest study caused female lipogenesis activity to increase by 570%, compared to no difference from baseline in males (Blanc et al., 2000b). In terms of similar observations in older persons, Bankoski et al. (2011) found that individuals aged 60.0 yrs and older were more likely to suffer from metabolic syndrome if they engaged in longer bouts of SB and had a greater percentage of time spent performing SB per day, independent of any PA they undertook in parallel. In aged ACP populations (74.6±6.5 yrs), positive associations between increased SB and fasting plasma glucose concentration, but no negative association between increased MVPA and fasting plasma glucose concentration or interaction between SB and MVPA plasma glucose concentration, was found (Gennuso et al., 2013). The lack of interaction between SB and MVPA highlights that the effects of SB and MVPA are independent of each other throughout the ageing process.

The findings of Gennuso et al. (2013) are supported in acute environments as the ACP lifestyle appeared to elicit effects on glucose regulation that were similar to the effects of prolonged SB (Peddie et al., 2013). During a 9-hour period, young participants (18.0 – 40.0 yrs) consumed a glucose solution at three hour intervals. Participants engaged in either; 9 hrs of prolonged sitting, 30.0 mins of MVPA then 8.50 hrs of sitting (ACP), or sitting interrupted every 30.0 mins by 1.00 mins 40.0 seconds (s) of walking (LIPA). Overall, plasma glucose incremental area under the curve (iAUC) was similar for prolonged sitting and ACP groups (48.8 mmol·l⁻¹·9h⁻¹ vs. 47.2 mmol·l⁻¹·9h⁻¹, respectively) while interrupted sitting produced lower plasma glucose concentrations (29.9 mmol·l⁻¹·9h⁻¹) (Peddie et al., 2013). Consequently highlighting that the PA guidelines may not ameliorate the deleterious consequences of SB. The UK, Canada, and Australia have recently updated the PA guidelines to recommend a reduction in SB. Although evidence for the effects of SB is increasing (see table 1.1 for a summary), no guidance is given as to the maximum amount of daily SB, how often SB should be interrupted, and methods to reduce SB.

Table 1.1. Sample of studies examining the effects of SB on markers of cardio-metabolic health in older persons.

Author	Age group (yrs)	Measure of PB	Measurement type	Outcome variable	Result
Bankoski et al. (2011)	71.0±8.0	Total SB time SB bouts SB breaks SB intensity	ActiGraph AM-7164 accelerometer	Metabolic syndrome	(**) 3.10 ↑ in metabolic syndrome development Odds Ratio (OR) from lowest to highest quartile of % of SB time of total wear time
Gennuso et al. (2013)	74.6±6.5	Total SB time	ActiGraph AM-7164 accelerometer	Fasting plasma glucose C-reactive protein	(**) 4 mg·dL ⁻¹ ↑ from lowest to highest quartile of SB hours 0.10 mg·dL ⁻¹ ↑ from lowest to highest quartile of SB hours
Healy et al. (2008b)	40-87	SB breaks	Actigraph WAM-7164	2-h plasma glucose Triglycerides BMI Waist circumference	(**) $r^2 = 0.14$ with ↑ in SB breaks, (**) $r^2 = 0.13$ with ↑ in SB breaks (**) $r^2 = 0.05$ with ↑ in SB break (**) $r^2 = 0.26$ with ↑ in SB breaks (all independent of age)
Dunstan et al. (2012)	45-65	MIPA or LIPA breaks in SB every 20 mins for 2 mins	NA	2-h plasma glucose	(N/A) LIPA ↓ 2-h plasma glucose by 1.7 mmol·l ⁻¹ , independent of age (N/A) MIPA ↓ 2-h plasma glucose by 2.0 mmol·l ⁻¹ , independent of age
Healy et al. (2007)	30-87	Total SB time Total LIPA time Total MIPA time	ActiGraph WAM 7164	2-h plasma glucose	(**) Total SB time ↑ 2-h plasma glucose ($r^2 = 0.18$), adjusted for age (**) Total LIPA time ↓ 2-h plasma glucose ($r^2 = 0.17$), adjusted for age (**) Total MIPA time ↓ 2-h plasma glucose ($r^2 = 0.18$), adjusted for age
Latouche et al. (2013)	45-65	MIPA or LIPA breaks in SB every 20 mins for 2 mins	NA	Dynein light chain 1 gene	(N/A) 1.6 fold ↑ with ↑ intensity breaks in SB

Table 1.1 continued.

Yamanouchi et al. (1992)	62-87	Bed ridden	NA	Glucose infusion rate	(N/A) Glucose infusion rate 12.2%↓ in bed ridden compared to controls
Carson et al. (2014)	20-79	SB breaks	Actical accelerometer	HDL-C Triglycerides Plasma glucose	(**) 10 extra SB breaks per day: 0.01 mmol·l ⁻¹ ↑ HDL-C 3.72%↓ 0.57%↓ (**) (all independent of age)
Fatouros et al. (2005)	69.8±3.8	6 months detraining	Subjective	Leptin	(N/A) 0.4 ng·ml ⁻¹ ↑ above pre-training baseline

** indicates that results were statistically significant after adjustment for MVPA. N/A indicates a study where the study design did not allow MVPA adjustments. NA indicates not applicable. ↑ indicates an increase in a cardio-metabolic marker. ↓ indicates a decrease in a cardio-metabolic marker.

The Benefits of Light Intensity Physical Activity

Interrupting long bouts of SB appears to be an important determinant of physical well-being. Irrespective of total SB, MVPA time, and age, individuals (40.0 – 87.0 yrs) who broke up SB on 673 occasions with LIPA (average break duration: 4.50 mins), over five days of accelerometer wear time, had a 0.880 mmol·l⁻¹ ($p < 0.05$) lower in 2-hour plasma glucose concentration compared to individuals who only had 506 breaks in SB. Reductions in triglycerides, body mass index (BMI), and waist circumference were also related to an increase in SB breaks (Healy et al., 2008b). This is further supported by a crossover study, which found 2-hour plasma glucose concentration, again independent of age, was decreased in middle-aged to older adults (45.0 – 65.0 yrs) when SB was interrupted every 20.0 mins with 2.00 mins of LIPA (1.70 mmol·l⁻¹·1h⁻¹ decline) or MVPA (2.00 mmol·l⁻¹·1h⁻¹ decline) compared to prolonged sitting (Dunstan et al., 2012). It appears that PA and SB plays an important role in the glucose-insulin axis as 53.0% of the variance in 2-hour plasma glucose concentration can be explained by SB ($r^2 = 0.18$), LIPA ($r^2 = 0.17$), and MVPA ($r^2 = 0.18$) (Healy et al., 2007). Therefore, because LIPA can be easily accumulated and prevents the deleterious effects of SB on additional cardiometabolic markers (e.g. LPL) (Bey and Hamilton, 2003; Healy et al., 2008b), PA interventions should aim to interrupt SB with LIPA.

The optimum length of SB bouts and LIPA interruptions has not yet been determined. Beneficial associations with interruptions in SB and plasma glucose concentration were still visible in young persons (25.9±5.30 yrs) when SB bouts were increased to 30.0 mins and interruption length was decreased to 1.00 mins 40.0 s (plasma glucose iAUC: interrupted, 29.9 mmol·l⁻¹·9h⁻¹, prolonged sitting, 48.8 mmol·l⁻¹·9h⁻¹, ACP, 47.2 mmol·l⁻¹·9h⁻¹) (Peddie et al., 2013). This shows that benefits in SB breaks still exist with relatively increased SB time and decreased SB interruption length. However, Peddie et al. (2013) interruption intervention caused a lower plasma glucose iAUC per hour compared to Dunstan et al. (2012) (3.32 mmol·l⁻¹·1h⁻¹, 5.10 mmol·l⁻¹·1h⁻¹, respectively), even though Dunstan et al. (2012) had shorter SB bouts and longer interruptions. This unexpected finding may be explained by Peddie et al. (2013) using a younger population than Dunstan et al. (2012) (25.9±5.3 yrs, 53.8±4.9 yrs, respectively) as glucose regulation declines with age (Gong and Muzumdar, 2012). As a result, PA guidelines may require different SB recommendations for different age groups.

Sedentary Behaviour and Physical Activity: Impact on Glucose Regulation

The interaction between SB and glucose regulation has been a popular topic due to the adverse effects hyperglycaemia has on the cardiovascular system. New recommended PA guidelines aimed at reducing SB by increasing LIPA could be crucial to the prevention of type 2 diabetes mellitus and subsequent CVD as SB and LIPA explain 35.0% of the variance in 2-hour plasma glucose concentration (Healy et al., 2007). However, relationships between SB and fasting plasma glucose are not commonly found in both self-report and objectively measured PB studies (Healy et al., 2007; Healy et al., 2008b; Dunstan et al., 2007). This suggests SB influences glucose regulation through skeletal muscle related mechanisms as 2-hour plasma glucose is characterised as a measure of skeletal muscle insulin resistance. Whereas fasting plasma glucose is more reflective of hepatic insulin resistance with sustained skeletal muscle insulin sensitivity (Abdul-Ghani et al., 2006).

It is thought that a reduction in facilitated glucose diffusion into skeletal muscle cells is brought about through a down-regulation of the glucose transporter, Glucose Transporter Type-4 (GLUT-4). In the *vastus lateralis*, ageing explains

7.80% and 26.0% of the variance in the decline of GLUT-4 concentration in males and females, respectively (Houmard et al., 1995). In addition, the ageing effect on GLUT-4 concentration also appears to be fibre type dependent, as a reduction in GLUT-4 is found with ageing in fast twitch fibres but not in slow twitch fibres of the *vastus lateralis* (Gaster et al., 2000). This decline in fast twitch GLUT-4 concentration may, in fact, be due to decreased participation in free-living MVPA alongside an increased engagement in SB. In line with the above observations, moderate intensity PA (MIPA) in older populations is found to increase GLUT-4 concentration, as it does in their younger counterparts (Cox et al., 1999). In support of the SB-GLUT-4 relationship, data shows that, seven young endurance runners aged 33.0 yrs, displayed a 17.5% reduction in gastrocnemius GLUT-4 concentration and a decrease in glucose disposal rates ($1.90 \text{ mg}\cdot\text{kg}\cdot\text{FFM}^{-1}\cdot\text{min}^{-1}$ decline), despite an increased insulin concentration of $4.20 \text{ }\mu\text{m}\cdot\text{ml}^{-1}$, after six days of bed rest (Vukovich et al., 1996). Thus, the relationship between GLUT-4 and free-living SB, which has been exhibited in young populations is likely to be present in older persons, though the latter is yet to be demonstrated and requires further research.

The evidence thus far suggests that longitudinal PA intervention in aged participants (60.0 - 72.0 yrs), causes an increase in *vastus lateralis* GLUT-4 concentration (Biensø et al., 2015). It appears that this effect on GLUT-4 could also be found in acute SB breaks. Breaking up SB with LIPA or MIPA increased the expression of the gene that encodes dynein light chain 1 (*DNLL1*). *DNLL1* plays a role in the transcription of GLUT-4 in middle aged to older adults (Latouche et al., 2013) and is thought to be part of a mechanism for GLUT-4 induced reduction in plasma glucose during SB breaks.

Insulin concentration may increase to compensate for the reduction in glucose uptake via the GLUT-4 pathway but consequently cause a decline in skeletal muscle insulin sensitivity. It should be noted that PA causes an increase in GLUT-4 translocation (Lund et al., 1995) and subsequently an increase in glucose clearance rate, independent of insulin (Lund et al., 1995). This increase in GLUT-4 translocation with PA (seen in the study of Lund et al. (1995), may explain why interrupting SB with bouts of PA, was shown to lower plasma glucose concentrations compared with prolonged sitting in previous studies (Peddie et al., 2013; Healy et al., 2008b). On the other hand, 30.0 mins of MIPA followed by 8.50 hrs of prolonged sitting produced plasma glucose iAUC results that were similar to 9.00 hrs of prolonged sitting (Peddie et al., 2013). Based on the discussions above, this short

bout of MIPA should have increased GLUT-4 concentration and hence impacted on the iAUC. However, this single bout of PA may not have been enough to sustain an increased concentration of GLUT-4 over the remaining 8.50 hrs of SB as GLUT-4 is predicted to have a half-life of just 8.00 – 10.0 hrs (Host et al., 1998).

Recently, Bailey and Locke (2014) found that SB breaks every 20.0 mins with 2.00 mins of light intensity walking reduced postprandial plasma glucose concentration (iAUC $18.5 \text{ mmol}\cdot\text{l}^{-1}\cdot 5\text{h}^{-1}$, reduction of $3.50 \text{ mmol}\cdot\text{l}^{-1}\cdot 5\text{h}^{-1}$) compared to prolonged sitting in young persons (24.0 ± 3.00 yrs). However, it was found that interrupting SB every 20.0 mins with 2.00 mins of standing (a LIPA) did not cause any beneficial decline in postprandial plasma glucose concentration (iAUC $22.2 \text{ mmol}\cdot\text{l}^{-1}\cdot 5\text{h}^{-1}$) compared to prolonged sitting (iAUC $22.0 \text{ mmol}\cdot\text{l}^{-1}\cdot 5\text{h}^{-1}$). Therefore, PA interventions should aim to interrupt SB with a movement based LIPA. In addition, objective measures of PA should distinguish between breaks in SB caused by standing and walking to increase the validity of epidemiological studies examining the association between PB and cardio-metabolic health.

Whilst glucose regulation declines with ageing (Gong and Muzumdar, 2012), PA interventions can protect against the ageing process. Bedridden, master athletes' (62.0 - 87.0 yrs), and healthy older adults' (76.0 ± 2.00 yrs) glucose infusion rates were compared to that of healthy young individuals and young athletes (20.0 ± 1.00 yrs). In ageing populations, glucose infusion rates were higher in master athletes and lower in bed ridden individuals than in healthy older adults. Compared to young individuals, the healthy older adult sub-populations had lower glucose infusion rates whereas master athletes had similar glucose infusion rates (Yamanouchi et al., 1992). Managing PA and SB is crucial in ageing populations to control glucose regulation, as the prevalence of diabetes mellitus is nearly seven fold greater in adults over 60.0 yrs compared to young populations (20.0 yrs) (Shaw et al., 2010). Although it is evident that frequent MVPA can protect against the effects of ageing on glucose control (Yamanouchi et al., 1992), it can be difficult for elderly populations to accumulate the recommended levels of MVPA. Therefore, future intervention studies, similar to the work of Peddie et al. (2013), Healy et al. (2008b), and Bailey and Locke (2014) should be conducted using ageing populations, whose waking hours are predominantly spent performing SB (Harvey et al., 2013), to examine the potential benefits of LIPA or sporadic MVPA (sMVPA, MVPA accumulated in bouts less than 10mins) breaks.

Sedentary Behaviour and Physical Activity: Impact on Cholesterol and Lipoprotein Lipase

Low density lipids (LDL-C) (normative value: $<3.00 \text{ mmol}\cdot\text{l}^{-1}$), high density lipids (HDL-C) (normative value: $>1.00 \text{ mmol}\cdot\text{l}^{-1}$), and triglycerides (normative value: $<1.70 \text{ mmol}\cdot\text{l}^{-1}$) are collectively known as total cholesterol (normative value: $<5.00 \text{ mmol}\cdot\text{l}^{-1}$) (National Health Service, 2013b). Decreasing LDL-C by $1.00 \text{ mmol}\cdot\text{l}^{-1}$ reduces the relative risk of cardiovascular events by 22.0% in populations aged 65.0 – 74.0 yrs, and by 16.0% in individuals over 75.0 yrs (Baigent et al., 2010). In conjunction, a $0.78 \text{ mmol}\cdot\text{l}^{-1}$ decline in triglyceride concentration can decrease the relative risk of CVD by 15.0% in males, and 9.0% in females (50.8 \pm 6.70 yrs) (Cui et al., 2001). Meanwhile, HDL-C has been shown to have a protective effect, as a $0.55 \text{ mmol}\cdot\text{l}^{-1}$ increase in HDL-C was associated with a decreased risk of coronary events by a factor of 1.70 and 1.95 in older adult (80.0 \pm 8.0 yrs) males and females, respectively (Aronow and Ahn, 1996). These findings are due to HDL-C role in transporting excessive cholesterol from peripheral tissues to the liver in a process known as reverse cholesterol transport (Sviridov and Nestel, 2002). LDL-C and triglycerides however, contribute to the pathogenesis of atherosclerosis. Increasing concentration of LDL-C can cause an accumulation within the arterial intimal space (Björnheden et al., 1996). These LDL-C particles become oxidised and bind to receptors on endothelial and smooth muscle cells causing apoptosis of smooth muscle cells (Björkerud and Björkerud, 1996), impaired nitric oxide (NO) synthesis (Keaney Jr et al., 1996) and oxidative stress (Cominacini et al., 2000). Macrophage secretion into the intimal space occurs to take up the oxidised LDL-C particles and consequently differentiate into foam cells (Rios et al., 2013). Triglyceride rich very-LDL-C remnants, resulting from partial hydrolysis of triglycerides by LPL, bond to cholesterol esters from HDL-C via cholesterol ester transfer proteins. These cholesterol-enriched, triglyceride-poor species accumulate and are up taken by macrophages, subsequently forming foam cells (Talayero and Sacks, 2011). LPL not only interacts with triglycerides during the pathogenesis of atherosclerosis but also promotes retention of LDL-C within the intimal space by binding LDL-C to proteoglycans, found on smooth muscle cells. This retention allows more time for LDL-C particle modification and subsequently, promotes uptake by macrophages (Li et al., 2014).

Whether LPL is antiatherogenic or proatherogenic is dependent on the cell/tissue that is expressing the enzyme (Mead and Ramji, 2002). Skeletal muscle, plasma and adipose tissue LPL is responsible for the clearance of lipoprotein particles and is therefore antiatherogenic, whereas endothelial LPL is proatherogenic (Mead et al., 1999; Mead and Ramji, 2002; Clee et al., 2000). Changes in LPL activity provides the best representation of the independence between MVPA and SB effect's on cardio-metabolic markers of health status. As shown in Sprague-Dawley rats, 6.0 hrs of SB was found to decrease LPL activity (relative to ambulatory controls) by 55.0%, in postural oxidative muscle fibres (soleus) while HDL-C concentration declined by 22.0%. Whereas, voluntary running ($56.0 \text{ m}\cdot\text{min}^{-1}$) caused no increase in relative LPL activity in oxidative muscle fibres. However, in glycolytic muscles (*vastus lateralis*), voluntary running caused an increase in relative LPL activity above that of ambulatory controls (Bey and Hamilton, 2003). The same authors (Bey et al., 2001) also reported a decrease in LPL activity in the soleus but not in the tibialis anterior with ageing in two strains of rat. It was stipulated that this ageing effect was visible in the soleus because it is a weight bearing muscle and LPL activity is dependent on muscle contractile activity (Hamilton et al., 1998). This apparent ageing effect on LPL activity in oxidative muscles was suggested to be due to a reduction in ambulatory movement with ageing and thus a reduction in postural muscle contractile activity (Hennig and Lømo, 1985). Therefore, the replacement of SB with LIPA may increase skeletal muscle LPL (antiatherogenic), subsequently reducing CVD risk.

In older adults (74.2 ± 0.5 yrs), individuals who engaged in more than 5 $\text{hrs}\cdot\text{day}^{-1}$ of LIPA had an average $0.23 \text{ mmol}\cdot\text{l}^{-1}$ (95%CI 0.07, 0.39, $p=0.002$) increase in HDL-C concentration and a lower ratio of total cholesterol to HDL-C (-0.92 [95%CI $-1.36, -0.48$], $p=0.003$) with a trend towards lower concentrations of LDL-C and triglycerides (Pescatello et al., 2000). Assessment of PA in this study was done using the Yale Physical Activity Survey, which despite being validated for use with older adults (Dipietro et al., 1993), may not necessarily provide an exact quantitative measurement of PA. It is possible that the use of a direct and objective measure of PA might have produced greater data reliability and hence improved the degree of statistical significance, and/or greater associations between LIPA and HDL-C. Objective measures of PB from 4935 Canadians (20.0 – 79.0 yrs) found an increase of 10 breaks in SB per day (break in SB were greater than 1.00 mins and classed as LIPA or MVPA) was associated with a $0.01 \text{ mmol}\cdot\text{l}^{-1}$ (95%CI 0.00, 0.02)

increase in HDL-C concentration and a $0.04 \text{ mmol}\cdot\text{l}^{-1}$ (95%CI -0.06, -0.01) decline in triglyceride concentration, independent of age. However, an increase in SB breaks was not associated with a decline in LDL-C. Conversely, a $0.21 \text{ mmol}\cdot\text{l}^{-1}$ (95%CI -0.380, 0.040) decline in LDL-C was associated with an hourly increase in MVPA (Carson et al., 2014). As a result, reducing SB by increasing LIPA appears to be partly beneficial in the management of cholesterol and LPL.

Sedentary Behaviour and Physical Activity: Impact on Ghrelin – Leptin – Adiponectin Axis

Ghrelin, leptin, and adiponectin were once considered to be single action hormones, mediating metabolism and hunger. However, their role in vascular function has become apparent and may forge the link between obesity, SB, and CVD. Ghrelin (hunger hormone) and adiponectin (glucose regulation and fatty acid breakdown) appear to play a preventative role in CVD and type 2 diabetes mellitus risk (Spranger et al., 2003; Nagaya et al., 2004). Leptin, known as the satiety hormone, is a product of the *OB* gene and is a regulator of several physiological processes. Leptin is thought to play a role in the pathogenesis of atherosclerosis due to leptin receptor gene deficient rats becoming morbidly obese but immune to atherosclerosis (Nishina et al., 1994). Leptin has a positive association with body fat mass and obese individuals display an exponential rise in circulating plasma leptin concentrations, more than likely due to the onset of leptin resistance (Wang et al., 2001). Normative values in normal weight populations ($6.60 \text{ ng}\cdot\text{ml}^{-1}$ – $18.8 \text{ ng}\cdot\text{ml}^{-1}$) are lower than those of obese populations ($28.6 \text{ ng}\cdot\text{ml}^{-1}$ – $34.8 \text{ ng}\cdot\text{ml}^{-1}$) highlighting the association between circulating plasma leptin concentration and fat mass in younger population (34.8 ± 4.60 yrs) (Kazmi et al., 2013). Interestingly, older adult populations also display higher fasting serum leptin levels than their younger counterparts ($4.30\pm 1.90 \text{ ng}\cdot\text{ml}^{-1}$, $1.25\pm 0.40 \text{ ng}\cdot\text{ml}^{-1}$, respectively, (Di Francesco et al., 2006)).

Due to leptin regulating the differentiation of bone marrow osteoprogenitor cells it is thought that high concentrations of leptin binding to endothelial leptin receptors could cause calcification of the intimal wall (Parhami et al., 2001). A 10.0% rise in plasma leptin concentration was associated with a 1.50% decline in arterial

compliance, on the other hand, no association was found between leptin concentration and flow mediated dilation (FMD) in healthy, normal weight adolescents (Singhal et al., 2002). This suggests endothelial dilators are not initially affected by increased leptin concentrations. However, leptin has also been associated with an increase in oxidative stress (Wang et al., 2013), which can cause nitric oxide synthase (eNOS) uncoupling and subsequently, downregulation of NO (Förstermann and Münzel, 2006). This mechanism may explain why negative associations between leptin concentration and endothelial dependent vasodilation exist in older adult populations (Gonzalez et al., 2013) due to oxidative stress being a mediator of declined FMD in populations older than 60.0 yrs (Taddei et al., 2001; Franzoni et al., 2005). Therefore, the effects of calcification appears to occur before the effects of oxidative stress in the development of vascular dysfunction and subsequently, CVD.

Taken together, both mechanisms could decrease vessel compliance and increase total peripheral resistance, which based on Darcy's law, decreases blood flow. A decline in blood flow increases the likelihood of fatty plaque build-up leading to the formation of atherosclerotic lesions at arterial bifurcation sites (VanderLaan et al., 2004).

In a controlled study, SB was simulated through seven days of head down bed rest with 16 participants (32.4 ± 1.90 yrs). The seven days of bed rest caused 40.0% and 20.0% increase in male and female plasma leptin concentration, respectively (Blanc et al., 2000a). Similarly, in an intervention study, six months of detraining following a resistance training intervention in older adults (69.8 ± 5.10 yrs) caused an increase in leptin concentrations that exceeded pre-LIPA and MIPA resistance training concentration. However, in vigorous intensity physical activity (VIPA) training group's leptin concentration did not return to baseline levels which suggests a PA intensity threshold (Fatouros et al., 2005). Therefore, further studies are warranted to explore the possible relationship between SB, PA, plasma leptin, and vascular health in young and older adult populations.

Conclusion

The body of literature surrounding SB is rapidly expanding and it has made the critical discovery that the cardio-metabolic effects of SB are distinct from MVPA. Therefore, individuals can be sufficiently active, based on the PA guidelines, but still sedentary for the remainder of the day. Epidemiological and crossover studies have shown a relationship between SB and cardio-metabolic markers associated with CVD. However, the objective measurement of SB is still a new concept. Future studies need to ensure that objective measurements of SB are able to distinguish sitting/lying from standing, and distinguish standing from LIPA that require movement. Although the use of pre-determined PA cut-off points, such as those derived by Freedson et al. (1998), are useful for between study comparisons, new research is needed to determine population-specific cut-off points, as no two populations are the same, physiologically. The need for population-specific cut-off points is especially important in ageing studies as most pre-determined thresholds were created using young or middle-aged adults and therefore, can cause misclassification of time spent in different PA intensities.

A threshold between couch potato populations and ACP has been established. However, what is not known is when an ACP becomes sufficiently active to counteract the effects of SB. A maximum threshold of SB needs to be established where the effects of SB on CVD risk are greatly reduced in individuals who report levels of SB that are below this maximum threshold. In older adults, engaging in more than 7 hrs·day⁻¹ of self-reported SB is associated with an increased risk of all-cause mortality (Chau et al., 2013). However, further confirmation of this possible threshold is required using more refined methodologies.

Laboratory controlled interventions have shown that breaking up SB with LIPA is a successful method to control cardio-metabolic markers associated with CVD, such as glucose regulation. Further studies are warranted to establish links between SB breaks and other cardio-metabolic markers as well as determining the optimum length of SB bouts and SB breaks to reduce CVD risk. More importantly, intervention studies that use older adult populations are required as older adult individuals are likely to engage in a greater amount of SB time than their young counterparts.

Overall, SB research is still in the early stages of development, and more specifically, has not been implemented throughout the spectrum of ages, in particular the older segment of the population (e.g. populations aged 75.0+ yrs). It is important that future research has a strong methodological framework in both epidemiological and intervention studies before any conclusions are implemented into the national health care initiatives.

Thesis Aims and Hypotheses

Based on the findings of the literature review (chapter 01), the aims of this thesis were 1) to illustrate how and what PB(s) older adults should increase/minimise engagement in to improve their cardiovascular/metabolic profile and thus hopefully improve their health-related quality of later life. 2) determine whether cardiovascular/metabolic markers are affected by increasing age after the age of 60 years and whether PB(s) can mediate any apparent ageing effects.

It was hypothesised that PB parameters representative of a greater engagement in SB would be detrimental to cardiovascular/metabolic parameters, whilst increased engagement in PA parameters would be beneficial to cardiovascular/metabolic parameters. Furthermore, it was hypothesised that a greater engagement in PA parameters would attenuate any age related changes in cardiovascular/metabolic parameters.

Chapter 02:

Monitoring Physical Behaviour in Older Adults

Part 1: 'Overview for Developing Accelerometer Cut-off Points Specific to Older Adult Populations.'

Part 2: 'Reliability and validity of the international physical activity questionnaire compared to calibrated accelerometer cut-off points in the quantification of sedentary behaviour and physical activity in older adults.'

Part 1: Overview for Developing Accelerometer Cut-off Points Specific to Older Adult Populations.

Accelerometer Calibration

The GENEActiv Original (GENEA, Activinsights Ltd, Kimbolton, UK) triaxial accelerometer (43.0 mm x 40.0 mm x 13.0 mm, 16.0 g), previously validated for objective measures of free living physical behaviour (PB) patterns (Esliger et al., 2011; Rowlands et al., 2014; Pavey et al., 2014), was chosen for objective measurement of older adult's free-living PB in the current thesis.

Twenty-four GENEActiv units underwent an initial static calibration process by recording the pull of gravity ($9.81 \text{ m}\cdot\text{s}^{-2}$ or 1.00 G) for one minute through each axis (van Hees et al., 2014) to determine any unit falling outside a 5.00% variability limit (Esliger and Tremblay, 2006) (figure 2.1.1). Only one GENEActiv unit fell outside of this limit (0.051 difference) and therefore, it was not used for the thesis. The initial configuration of GENEActiv required each unit to undergo a three-hour charging period. This process has to be repeated every two months, during the data collection phase, to protect the longevity of the GENEActiv battery.



Figure 2.1.1. The difference between actual force of gravity (1 G) and GENEActiv measured force of gravity in both directions of the x, y, and z axes. Dotted line represents the 5% variability limit stipulated by Esliger and Tremblay (2006).

Accelerometer Configuration

To prepare a GENE unit for a monitoring session, it was inserted into the Universal Serial Bus (USB) docking station (ActivInsights Ltd, Kimbolton, United Kingdom), which was connected to a ProBook 4320s laptop (Hewlett-Pickard, California, USA). Successful GENE connection was confirmed by the unique GENE unit code being displayed as a connect device in the GENEActiv Computer Software (ActivInsights Ltd, Kimbolton, United Kingdom). GENE units were only used for monitoring if the battery status was $\geq 80\%$, as this was the minimum requirement for continuous weeklong monitoring. Within the 'Config. Setup' (figure 2.1.2) Menu option, 'Measurement Frequency' was set at 60 Hertz (Hz) over a range of ± 8 Gravity (g) at a 12-bit (3.9 Milli-Gravity [mg]) resolution, as this allowed for a maximum of 12 continuous monitoring days. 'Time Setup' was always set as 'Local PC Time' and the same computer was always used for the GENE configuration to ensure that time stamping within the raw data file reflected the actual time of day and was consistent between participants. 'Recording Start Mode' was set 'At Future Time' for 00:00 the following day to allow ease of automated analysis and between person comparisons. The unique identification code of the participant was inserted into the 'Subject Code', as multiple participants were monitored at the same time; this ensured that GENE data was not matched to the wrong participant. 'Body Location' displayed which thigh the GENE was placed on. This was the dominant leg of the participant, determined as the participant's standing leg preference in a single leg postural balance attempt. It is pertinent to state that when the researcher removed the GENE after the monitoring week, the thigh that the GENE was placed on was again noted, to confirm that the 'Body Location' selected during the configuration was correct. Once the 'Config. Setup' information was completed, a GENE unit was selected from the 'Devices' list and then 'Erased and Configured'.

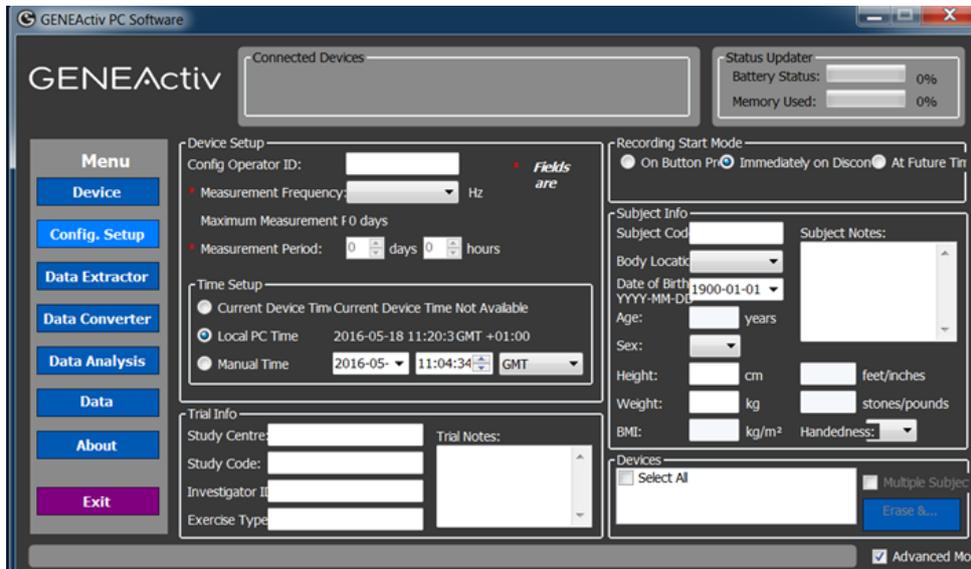


Figure 2.1.2. 'Config. Setup' stage of the GENEActiv Computer Software, which was used to prepare a GENEActiv for monitoring.

Fitting the Accelerometer

Pilot testing revealed that the GENEActiv can cause a degree of skin irritation during weeklong monitoring as a result of the sharp edges that are present on the back of the unit. To overcome this, a 35 × 55 × 7 mm soft sponge was attached to the back of the GENEActiv using two micropore strips (3M, Minnesota, USA) placed horizontally and vertically around the GENEActiv and sponge (figure 2.1.3). However, micropore tape did not cover the 'gold prongs', which are used to download the raw data, as residue from the tape may cause download errors when the GENEActiv is connected to the USB docking station.



Figure 2.1.3. How the sponge was connected to GENEActiv to prevent sharp edges from causing skin irritation.

GENEA was mounted against the thigh of the dominant leg (anterior, 50% of femur length from greater trochanter to lateral femoral condyle) using two Tegaderm Films 1626W (100 mm x 120 mm) (3M, Minnesota, USA), which were placed horizontally and vertically over the GENEAs. At this time, participants were asked to place a hand on either side of their thigh and pull downwards to stretch the skin, this allowed a better contact/adhesion between the skin and the Tegaderm Films and minimised feelings of restriction during movement due to the attachment of GENEAs. The gold 'prongs' of the GENEAs facing downwards and the on/off light being visible confirmed correct orientation of the GENEAs axes. A thigh-mounted accelerometer is considered the gold standard for sedentary behaviour (SB) time measurement (P. Kelly et al., 2016; Steeves et al., 2015; Edwardson et al., 2016) and therefore, was the chosen placement (as opposed to the wrist-worn option that GENEAs was originally designed for). This is due to the change in thigh orientation that is common in the transition from standing to seated or reclined postures and *vice versa*. Using this knowledge, analysis software can determine posture relative to the Earth's surface by examining the static acceleration of the GENEAs axes (figure 2.1.4).

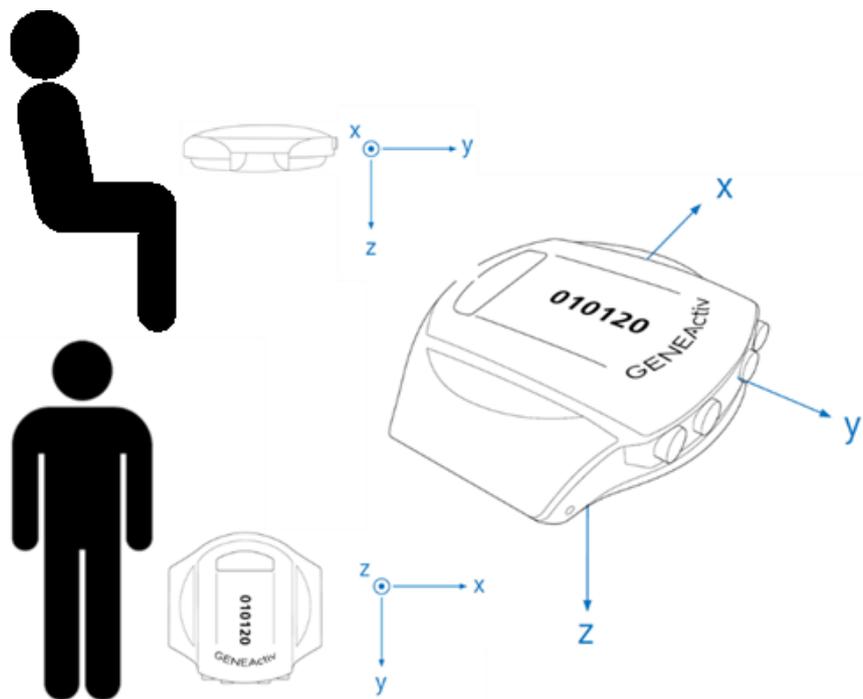


Figure 2.1.4. The distinct orientation of the GENEAs axes that allows posture to be identified through the static acceleration of gravity within an axis when GENEAs is thigh mounted. Picture adapted from *Activinsights (2012)*.

Accelerometer Care during the Monitoring Week

In an attempt to reduce data skewing, GENE surveillance was postponed until a later date if the participant had a 'planned' holiday or long journey that was not considered part of their 'typical' week. Participants were provided with two spare Tegaderm films in case the original films began to peel away from the skin. Participants were instructed not to remove the existing film as this could cause the GENE to be disrupted. Instead, a spare film should have to be placed over the part of the existing film that was peeling away from the skin. If fluid began to accumulate within the air bubble that was present around the GENE, participants were instructed to cut a small hole in the bottom of the air bubble to allow the fluid to drain and then place a spare film over the top to seal the air bubble once again. This was done purely for skin hygiene reasons, as the presence of fluid would not affect the GENE. In the rare case that GENE came away from the skin, participants were asked to write down on the sleep diary at what date and time they noticed the GENE had been disturbed and at what date and time they reattached GENE using the spare films. This allowed the researcher to then decide whether this period or day should be removed from the data analysis or whether a second monitoring week would be required.

A sleep diary was provided to participants so they could self-report the time they woke up in the morning and what time they turned the lights off to go to sleep at night for each of the monitoring days. These times would then be used during the analysis of the GENE data to help identify sleeping hours throughout the monitoring week. In cases where the sleeping diary had missing times, the epoched data was manually assessed to determine at what time prolonged SB occurred or ended. If this time point was similar to the self-reported 'go to sleep time' or 'wake-up time' of other days then it was assumed, at the researcher's discretion, that this was the 'go to sleep time' or 'wake-up time' for the respective day.

Analysis of Accelerometer Data

Following weeklong surveillance, the raw data was downloaded from the GENE by connecting it to the GENEActiv Computer Software using the USB

docking station. Using the 'Data Extractor' stage, downloaded raw data was saved as a .bin file to the participant's data folder. The download period normally lasted approximately 15 minutes. Successful download was confirmed with a pop-up message stating no errors had occurred. The .bin file was then smoothed in the 'Data Converter' stage into 10-second (s) epochs. 10 s epochs were chosen over the commonly used 60 s epoch to increase the sensitivity to changes in a participant's PB and allow for further smoothing, if needed for direct comparison with other studies (Atkin et al., 2012; Aguilar-Farías et al., 2014; Ayabe et al., 2013). Short epochs are recommended as a small accumulation of high intensity movement (counts per minute, G values, sum of vector magnitude) within an epoch that consists mostly of SB could cause a larger average output for the epoch and subsequently, lead to an over/underestimation of the PB intensity (Gabriel et al., 2010; Ayabe et al., 2013). 'Temperature Compensation' was not applied to the data as it has shown to add no significant improvement to the calibration of GENE A in the United Kingdom (van Hees et al., 2014). The smoothed data files were saved as .csv files to allow analyses within Office Excel 2013 (Microsoft Corporation, Washington, USA). The Cheshire Algorithm for Sedentarism (CAS) (Wullems et al., 2015) was used for off-line analyses of GENE A data (figure 2.1.5).

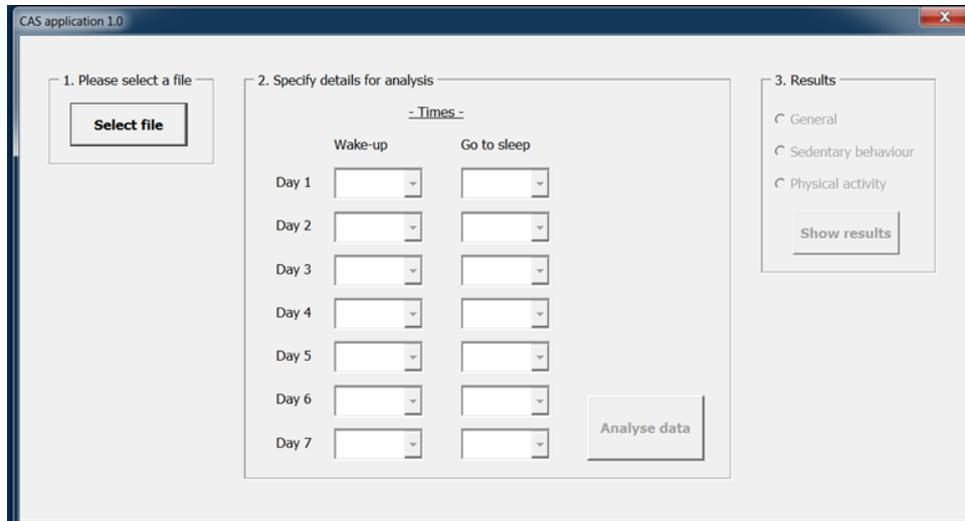


Figure 2.1.5. Data analysis page of the CAS application that was used for GENE A data analyses.

The Cheshire Algorithm for Sedentarism

The novel CAS software uses concepts that are similar to that of the 'Sedentary Sphere' where the mean epoch G value of each x, y, and z axis were compared to one another to recognise posture (Rowlands et al., 2014). For example, if the y-axis G value was the lowest of the three axes (-1 G), the participant was standing up. If the z-axis G value was the lowest of the three axes (-1 G), the participant was sitting down or supine. If the x-axis G value was the lowest of the three axes (-1 G), the participant was lying sideways. Following posture identification (classified as SB or PA) the CAS then uses cut-off points that were locally validated against the energy expenditure of older adults (see Accelerometer Cut-off Point Development below) to determine physical activity (PA) intensity. Energy expenditure was represented as metabolic equivalent tasks (METs), which is the equivalent of oxygen utilisation ($\text{ml}\cdot\text{kg}\cdot\text{min}^{-1}$) as a multiple of resting metabolic rate (RMR). The commonly applied METs denominator of $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$, which represents the RMR of a 70 kg, 40 year old male (Byrne et al., 2005) was substituted for the participant's RMR, within the current thesis, as this has been found to be lower than $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$ in older adults (Ryan et al., 2015b). Therefore, using RMR derived METs should have reduced the likelihood of PA intensity misclassification and also account for individual difference in physical fitness (Ryan et al., 2015b; Ryan et al., 2015a). The summative G value used within the CAS software is referred to as Residual G, as it uses the standard deviations of the axes to determine overall movement. Residual G is similar to that of total movement (Onambele et al., 2006) and is calculated using equation 2.1.1. The Residual G cut-off points of a 10 s epoch for a mixed sex older adult population were 0.057 Residual G for SB – light intensity PA (LIPA) (1.50 METs, PA below this threshold was classified as standing) and 0.216 Residual G for LIPA – moderate to vigorous PA (MVPA) (3.00 METs). The CAS provided several parameters for both SB and PA. Details of these parameters are provided in table 2.1.1.

Equation 2.1.1. Residual G calculation.

$$\text{Residual G} = \sqrt{(\text{SD } x^2 + \text{SD } y^2 + \text{SD } z^2)}$$

Where: x is the medio-lateral axis

y is the vertical axis

z is the anterior-posterior axis

SD is standard deviation

$\sqrt{\quad}$ is square root

Table 2.1.1. Definitions of the PB parameters provided by the CAS.

Terminology	Unit	Definition
Physical Behaviour		
Sedentary Behaviour (SB)	hrs·day ⁻¹	The mean amount of SB, of any length, that is accumulated in a 24hr day. Any waking behaviour characterised by a seated or reclined posture (Tremblay et al., 2010).
Standing	hrs·day ⁻¹	The mean amount of standing, of any length, that is accumulated in a 24hr day. Any standing posture that elicits little to no movement and expends ≤1.50 METs of energy (Tremblay et al., 2010).
Light Intensity Physical Activity (LIPA)	hrs·day ⁻¹	The mean amount of LIPA, of any length, that is accumulated in a 24hr day. Any standing posture that elicits 1.50 - <3.00 METs (Tremblay et al., 2010).
Sporadic Moderate to Vigorous Physical Activity (sMVPA)	hrs·day ⁻¹	The mean amount of MVPA, of any length, that is accumulated in bouts <10.0 mins in a 24hr day (Department of Health Social Services and Public Safety, 2011). Any standing posture that elicits ≥3.00 METs (Tremblay et al., 2010).
≥10 Minute Moderate to Vigorous Physical Activity (₁₀ MVPA)	hrs·day ⁻¹	The mean amount of MVPA that is accumulated in bouts ≥10.0 mins in a 24hr day (Department of Health Social Services and Public Safety, 2011). Any standing posture that elicits ≥3.00 METs (Tremblay et al., 2010).

Table 2.1.1 continued.

Patterns of Physical Behaviour		
SB Parameters		
SB Breaks	$n \cdot \text{day}^{-1}$	The mean amount of SB Breaks in a 24hr day. ≥ 2.00 mins of continuous PA (Benatti and Ried-Larsen, 2015) that follows ≥ 1.00 of SB (Messinger-Rapport et al., 2003). NB – Every day starts in a sedentary state.
<5min SB Bout	$n \cdot \text{day}^{-1}$	The mean amount of SB bouts that consist of <5.00 mins in a 24hr day (Saunders et al., 2013). SB bout starts after ≥ 2.00 mins of PA is followed by ≥ 1.00 mins SB and ends with an 'SB Breaks'. Contains SB and <2.00 mins of PA.
≥ 5 min SB Bout	$n \cdot \text{day}^{-1}$	The mean amount of SB bouts that consist of ≥ 5.00 mins in a 24hr day (Saunders et al., 2013). SB bout starts after ≥ 2.00 mins of PA is followed by ≥ 1.00 mins SB and ends with an 'SB Breaks'. Contains SB and <2.00 mins of PA.
True Mean SB Bout	$\text{mins} \cdot \text{day}^{-1}$	SB time (≥ 1.00 mins) between PA bouts of ≥ 2.00 mins are LOG transformed to normally distribute the data and then anti-logged to find the "True Mean" (Chastin et al., 2015c). Mean amount in a 24hr day.
Alpha	$\alpha \cdot \text{day}^{-1}$	How steeply the number of SB bouts decreases with increasing SB bout duration in a power-law distribution (Chastin et al., 2015c). Mean amount in a 24hr day.
W50%	$\text{mins} \cdot \text{day}^{-1}$	50% of SB time in a 24hr day is accumulated by SB bouts of this specific length or shorter (Chastin et al., 2015c). Mean amount in a 24hr day.
SB%	$\% \cdot \text{waking hrs} \cdot \text{day}^{-1}$	The mean percentage of waking hours that is spent performing 'SB' of any length in a 24hr day.

Table 2.1.1 continued.

<i>PA Parameters</i>		
PA Bouts	$n \cdot \text{day}^{-1}$	The mean amount of bouts that consist of ≥ 2.00 mins of continuous PA (Benatti and Ried-Larsen, 2015) followed by ≥ 1.00 mins of continuous SB (Messinger-Rapport et al., 2003) in a 24hr day. PA bout starts after 'SB Breaks' and ends with an SB bout. Contains PA and < 1.00 mins of continuous SB.
Daily Sum of PA Bout time	$\text{mins} \cdot \text{day}^{-1}$	The mean amount of time that spent in 'PA Bouts' in a 24hr day.
True Mean PA Bout	$\text{mins} \cdot \text{day}^{-1}$	PA time (≥ 2.00 mins) between SB bouts of ≥ 1.00 mins are LOG transformed to normally distribute the data and then anti-logged to find the "True Mean" (Chastin et al., 2015c). Mean amount in a 24hr day.
$_{10}\text{MVPA}$ Bouts	$n \cdot \text{day}^{-1}$	The mean amount of bouts that consist of ≥ 10.0 mins MVPA in a 24hr day.
Total Week $_{10}\text{MVPA}$	$\text{hrs} \cdot \text{week}^{-1}$	The sum amount of ' $_{10}\text{MVPA}$ Bouts' time that is accumulated in a monitoring week (7 days).
Standing%	$\% \cdot \text{waking}$ $\text{hrs} \cdot \text{day}^{-1}$	The mean percentage of waking hours that is spent performing 'Standing' in a 24hr day.
LIPA%	$\% \cdot \text{waking}$ $\text{hrs} \cdot \text{day}^{-1}$	The mean percentage of waking hours that is spent performing 'LIPA' in a 24hr day.
sMVPA%	$\% \cdot \text{waking}$ $\text{hrs} \cdot \text{day}^{-1}$	The mean percentage of waking hours that is spent performing 'sMVPA' in a 24hr day.
$_{10}\text{MVPA}\%$	$\% \cdot \text{waking}$ $\text{hrs} \cdot \text{day}^{-1}$	The mean percentage of waking hours that is spent performing ' $_{10}\text{MVPA}$ ' in a 24hr day.

Accelerometer Cut-off Point Development

A sub-population of the thesis participants who were recruited for chapter 03 - 05 agreed to complete the GENE A cut-off point development study. Thus, 20 participants (5 females < 75.0 yrs, 5 females ≥ 75.0 yrs, 4 males < 75.0 yrs, 6 males ≥ 75.0 yrs) underwent laboratory controlled activities to produce PB cut-off points for the weeklong free-living data collection. The Manchester Metropolitan University Exercise and Sport Science Sub Committee granted ethical approval and participants signed informed consent forms prior to data collection.

Participants were fitted with a GENE A on both upper legs (anterior, 50% of greater trochanter to lateral femoral condyle length) and a heart rate monitor (Polar

H7 monitor, Polar Electro, Kempele, Finland). The two GENE units plus heart rate monitor were used as a reliability and validity measure. Video monitoring took place to assess the agreement between real-world behaviours and GENE recognised PB intensity. Participants completed the protocol highlighted in figure 2.1.6 whilst wearing a mouthpiece connected to a Douglas bag, to collect exhaled gas samples.

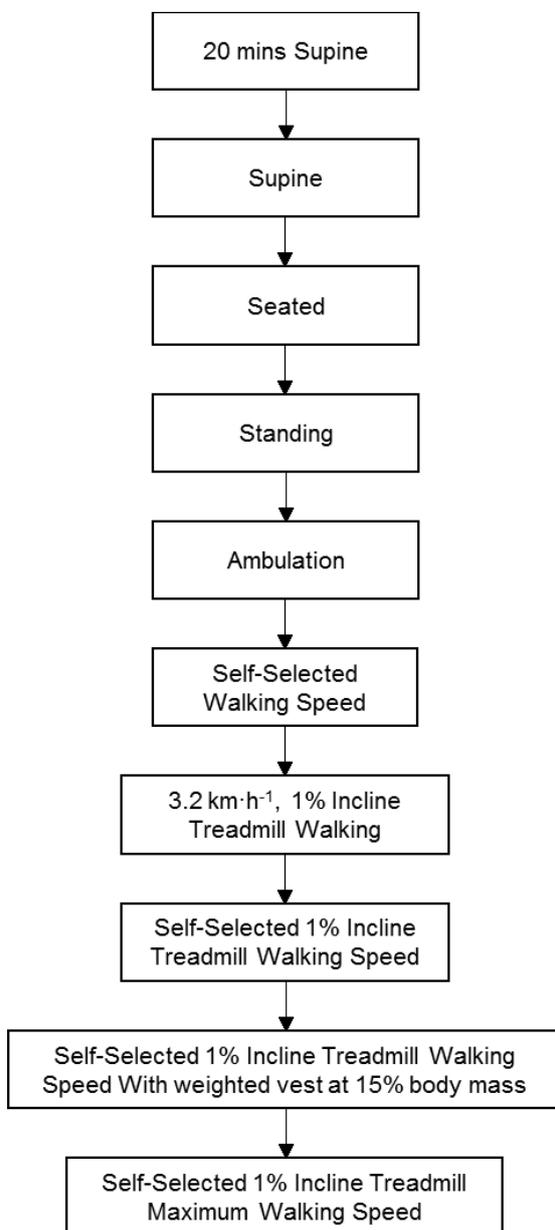


Figure 2.1.6. Cut-off points calibration protocol. Heart rate and two expired gas samples were collected during the last two minute of each four-minute stage. Ambulation required the participant to step 30 cm side to side at the pace of a 30 beats per minutes (30 steps per minute) metronome app (Logan Gauthier, Huntsville, USA), touching their feet together either side of the 30 cm line. Maximum walking speed for the final stage was set at 6.50 km·h⁻¹ based on a previous protocol performed by a similar population (Miller et al., 2010).

Two exhaled gas samples (one minute each) were collected in the last two minutes of each four minute stage using Douglas bags, and later analysed through

indirect calorimetry (Servomex 5200, Servomex, Crowborough UK). The mean oxygen utilisation of the two exhaled gas samples for each stage were divided by the lowest of the two oxygen utilisation readings from the seated stage (to represent RMR) to calculate MET conversion (referred to as measured METs). The lowest reading of the two seated oxygen utilisation samples was selected instead of the mean to account for hyperventilation, which is common within exhaled gas sample collection that uses a mouthpiece method (Scott, 1993). In a further attempt to reduce the likelihood of hyperventilation, participants were asked to wear the mouthpiece for five-minutes prior to the start of the four-minute supine stage so they could become accustomed to the unfamiliar procedure. Participant's heart rate was monitored between each stage; only when their heart rate had returned to baseline (10% range) were they allowed to begin the next stage. This was done to protect against any fatiguing effect that could carry over into subsequent stages and thus, could influence both GENE A and oxygen utilisation readings.

GENEA data was downloaded into Office Excel 2013 (Microsoft Corporation, Washington, USA) and time-matched to the heart rate and measured METs for each stage. Heart rate was used as a validity measure to ensure that any increase in GENE A Residual G or measured METs was a result of increasing physical task demand (figure 2.1.7). Residual G cut-off points were then determined, to represent the standard MET thresholds (SB-LIPA, 1.5 METs; LIPA-MVPA, 3.0 METs), using a single power regression trend line (equation 2.1.2).

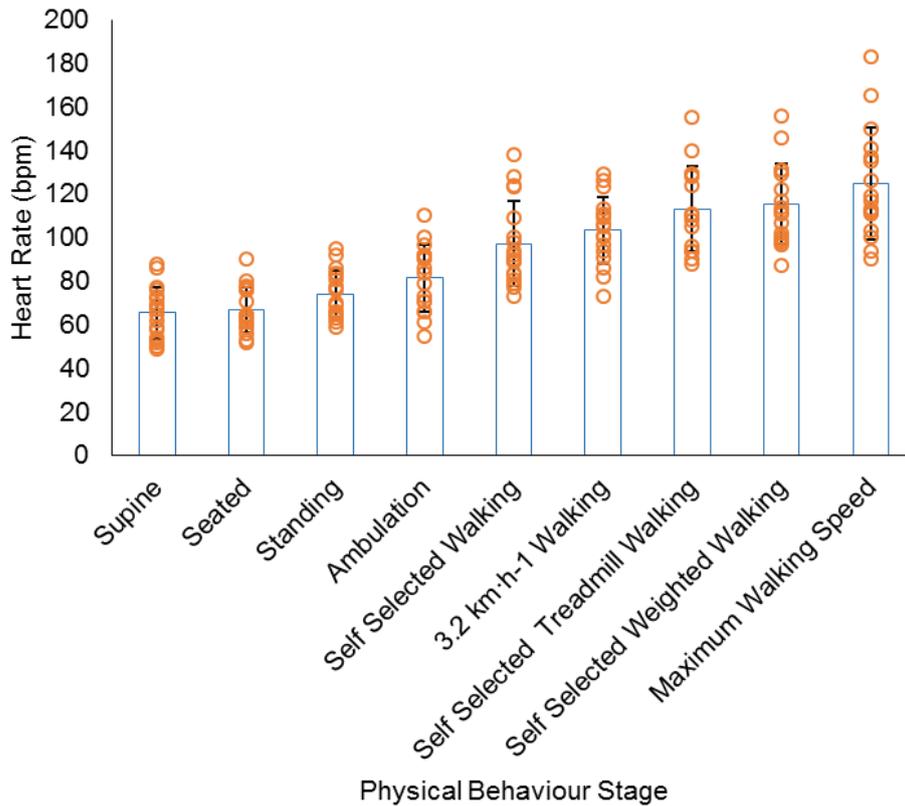


Figure 2.1.7. The mean heart rate of the participants' during each physical behaviour stage. Blue bars represent mean heart rate. Error bars represent heart rate standard deviation per stage. Open circles represent the heart rate of each participant per stage.

Equation 2.1.2. Accelerometer Cut-off Point Equation.

$$\text{Residual G} = 0.0262 \times \text{Measured METs}^{1.9204}$$

Measured METs explained 89% of the variance in Residual G (figure 2.1.7), which was similar to that of other cut-off point development research (Matthew, 2005) and therefore, deemed that GENE A Residual G was sufficient for quantifying energy expenditure in older adult populations. More importantly, the GENE A posture recognition and Residual G cut-off points could accurately classify the time spent engaging in SB and PA intensity as shown by a Cohen's Kappa value of 1.00 (95% confidence interval [CI] 1.00, 1.00, $p < 0.001$) and 0.89 (95%CI 0.49, 1.31, $p < 0.001$), respectively. Therefore, this validation procedure increased the confidence that the results provided by the CAS were representative of the true PB(s) performed within the participant's free-living monitoring week.

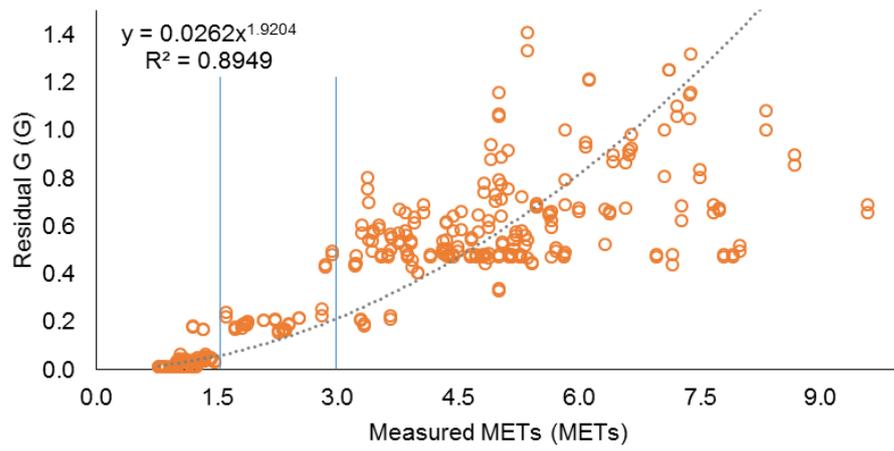


Figure 2.1.8. The association between Measured METs and Residual G for each PB stage within the participants' calibration protocols. Grey dotted line illustrates the power regression trend line. Blue lines illustrate the 1.5 and 3.0 METs thresholds normally used to classify PB intensity.

Part 2: Reliability and validity of the international physical activity questionnaire compared to calibrated accelerometer cut-off points in the quantification of sedentary behaviour and physical activity in older adults.

Introduction

The International Physical Activity Questionnaire (IPAQ) is used worldwide to indirectly evaluate an individual's volumes of sedentary behaviour (SB) and physical activity (PA) throughout a seven-day week (Craig et al., 2003). Its potential use to measure SB and PA has become essential in recent years due to the emerging understanding of the independent effects of SB and moderate to vigorous PA (MVPA) on health-related factors (Bey and Hamilton, 2003; Bankoski et al., 2011).

Sedentary behaviour is defined as any seated or reclined posture (e.g. sitting, lying down, and driving) that expends 1.50 or less Metabolic Equivalent Tasks (METs), while MVPA is any activity that expends 3.00 or more METs (Tremblay et al., 2010). One MET is equal to Resting Metabolic Rate (RMR) or $3.50 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$ of oxygen utilisation, which is reported to reflect a 70 kg young person's RMR (Byrne et al., 2005). In light of the independence between SB and MVPA, it is now possible to be highly sedentary whilst also being physically active ($\geq 2.5 \text{ hrs}\cdot\text{week}^{-1}$ of MVPA accumulated in bouts ≥ 10 continuous minutes). Therefore, people can be categorised into four physical behaviour (PB) (Bussmann and van den Berg-Emons, 2013) groups: highly sedentary and physically inactive (couch potato), highly sedentary but physically active (active couch potato, ACP), low sedentary and physically inactive (ambulator), and finally, low sedentary and physically active (active ambulator).

MET thresholds are commonly applied to accelerometer data (Freedson et al., 1998; Miller et al., 2010) to allow for an objective measure of free-living SB and MVPA. Accelerometry is still a new concept within free-living monitoring, but is

deemed a valid determination of SB and MVPA (Montoye et al., 2014). Nevertheless, in large epidemiological studies, the use of accelerometers can be costly and time consuming.

By contrast, self-report measures such as the IPAQ are readily available, very cost-effective and can generate large data sets. The IPAQ has previously undergone reliability and validity assessments in young to middle aged adults ($n=2721$, 54% female, 36.8 ± 7.9 years) (Craig et al., 2003). Reliability is the extent to which results are consistent over time whilst validity determines whether a tool truly measures what it is intended to measure (Golafshani, 2003). However, few studies have assessed its validity of the IPAQ in populations over 60 years of age (Tomioka et al., 2011; Hurtig-Wennlöf et al., 2010; Cerin et al., 2012). These aforementioned studies used populations that ranged from 54 – 325 participants (49% - 58% female) to validate the IPAQ against waist mounted accelerometer measures, which may not be as accurate as thigh mounted accelerometers when determining posture (Edwardson et al., 2016). Sex differences have been reported within IPAQ data sets as well as in hip-mounted accelerometer measures (Tomioka et al., 2011), which suggests that the IPAQ results (Tomioka et al., 2011) were not due to sex differences in social desirability or approval (Hebert et al., 1997). To the author's knowledge no study, to date, has investigated the reliability and validity of the self-administered IPAQ Long-Form, English (last 7 days format) against thigh-mounted accelerometer derived data, using an older community-dwelling participant sample. Assessing the quality of the IPAQ across countries and age groups is essential to ensure the IPAQ is adaptable across different social, sex, and ethnic groupings.

Therefore, the objectives of this study were to compare two IPAQ Long-Form, English (last 7 days format) data sets, collected a week apart, and to compare the results of the IPAQ to accelerometer measures of free-living SB and MVPA. The aim was to provide a reliability and validity record for IPAQ measures of SB and MVPA in middle-class (e.g. coming from a sub-region characterised by proximity to several universities, low claimant unemployment rate, high proportion of managerial and professional posts), semi-rural UK-based older-adults. It was hypothesised that: 1. The IPAQ would be reliable. 2. Owing to a bias in the IPAQ to questions relating to PA rather than SB *per se*, the IPAQ may in fact underestimate SB and overestimate MVPA time. Thus, the second hypothesis was that the IPAQ may not

be valid in quantifying SB and MVPA time. 3. Sex bias would not occur in both the reliability, validity and PB classification of the IPAQ.

Methods

Eighty-nine community-dwelling retired (and/or in voluntary employment) older participants (73.7 ± 6.3 years, 60 – 89 years, 54% female) who were independently mobile (i.e. no walking aids), did not suffer from an untreated cardiovascular disease, did not sustain an injury within the last three months, did not suffer from any mental impairment, and were deemed generally healthy, volunteered to participate in the study. These participants were recruited primarily from older adult community groups within the Cheshire East Borough, England. The Manchester Metropolitan University: Exercise and Sports Science Ethics Sub-Committee granted ethical approval. Informed consent was obtained prior to participation in the study. On recruitment, it was made clear to the participants that their inclusion within the study would require their PB to remain habitual for at least the two weeks covered by the study protocol (see below).

First Laboratory Visit

During the first visit to the laboratory, 76 participants successfully completed the self-administered IPAQ Long-Form, English (last 7 days format), with the remaining participants returning incomplete questionnaires which were therefore not analysed. Participants' demographics were collected and were then fitted with a commercially available thigh mounted (anterior aspect, at 50% of greater trochanter to femoral lateral condyle distance) triaxial accelerometer (GENEA, GENEActiv Original, Activinsights Ltd, Kimbolton, UK) on their dominant thigh, for a free-living week (7 consecutive days). Standing leg preference during a single leg balance exercise determined leg dominance. Two waterproof adhesive patches (3M Tegaderm Film, North Ryde, Australia) were used to mount GENEActiv. Height (precision: 0.01 m) and mass (precision: 0.1 kg) were measured with a stadiometer

(Harpenden, Holtain, Croswell, Wales) and electronic weighing scales (Salter, Kent, England).

GENEA recorded at a 60.0 Hertz (Hz) frequency and data were smoothed using 10 second (s) epochs. The chosen GENE output was Residual G (equation 2.2.1), adapted from previous work on total movement analysis in older persons (Onambele et al. (2006) and termed the Cheshire Algorithm for Sedentarism (CAS, Chapter 02: Part 1) (Wullems et al., 2015).

Equation 2.2.1. Residual G calculation.

$$\text{Residual G} = \sqrt{(\text{SD } x^2 + \text{SD } y^2 + \text{SD } z^2)}$$

Where: x is the medio-lateral axis

y is the vertical axis

z is the anterior-posterior axis

SD is standard deviation

$\sqrt{\quad}$ is square root

Posture (SB or PA classification) was determined by comparing the mean epoch G values for the x , y , and z axes to one another (Rowlands et al., 2014). For example, if the y -axis G value was the lowest of the three axes (-1 G), the participant was standing up. If the z -axis G value was the lowest of the three axes (-1 G), the participant was sitting down or supine. If the x -axis G value was the lowest of the three axes (-1 G), the participant was lying sideways. The SB-light intensity PA (LIPA) (1.5 METs) Residual G cut-off point was 0.057 Residual G and the LIPA-MVPA (3.0 METs) cut-off point was 0.216 Residual G. These cut-off points were derived from a systematic validation of the GENE against expired gas samples of older adults collected during a laboratory-based activity calibration protocol, where nine PB functions (i.e. 1-supine, 2-sitting, 3-standing quietly, 4-self-selected ground walking, 5-brisk walk on treadmill, 6-3.5 km·hr⁻¹ walk on treadmill, 7-self-selected walk on treadmill, 8-self-selected weighted-vest treadmill walking [at 15% of body weight], 9-repeated side-stepping) were monitored with concurrent gas analyses, heart rate, motion analysis and accelerometer output (Chapter 02: Part 1). There was a strong explained variance between Residual G and METs ($r^2=0.89$, $p<0.001$). GENE posture recognition, Residual G cut-off points and MET thresholds had a strong agreement in posture (Cohen's kappa = 1.00, $p<0.001$) and PA intensity

(Cohen's kappa = 0.81, $p < 0.001$) identification (Chapter 02: Part 1). During in house calibration, it was found that normalising one MET to the RMR of the participant would help account for individual differences in physical fitness (Ryan et al., 2015a; Ryan et al., 2015b) and therefore make the Residual G cut-off points more ecologically valid.

Second Laboratory Visit

Following a week (uninterrupted 7 days of 24-hour-long accelerometer wear) of GENEa wear-time, 86 participants completed another self-administered IPAQ Long-Form, English (last 7 days format) within 8 – 11 days of the first laboratory visit. Scoring of the IPAQ was completed as described previously (IPAQ Research Committee, 2005). Due to older adults completing small amounts of MVPA (Craig et al., 2009), IPAQ measures of moderate PA and vigorous PA were combined to make $_{10}$ MVPA (MVPA accumulated in bouts ≥ 10 continuous minutes).

To allow direct comparison with the IPAQ, total week $_{10}$ MVPA (hours over a week) as well as total week sporadic MVPA (sMVPA, accumulated in bouts < 10 continuous minutes, hours over a week), were used as the chosen output for GENEa. For SB, total week SB (hours over a week) was the chosen output of the IPAQ and GENEa, with the former including transport and other sitting time. For participants who had less than seven days of valid GENEa data (three participants [1 male and 2 females] only had 6 valid days [i.e. full 24 hour-long data set]), the mean daily engagement in SB, $_{10}$ MVPA, and sMVPA of each participant's six valid days was added to the sum of their valid days to complete their total week (7 day) SB, $_{10}$ MVPA, and sMVPA data set. PB classification definitions are provided in table 2.2.1. Only waking hours (determined with self-reported sleeping hours, lights-off time and wake-up time) are analysed for the purpose of this chapter.

Table 2.2.1. Physical behaviour group classification.

	Total Week SB daily mean $\geq 8 \text{ h} \cdot \text{day}^{-1}$	Total Week SB daily mean $< 8 \text{ h} \cdot \text{day}^{-1}$
Total Week $_{10}$ MVPA $< 2.5 \text{ h} \cdot \text{week}^{-1}$	Couch Potato	Ambulator
Total Week $_{10}$ MVPA $\geq 2.5 \text{ h} \cdot \text{week}^{-1}$	Active Couch Potato	Active Ambulator

Statistical Analyses

SPSS Statistics 21 (International Business Machines Corporation, New York, USA) was used for statistical analyses. Those who were missing first or second visit IPAQ data were removed from the relative and absolute between day IPAQ reliability assessments. Meanwhile individuals only missing first visit IPAQ data were included in the concurrent validity and PB classification agreement assessments against GENE data. For hypothesis one, related to between day IPAQ reliability, with the data being non-parametric (based on the outcome of the Kolmogorov-Smirnov and Levene's tests outcomes), Spearman rho correlations were used to examine the association between the first and second visit IPAQ data (for total week SB and total week ₁₀MVPA). To illustrate the absolute reliability between first laboratory visit and second laboratory visit IPAQ data sets, Bland-Altman plots were used.

For hypothesis two, related samples t-tests (or Wilcoxon Signed Rank tests for non-parametric data sets) and Spearman rho correlations were used to assess the validity of the IPAQ against GENE measures of total week SB, total week ₁₀MVPA, and total week sMVPA. To illustrate the concurrent validity of the IPAQ against GENE, Bland-Altman plots were used.

For hypothesis three, independent samples t-tests (or Mann Whitney-U tests for non-parametric data sets) were used to examine sex differences in total week SB, total week ₁₀MVPA, and total week sMVPA. These sex differences were carried out for both IPAQ and GENE data sets. To identify sex differences in correlation coefficients and correlation slopes for reliability and validity assessments, Fisher's z transformation tests were performed. Differences in the residuals within the systematic bias between sexes were assessed with independent samples t-tests (or Mann Whitney-U for non-parametric data sets). Cohen's Kappa test was used to assess PB classification agreement between the IPAQ and GENE. Whilst Pearson's Chi Squared was used to assess the effect of sex on PB classification.

Significance was set at $p \leq 0.05$. Explained variance was expressed as weak (< 0.40), moderate (≥ 0.40), or strong (≥ 0.60). Data are presented as Mean \pm Standard Deviation (SD), or for non-parametric data, Median (Interquartile Range [IR]).

Results

Seventy-three participants (54% females) were included in the reliability assessment while 86 participants (52% females) were included in the validity assessment of the IPAQ (table 2.2.2). Two females were then excluded from the validity assessment due to GENE data errors.

Table 2.2.2. Participant demographics and breakdown of IPAQ completion.

Group	Male (n = 41)	Female (n = 48)	Pooled (n = 89)
<i>Completed</i>	(n = 33)	(n = 40)	(n = 73)
Age (yrs)	74.6 ± 6.0	72.8 ± 6.8	73.7 ± 6.5
Height (m)	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Mass (kg)	79.8 ± 11.0	71.4 ± 12.5	75.2 ± 12.5
BMI (kg·m ²)	27.6 ± 4.0	28.3 ± 5.0	28.0 ± 4.6
<i>Missing first visit IPAQ</i>	(n = 8)	(n = 5)	(n = 13)
Age (yrs)	76.1 ± 5.2	72.3 ± 5.7	74.3 ± 5.4
Height (m)	1.70 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
Mass (kg)	85.1 ± 8.9	70.0 ± 20.5	80.4 ± 15.6
BMI (kg·m ²)	28.5 ± 3.7	27.2 ± 8.7	27.9 ± 5.8
<i>Missing second visit IPAQ</i>	(n = 0)	(n = 3)	(n = 3)
Age (yrs)		69.0 ± 6.1	69.0 ± 6.1
Height (m)		1.7 ± 0.1	1.7 ± 0.1
Mass (kg)		85.3 ± 14.1	85.3 ± 14.1
BMI (kg·m ²)		31.1 ± 5.9	31.1 ± 5.9

BMI Body mass index.

Relative Reliability of the IPAQ

Within the pooled population, a weak correlation between first and second visit IPAQ data was present for total week SB (figure 2.2.1a). For total week ₁₀MVPA, moderate correlations were found between first and second visit IPAQ data for the pooled population (figure 2.2.1b). Interestingly, the majority of data points were situated below the line of unity for total week SB and total week ₁₀MVPA; suggesting that there was a trend for participants to report a lesser amount of time spent performing total week SB and total week ₁₀MVPA in the second visit IPAQ compared to the first visit IPAQ.

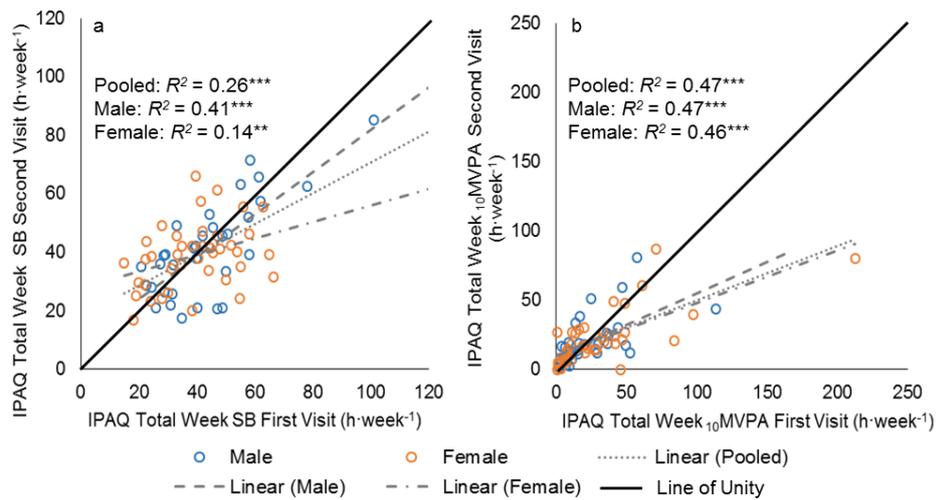


Figure 2.2.1 a) Spearman rho correlation between first and second visit IPAQ total week SB. b) Spearman rho correlation between first and second visit IPAQ total week $_{10}MVPA$. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Absolute Reliability of the IPAQ

A Bland-Altman plot (figure 2.2.2a) of total week SB difference between first visit and second visit IPAQ for the pooled population showed small systematic bias (non-significant). However the 95% confidence intervals (95%CI) suggested large inter-individual random error ($b=1.51 \pm 25.9 \text{ h} \cdot \text{week}^{-1}$). Systematic bias was similar as total week SB mean increased, suggesting no proportional bias ($p=0.21$).

For total week $_{10}MVPA$, a Bland-Altman plot (figure 2.2.2b) of total week $_{10}MVPA$ difference between first visit and second visit IPAQ for the pooled population showed systematic bias ($b=6.37 \pm 22.7 \text{ h} \cdot \text{week}^{-1}$, $p=0.025$). It is notable that heteroscedasticity was present for the pooled population (Kolmogorov-Smirnov, $p < 0.001$). Thus, proportional bias was present due to the difference between first and second visit IPAQ total week $_{10}MVPA$ data increasing as the mean of first and second visit IPAQ total week $_{10}MVPA$ data increased ($p < 0.001$).

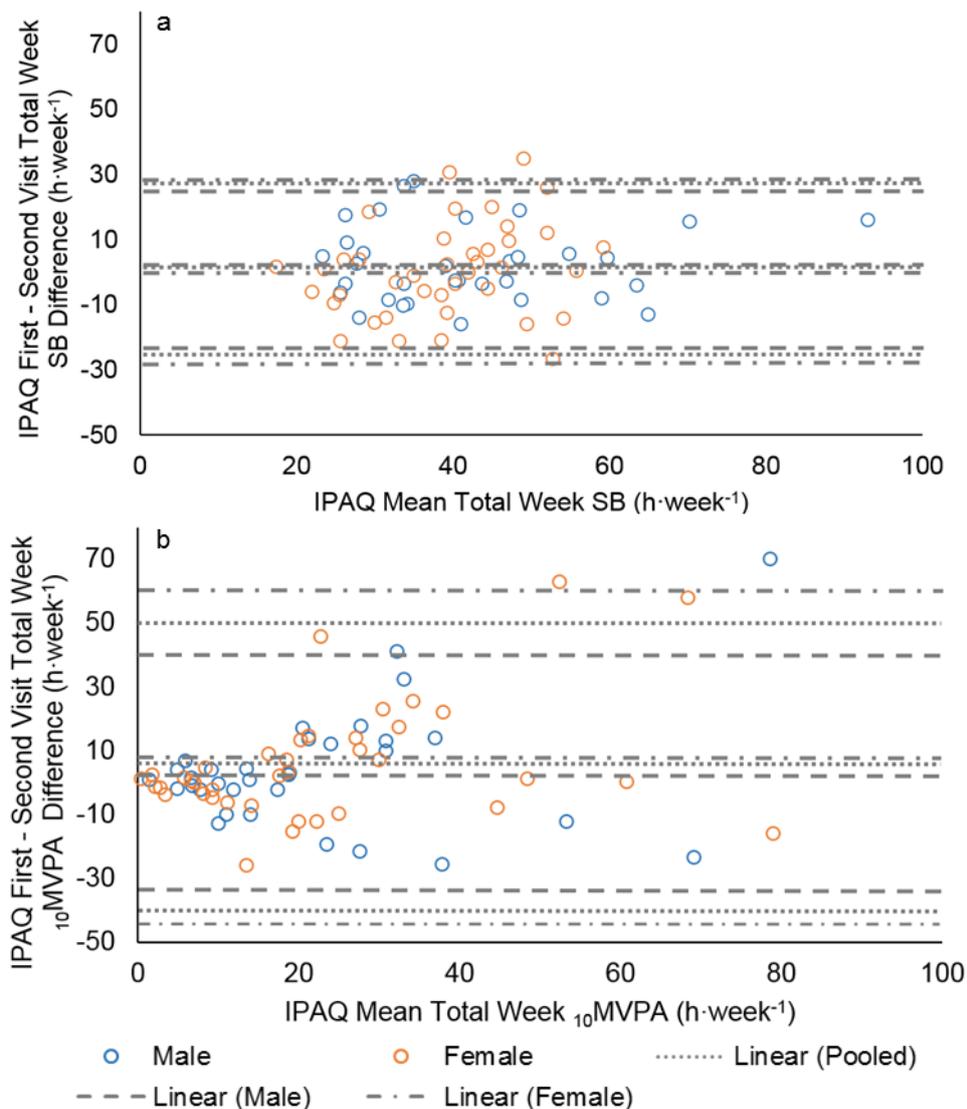


Figure 2.2.2 a) Bland-Altman plot of the difference between First and Second Visit IPAQ data against the Mean of the First and Second Visit IPAQ data for total week SB. b) Bland-Altman plot of the difference between First and Second Visit IPAQ data against the Mean of the First and Second Visit IPAQ data for total week ₁₀MVPA. Lines represent systematic bias and 95%CI for pooled, male, and female populations.

Concurrent Validity of the IPAQ

Total week SB measured by the IPAQ was 41% ($p \leq 0.05$) less than that of GENEAs while total week ₁₀MVPA measured by the IPAQ was 15.7 fold ($p \leq 0.05$) greater than that of GENEAs. Interestingly, IPAQ total week ₁₀MVPA was similar to GENEAs sMVPA (21.6 ± 19.4 h·week⁻¹, Median 16.6(8.63) h·week⁻¹, 18.5 ± 5.63 h·week⁻¹, Median 18.3(15.1) h·week⁻¹, $p > 0.05$, respectively) (figure 2.2.3).

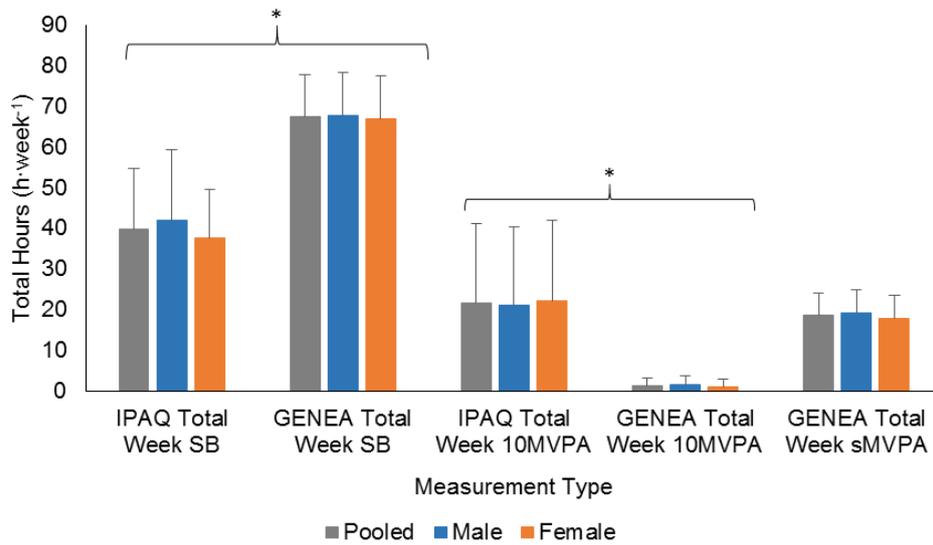


Figure 2.2.3. Comparison between IPAQ and GENE A measures of total week SB, total week ₁₀MVPA, and GENE A total week sMVPA. * $p \leq 0.05$.

The pooled population showed a weak correlation between IPAQ and GENE A measures of total week SB (figure 2.2.4a). Additionally, the majority of data points were situated below the line of unity; suggesting a trend for the IPAQ to under report total week SB when compared to GENE A measures. This is also supported by a Bland Altman (figure 2.2.5a) plot that suggested all but two participants ($11.4 \text{ h} \cdot \text{week}^{-1}$, $30.8 \text{ h} \cdot \text{week}^{-1}$) under-reported their total week SB using the IPAQ (pooled: $b = -27.6 \pm 26.5 \text{ h} \cdot \text{week}^{-1}$, $p < 0.001$). Proportional bias was present in the pooled population ($p \leq 0.001$).

No significant correlation ($p > 0.05$) between IPAQ and GENE A measures of total week ₁₀MVPA was found for the pooled population (figure 2.2.4b). Heteroscedasticity was present in total week ₁₀MVPA data sets ($p \leq 0.001$). A Bland-Altman revealed systematic bias that suggested the IPAQ over reported total week ₁₀MVPA compared to GENE A (figure 2.2.5b, $b = 20.3 \pm 19.6 \text{ h} \cdot \text{week}^{-1}$, $p \leq 0.001$). The presence of proportional bias suggested the difference between IPAQ and GENE A measures of total week ₁₀MVPA increase as the mean of IPAQ and GENE A total week ₁₀MVPA increase ($p \leq 0.001$).

A weak correlation between IPAQ total week ₁₀MVPA and GENE A total week sMVPA was present for pooled populations (figure 2.2.4c). Heteroscedasticity, the variance of the error in the IPAQ was predicted by a GENE A variable (Kaltenbach, 2012), was present for both total week ₁₀MVPA and sMVPA data sets ($p \leq 0.001$).

Interestingly, a Bland-Altman test suggested there was no systematic bias between IPAQ total week $_{10}$ MVPA and GENEAs total week sMVPA measures (figure 2.2.5c, $b=3.13\pm 17.5$ h \cdot week $^{-1}$, $p=0.11$). However, proportional bias was present ($p\leq 0.001$).

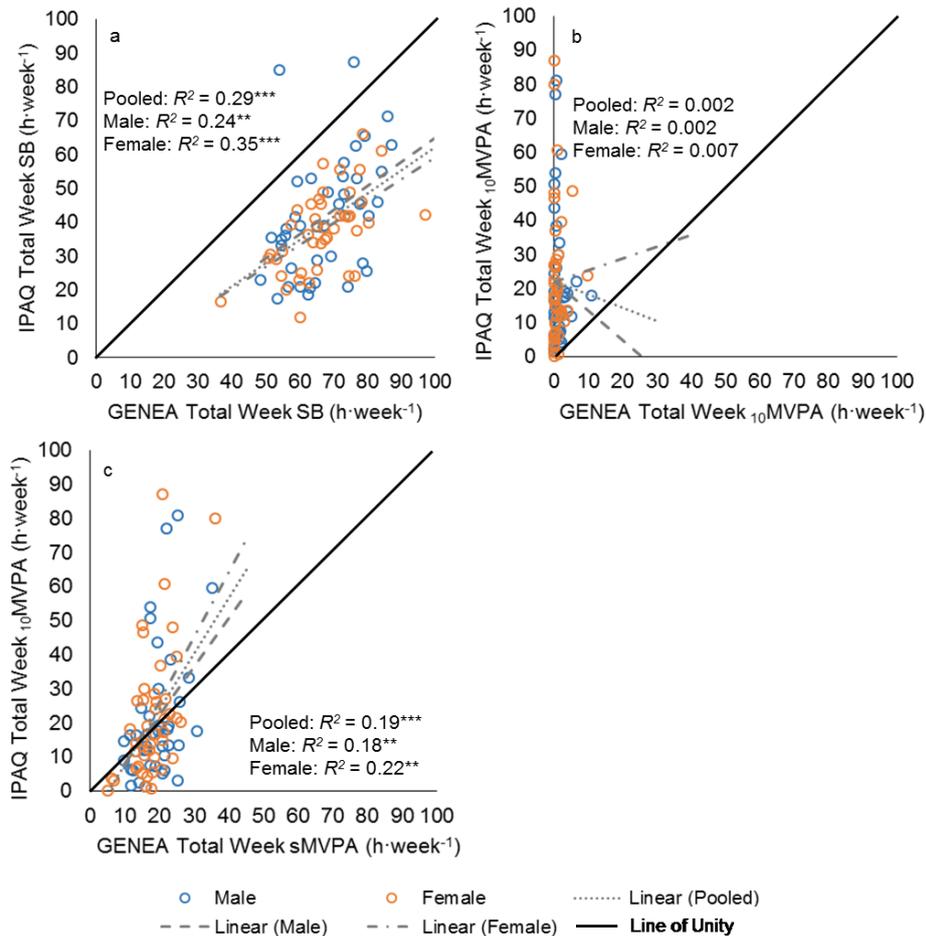


Figure 2.2.4 a) Spearman rho correlation between IPAQ and GENEAs measures of total week SB. b) Spearman rho correlation between IPAQ and GENEAs measures of total week $_{10}$ MVPA. c) Spearman rho correlation between IPAQ total week $_{10}$ MVPA and GENEAs total week sMVPA. * $p\leq 0.05$, ** $p\leq 0.01$, *** $p\leq 0.001$.

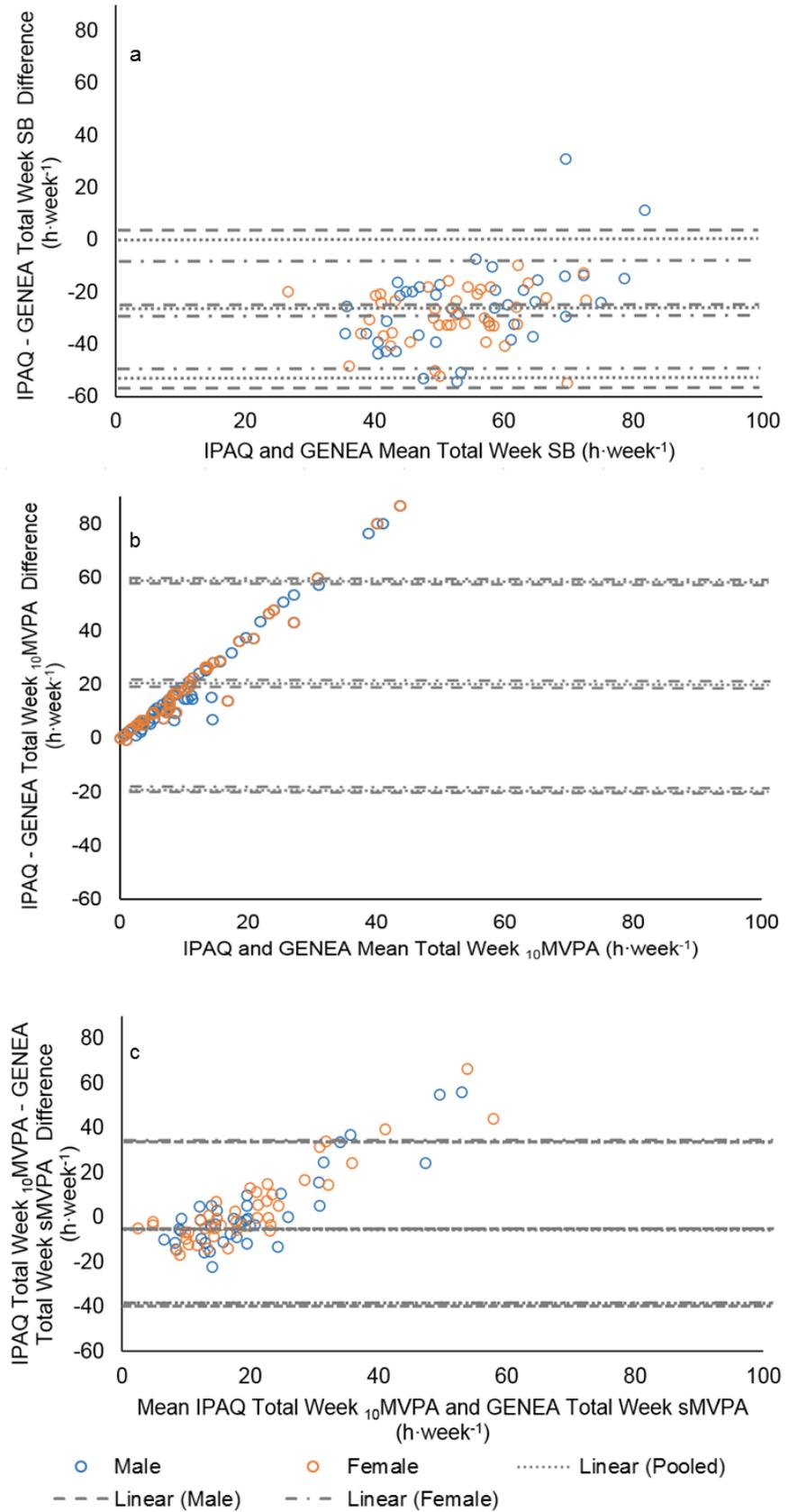


Figure 2.2.5 a) Bland-Altman plot of IPAQ and GENE A total week SB. b) Bland-Altman plot of IPAQ and GENE A total week₁₀MVPA. c) Bland-Altman plot of IPAQ total week₁₀MVPA and GENE A total week sMVPA. Lines represent systematic bias and 95%CI for pooled, male, and female populations.

Sex Differences

A moderate and weak correlation was found between first and second visit IPAQ total week SB data sets for males and females, respectively (figure 2.2.1a). These correlations were not significantly different from one another ($z=0.62$, $p>0.05$). Additionally, male and female correlation slopes were similar ($z=1.48$, $p>0.05$). A Bland-Altman plot illustrated that the systematic bias between first and second visit IPAQ total week SB was non-significant for males and females ($b=2.91\pm 22.8$ h·week⁻¹, $b=0.61\pm 28.2$ h·week⁻¹, $p>0.05$, respectively). Proportional bias was not present for either group (figure 2.2.2a). There was no significant difference between the systematic bias of males and females ($p=0.53$).

Moderate correlations between first and second visit IPAQ total week ₁₀MVPA data sets were found for males and females (figure 2.2.1b). These correlations were not significantly different ($z=0.02$, $p>0.05$). However, male and female correlation slopes were significantly different ($z=-2.20$, $p<0.05$). A Bland-Altman plot illustrated that the systematic bias between first and second visit IPAQ total week ₁₀MVPA was non-significant within males and females (figure 2.2.2b, $b=3.64\pm 18.8$ h·week⁻¹, $b=8.62\pm 27.1$ h·week⁻¹, $p>0.05$, respectively). The systematic bias was not different between males and females ($p=0.68$). Proportional bias was present for females ($p\leq 0.001$) but not for males ($p=0.08$) (figure 2.2.2b).

No sex differences were present within measures of total week SB, ₁₀MVPA, or sMVPA for second visit IPAQ and GENE A (figure 2.2.3). Differences between IPAQ and GENE A measures of total week SB, ₁₀MVPA were found for males and females ($p\leq 0.05$). No difference between IPAQ total week ₁₀MVPA and GENE A sMVPA was found for males and females (figure 2.2.3).

Weak correlations were found between IPAQ and GENE A total week SB within males and females (figure 2.2.4a), though there was no difference either between these correlations ($z=-0.25$, $p>0.05$) or male and female correlation slopes ($z=0.22$, $p>0.05$). A Bland-Altman plot (figure 2.2.5a) illustrated that there was systematic bias between IPAQ and GENE A total week SB for males and females ($b=-25.9\pm 31.4$ h·week⁻¹, $p<0.05$, $b=-29.3\pm 20.7$ h·week⁻¹, $p<0.05$, respectively). No difference in systematic bias was found between sexes ($p=0.25$). Proportional bias was present for males ($p\leq 0.001$) but not females ($p=0.32$).

No correlation was found between IPAQ and GENEAtotal week₁₀MVPA within males and females (figure 2.2.4b). The correlations coefficients and slopes did not differ between sexes ($z=-0.07$, $p>0.05$, $z=-0.54$, $p>0.05$, respectively). A Bland-Altman plot illustrated that systematic bias was present for the difference between IPAQ and GENEAtotal week₁₀MVPA within males and females (figure 2.2.5b, $b=19.3\pm 19.8$ h·week⁻¹, $p\leq 0.001$, $b=21.2\pm 19.6$ h·week⁻¹, $p\leq 0.001$, respectively). Systematic bias was not different between sexes ($p=0.44$). Proportional bias was present for both sexes ($p\leq 0.001$).

Weak correlations between IPAQ total week₁₀MVPA and GENEAtotal week_sMVPA were present for males and females ($r^2=0.18$, $p\leq 0.01$, $r^2=0.22$, $p\leq 0.01$). These correlations were not different between sexes ($z=-0.09$, $p>0.05$). Male and female correlation slopes were non-significantly different ($z=-0.70$, $p>0.05$). A Bland-Altman plot (figure 2.2.5c) illustrated no systematic bias between IPAQ total week₁₀MVPA and GENEAtotal week_sMVPA for males and females ($b=2.02\pm 17.7$ h·week⁻¹, $p=0.47$, $b=4.17\pm 17.4$ h·week⁻¹, $p=0.12$, respectively). Systematic bias was not different between sexes ($p=0.41$). Proportional bias was present for both sexes ($p\leq 0.001$).

The IPAQ and GENEAtotal week₁₀MVPA exhibited low agreement in the PB classification of the pooled ($k=-0.01$, $p=0.55$), male ($k=-0.03$, $p=0.28$), and female ($k=0.01$, $p=0.69$) populations (table 2.2.3). Only 2% of PB levels were correctly classified by the IPAQ in the pooled, male, and female populations (table 2.2.3). Sex was without effect on the ability for the IPAQ to correctly classify PB levels ($\text{Chi}^2=0.001$, $p=0.97$).

Table 2.2.3. Physical behaviour classification of the IPAQ compared against that of GENE A.

IPAQ	GENEA				IPAQ Total
	Couch Potato	ACP	Ambulator	Active Ambulator	
Couch Potato	NC	NC	NC	NC	NC
ACP	9 (7 M, 2 F)	1 (0 M, 1 F)	NC	NC	10 (7 M, 3 F)
Ambulator	5 (2 M, 3 F)	NC	NC	NC	5 (2 M, 3 F)
Active Ambulator	47 (19 M, 28 F)	10 (7 M, 3 F)	11 (5 M, 6 F)	1 (1 M, 0 F)	69 (32 M, 37 F)
GENEA Total	61 (28 M, 33 F)	11 (7 M, 4 F)	11 (5 M, 6 F)	1 (1 M, 0 F)	84 (41 M, 43 F)

NC Not classified based on their IPAQ data, participants were not classified into the group in question. M Males. F Females. $p \leq 0.05$.

Discussion

The objectives of this study were to compare two self-administered IPAQ Long-Form, English (last 7 days format) documents completed a week apart by a middle-class older adult UK population and to evaluate the results of the IPAQ to GENE A measures of free-living SB and MVPA. The aims were to 1) Assess the reliability and validity of the IPAQ for SB and MVPA in these comparatively older-old, community-dwelling persons and 2) Determine any sex differences within the reliability and validity assessments. It was hypothesised that the IPAQ would provide a reliable (i.e. repeatable) measure of total week SB and 10MVPA in relatively older persons but may not provide acceptable levels of external validity (i.e. when absolute data are compared against GENE A data sets). Additionally, it was thought that sex differences would not influence reliability and validity assessments of the IPAQ. The results of the current study suggest that two hypotheses can be upheld: the IPAQ does not provide acceptable levels of validity when compared to GENE A measures of total week SB and 10MVPA in older persons. As expected, sex differences did not influence reliability and validity assessments of the IPAQ. In addition to these accepted hypotheses, the IPAQ showed low reliability/repeatability qualities in a community-dwelling UK older adult population.

Reliability of the IPAQ

Relative reliability of the IPAQ exhibited a significant repeated measures correlation coefficient ($r = 0.59$; $r^2 = 0.26$, $p < 0.001$) for total week SB. This is lower than that reported in the relatively younger population (36.8 ± 7.93 years) used in the original 12-country IPAQ study (Craig et al., 2003) ($r = 0.83 \pm 0.06$, $p \leq 0.05$) and lower than the UK population ($r = 0.84$, $p \leq 0.05$) used in the aforementioned study. Whether the geographical location and therefore socio-economic factors of the participants (current study: Cheshire East Borough, vs. Craig et al. (2003): Bristol) played a role in the differences between studies is unknown. It could be argued that, unlike the participants in the current study, the adults in the study of Craig et al. (2003) were still employed and therefore, had a predictable structure in their day-to-day lives. However, older adults have been shown to also have similarly predictable daily routines (Monk et al., 1992), which they highly value (Belza et al., 2004). Furthermore, the lack of systematic bias (figure 2.2.2a) in the difference between first and second visit IPAQ total week SB may be highlighting this predictability in daily routines (Monk et al. (1992). On the other hand, large 95%CI ($b = 1.51 \pm 25.9$ h·week⁻¹) questions the reliability of the IPAQ.

The IPAQ measure of total week ₁₀MVPA also displayed a moderate relative reliability coefficient ($r = 0.69$, $r^2 = 0.47$, $p < 0.001$) but showed systematic bias ($b = 6.37 \pm 22.7$ h·week⁻¹, $p = 0.025$), thereby putting in question the reliability of the IPAQ for quantifying absolute MVPA in older persons. Unfortunately, the current study data cannot be compared with the 12-country IPAQ study as the previous study did not provide a suitable comparable measure of MVPA (Craig et al., 2003). However, the results of the current study are similar to previous IPAQ – Long Form reliability measures in Belgian older adults ($r = 0.63$) (Van Holle et al., 2015) and outperformed the reliability of the IPAQ – Long Form, Chinese (7 day format), which found no significant between week correlation (Cerin et al., 2012). The presence of positive systematic bias (figure 2.2.2b) and the majority of data points below the line of unity for total week ₁₀MVPA (figure 2.2.1b) may illustrate a learning effect following the completion of the first visit IPAQ or greater awareness of ₁₀MVPA engagement. The greater awareness may be a result of a weeklong lifestyle surveillance by GENE. However, it is hoped that the discrete and unrestrictive

placement of GENE (mid-thigh) would minimise any effect on the participant's awareness of lifestyle.

Validity of the IPAQ

For measurement of time spent performing SB or MVPA, accelerometry is considered the gold standard (P. Kelly et al., 2016). Moreover, thigh-mounted accelerometry is deemed the most suitable for SB measures due to the change in thigh orientation that is common to the transition from standing to seated or reclined postures (Steeves et al., 2015). A strength of the current study is that it used a thigh-mounted triaxial GENE that records the amount of static acceleration due to gravity. Using this knowledge, the in-house developed algorithm (CAS) (Wullems et al., 2015) can calculate thigh orientation relative to the Earth's surface to accurately determine whether the participant is standing, sitting down, lying sideways, or prone. Furthermore, unlike earlier research that applied accelerometer cut-points, which were not validated for the population being studied, the CAS uses cut-off points validated against the energy expenditure (METs) of aged-matched older adults (in our laboratories, Chapter 02: Part 1) to determine the time spent performing different PA intensities.

Participants had great difficulty in reporting total week SB using the IPAQ and often spent a few minutes formulating an answer. Although, significant correlations between IPAQ and GENE for total week SB were present (figure 2.2.4a), differences in average time (figure 2.2.3) and systematic bias (figure 2.2.5a, $27.6 \pm 26.5 \text{ h} \cdot \text{week}^{-1}$, $p < 0.001$) revealed that the IPAQ underestimated total week SB by 41%. Only two participants over-reported their amount of total week SB (+11.4 and +30.8 $\text{h} \cdot \text{week}^{-1}$). This underestimation is consistent with another IPAQ validity study (hip mounted accelerometer) ($b = 27.4 \pm 3.46 \text{ h} \cdot \text{week}^{-1}$, $p \leq 0.001$) that used older adults ($n = 94$, 65-85 years) (Cerin et al., 2012). Under-reporting is a common problem, qualitative research has shown that the sitting questions create confusion, as "*sitting on a weekday?*" does not provide details on which 'day' to report (Van Uffelen et al., 2011; Heesch et al., 2010). Sedentary behaviour is viewed as a negative lifestyle choice by older adults (Mcewan et al., 2016). Therefore, participants may have reported the day that had the least amount of SB to appear socially desirable. This is supported by the negative association found between

social desirability and self-reported SB in other populations (Jago et al., 2007). Alternatively, this difficulty in recall may also be due to the sporadic nature of SB and, unlike MVPA, engagement in other behaviours that occur at the same time as SB (e.g. eating, reading, and driving). Qualitative data was not the focus of this study; however, participant's recollection of SB often came in the form of TV viewing therefore, SB such as eating may have been overlooked.

For total week $_{10}$ MVPA, a large systematic bias was also present as participant's IPAQ data over-reported by $20.3 \pm 19.8 \text{ h} \cdot \text{week}^{-1}$ ($p < 0.001$) equivalent to, on average, 15.7 fold greater than total week $_{10}$ MVPA time. This overestimation is consistent with previous IPAQ research that used accelerometry (hip mounted) in older adults (Cerin et al., 2012; Van Holle et al., 2015; Grimm et al., 2012). This, like SB, may be a result of social desirability as older adults view PA as a positive lifestyle choice (Belza et al., 2004). Alternatively, participants may be reporting MVPA bouts that were less than 10 continuous minutes in duration. The results of the current study revealed weak correlations between IPAQ total week $_{10}$ MVPA and GENEА total week sMVPA (figure 2.2.4c, $r^2 = 0.19$, $p \leq 0.001$). However, there was a small non-significant systematic bias between the two aforementioned variables (figure 2.2.5c, $b = 3.12 \pm 17.5$, $p = 0.11$) and no statistical difference between the population means of these two variables (figure 2.2.3). Heesch et al. (2010) suggested that the structure of the questions may be leading to this problem as the, "*report activities lasting ≥ 10 minutes per session*" instruction is included in the 'number of days the activity was performed' question and not the following question relating to the duration of one of those activities. This could be interpreted that the 10-minute criterion only relates to the frequency of the activity per week and not the duration of that activity.

Sex Differences

There was only one incident of a sex difference in the reliability of PA measures. For the relative reliability of total week $_{10}$ MVPA, males correlation slope was found to be greater than females (figure 2.2.1b, $z = -2.20$, $p < 0.05$). Males and females had similar levels of GENEА measured total week $_{10}$ MVPA, sMVPA, and SB (figure 2.2.3) therefore, it seems unlikely that one of the groups performed tasks

that were not as memorable during the monitoring week (Kriska, 2000). Unfortunately, participants did not wear GENEAs prior to the completion of the first visit IPAQ. Therefore, we cannot be sure whether this suggested sex difference was a result of changeable memory within groups or actual inter-week behaviour change (P. Kelly et al., 2016).

As a result of the IPAQ overestimating total week $_{10}$ MVPA and underestimating total week SB, the majority of participants were placed into a higher PB classification than they actually were (table 2.2.3). A previous attempt to correctly classify older adults (66 – 85 years) as physically active had found an 81% ($k=0.448$, $p<0.001$) agreement between IPAQ and waist mounted accelerometer data (Hurtig-Wennlöf et al., 2010). For direct comparison, of the 12 participants that were physically active in the current study, based on GENEAs data (ACP and Active Ambulator), 100% of those participants were correctly classified as physically active by the IPAQ. However, an additional 62 participants were also classed as physically active by the IPAQ causing only 14% of participants to be classified as physically inactive by the IPAQ. Overall, the IPAQ was only able to correctly classify 2% ($k=-0.01$, $p=0.55$) of the participants into one of the four PB groups for the pooled, male and female populations, causing no participants to be classed as a Couch Potato (table 2.2.3). In addition, sex had no effect on the ability of the IPAQ to correctly classify PB levels ($\chi^2=0.001$, $p=0.97$).

Study Limitations

The age of the participants in our current study ranged from 60 – 89 years, which only has a 9 year overlap with population for whom the IPAQ was originally validated for (15 – 69 years) (Craig et al., 2003). Arguably, the reduced reliability of the IPAQ, could be partially assigned to this fact. Notably however previous studies have not made this distinction and have indiscriminately used the IPAQ in similarly much older population (Milanović et al., 2013) and yet have not reported any necessity to improve the uptake of the questionnaire through population stratification by decade of age, thereby supporting our current design that includes persons aged 60 through to 89 years.

Conclusion

This is one of the first studies to use thigh-mounted accelerometry (considered the gold standard for SB and MVPA measurement) as a validator for the self-administered IPAQ – Long Form, English (last 7 days format) in older adults. The results of the current study suggest that use of the self-administered IPAQ – Long Form, English (last 7 days format) in the ‘older-old’ adults shows little relative and absolute reliability when re-tested in an 8 – 11 day window, even after accounting for the fact that there two weeks of monitoring where self-reporting as ‘typical’. A Bland-Altman plot that revealed total week SB is underestimated by the IPAQ supports a weak correlation found between the IPAQ and GENEAs measures of total week SB. This suggests that the IPAQ may not be suitable for quantitatively measuring SB in older adults. Similarly, the IPAQ overestimated the amount of total week $_{10}$ MVPA and this was likely due to participants reporting MVPA bouts that were less than 10 continuous minutes, as there was no difference or systematic bias between IPAQ total week $_{10}$ MVPA and GENEAs total week sMVPA measures. Therefore, the IPAQ may not be suitable for measuring MVPA that consists of bouts at least 10 continuous minutes in duration. Due to underestimation of SB and overestimation of $_{10}$ MVPA by the IPAQ, only 2% of participants were correctly categorised into one of the four PB groups. Interestingly, no difference in reliability or validity measures were found between sexes apart from correlation slope measures of total week $_{10}$ MVPA reliability. Based on these results, it is suggested that the IPAQ should not be used as a monitoring technique to qualitatively classify or quantitatively measure habitual PB in older adults. Research aiming to monitor PB should use an objective measurement technique where possible. The objective of future research should be to increase the number and precision of the questions in the self-administered IPAQ Long-Form, English (last 7 days format), specifically addressing SB, to make it more relevant to older adults (e.g. changing examples of activity), with the aim to improve its reliability and validity.

Chapter 03:

Segregating the distinct effects of sedentary behaviour and physical activity on older adults' cardiovascular structures and functions.

Part 1: *'Linear regression analysis approach.'*

Part 2: *'Isotemporal substitution approach.'*

Part 3: *'3-Dimensional heat mapping approach.'*

Part 1: Linear regression analysis approach.

Introduction

Cardiovascular related deaths in the UK increase ~1.8 fold per decade between the ages of 55 – 85+ years (yrs) (Townsend et al., 2015). This dramatic increase is likely to augment the socioeconomic burden as people over the age of 60 are forecast to account for 25% of the population by the year 2035 (Office for National Statistics, 2012a). Physical activity (PA), more specifically moderate-vigorous PA (MVPA), has been shown to be successful in the risk reduction and treatment of cardiovascular diseases (CVD) and therefore, is recommended in government policies. It is recommended by the UK government that older adults engage in bouts of at least 10 continuous minutes (mins) of MVPA that accumulate to a minimum of 150 mins over a seven-day week (Department of Health Social Services and Public Safety, 2011). In 2011, the government PA recommendations were updated to highlight the need to also avoid bouts of prolonged sedentary behavior (SB), in light of the increased awareness of the independent effects of SB on health (Bey and Hamilton, 2003; Gennuso et al., 2013; Gennuso et al., 2015). However, this initial recommendation was made in the absence of any clear evidence of the metabolic and/or circulatory consequences of prolonged SB in older adults. As such, it was not possible to provide a quantitative recommendation for SB time. Timely and recent evidence highlights the degree to which increments in SB time and other SB measures (e.g. breaks in SB) affect cardiovascular health independent of MVPA engagement (Thosar et al., 2014; Stamatakis et al., 2012; Gennuso et al., 2013; Gennuso et al., 2015; Rezende et al., 2016; García-Hermoso et al., 2015). Thus, it has been proposed that low intensity PA (standing, and light intensity PA [LIPA]) could be used to reduce SB time and improve health (Bailey and Locke, 2014; Peddie et al., 2013), either directly or indirectly.

With technological improvements, it is now possible to accurately quantify physical behaviour (PB) levels (SB and PA time). Thigh-mounted triaxial accelerometers are considered the gold standard for SB time quantification as posture can be determined through recognising the positional orientation of the upper leg relative to the Earth's surface and monitoring can be carried out in real-

time over a number of days. However, few key cardiovascular parameters have been mapped against this gold standard method of SB quantification (Brocklebank et al., 2015; Hajduk and Chaudhry, 2016).

Therefore, the goal of the present study was to determine the degree of association between thigh-mounted accelerometer measures of habitual PB and key cardiovascular parameters in older adults. The objectives of this study were threefold: 1) determine whether measures of daily PB predict older adults' cardiovascular profile; 2) determine which measures of PB patterns are better predictors of cardiovascular profile; 3) highlight any effects of SB on cardiovascular profile that are independent of MVPA and vice versa. The aim of this study was to provide an evidence-based recommendation for the parameter of PB that is the most prolific predictor of cardiovascular profile. It was broadly hypothesised that SB would be independently associated with certain cardiovascular parameters so that an objective lifestyle recommendation of SB may have to consider both amount and pattern of SB accumulation to ultimately improve health. In addition, it was hypothesised that PA, other than MVPA, could improve cardiovascular profile, thus creating an evidence-base for a recommendation for the spectrum of PA intensity accumulation specific to older adults.

Methods

Ninety-three older participants (73.8 ± 6.23 yrs, 60 – 89 yrs, 55% female) whose screening questionnaire revealed as independently mobile (did not require a wheelchair or Zimmer frame), did not suffer from an untreated cardiovascular disease, had not sustained an injury within the preceding three months, had not ever/recently suffered from a neurological disease that impaired motor control or academic ability, and were not diabetic, were recruited for the study. These participants were recruited primarily from older adult community groups within the Cheshire East Borough, England. The local University (Manchester Metropolitan University) Ethics Sub-Committee granted ethical approval. Participant approval was acquired through written informed consent. Participants visited the laboratory on two occasions separated by a minimum of seven days, the details of which are outlined below.

First Laboratory Visit

On the first visit participant demographics were collected (table 3.1.1) and test protocol familiarisation was conducted. Height and mass were measured with a stadiometer (Harpenden, Holtain, Croswell, Wales) and electronic weighing scales (Salter, Kent, England). Hydration was assessed during the second visit as outlined below (see Second Laboratory Visit). Participants were fitted with a commercially available thigh mounted (anterior aspect, at 50% of greater trochanter to femoral condyle distance) triaxial accelerometer (GENEA, GENEActiv Original, Activinsights Ltd, Kimbolton, UK) using two waterproof adhesive patches (3M Tegaderm Film, North Ryde, Australia) on their dominant leg, which remained in place for seven consecutive, free-living days. Standing leg preference during a single leg balance exercise, determined leg dominance. GENEActiv data (60.0 Hertz [Hz] frequency) were smoothed using 10 second (s) epochs. Residual G was selected as the GENEActiv output (equation 3.1.1), adapted from previous work on total movement analysis in older persons (Onambele et al., 2006) and termed the Cheshire Algorithm for Sedentarism (CAS, Chapter 02: Part 1).

Table 3.1.1. Demographics for pooled, male, and female populations. Data presented as mean (standard deviation) unless stated otherwise.

Variable	Pooled	Male	Female
Age (yrs)	73.8 (6.22)	74.9 (5.77)	72.8 (6.48)
Height (m)	1.65 (0.08)	1.71 (0.07)	1.59 (0.06)***
Mass (kg)	75.9 (13.1)	81.1 (10.6)	71.7 (13.4)***
BMI (kg·m ²)	27.9 (4.71)	27.6 (3.90)	28.1 (5.32)
Primary CVD Meds (%)†	48.0	55.0	43.0
(in)direct CVD Meds (%)‡	59.0	55.0	63.0
Hydration (%·body mass ⁻¹)	50.6 (7.15) _m	53.8 (5.08) _m	46.4 (9.70) _m ***

† Participants are currently prescribed an amount of medication that reduces the risk or treats CVD (i.e. statins, warfarin). ‡ Participants are currently prescribed a medication that may affect the cardiovascular system either directly or as a side effect. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Equation 3.1.1. Residual G calculation.

$$\text{Residual G} = \sqrt{(\text{SD } x^2 + \text{SD } y^2 + \text{SD } z^2)}$$

Where: x is the medio-lateral axis

y is the vertical axis

z is the anterior-posterior axis

SD is standard deviation

$\sqrt{\quad}$ is square root

The SB-LIPA (1.50 Metabolic Equivalent Tasks [METs]) cut-off point was 0.057 Residual G and the LIPA-MVPA (3.00 METs) cut-off point was 0.216 Residual G. To obtain these cut-off points, a systematic validation of the GENE A against expired gas during a laboratory-based activity calibration protocol, was carried out in a sub-sample of older adults ($n=20$, Chapter 02: Part 1). Thus, nine PB functions (i.e. 1-lying down, 2-sitting, 3-standing quietly, 4-repeated side-stepping 5-self-selected speed ground walking, 6-3.5 km·hr⁻¹ walk on treadmill, 7-self-selected speed walk on treadmill, 8-self-selected speed weighted-vest treadmill walking [at 15% of body weight], 9-self-selected speed brisk walk on treadmill) were monitored with concurrent gas analyses, heart rate, motion analysis and accelerometer output. The scatter plot exhibited a strong explained variance between Residual G (GENEA) and METs (expired gas) ($r^2=0.89$, $p<0.001$). Postural identification using accelerometer axes orientation, similar to that developed by Rowlands et al. (2014), showed perfect agreement with known time spent performing SB (6.00 mins) and PA (21.0 mins) (Cohen's kappa=1.00 [95% confidence interval (CI) 1.0, 1.0], $p<0.001$). Residual G cut-off points and MET thresholds had a strong agreement for PB intensity identification (Cohen's kappa=0.81 [95%CI 0.49, 1.31], $p<0.001$). To help account for individual differences in physical fitness, one MET was equal to the resting metabolic rate (RMR) of the participant. Participants were provided with a self-report sleep diary (wake-up time, lights-off, go to sleep time, naps not included) and were requested to complete it throughout the GENE A data collection week. GENE A outcome variables and definitions are provided in table 3.1.2. Previous GENE A findings have suggested that 6 days of accelerometer wear time are required to provide reliable estimates of PB (Dillon et al., 2016). Therefore, three

participants were removed from the analyses for not having sufficient accelerometer data (≥ 6 days). Hydration guidance was provided as participants were asked to arrive at the second laboratory visit in a fasted state (> 8.00 hrs) but also hydrated as this could influence vascular parameters.

Table 3.1.2. Definitions of the PB parameters provided by the CAS.

Terminology	Unit	Definition
PB		
Sedentary Behaviour (SB)	hrs·day ⁻¹	The mean amount of SB, of any length, that is accumulated in a 24hr day. Any waking behaviour characterised by a seated or reclined posture (Tremblay et al., 2010).
Standing	hrs·day ⁻¹	The mean amount of standing, of any length, that is accumulated in a 24hr day. Any standing posture that elicits little to no movement and expends ≤ 1.50 METs of energy (Tremblay et al., 2010).
Light Intensity Physical Activity (LIPA)	hrs·day ⁻¹	The mean amount of LIPA, of any length, that is accumulated in a 24hr day. Any standing posture that elicits 1.50 - < 3.00 METs (Tremblay et al., 2010).
Sporadic Moderate to Vigorous Physical Activity (sMVPA)	hrs·day ⁻¹	The mean amount of MVPA, of any length, that is accumulated in bouts < 10.0 mins in a 24hr day (Department of Health Social Services and Public Safety, 2011). Any standing posture that elicits ≥ 3.00 METs (Tremblay et al., 2010).
≥ 10 Minute Moderate to Vigorous Physical Activity (₁₀ MVPA)	hrs·day ⁻¹	The mean amount of MVPA that is accumulated in bouts ≥ 10.0 mins in a 24hr day (Department of Health Social Services and Public Safety, 2011). Any standing posture that elicits ≥ 3.00 METs (Tremblay et al., 2010).
Patterns of PB		
SB Parameters		
SB Breaks	n·day ⁻¹	The mean amount of SB Breaks in a 24hr day. ≥ 2.00 mins of continuous PA (Benatti and Ried-Larsen, 2015) that follows ≥ 1.00 of SB (Messinger-Rapport et al., 2003). NB – Every day starts in a sedentary state.

Table 3.1.2 continued.

<5min SB Bout	$n \cdot \text{day}^{-1}$	The mean amount of SB bouts that consist of <5.00 mins in a 24hr day (Saunders et al., 2013). SB bout starts after ≥ 2.00 mins of PA is followed by ≥ 1.00 mins SB and ends with an 'SB Breaks'. Contains SB and <2.00 mins of PA.
$\geq 5\text{min SB Bout}$	$n \cdot \text{day}^{-1}$	The mean amount of SB bouts that consist of ≥ 5.00 mins in a 24hr day (Saunders et al., 2013). SB bout starts after ≥ 2.00 mins of PA is followed by ≥ 1.00 mins SB and ends with an 'SB Breaks'. Contains SB and <2.00 mins of PA.
True Mean SB Bout	$\text{mins} \cdot \text{day}^{-1}$	SB time (≥ 1.00 mins) between PA bouts of ≥ 2.00 mins are LOG transformed to normally distribute the data and then anti-logged to find the "True Mean" (Chastin et al., 2015c). Mean amount in a 24hr day.
Alpha	$\alpha \cdot \text{day}^{-1}$	How steeply the number of SB bouts decreases with increasing SB bout duration in a power-law distribution (Chastin et al., 2015c). Mean amount in a 24hr day.
W50%	$\text{mins} \cdot \text{day}^{-1}$	50% of SB time in a 24hr day is accumulated by SB bouts of this specific length or shorter (Chastin et al., 2015c). Mean amount in a 24hr day.
SB%	$\% \cdot \text{waking hrs} \cdot \text{day}^{-1}$	The mean percentage of waking hours that is spent performing 'SB' of any length in a 24hr day.
<i>PA Parameters</i>		
PA Bouts	$n \cdot \text{day}^{-1}$	The mean amount of bouts that consist of ≥ 2.00 mins of continuous PA (Benatti and Ried-Larsen, 2015) followed by ≥ 1.00 mins of continuous SB (Messinger-Rapport et al., 2003) in a 24hr day. PA bout starts after 'SB Breaks' and ends with an SB bout. Contains PA and <1.00 mins of continuous SB.
Daily Sum of PA Bout time	$\text{mins} \cdot \text{day}^{-1}$	The mean amount of time that spent in 'PA Bouts' in a 24hr day.
True Mean PA Bout	$\text{mins} \cdot \text{day}^{-1}$	PA time (≥ 2.00 mins) between SB bouts of ≥ 1.00 mins are LOG transformed to normally distribute the data and then anti-logged to find the "True Mean" (Chastin et al., 2015c). Mean amount in a 24hr day.
$_{10}\text{MVPA Bouts}$	$n \cdot \text{day}^{-1}$	The mean amount of bouts that consist of ≥ 10.0 mins MVPA in a 24hr day.

Table 3.1.2 continued.

Total Week ₁₀ MVPA	hrs·week ⁻¹	The sum amount of ' ₁₀ MVPA Bouts' time that is accumulated in a monitoring week (7 days).
Standing%	%·waking hrs·day ⁻¹	The mean percentage of waking hours that is spent performing 'Standing' in a 24hr day.
LIPA%	%·waking hrs·day ⁻¹	The mean percentage of waking hours that is spent performing 'LIPA' in a 24hr day.
sMVPA%	%·waking hrs·day ⁻¹	The mean percentage of waking hours that is spent performing 'sMVPA' in a 24hr day.
₁₀ MVPA%	%·waking hrs·day ⁻¹	The mean percentage of waking hours that is spent performing ' ₁₀ MVPA' in a 24hr day.

Second Laboratory Visit

Participants arrived for the second laboratory visit in a fasted, hydrated state. Where appropriate, participants were asked to refrain from taking medication until testing had been completed. All participants refrained from taking medication prior to the completion of the laboratory tests. A standardised meal (43.0% carbohydrate, 43.0% protein, 14.0% fat) was provided to participants before commencing the testing session.

A three lead electrocardiogram (ECG) was fitted to participants to allow for R-gated artery analysis and resting heart rate measures. The skin was cleaned with an alcohol wipe prior to electrode (BlueSensor M, Ambu, Copenhagen, Denmark) placement. Participants began testing by resting in the supine position for 15.0 mins to minimise any impact of orthostatic changes. Room temperature and light intensity were maintained at 22.0°C and 20.0 lm·ft² (Sekonic Studio Deluxe III L-398A Light Meter, Sekonic, Newcastle Under Lyme, UK), respectively, in order to minimise any impact of environmental ambience variations. Supine blood pressure (BP) (M2 HEM-7121-F, OMRON, Hoofddorp, The Netherlands) was assessed three times to obtain an average systolic BP, diastolic BP, and pulse pressure.

Hydration was assessed using bioelectrical impedance analysis (BIA) (BodyStat 1500, BodyStat, Douglas, UK). The BIA assessed total body water as a percentage of total body mass using the manufacturer's own algorithms that accounted for sex, age (yrs), height (cm), and body mass (kg). BIA has been shown

to be a reliable (Shanholtzer and Patterson, 2002) and valid (Ritz, 2001) method for hydration assessment. Participants were hydrated if total body water, as a percentage of total body mass, was 55.0% - 65.0% for males or 50.0% - 60.0% for females.

Baseline Vascular Assessment

Ultrasound assessments were performed using an echo Doppler ultrasound machine (model AU5; Esaote, Genova, Italy) with a 7.50 MHz broadband linear array transducer in brightness or B-mode with an angle of insonation of 60.0° (Harris et al., 2010) (B gain: 75.0, Doppler gain: 49.0, CFM gain: 47.0, depth of penetration: 49.3 mm, depth of focus: 27.0 – 31.0). Live streaming of all assessments were collected on a Hewlett-Packard computer running video capture software (Premier 6.0, Adobe Systems, San Jose, USA) through an analogue to digital converter (Pinnacle, Corel Inc., Ottawa, Canada) at 25.0 Hz. The depth of the transducer penetration was noted to allow for video scaling during off-line analyses using Brachial Analyzer (no Bland-Altman bias in reliability, and low pixel error in synthetic data analysis (Sonka et al., 2002)) and Carotid Analyzer (Medical Imaging Application LLC, Iowa, USA), which has shown excellent validity compared to previous methods ($r^2=0.98$, $p<0.001$, Bland-Altman bias 0.04, $p=0.82$) (Mancini et al., 2004). Participants were supine for left common carotid artery, right brachial artery, and prone for left popliteal artery baseline assessments. Video recordings were collected over ten cardiac cycles (Harris et al., 2010; Gill, 1985) for the assessment of systemic peak blood velocity, intima-media thickness (IMT), artery diameter, calculation of shear rate, and resistance index (RI) (table 3.1.3). All structural measures were obtained in a 10 mm region of interest (ROI), which was 10 mm distal to the carotid bulb in the anterior (AL) and posterior longitudinal (PL) plane (figure 3.1.1) and 10 mm distal to the superior medial genicular bifurcation for the popliteal artery (Touboul et al., 2012). Artery diameter measures were filtered using automated R-gating to ensure artery diameter was measured during the end-diastolic phase. Remaining frame-to-frame measurements were filtered from final analysis if they did not use at least 70% of the ROI to measure artery diameter and/or were more than one standard deviation (SD) from the mean artery diameter. All automated measures were assessed for errors by one researcher. Measurement

of carotid, popliteal and brachial IMT was performed on the far wall as this is shown to truly reflect anatomic intima-media layer (Wong et al., 1993; Pignoli et al., 1986). Previous validation of ultrasound showed 6.52% underestimation of carotid far wall IMT compared to histological measures whereas near wall IMT had a 25.3% overestimation in autopsies of 36 males (69.0±8.0 yrs) with an intraobserver error of 5.4±4.3% (Wong et al., 1993). These cardiovascular parameters were selected as they have previously been associated with CVD risk (Bots et al., 1997; Van Dijk et al., 2001; Padilla et al., 2009) and to distinguish any limb specific associations between PB and cardiovascular parameters.

Table 3.1.3. Outline of dependent variables assessed using ultrasound.

Variable	Definition	Equation
Blood Velocity	The mean peak speed of blood cell movement through the vessel. Determined using five Doppler waveforms.	N/A
Shear Rate	An estimate of shear stress, which does not account for blood viscosity.	$8 \times (\text{Blood Velocity} \div \text{Artery Diameter [mm]})$
Resistance Index	Represents the resistance of the vascular bed distal to the interrogated vessel.	$(\text{Peak Systolic Velocity} - \text{End-Diastolic Velocity}) \div \text{Peak Systolic Velocity}$
Intima-Media Thickness	Distance from the lumen-intima interface to the media-adventitia interface of the far wall.	N/A
Artery Diameter	Perpendicular measurement from the far wall media-adventitia interface to the near wall lumen-intima interface.	N/A

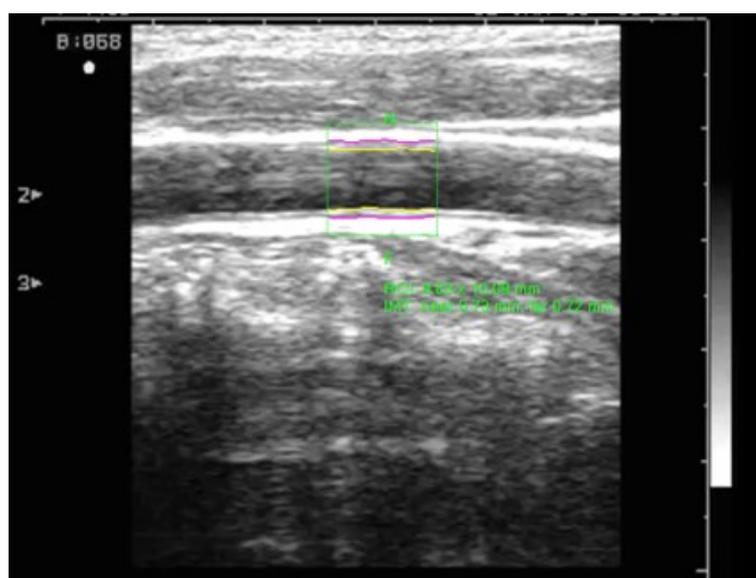


Figure 3.1.1. Screenshot of Carotid Analyzer automated IMT analysis. Green box represents the region of interest, purple line represents the media-adventitia interface, and the yellow line represents the lumen-intima interface.

Intra-day and inter-day coefficient of variation (CV) were calculated from seven participants. Inter-day CV were 4.47%, 1.57%, and 5.33% for brachial, carotid, and popliteal artery diameter respectively, whilst intra-day CV were 4.97%, 2.34%, and 4.03% for brachial, carotid, and popliteal artery diameter, respectively. Inter-day CV were 1.45%, 7.91%, and 11.3% for brachial, carotid, and popliteal IMT respectively, whilst intra-day CV were 3.04%, 3.40%, and 7.04% for brachial, carotid, and popliteal IMT, respectively. Artery diameter and IMT CV should be sensitive enough to detect PA related changes as three months of aerobic leg exercise caused a 9.0% increase in diameter and a 16.0% reduction in IMT (Dinenno et al., 2001). Blood Velocity CV was below 20.0% for inter-day and intra-day measures of all three arteries. Baseline Shear Rate CV was below 16.0% for inter-day and intra-day measures of all three arteries. Both blood velocity and shear rate CV should be sensitive enough to detect changes caused by PA as MVPA has been shown to increase blood velocity and shear rate by 39.8% and 43.7%, respectively (Thijssen et al., 2009). Inter-day and intra-day CV was 5.75% and 11.1% for carotid RI, respectively. RI CV could be sensitive enough to detect PA related changes as exercised individuals display a 6.94% lower RI compared to sedentary individuals (Azhim et al., 2007).

Additional Measures: Flow Mediated Dilation

Supine blood pressure was assessed three times (separated by 30.0 s) on the contralateral arm using an automated blood pressure sphygmomanometer (BP 710, Omron, Kyoto, Japan). The highest systolic blood pressure was used as a reference pressure for the Flow Mediated Dilation (FMD) protocol. Participants were supine while the right arm was extended and abducted at an internal angle of 80.0° to keep the measurement area below heart level. The arm was supported under the elbow and hand by two cradles to keep the arm level with the body to minimise any effects of gravity (Harris et al., 2010). A manual blood pressure sphygmomanometer was placed around the forearm (20.0 mm distal of the antecubital fossa). To locate the brachial artery, the ultrasound transducer was placed distally, at 65.0% of upper arm length (acromion process to radial head) in the medial aspect. Once the brachial artery was located and artery walls were clearly identified, a mark was made on the skin using a waterproof pen, and the ultrasound transducer was secured in place

with a clamp manufactured by the university's technical team at Cheshire (figure 3.1.2). The variables outlined in table 3.1.3 were collected over ten cardiac cycles for the brachial artery prior to pressure cuff inflation as baseline measures.



Figure 3.1.2. Flow mediated dilation equipment set-up.

The manual blood pressure sphygmomanometer was inflated 25.0 mm Hg above maximum systolic blood pressure for 5.0 mins (Harris et al., 2010) to reduce blood flow to the hand and wrist. Due to the ultrasound equipment not possessing dual functionality, the ultrasound was changed to Doppler mode 10.0 s after pressure release to allow for the assessment of blood velocity at 15.0 s post deflation. Once five blood velocity waveforms were obtained the ultrasound was changed back to B-mode to capture the change in artery diameter (approximately 30.0 s post deflation). Live stream recordings were captured for 2.0 mins post deflation when a final blood velocity measure was obtained. Off-line artery diameter analyses were performed using Brachial Analyzer (Medical Imaging Application LLC, Iowa, USA) as described above. FMD was subsequently calculated using equations 3.1.1 – 3.1.3.

Equation 3.1.1. Unscaled FMD.

$$\text{Unscaled FMD} = ([\text{Peak artery diameter post pressure release} - \text{Baseline artery diameter}] \div \text{Baseline artery diameter}) \times 100$$

Equation 3.1.2. Allometrically Scaled FMD.

$$\text{Allometrically Scaled FMD} = \left(\left[\frac{\text{Peak artery diameter post pressure release} - \text{Baseline artery diameter}^{0.87}}{\text{Baseline artery diameter}} - 1 \right] \div \text{Baseline artery diameter} \right) \times 100$$

Equation 3.1.3. FMD normalised to post deflation shear rate.

$$\text{FMD:SR} = \text{Unscaled FMD} \div \text{Shear rate 15.0 s post deflation}$$

Flow Mediated Dilation data were removed from the main content of the chapter as many participants had a negative FMD (vasoconstriction) and the associations with PA parameters were questionable. Due to the questionable data, the demographics and results for FMD are provided in the Appendix Chapter 03, table A3.1.1-5.

Statistical Analyses

SPSS Statistics 22 (International Business Machines Corporation, New York, USA) was used for statistical analyses. Firstly, bivariate linear regression models were used to examine any association between PB (measured in hrs·day⁻¹ only), covariables (including hydration status, amount of prescribed medication that primarily targets cardiovascular disease, total of prescribed medication that could (in)directly influence cardiovascular profile), and cardiovascular parameters. Hydration status was used as a covariate as this has been shown to effect artery diameter (Chen et al., 2007) whilst medication use was used as a covariate as it has been shown to effect cardiovascular parameters (Furberg et al., 1994; Williams et al., 2006; Dernellis and Panaretou, 2002). Hydration, primary CVD meds, and (in)direct CVD meds were used for covariate adjustment if a forced entry linear regression analysis had shown that that they are associated with the respective cardiovascular parameter. If two or more PB(s) or covariate parameters showed predictive qualities for a cardiovascular profile, a stepwise multivariate linear regression was used to assess the interactions between multiple PB(s) (measured in hrs·day⁻¹ only) and/or covariate parameters and their combined association with cardiovascular parameters. Bivariate linear regression models were also used to examine the associations between patterns of PB and cardiovascular parameters. Cardiovascular variables were natural LOG transformed if they were non-normally distributed (Kolmogorov-Smirnov or Shapiro-Wilk, $p \leq 0.05$).

GENEA outliers were identified using box and whisker plots and subsequently removed. The aforementioned statistical tests were then re-performed to determine whether the GENE outliers were influencing the statistical outcomes.

Statistical significance was set at $p \leq 0.05$. Data are presented as Mean (SD) or Median (Interquartile Range [IR]) if parametricity was violated, unless stated otherwise.

Results

Physical Behaviour Profile

After discounting the participants with insufficient accelerometer data (<6 days), the remaining participants' PB(s) and patterns of PB parameters are outlined in table 3.1.4.

Table 3.1.4. Physical behaviour and patterns of PB demographics for pooled, male, and female populations with and without outlier data.

Variable	Outliers Included	Outliers Removed	Outlier Data[†]	Male[†]	Female[†]
SB ^a (hrs·day ⁻¹)	9.65 (1.46)	9.68 (1.30)	43. 5.24 84. 13.9	9.69 (1.47)	9.61 (1.47)
Standing ^b (hrs·day ⁻¹)	1.11 (0.44)	1.10 (0.40)	56. 2.89	1.11 (0.43)	1.12 (0.45)
LIPA ^c (hrs·day ⁻¹)	1.97 (0.63)	-	-	1.92 (0.65)	2.01 (0.61)
sMVPA ^d (hrs·day ⁻¹)	2.63 (0.78)	2.57 (0.63)	33. 5.05 43. 5.14 52. 4.42 84. 0.74	2.72 (0.80)	2.55 (0.76)
₁₀ MVPA ^e (hrs·day ⁻¹)	0.10 (0.23) _m	0.08 (0.19) _m	11. 0.93 12. 1.40 30. 1.54 36. 0.78 76. 0.71	0.15 (0.31) _m	0.05 (0.15) _m
SB Breaks ⁱ (n·day ⁻¹)	22.1 (3.43)	22.6 (3.10)	62. 13.1	22.5 (3.86)	21.7 (3.39)
<5min SB Bout ^j (n·day ⁻¹)	6.38 (1.86)	-	-	6.54 (1.92)	6.22 (1.95)
≥5min SB Bout ^k (n·day ⁻¹)	16.5 (2.32)	-	-	16.8 (2.51)	16.2 (2.22)

Table 3.1.4 continued.

True Mean SB	29.8 (12.5) _m	29.4 (11.8) _m	13. 53.9	30.0 (7.76) _m	32.1 (16.1) _m
Bout [†]			62. 70.3		
mins·day ⁻¹			84. 67.5		
Alpha ^m	1.44 (0.03)	-	-	1.44 (0.03)	1.44 (0.03)
(α ·day ⁻¹)					
W50% ⁿ	51.9 (19.2) _m	52.7 (17.5)	8. 94.6	51.0 (19.2) _m	53.1 (23.0) _m
(mins·day ⁻¹)					
PA Bouts ^o	22.1 (3.43)	22.6 (3.10)	62. 13.1	22.5 (3.86)	21.7 (3.39)
(<i>n</i> ·day ⁻¹)					
Daily Sum of PA	344 (94.8)	-	-	340 (155) _m	341 (137) _m
Bout Time ^p					
(mins·day ⁻¹)					
True Mean PA	15.9 (4.96)	15.3 (4.25)	43. 33.3	16.0 (5.04)	16.1 (4.84)
Bout ^q					
mins·day ⁻¹					
₁₀ MVPA Bouts ^r	0.28 (0.71) _m	0.28 (0.57) _m	2. 2.14	0.57 (1.00) _m	0.53 (0.57) _m
(<i>n</i> ·day ⁻¹)			4. 2.00		
			52. 2.00		
			57. 2.29		
Total Week	0.57 (1.36) _m	0.52 (1.25) _m	2. 3.85	0.81 (1.93) _m	0.41 (1.08) _m
₁₀ MVPA ^s			4. 3.60		
(hrs·week ⁻¹)			26. 3.83		
			30. 10.8		
			52. 3.43		
			57. 3.92		
SB% ^t	62.2 (9.57)	-	-	61.8 (9.78)	62.2 (9.68)
(%·waking hrs·day ⁻¹)					
Standing% ^u	7.20 (2.83)	7.06 (2.39)	56. 18.6	7.07 (2.74)	7.24 (2.90)
(%·waking hrs·day ⁻¹)			73. 15.0		
LIPA% ^v	12.6 (3.90)	-	-	12.2 (3.97)	12.9 (3.82)
(%·waking hrs·day ⁻¹)					
sMVPA% ^w	16.9 (4.99)	16.9 (4.00)	33. 30.4	17.3 (4.94)	16.5 (5.05)
(%·waking hrs·day ⁻¹)			43. 35.6		
			52. 28.7		
			70. 6.27		
			84. 4.70		
₁₀ MVPA% ^x	0.64 (1.52) _m	0.47 (1.11) _m	11. 5.80	0.98 (1.82) _m	0.40 (1.00) _m
(%·waking hrs·day ⁻¹)			12. 8.61		
			30. 8.94		
			36. 4.74		
			76. 4.39		

c, j, m, p, t, v Did not have outliers. † Subscript numbers represents which participant had outlier data. † Demographics calculated using data with outliers included. _m Median (IR). *** $p \leq 0.05$, ** $p \leq 0.01$, * $p \leq 0.001$.

Cardiovascular Profile

Cardiovascular characteristics are outlined in table 3.1.5. Measurement of carotid PL and popliteal variables was performed on a sub-population ($n=45$, age: 73.6(7.17) yrs, male: 22, female: 23).

Table 3.1.5. Cardiovascular parameter demographics for pooled, male, and female populations. Data presented as mean (SD), unless stated otherwise.

Variable	Pooled	Male	Female
Systolic BP (mmHg)	139 (17.0)	137 (16.1)	140 (17.8)
Diastolic BP (mmHg)	71.4 (11.8) _m	72.0 (8.98)	73.5 (8.46)
	4.28 (0.11)	4.26 (0.11)	4.29 (0.11)
Pulse Pressure (mmHg)	66.5 (12.8)	65.4 (12.4)	67.4 (13.3)
Heart Rate (bpm)	63.4 (8.92)	62.0 (9.27)	64.6 (8.53)
Brachial			
Baseline Diameter (mm)	4.14 (0.71)	4.60 (0.67)	3.77 (0.51) ^{***}
Baseline Blood Velocity (mm·s ⁻¹)	309 (113) _m	301 (137)	323 (107)
	5.73 (0.28)	5.75 (0.27)	5.72 (0.28)
Baseline Shear Rate (·s ⁻¹)	602 (262) _m	558 (286) _m	645 (213) _m *
	6.41 (0.32)	6.32 (0.30)	6.49 (0.33)*
IMT (mm)	0.51 (0.11) _m	0.59 (0.14) _m	0.46 (0.19) _m *
	-0.67 (0.36)_m	-0.52 (0.27)_m	-0.77 (0.39)_m*
Carotid			
AL Artery Diameter (mm)	7.32 (0.85)	7.70 (0.85)	6.99 (0.71) ^{***}
AL IMT (mm)	0.76 (0.15) _m	0.76 (0.18) _m	0.77 (0.13) _m
	-0.25 (0.15)	-0.24 (0.15)	-0.26 (0.15)
AL Blood Velocity (mm·s ⁻¹)	351 (120) _m	372 (116) _m	335 (95.2) _m
	5.87 (0.27)	5.92 (0.27)	5.83 (0.26)
AL Shear Rate (·s ⁻¹)	412 (122)	411 (118)	412 (127)
AL RI	0.71 (0.11) _m	0.73 (0.09)	0.72 (0.08)
	-0.32 (0.11)	-0.31 (0.14)_m	-0.33 (0.15)_m
PL Artery Diameter (mm)	7.21 (0.54)	7.37 (0.35)	7.09 (0.64)

Table 3.1.5 continued.

PL IMT (mm)	0.80 (0.13)	0.81 (0.10)	0.80 (0.15)
<i>Popliteal</i>			
Artery Diameter (mm)	6.35 (1.21)	6.84 (2.18) _m	6.14 (1.28) _m **
IMT (mm)	0.78 (0.14)	0.80 (0.16)	0.76 (0.11)
Blood Velocity (mm·s ⁻¹)	255 (79.1) _m	265 (71.7) _m	240 (122) _m
	5.60 (0.32)	5.58 (0.27)_m	5.48 (0.42)_m
Shear Rate (·s ⁻¹)	334 (168) _m	312 (173) _m	360 (240) _m
	5.85 (0.38)	5.73 (0.33)	5.97 (0.39)*

_m Median (IR). **Bold** data has been natural LOG transformed. BMI Body mass index. BP Blood pressure. IMT Intima media thickness. AL Anterior longitudinal plane. PL Posterior longitudinal plane. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Physical Behaviour Predicted Cardiovascular Profile (Bivariate)

SB showed no predictive qualities for cardiovascular parameters. Meanwhile PA variables showed a number of associations (4 out of 19) with cardiovascular parameters (table 3.1.6). Notably, an hour per day increase in low intensity PA (Standing, and LIPA) is sufficient to stimulate vascular adaption of popliteal artery diameter (Standing: -0.75 [95%CI -1.41, -0.09] mm) and IMT (LIPA: -0.09 [95%CI -0.15, -0.03] mm). In addition, an hour increase in sMVPA was also sufficient to reduce popliteal IMT (-0.06 [95%CI -0.12, 0.002] mm) and resting heart rate (-3.36 [95%CI -5.67, -1.05] bpm).

Table 3.1.6. Bivariate and multivariate stepwise linear regressions between PB, covariates and cardiovascular parameters.

Variable	Model	b	-95% CI	+95% CI	p	r ² adj.	P. Corr.	
Systolic BP	SB ^a	-0.39	-2.85	2.06	0.75	-0.01		
	Standing ^b	-1.27	-9.38	6.82	0.75	-0.01		
	LIPA ^c	-0.75	-6.45	4.94	0.79	-0.01		
	sMVPA ^d	1.02	-3.58	5.64	0.65	-0.01		
	₁₀ MVPA ^e	1.44	-11.7	14.6	0.82	-0.01		
	Hydration ^h	-0.65	-1.14	-0.17	0.01	0.06		
LOG	SB ^a	0.001	-0.01	0.01	0.93	-0.01		
Diastolic BP	Standing ^b	-0.03	-0.09	0.01	0.15	0.01		
	LIPA ^c	-0.01	-0.05	0.02	0.39	-0.003		
	sMVPA ^d	0.01	-0.01	0.04	0.37	-0.002		
	₁₀ MVPA ^e	-0.01	-0.10	0.08	0.81	-0.01		
	Hydration ^h	-0.004	-0.007	0.00	0.03	0.04		
Pulse Pressure	SB ^a	-0.45	-2.31	1.39	0.62	-0.01		
	Standing ^b	1.54	-4.56	7.64	0.61	-0.01		
	LIPA ^c	0.61	-3.68	4.91	0.77	-0.01		
	sMVPA ^d	0.17	-3.30	3.65	0.92	-0.01		
	₁₀ MVPA ^e	2.48	-7.44	12.4	0.62	-0.01		
	Hydration ^h	-0.39	-0.75	-0.02	0.03	0.03		
Heart Rate	SB ^a	1.10	-0.16	2.37	0.08	0.02		
	Standing ^b	-5.59	-9.67	-1.52	0.01	0.06		
	LIPA ^c	-2.74	-5.67	0.18	0.06	0.02		
	sMVPA ^d	-3.36	-5.67	-1.05	0.01	0.07		
	₁₀ MVPA ^e	-6.66	-13.4	0.10	0.05	0.03		
			-6.02^d	-9.54^d	-2.50^d	0.001^d		
			-8.50^b	-13.4^b	-3.57^b	0.001^b		-0.34^d
		MR ^{dbae}	-3.01^a	-5.23^a	-0.78^a	0.01^a	0.19	-0.34^b
			-6.84^e	-13.1^e	-0.53^e	0.03^e		-0.28^a
						0.00^{dbae}		-0.22^e
Brachial								
Artery Diameter	SB ^a	0.002	-0.10	0.10	0.97	-0.01		
	Standing ^b	0.08	-0.26	0.43	0.63	-0.01		
	LIPA ^c	0.02	-0.22	0.26	0.88	-0.01		
	sMVPA ^d	0.05	-0.14	0.25	0.59	-0.01		
	₁₀ MVPA ^e	0.02	-0.54	0.58	0.94	-0.01		
	Primary CVD		0.09	0.01	0.19	0.03	0.03	
Meds ^f	Hydration ^h	0.02	0.003	0.04	0.02	0.04		
			0.02^h	0.00^h	0.04^h	0.01^h		
			0.10^f	0.01^f	0.19^f	0.03^f	0.09	
						0.01^{hf}	0.26^h	
							0.22^f	

Table 3.1.6 continued.

LOG Blood	SB ^a	-0.01	-0.04	0.03	0.80	-0.01
Velocity	Standing ^b	-0.06	-0.14	0.12	0.92	-0.01
	LIPA ^c	-0.03	-0.12	0.06	0.50	-0.01
	sMVPA ^d	0.04	-0.03	0.11	0.28	0.002
	₁₀ MVPA ^e	0.03	-0.18	0.25	0.75	0.001
	LOG Shear	SB ^a	-0.002	-0.05	0.04	0.94
Rate	Standing ^b	-0.04	-0.20	0.11	0.58	-0.01
	LIPA ^c	-0.04	-0.15	0.06	0.41	-0.004
	sMVPA ^d	0.02	-0.06	0.11	0.57	-0.01
	₁₀ MVPA ^e	0.01	-0.24	0.27	0.91	-0.01
	LOG IMT	SB ^a	0.01	-0.02	0.03	0.77
	Standing ^b	0.04	-0.06	0.16	0.37	-0.002
	LIPA ^c	0.01	-0.06	0.09	0.68	-0.01
	sMVPA ^d	0.003	0.06	0.06	0.92	-0.01
	₁₀ MVPA ^e	-0.06	-0.24	0.11	0.48	-0.01
	Carotid					
AL Artery	SB ^a	0.07	-0.05	0.19	0.26	0.003
Diameter	Standing ^b	-0.12	-0.54	0.28	0.53	-0.01
	LIPA ^c	-0.22	-0.50	0.06	0.12	0.01
	sMVPA ^d	0.01	-0.22	0.23	0.96	-0.01
	₁₀ MVPA ^e	0.33	-0.33	1.00	0.32	0.00
	Primary CVD Meds ^f	0.11	0.003	0.22	0.04	0.03
LOG AL IMT	SB ^a	0.001	-0.02	0.02	0.91	-0.01
	Standing ^b	0.05	-0.02	0.12	0.17	0.01
	LIPA ^c	0.01	-0.03	0.07	0.50	-0.01
	sMVPA ^d	0.002	-0.04	0.04	0.91	-0.01
	₁₀ MVPA ^e	0.06	-0.06	0.18	0.32	0.00
	LOG AL	SB ^a	-0.02	-0.05	0.02	0.32
Blood Velocity	Standing ^b	-0.003	-0.13	0.12	0.95	-0.01
	LIPA ^c	0.03	-0.05	0.12	0.47	-0.01
	sMVPA ^d	0.04	-0.03	0.11	0.25	0.004
	₁₀ MVPA ^e	0.11	-0.10	0.32	0.30	0.001
	AL Shear	SB ^a	-9.64	-27.4	8.16	0.28
Rate	Standing ^b	2.95	-56.4	62.3	0.92	-0.01
	LIPA ^c	21.8	-19.8	63.5	0.30	0.001
	sMVPA ^d	14.5	-18.9	48.1	0.38	-0.003
	₁₀ MVPA ^e	20.7	-76.2	117	0.67	-0.01
	LOG AL RI	SB ^a	0.001	-0.01	0.01	0.90
	Standing ^b	0.02	-0.03	0.07	0.42	-0.004
	LIPA ^c	0.01	-0.03	0.04	0.74	-0.01
	sMVPA ^d	-0.01	-0.04	0.02	0.45	-0.01
	₁₀ MVPA ^e	-0.05	-0.14	0.03	0.24	0.004

Table 3.1.6 continued.

PL Artery	SB ^a	0.07	-0.06	0.20	0.28	0.01
Diameter	Standing ^b	-0.07	-0.42	0.27	0.65	-0.02
	LIPA ^c	-0.17	-0.46	0.13	0.25	0.01
	sMVPA ^d	-0.19	-0.48	0.09	0.17	0.03
	₁₀ MVPA ^e	0.27	-0.77	1.32	0.59	-0.02
PL IMT	SB ^a	-0.01	-0.05	0.01	0.24	0.01
	Standing ^b	-0.01	-0.09	0.07	0.88	-0.03
	LIPA ^c	0.02	-0.05	0.09	0.52	-0.02
	sMVPA ^d	0.01	-0.05	0.08	0.64	-0.02
	₁₀ MVPA ^e	0.10	-0.15	0.35	0.41	-0.01
Popliteal						
Artery	SB ^a	0.14	-0.10	0.40	0.24	0.01
Diameter	Standing ^b	-0.75	-1.41	-0.08	0.02	0.08
	LIPA ^c	-0.35	-0.91	0.20	0.21	0.01
	sMVPA ^d	0.18	-0.37	0.74	0.51	-0.01
	₁₀ MVPA ^e	0.89	-1.19	2.98	0.39	-0.01
IMT	SB ^a	0.02	-0.003	0.05	0.07	0.05
	Standing ^b	-0.06	-0.15	0.01	0.11	0.03
	LIPA ^c	-0.09	-0.15	-0.03	0.004	0.15
	sMVPA ^d	-0.06	-0.12	-0.002	0.04	0.07
	₁₀ MVPA ^e	-0.07	-0.32	0.17	0.54	-0.01
	MR ^c	-0.09	-0.15	-0.03	0.004	0.15
LOG Blood Velocity	SB ^a	0.03	-0.03	0.10	0.36	-0.004
	Standing ^b	-0.12	-0.31	0.06	0.18	0.01
	LIPA ^c	-0.06	-0.22	0.08	0.37	-0.01
	sMVPA ^d	-0.08	-0.23	0.06	0.25	0.01
	₁₀ MVPA ^e	-0.51	-1.06	0.03	0.06	0.05
	Hydration ^h	-0.01	-0.02	-0.01	0.002	0.19
LOG Shear Rate	SB ^a	0.01	-0.07	0.08	0.90	-0.02
	Standing ^b	0.01	-0.21	0.22	0.96	-0.02
	LIPA ^c	-0.01	-0.18	0.17	0.95	-0.02
	sMVPA ^d	-0.11	-0.28	0.06	0.20	0.01
	₁₀ MVPA ^e	-0.66	-1.29	-0.03	0.04	0.07
	Hydration ^h	-0.01	-0.03	-0.004	0.01	0.12
	MR ^h	-0.01	-0.03	-0.004	0.01	0.12

Bold font highlights significant ($p \leq 0.05$) bivariate and multivariate stepwise linear regression models. Hydration Change per percent increase in total body water. Primary CVD Meds Change per one unit increase in the number of medications directly targeting CVD risk. (in)direct CVD Meds Change per one unit increase in the number of medications (in)directly targeting CVD risk. MR Multivariate stepwise linear regression model. Superscript letters represent which, and what order PB variables are included in the multivariate model. b Change in cardiovascular variable per unit increase in GENE variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. p Significance value. r^2 adj. Adjusted explained variance. P. Corr. Partial Correlation.

When GENE outliers were removed from the data (table 3.1.7), SB was found to be a predictor of heart rate (1.58 [95%CI 0.17, 2.99] bpm) (thus predicting 1 out of 19 cardiovascular parameters). Standing was also found to be a predictor of popliteal IMT (-0.13 [95%CI -0.22, -0.03] mm) with the removal of outliers (thus predicting 3 out of 19 cardiovascular parameters). LIPA as a predictor had no outliers (1 out of 19 predictions for cardiovascular parameters). sMVPA was no longer a predictor of popliteal IMT when outliers were removed (thus now predicting 1 out of 19 cardiovascular parameters). ₁₀MVPA was no longer a predictor of popliteal shear rate following the removal of outliers (thus predicting no cardiovascular parameters).

Table 3.1.7. Change in bivariate and multivariate stepwise linear regressions models following the removal of PB outliers.

Variable	Model	b	-95% CI	+95% CI	p	r ² adj.	P. Corr.	
Heart Rate	SB ^a	1.10	-0.16	2.37	0.08	0.02		
	Standing ^b	-5.59	-9.67	-1.52	0.01	0.06		
	LIPA ^c	-2.74	-5.67	0.18	0.06	0.02		
	sMVPA ^d	-3.36	-5.67	-1.05	0.01	0.07		
	₁₀ MVPA ^e	-6.66	-13.4	0.10	0.05	0.03		
	MR ^{dbae}		-6.02^d	-9.54^d	-2.50^d	0.001^d		-0.34^d
			-8.50^b	-13.4^b	-3.57^b	0.001^b		-0.34^b
			-3.01^a	-5.23^a	-0.78^a	0.01^a	0.19	-0.28^a
			-6.84^e	-13.1^e	-0.53^e	0.03^e		-0.22^e
						0.00^{dbae}		
Heart Rate	SB ^a	1.58	0.17	2.99	0.02	0.04		
	Standing ^b	-6.77	-11.2	-2.28	0.004	0.08		
	LIPA ^c	-2.74	-5.67	0.18	0.06	0.02		
	sMVPA ^d	-4.69	-7.54	-1.84	0.002	0.10		
	₁₀ MVPA ^e	-6.86	-19.3	5.59	0.27	0.002		
	MR ^{db}		-3.61^d	-6.60^d	-0.63^d	0.01^d		-0.26^d
			-5.52^b	-10.3^b	-0.71^b	0.02^b	0.15	-0.25^b
						0.000^{db}		
	Excluded ^{ae}		-0.25 ^a			0.14 ^a		-0.16 ^a
			-0.04 ^e			0.68 ^e		-0.04 ^e

Table 3.1.7 continued.

Brachial							
Artery	SB ^a	0.002	-0.10	0.10	0.97	-0.01	
Diameter	Standing ^b	0.08	-0.26	0.43	0.63	-0.01	
	LIPA ^c	0.01	-0.22	0.26	0.88	-0.01	
	sMVPA ^d	0.05	-0.14	0.25	0.59	-0.01	
	₁₀ MVPA ^e	0.02	-0.54	0.58	0.94	-0.01	
	Primary CVD	0.09	0.01	0.19	0.03	0.03	
	Meds ^f						
	Hydration ^h	0.02	0.003	0.04	0.02	0.04	
	MR ^{hf}	0.02^h	0.01^h	0.04^h	0.01^h		0.26^h
		0.10^f	0.01^f	0.19^f	0.03^f	0.09	0.22^f
					0.01^{hf}		
	SB ^a	-0.01	-0.13	0.10	0.77	-0.01	
	Standing ^b	-0.04	-0.42	0.34	0.82	-0.01	
	LIPA ^c	0.01	-0.22	0.26	0.88	-0.01	
	sMVPA ^d	0.12	-0.12	0.37	0.30	0.001	
	₁₀ MVPA ^e	0.20	-1.25	0.84	0.69	-0.01	
	Primary CVD	0.08	-0.01	0.18	0.07	0.02	
	Meds ^f						
	Hydration ^h	0.02	0.003	0.04	0.02	0.04	
	MR ^h	0.02	0.001	0.04	0.03	0.04	0.23
	Excluded ^f	0.22			0.06		0.22
Popliteal							
IMT	SB ^a	0.02	-0.003	0.05	0.07	0.05	
	Standing ^b	-0.06	-0.15	0.01	0.11	0.03	
	LIPA ^c	-0.09	-0.15	-0.03	0.004	0.15	
	sMVPA ^d	-0.06	-0.12	-0.002	0.04	0.07	
	₁₀ MVPA ^e	-0.07	-0.32	0.17	0.54	-0.01	
	MR ^c	-0.09	-0.15	-0.03	0.004	0.15	
	SB ^a	0.02	-0.01	0.06	0.10	0.03	
	Standing ^b	-0.13	-0.22	-0.03	0.01	0.14	
	LIPA ^c	-0.09	-0.15	-0.03	0.004	0.15	
	sMVPA ^d	-0.06	-0.13	0.004	0.06	0.05	
	₁₀ MVPA ^e	0.01	-0.27	0.28	0.96	-0.02	
	MR ^c	-0.11	-0.17	-0.04	0.001	0.21	

Table 3.1.7 continued.

LOG	SB ^a	0.01	-0.07	0.08	0.90	-0.02
Shear	Standing ^b	0.01	-0.21	0.22	0.96	-0.02
Rate	LIPA ^c	-0.01	-0.18	0.17	0.95	-0.02
	sMVPA ^d	-0.11	-0.28	0.06	0.20	0.01
	₁₀ MVPA ^e	-0.66	-1.29	-0.03	0.04	0.07
	Hydration ^h	-0.01	-0.03	-0.004	0.01	0.12
	MR ^h	-0.01	-0.03	-0.004	0.01	0.12
	SB ^a	0.01	-0.07	0.08	0.90	-0.02
	Standing ^b	0.01	-0.21	0.22	0.96	-0.02
	LIPA ^c	-0.01	-0.18	0.17	0.95	-0.02
	sMVPA ^d	-0.11	-0.28	0.06	0.20	0.01
	₁₀ MVPA ^e	-0.65	-1.39	0.08	0.08	0.04
	Hydration ^h	-0.01	-0.03	-0.004	0.01	0.12

Bold font highlights significant ($p \leq 0.05$) bivariate and multivariate stepwise linear regression models. Orange shading highlights regression models following outlier removal. Hydration Change per percent increase in total body water. Primary CVD Meds Change per one unit increase in the number of medications directly targeting CVD risk. (in)direct CVD Meds Change per one unit increase in the number of medications (in)directly targeting CVD risk. MR Multivariate stepwise linear regression. Superscript letters represent which, and what order PB variables are included in the multivariate model. b Change in cardiovascular variable per unit increase in GENE A variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. p Significance value. r^2 adj. Adjusted explained variance. P. Corr. Partial Correlation.

Physical Behaviour Predicted Cardiovascular Profile (Multivariate)

Resting heart rate had the most PB predictors, excluding only LIPA from the model. Standing and sMVPA each explained 12.2% of the variance in heart rate whilst controlling for the other PB parameters in the model. This was the largest partial correlation of the PB parameters included in the prediction of heart rate. There were no other cardiovascular parameters that could be predicted using multiple PB parameters (table 3.1.6).

With the removal of GENE A outliers, SB and ₁₀MVPA were removed from the heart rate regression model (table 3.1.7).

Patterns of Physical Behaviour Predict Cardiovascular Profile

The predictive quality of cardiovascular parameters using patterns of PB are displayed in table 3.1.7. W50%, SB%, and alpha appear to be the most common predictors within the SB category, showing predictive qualities for three, two, and two cardiovascular parameters (out of 19), respectively (table 3.1.8). Within the PA category, daily sum of PA bout time showed predictive qualities for two cardiovascular markers (out of 19), with PA bouts, true mean PA bout, Standing%, LIPA%, sMVPA%, and ₁₀MVPA% all showing predictive qualities for one cardiovascular parameter (out of 19) (table 3.1.8). Please note that only significant associations are displayed in table 3.1.7 to reduce its size. The complete results tables can be found in the Appendix Chapter 03, table A3.1.6.

Table 3.1.8. Bivariate linear regressions models between patterns of PB and cardiovascular parameters. Note that neither Systolic BP, Pulse Pressure, Brachial; Artery Diameter, LOG Blood Velocity, LOG Shear Rate, LOG IMT, Carotid; AL Artery Diameter, LOG AL IMT, LOG AL Blood Velocity, LOG AL Shear Rate, LOG AL RI, and PL Artery Diameter showed any significant model with the 16 patterns of PB of interest and hence these models are not shown. Note that only significant models between patterns of PB and cardiovascular variables are shown.

Variable	Model	b	-95% CI	+95% CI	p	r ² adj.
LOG Diastolic BP	Alpha	0.73	0.10	1.37	0.02	0.04
Heart Rate	SB Breaks	-0.63	-1.13	-0.13	0.01	0.05
	<5min SB Bout	-1.34	-2.27	-0.40	0.01	0.07
	True Mean SB Bout	0.17	0.01	0.35	0.04	0.03
	W50%	0.19	0.17	0.31	0.001	0.10
	PA Bouts	-0.63	-1.13	-0.13	0.01	0.05
	Daily Sum of PA Bout Time	-0.03	-0.05	-0.01	0.001	0.10
	SB%	0.27	0.08	0.46	0.01	0.07
	Standing%	-0.80	-1.45	-0.15	0.01	0.05
	sMVPA%	-0.41	-0.78	-0.04	0.02	0.04
Carotid						
PL IMT	Alpha	1.60	0.29	2.90	0.01	0.14
Popliteal						
Artery Diameter	Standing%	-0.12	-0.22	-0.01	0.02	0.09

Table 3.1.8 continued.

IMT	W50%	0.003	0.00	0.01	0.04	0.07
	Daily Sum of					
	PA Bout	-0.001	-0.001	0.00	0.001	0.13
	Time					
	True Mean					
	PA Bout	-0.01	-0.01	-0.001	0.02	0.09
	SB%	0.01	0.001	0.01	0.01	0.11
	LIPA%	-0.01	-0.02	-0.004	0.01	0.13
LOG Blood						
Velocity	W50%	0.01	0.00	0.01	0.03	0.07
LOG Shear						
Rate	₁₀ MVPA%	-0.10	-0.20	-0.001	0.04	0.06

Significant bivariate linear regressions ($p \leq 0.05$). b Change in cardiovascular variable per unit increase in GENEVA variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. p Significance value. r^2 adj. Adjusted explained variance.

After the removal of outliers (table 3.1.9), W50%, SB%, and alpha remained the best predictors of cardiovascular parameters within the SB category (three, two, and two out of 19, respectively), showing no change in prediction quality. Within the PA category, true mean PA bout, daily sum of PA bout time, and total week ₁₀MVPA showed the most predictive qualities for cardiovascular parameters (two each), followed by PA bouts, ₁₀MVPA bouts, Standing%, LIPA%, and sMVPA% (one each) (table 3.1.9).

Table 3.1.9. Change in bivariate linear regression models between patterns of PB and cardiovascular parameters after patterns of PB outliers were removed.

Variable	Model	b	-95% CI	+95% CI	p	r ² adj.
Heart Rate	True Mean	-0.30	-0.68	0.07	0.11	0.01
	PA Bout	-0.41	-0.82	-0.01	0.04	0.03
Popliteal						
Artery	Standing%	-0.12	-0.22	-0.01	0.02	0.09
		-0.11	-0.24	0.02	0.10	0.03
Diameter	Standing%	-0.01	-0.02	0.004	0.17	0.02
		-0.01	-0.03	-0.004	0.01	0.11
LOG Blood	Total Week	-0.07	-0.15	0.01	0.06	0.05
	Velocity	-0.10	-0.18	-0.01	0.01	0.10
LOG Shear	¹⁰ MVPA	-0.15	-0.34	0.03	0.10	0.03
	Rate	-0.27	-0.50	-0.04	0.01	0.10
LOG Shear	Total Week	-0.09	-0.18	0.001	0.05	0.06
	Rate	-0.13	-0.22	-0.03	0.01	0.14
LOG Shear	¹⁰ MVPA%	-0.10	-0.20	-0.001	0.04	0.06
	Rate	-0.09	-0.21	0.01	0.09	0.04

Bold font highlights significant ($p \leq 0.05$) bivariate linear regressions. Orange shading highlights regression models following outlier removal. b Change in cardiovascular variable per unit increase in GENE variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. p Significance value. r² adj. Adjusted explained variance.

Discussion

The purpose of the current study was to address three objectives: 1) determine whether measures of daily PB predict older adults' cardiovascular profile; 2) determine which measures of PB patterns are better predictors of cardiovascular profile; 3) highlight any effects of SB on cardiovascular profile that are independent of MVPA and vice versa. It was broadly hypothesized that a number of cardiovascular parameters will be uniquely sensitive to SB, and others to MVPA.

Physical Behaviour Predicts Cardiovascular Parameters

A lack of predictive qualities of PB relative to brachial and carotid parameters suggests that the effects of PB may either be site specific or indicative of a remodeling process, which masks any adaptation to changes in PB in older adults. Interestingly, predictive qualities relative to popliteal parameters were seen with low intensity PA (standing and LIPA), suggesting that low intensity PB could reduce popliteal parameters that are associated with CVD (Burke et al., 1995). Within bivariate regression models both an hour increase in standing and LIPA led to a 0.14 and 0.09 mm reduction in popliteal IMT, respectively, whilst LIPA was the only PB variable included in the multivariate stepwise regression model that suggested popliteal IMT would decrease by 0.112 mm per hour increase in LIPA. This finding is consistent with a training study that found popliteal IMT decreased by 0.038 mm over 12 weeks (18 hours) of LIPA (30% heart rate reserve), which equated to a 0.002 mm reduction in popliteal IMT per hour of LIPA (Green et al., 2010). The results of the current study would have strong implications in older adults who struggle to accumulate sufficient $_{10}$ MVPA, as they may find it easier to accumulate LIPA.

Which Physical Behaviour Pattern is the Best Predictor of Cardiovascular Profile?

With improvements in the objective measurement of SB, the focus of research has shifted to the patterns in which SB is accumulated rather than total SB time per se. In particular, SB breaks has become a heavily researched parameter (Carson et al., 2014; Chastin et al., 2015a) especially in acute interventions (Peddie et al., 2013; Bailey and Locke, 2014; Dunstan et al., 2012; Holmstrup et al., 2014). Within the current data, SB breaks only had predictive qualities for resting heart rate, whilst other patterns of SB, W50% and alpha showed more predictive qualities for cardiovascular parameters (three and two, respectively). W50% and alpha were first introduced by Chastin and Granat (2010) to create a more sensitive measure of change in SB accumulation as SB breaks can be similar when W50% and alpha are significantly different between the pre and post phases of an intervention, or between groups (Chastin et al., 2015c; Ryan et al., 2016). W50% is the usual SB

bout length that would accumulate 50.0% of total SB time if all of the SB bouts of that length and shorter/greater were accumulated (Chastin and Granat, 2010). W50% had predictive qualities for popliteal IMT as every minute increase in W50%, increased popliteal IMT by 0.003 mm, which may have CVD implications as those with a history of CVD have exhibited a 0.04 (95% CI 0.03, 0.04) mm increase in popliteal IMT than those with no history (Burke et al., 1995). Thus, as little as a ten-minute increase in W50% could lead to CVD complications.

Alpha is a unit-less power-law distribution that displays the increase in SB bouts as SB bout duration decreases (Chastin et al., 2015c). Diastolic BP and carotid PL IMT showed predictive associations with alpha while W50% did not in the current study. The direction of these predictive models suggested that an increase in alpha (more SB bouts, shorter duration) would result in an increase in diastolic BP and carotid PL IMT. This is opposite to what would be intuited as brachial diastolic BP has been found to be similar between supine and orthostatic postures (Gemignani et al., 2012). On the other hand, orthostatic posture increases carotid circumferential wall tension, compared to supine posture, and is associated with an increase in carotid plaque formation, which can be expressed as an increased IMT. This suggests that the association between alpha and carotid PL IMT may be a result of more PA (orthostatic posture) due to a reduction SB bout length. The large 95%CI (0.29, 2.90 mm) in the current data may highlight the need for further data to confirm or otherwise, the reported association between alpha and carotid PL IMT since a 0.10 mm increase in IMT can increase the relative risk of stroke by 18.0% (Lorenz et al., 2007). The increase in diastolic BP with increasing alpha may hold true as more bouts of a shorter duration would indicate that the older person causes this offset by engaging in more PA, where the arms are likely hanging by their side (as may be the case in gentle strolling or even chair-based exercise). In other words, there may have been PA not captured by the thigh-mounted accelerometer in the current study. This may cause a hydrostatic pressure that would subsequently increase blood pressure (Padilla et al., 2009). However, the lack of association between standing and diastolic BP within the current data does not support this idea.

The current findings suggest that W50% should be the preferred SB pattern of PB parameter for predicating cardiovascular risk because it has the most predictive qualities (three). In addition, W50% is presented in minutes, which can be easily understood and explained in a 'real-world' therapeutic (clinical or lifestyle) intervention settings.

Within PA patterns of PB, true mean PA bout length (Chastin and Granat, 2010), showed the most predictive qualities for cardiovascular parameters (two) where 'true mean' refers to the mean duration of a PA bout succeeding anti-log transformations of previously LOG transformed non-normally distributed PA bout lengths (Chastin and Granat, 2010). This adds strength to the argument that it is not the number of SB breaks that is the most important, but the complex interaction between those SB breaks and the duration of individual SB breaks. The predictive qualities of total week $_{10}$ MVPA (two) and the number of $_{10}$ MVPA bouts (one) within the current study support the government's use of a total $_{10}$ MVPA recommendation accumulated in, at least, ten minute bouts within their PA guidelines for older adults (Department of Health Social Services and Public Safety, 2011).

Independent Physiological Effects of Sedentary Behaviour and Moderate to Vigorous Physical Activity

The basis of SB physiology stems from the apparently independent effects of SB and MVPA on health status (Bey and Hamilton, 2003; Gennuso et al., 2013). Prior to the removal of outliers, MVPA (sMVPA or $_{10}$ MVPA) showed predictive qualities for resting heart rate, popliteal IMT, and popliteal LOG shear rate, whereas SB did not within bivariate regression models. After the removal of outliers, sMVPA only showed predictive qualities for heart rate however, SB now displayed predictive qualities for heart rate too. This could infer that the effects of SB and MVPA on heart rate may not be independent. Furthermore, SB is excluded whilst sMVPA is included in the multivariate predictive model for heart rate suggesting SB does not add any further strength to the predictive model for heart rate, this could infer that SB and sMVPA use the same mechanistic pathways to affect heart rate. MVPA is known to reduce resting heart rate through increases in stroke volume (Hagberg et al., 1983) and reduction in peripheral resistance (Hambrecht et al., 2000) whilst SB does the opposite (Spaak et al., 2005; Levine et al., 1997). The results of the current study support the idea that SB and MVPA effect resting heart rate indirectly by impacting on stroke volume and total peripheral resistance.

The predictive qualities of W50% and alpha were just as prevalent as those of true mean PA bout length, daily sum of PA bout time, and total week $_{10}$ MVPA. Of

the six cardiovascular markers these patterns of PB parameters predicted, three of them were only associated with one pattern of PB variable. This may infer that SB and PA parameters are physiologically independent and as such, warrants the need for future studies to include multiple PB parameters to be able to fully assess the effect of PB on health.

The main strength of this study is the use of a thigh-mounted accelerometer, which allows for accurate posture classification. However, one PB variable this study did not measure is seated/reclined PB eliciting >1.50 METs (as would occur in seated exercise training programs for instance, a modality of exercise of particular prevalence in frail older persons (Yates and Dunnagan, 2001). Arguably, it is unlikely that this classification of PB would be prevalent within independent living older adults and therefore its absence from our current PB stratification would be minimal in this type of population. It is however notable that owing to the age group of our study participants, the sample was skewed towards low adherence to $_{10}$ MVPA and hence any relationship assessment up to that level of PA intensity would be incomplete.

Conclusion

The purpose of this study was to determine which measures of PB display predictive qualities for cardiovascular variables so future research could justify the use of specific PB parameters as dependent variables within intervention studies. The main strength of this study is the use of a thigh-mounted accelerometer, which allows for accurate posture classification. Overall, the present study displayed that all PB measures ($\text{hrs}\cdot\text{day}^{-1}$), excluding $_{10}$ MVPA, showed predictive qualities for at least one cardiovascular variable (figure 3.1.3). Within patterns of PB, W50% and total week MVPA, daily sum of PA time, and true mean PA bout length were the best predictors of cardiovascular parameters (figure 3.1.3). The results suggest patterns of PB are more prolific predictors of cardiovascular health status than total PB measured in hours per day. SB and MVPA PB measures showed different and unique predicative qualities for cardiovascular parameters. This observation further supports the notion that both SB and MVPA engagements need to be considered in future PB research and/or lifestyle recommendations. Finally, increasing standing

and LIPA engagement showed predictive qualities for popliteal IMT reduction. We propose this to be one of the most clinically relevant findings from our current work as it suggests that older adults do not have to engage in MVPA (which they have, in any case, shown poor long-term compliance to), in order to gain health benefits.

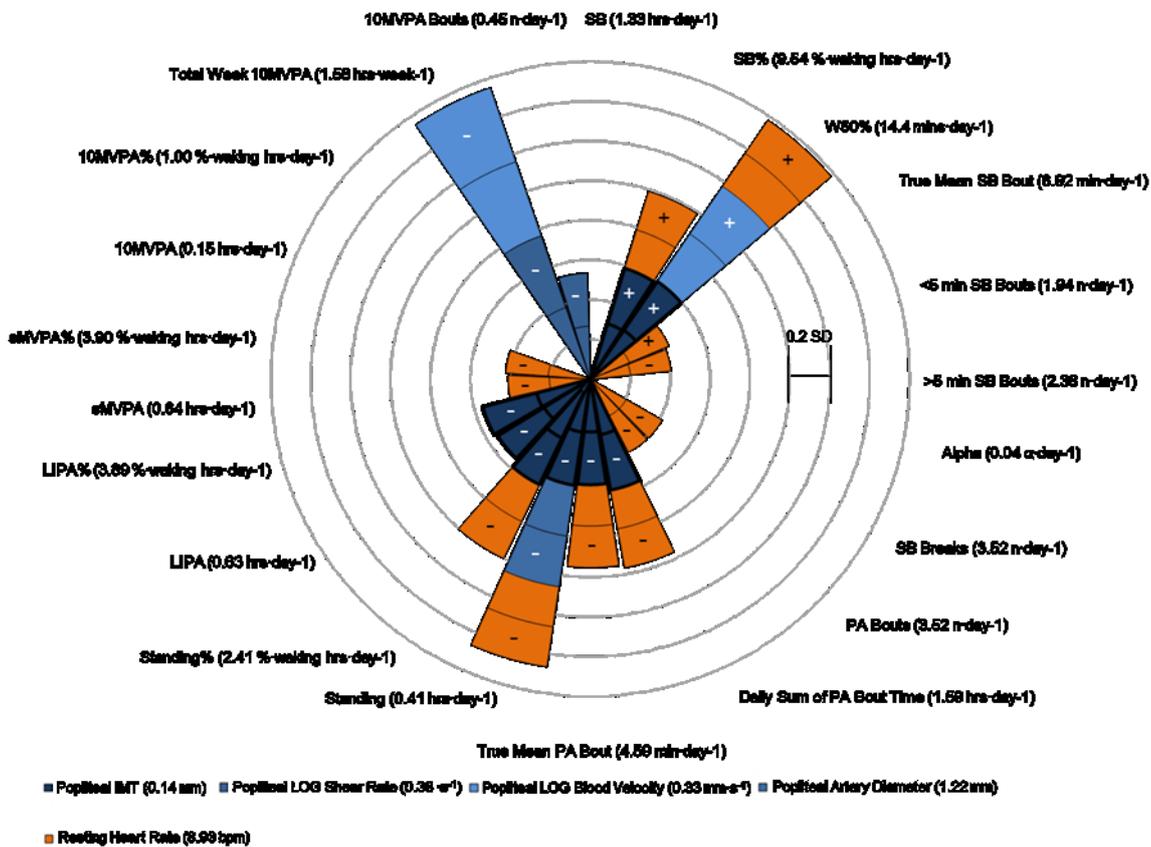


Figure 3.1.3. Summary of significant ($p \leq 0.05$) bivariate linear regressions displaying the SD change in cardiovascular parameter z-score per SD increase in PB parameter z-scores. Black lines indicate -95%CI, beta coefficient, and +95%CI from centre to outer perimeter. - indicates a negative association. + indicates a positive association. Graph is standardised to stack the regression models on top of the preceding model. E.g. the +95%CI line for the W50% and popliteal LOG blood velocity model also represents the -95%CI for the W50% and resting heart rate model. Data in brackets represents the standard deviation for the respective parameter. Models created using GENEAs outliers removed data.

Part 2: Isotemporal substitution approach.

Introduction

It is becoming evident that sedentary behaviour (SB) affects a number of physiological parameters independent of the amount of moderate to vigorous intensity physical activity (MVPA) engagement (Gennuso et al., 2013; Bey and Hamilton, 2003). With time being finite within a day (i.e. 24 hour endpoint), engagement in one physical behaviour (PB) (Busmann and van den Berg-Emons, 2013) will offset the amount of time that can be spent performing another. Standard regression modelling fails to recognise the time constraints and therefore the use of multiple measures of PB within a regression model will not account for the time that is displaced by engaging in a specific bout of PB.

Isotemporal substitution modelling (ISM) recognises that time is finite by including a measure of total PB (e.g. sum of waking hours SB and physical activity [PA]), which is kept constant and therefore, provides the opportunity to substitute one PB for another, thereby reflecting the realities of daily life (Mekary et al., 2013). Rather than prediction, *per se*, ISM reflects the decisions people have made (e.g. prolonged SB) and offers an extrapolation of what would happen should they decided to do something different (e.g. MVPA). Therefore, this analysis may be more advantageous to public health PB action plans, as it clearly illustrates what will happen to markers of health if habitual PB levels and/or patterns are changed. In older adult populations, ISM has mainly been used to assess the effect on cardio-metabolic (van der Berg et al., 2017; Yates et al., 2015; Hamer et al., 2014) rather than cardiovascular parameters (Wellburn et al., 2015). However when cardiovascular parameters have been assessed, it has demonstrated promising results, for instance, suggesting the substitution of SB for light intensity PA (LIPA) would reduce the relative risk of cardiovascular disease (CVD) prevalence within older adult cohorts (Wellburn et al., 2015). Light intensity PA is a promising intervention to reduce SB for older adult populations as it can arguably prove to be easier (in comparison to MVPA) to comply with, and can be accumulated to consist the greater majority of a 24-hour simplex (Craig et al., 2009).

The ten-minute minimum threshold for an MVPA bout ($_{10}$ MVPA), highlighted in the PA guidelines (Department of Health Social Services and Public Safety, 2011), to show clinically beneficial outcomes, has not been examined using ISM. If sporadic MVPA (sMVPA, MVPA accumulated in bouts of less than 10 continuous minutes) has beneficial effects on cardiovascular profile, this alternative mode of accumulating MVPA would likely allow older adults to improve their health within their physical capacities, and maintain this PB profile in the long term. Therefore, the objective of this study was to simulate the degree to which the substitution of SB and lower intensity PA for MVPA would have positive effects on cardiovascular profile markers and *vice versa*, in older adults. The aim was to provide a time-constrained, alternative to bivariate/multivariate regression modelling tool, to predict how changes in PB may affect the cardiovascular profile of older adults. It was hypothesised that substituting SB for any intensity of PA would improve cardiovascular parameters and that substituting a PB for a higher intensity would improve cardiovascular profile. It was also hypothesised that substituting SB for $_{10}$ MVPA would have a greater effect on cardiovascular parameters than seen with sMVPA substitutions.

Methods

This methods section follows that of Chapter 03 Part 1, using the same participants and data collection procedures. The descriptives of the measured parameters are outlined in table 3.2.1 as a reminder of the assessed cardiovascular and PB parameters.

Table 3.2.1. Demographics, cardiovascular, and PB parameters of the sample population.

Variable	Pooled
Age (yrs)	73.8 (6.22)
Height (m)	1.65 (0.08)
Mass (kg)	75.9 (13.1)
BMI (kg·m ²)	27.9 (4.71)
Primary CVD Meds (%)†	48.0
(in)direct CVD Meds (%)‡	59.0
Hydration (%)	50.6 (7.15) _m
Systolic BP (mmHg)	139 (17.0)
Diastolic BP (mmHg)	71.4 (11.8) _m
	4.28 (0.11)
Pulse Pressure (mmHg)	66.5 (12.8)
Heart Rate (bpm)	63.4 (8.92)
Brachial	
Baseline Diameter (mm)	4.14 (0.71)
Baseline Blood Velocity (mm·s ⁻¹)	309 (113) _m
	5.73 (0.28)
Baseline Shear Rate (·s ⁻¹)	602 (262) _m
	6.41 (0.32)
IMT (mm)	0.51 (0.11) _m
	-0.67 (0.36)_m
Carotid	
AL Artery Diameter (mm)	7.32 (0.85)
AL IMT (mm)	0.76 (0.15) _m
	-0.25 (0.15)
AL Blood Velocity (mm·s ⁻¹)	351 (120) _m
	5.87 (0.27)
AL Shear Rate (·s ⁻¹)	412 (122)
AL RI	0.71 (0.11) _m
	-0.32 (0.11)
PL Artery Diameter (mm)	7.21 (0.54)
PL IMT (mm)	0.80 (0.13)

Table 3.2.1 continued.

Popliteal	
Artery Diameter (mm)	6.35 (1.21)
IMT (mm)	0.78 (0.14)
Blood Velocity (mm·s ⁻¹)	255 (79.1) _m
	5.60 (0.32)
Shear Rate (·s ⁻¹)	334 (168) _m
	5.85 (0.38)
Physical Behaviour	
SB (hrs·day ⁻¹)	9.68 (1.30)
Standing (hrs·day ⁻¹)	1.10 (0.40)
LIPA (hrs·day ⁻¹)	1.95 (0.60)
sMVPA (hrs·day ⁻¹)	2.58 (0.66)
₁₀ MVPA (hrs·day ⁻¹)	0.08 (0.18) _m
Total PB (hrs·day ⁻¹)	15.4 (4.77) _m

m Median (Interquartile Range). † Participants are currently prescribed an amount of medication that reduces the risk or treats CVD (i.e. statins, warfarin). ‡ Participants are currently prescribed a medication that may affect the cardiovascular system either directly or as a side effect. **Bold** data has been natural LOG transformed. BMI Body mass index. BP Blood pressure. IMT Intima media thickness. AL Anterior longitudinal plane. PL Posterior longitudinal plane. SB Sedentary behaviour, LIPA Light intensity physical activity, sMVPA Sporadic moderate to vigorous intensity physical activity (accumulated in bouts < 10 mins), ₁₀MVPA 10 minute moderate to vigorous intensity physical activity (accumulated in bouts ≥ 10 mins), Total PB Total physical behaviour, BMI – body mass index.

Statistical Analyses

SPSS version 22 (IBM, New York, USA) was used for statistical analyses. Pearson correlation was used to assess multicollinearity between PB parameters and total PB, no adjustment was made to the data if multicollinearity was present. Isotemporal substitution regression modelling (forced entry) was implemented to examine the impact of one hour of PB substitution (Mekary et al., 2013). Isotemporal substitution modelling is performed by removing the substitution PB from the forced entry linear regression model (i.e. substitute SB model: Intercept + (β_1 x Standing) + (β_2 x LIPA) + (β_3 x sMVPA) + (β_4 x ₁₀MVPA) + (β_5 x Total PB) + Covariates + Error).

Significant PB predictors within the ISM illustrate that replacing one hour of the substituted PB (as data is measured in $\text{hrs}\cdot\text{day}^{-1}$) with the significant PB would have an effect on the respective cardiovascular parameter (magnitude of unit change illustrated by beta coefficient and 95% confidence intervals [95%CI]). Including total PB at the end of the ISM represents the time-constrained hours within a waking hours day, which standard linear regression modelling does not account for. Partition regression modelling (forced entry) was used to examine the effect of adding PB to the prediction model. Both ISM and partition models were conducted without (Model 1) and with (Model 2) adjustment for covariates to determine how hydration status and medication affect the relationship between PB and cardiovascular profile. Hydration status was used as a covariate as this has been shown to effect artery diameter (Chen et al., 2007) whilst medication use was used as a covariate as it has been shown to effect cardiovascular parameters (Furberg et al., 1994; Williams et al., 2006; Dernellis and Panaretou, 2002). Hydration, primary CVD meds, and (in)direct CVD meds were used for covariate adjustment if a forced entry linear regression analysis had shown that that they are associated with the respective cardiovascular parameter. Cardiovascular data were natural LOG transformed if they violated normal distribution. Data are presented as beta coefficient (95%CI) unless otherwise stated.

Results

Isotemporal Substitution Modelling

Isotemporal substitution showed that changes in PB levels would significantly affect three out of the 19 assessed cardiovascular parameters (Appendix Chapter 03 table A3.2.1-19), these being resting heart rate, carotid AL artery diameter, and popliteal artery diameter. The significant substitutions are shown in figure 3.2.1.

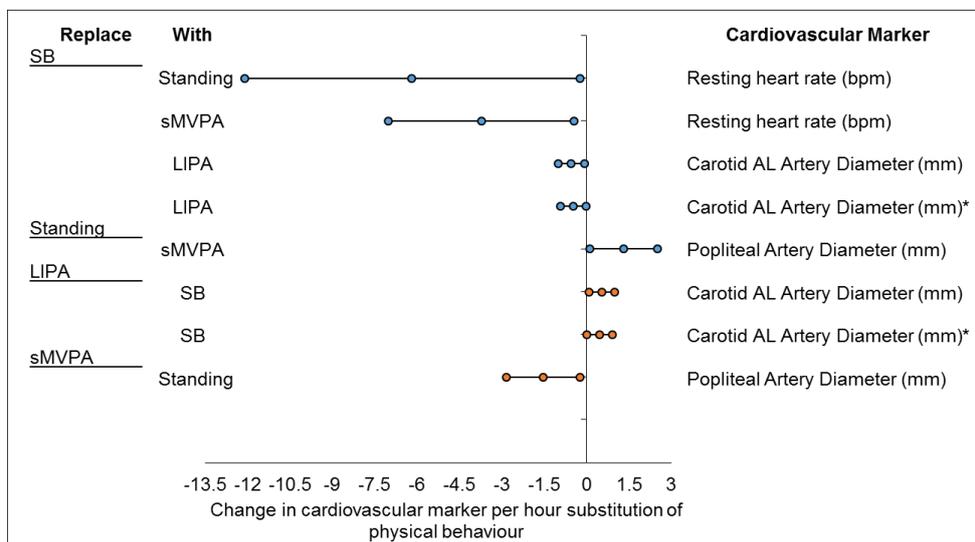


Figure 3.2.1 Significant PB ISM and their impact on cardiovascular parameters. Markers indicate (left to right) -95%CI, beta coefficient, and +95%CI. * Normalised for Primary CVD Medication. SB Sedentary behaviour, LIPA Light intensity PA, sMVPA Sporadic moderate to vigorous intensity PA (accumulated in bouts <10 mins), ₁₀MVPA 10 minute moderate to vigorous intensity PA (accumulated in bouts ≥10 mins), Carotid AL artery diameter Carotid anterior longitudinal plane artery diameter. Blue circles indicate a positive effect on cardiovascular profile. Orange circle indicates a negative effect on cardiovascular profile.

Substitution of SB with Standing and sMVPA was suggested to reduce resting heart rate (figure 3.2.1, -6.20 [-12.1, -0.22] bpm, -3.72 [-7.01, -0.44] bpm, respectively) which, is clinically relevant as a 5 bpm increase in resting heart rate increases the risk of cardiovascular mortality by 3% (2.0, 4.0) (Li, 2015). After the substitution of SB with LIPA, carotid AL artery diameter was predicted to reduce (figure 3.2.1, -0.54 [-1.00, -0.07] mm) and *vice versa* (figure 3.2.1, 0.54 [0.08, 1.00] mm), which is clinically relevant as a 0.78 mm increase is associated with a 2.1 (1.3, 3.3) hazard ratio risk of all-cause mortality (Van Dijk et al., 2001). Substitution of Standing with sMVPA (figure 3.2.1, 1.31 [0.11, 2.51] mm) would increase popliteal artery diameter and *vice versa* (figure 3.2.1, -1.52 [-2.83, -0.22] mm). This result is clinically relevant as an 8-week interval training program increased popliteal artery diameter by 0.14 mm per hour of training (Madsen et al., 2015) as well as the popliteal artery diameter of healthy controls being 0.6 mm ($p=0.11$) larger than those with coronary artery disease (males aged 40 – 70 years) (Angerer et al., 2001).

Within model 2, the results for all cardiovascular variables remained the same after covariate adjustment suggesting that co-variables had no effect on the relationship between PB and cardiovascular profile.

Partition Model

Interestingly, engagement in Standing (-8.08 mmol·l⁻¹ [-14.26, -1.90]) would reduce resting heart rate by a greater magnitude compared to engagement in sMVPA (-5.60 mmol·l⁻¹ [-9.70, -1.49]) (Appendix Chapter 03 table A3.2.1-19).

Increasing levels of SB and Standing would increase carotid AL IMT by 1.05 (1.00, 1.11) mm and 1.13 (1.01, 1.26), mm, respectively (Appendix Chapter 03 table A3.2.1 - 19).

Multicollinearity

The largest correlation coefficient within the multicollinearity matrix was between SB, LIPA and sMVP ($r=-0.69$) whilst the remaining variables only had weak correlations suggesting low influence of collinearity on the results (table 3.3.2).

Table 3.2.2. Collinearity statistics for PB parameters.

	SB	Standing	LIPA	sMVPA	₁₀MVPA	Total PB
SB	-	-0.58***	-0.69***	-0.69***	-0.23*	0.32**
Standing		-	0.64***	0.35**	0.01	0.24*
LIPA			-	0.45***	-0.02	0.13
sMVPA				-	0.19	0.23*
₁₀ MVPA					-	0.05

Pearson Correlations. *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$.

SB Sedentary behaviour, LIPA Light intensity PA, sMVPA Sporadic moderate to vigorous intensity PA (accumulated in bouts <10 mins), ₁₀MVPA 10 minute moderate to vigorous intensity PA (accumulated in bouts ≥10 mins), Total PB Total physical behaviour.

Discussion

The objective of this study was to determine whether the substitution of SB and lower intensity PA with MVPA would have positive effects on cardiovascular health and *vice versa*, in older adults. The aim was to provide a time-constrained, alternative to bivariate/multivariate regression modelling, to simulate how changes in PB would affect the cardiovascular profile of older adults. It was hypothesised that substituting SB with any intensity of PA would improve cardiovascular parameters and that substituting a PB with a higher intensity would improve cardiovascular profile. It was also hypothesised that substituting SB with $_{10}$ MVPA would have a greater effect on cardiovascular parameters than seen with sMVPA substitutions.

Heart rate is controlled by the central nervous system, which is compromised of the sympathetic and parasympathetic pathways. The simulation of the replacement of SB with Standing or sMVPA reduced resting heart rate. Physiologically, this could be achieved through improved baroreceptor function, which naturally declines with age (Gribbin et al., 1971). Given that six weeks of yoga (consisting mainly of static postures [and breathing exercises]) has been reported to improve high frequency baroreceptor sensitivity, and to reduce resting heart rate in older adults (whereas prolonged aerobic training did not) (Bowman et al., 1997), a similar effect may be at play in the Standing PB within our current modelling. High frequency baroreceptors represent the sympathetic nervous system, suggesting that vasoconstriction response was improved to counteract the natural fall in blood pressure with standing activities (Hill, 1895). Subsequently, increased vasoconstriction would increase venous return and stroke volume, which would result in the need for a lower heart rate to maintain resting cardiac output. On the other hand, the modelling of reduction in heart rate through increased sMVPA may be achieved via improvements in the parasympathetic pathway. Interval training consisting of nine, 5-minute repeated bouts at 65% of maximum heart rate (MVPA) over 14 weeks improved markers of parasympathetic activity (PNN50 [percentage of successive normal sinus RR intervals >50.0 ms] and RMSSD [root mean square of the successive normal sinus RR interval difference]) and subsequently decreased 24-hour mean heart rate within older adults (Pichot et al., 2005). Therefore, the simulations from real data in our current study suggest that reducing SB with PA, such as Standing (arguably easy to accumulate, due to the increased perceived

barriers to exercise that occur with increasing age (O'Neill and Reid, 1991)), could yield health benefits. However, engagement in MVPA is also important, as it would appear that different pathways are targeted by the two distinct PA intensities.

The reduction in resting heart may also be a result of vascular remodelling within compliant blood vessels such as the carotid and popliteal arteries, but not the stiffer brachial artery. With ageing, artery diameter increases as elastin stiffness decreases causing the load bearing to shift to collagen fibres within the vascular smooth muscle (O'rourke and Hashimoto, 2007). This structural change may not be due solely to ageing but also due to increased SB, as the substitution of LIPA with SB increased carotid AL artery diameter in our modelling. The opposite association was shown when the reverse substitution between SB and LIPA was made. These inferences are in line with previous older adult research which found an increase and decrease in carotid-femoral pulse wave velocity with increased engagement in LIPA and SB, respectively (Gando et al., 2010).

The increase in arterial stiffness with ageing is also a determinant for the fall in orthostatic blood pressure, which begins before baroreceptor mediated reflexes (Gross, 1970). Orthostatic posture increases lower limb blood pressure, which subsequently leads to an increase in total peripheral resistance and declined cardiac output. With the substitution of standing with sMVPA, popliteal artery diameter increased. This, in line with Poiseuille's Law of flow, would decrease local blood pressure and thus total peripheral resistance. However, sMVPA engagement would also acutely increase blood flow (Krustrup et al., 2004). Blood flow declines with age in the legs due to increased sympathetic activity (Dinenno et al., 1999), the latter which could increase total peripheral resistance. Training interventions within physically inactive have shown that the acute vascular responses to interval training (MVPA bouts <10 mins, representative of sMVPA) stimulates baroreceptor activity (Pichot et al., 2005) and increased artery diameter (Madsen et al., 2015), subsequently leading to improved popliteal endothelial function and distensibility (Rakobowchuk et al., 2008). Overall, our results suggest that older adults who cannot sustain MVPA for 10 continuous minutes can still attain vascular adaptations (reduced resting heart rate and increased popliteal artery diameter) that mediate improved health through engaging in sMVPA.

Conclusion

The ISM of the current study suggested that an accumulation of MVPA bouts, that are shorter than the recommended 10-minute minimum, would improve cardiovascular parameters (including resting heart rate and popliteal artery diameter), with lower intensity PA also influencing cardiovascular parameters. Our findings are therefore a promising avenue for lifestyle interventions in older adults in order to reduce the ageing effects on cardiovascular health, especially those end-users who cannot engage or sustain sufficient MVPA to be classed as physically active. The replacement of SB with PA influenced two of the 19 (resting heart rate, and carotid AL artery diameter) whilst the replacement of sMVPA with a lower intensity PB influenced one (popliteal artery diameter) cardiovascular parameter(s). The current findings suggested that the reduction of SB is just as important as the need to be physically active for older adults.

Finally, the current study illustrates the usefulness of ISM in simulating the different effects (and/or physiological pathways) that a PB outcome of interest, may have on a unique (or a set of) cardiovascular parameter(s), dependent on the PB it is displacing. This is the first study, to the authors' knowledge, to demonstrate changes in cardiovascular structure within an isothermal substitution model for an older adult cohort.

Intervention studies are needed to determine the time course of the suggested temporal changes shown in isothermal substitution modelling in older adult populations.

Part 3: 3-Dimensional heat mapping approach.

Introduction

Since 2003, it has become increasingly evident that sedentary behaviour (SB) has physiological effects that are independent from those of moderate to vigorous physical activity (MVPA) (Bey and Hamilton, 2003), resulting in an approximate 20.0% per annum increase in SB research (PubMed, key word: sedentary behaviour, date accessed: 02 February 2, 2017). In particular, SB appears to be a strong mediator of cardiovascular disease (CVD), as recent meta-analysis have suggested that increased SB carries a 71% (95% confidence interval [CI] 8.0, 148) increase in the relative risk of CVD mortality (Wilmot et al., 2012). Predictive models have mainly focused on using MVPA as a covariate for SB however, few have used light intensity physical activity (LIPA) (Buman et al., 2010; Healy et al., 2007; Fanning et al., 2016), which is hypothesised to be a method of SB reduction (Bailey and Locke, 2014), as a covariate. Recently, it has been argued that different intensities of physical behaviour (PB) (Bussmann and van den Berg-Emons, 2013) should be considered as co-dependents, rather than independents, as engagement in one form of PB will offset the amount of time that can be engaged in other forms of PB within a day (Rosique-Esteban et al., 2017). Furthermore, the physiological effects of SB independent from MVPA (Gennuso et al., 2013) may be altered by other PB engagement within the remaining hours of the day, that are not considered in traditional statistical analyses and therefore, these do not represent a 'real-world' environment (Chastin et al., 2015b). Currently, the UK government does not recommend a threshold for the maximum amount of time an adult should engage in SB. Critically, further investigation is therefore required to determine the complex interaction between SB and the different intensities of physical activity (PA) on health parameters. These studies are integral to the formulation and prescription of meaningful and/or justifiable health recommendations. For older adults, the inclusion of quantitative SB and LIPA advice in the government PA recommendations is essential as less than 5.0% of older adults attain sufficient MVPA (Craig et al., 2009) therefore, other avenues to improve health need to be highlighted which, may be more attainable for frail older adults.

The objectives of the current study are to examine whether; 1) any SB effects on older adult cardiovascular profile are present after accounting for LIPA and $_{10}$ MVPA (MVPA accumulated in bouts of ≥ 10 minutes [mins]). 2) there is consistently one or several PB profiles that display unfavourable measures of cardiovascular parameters. 3) greater engagement in one PB can offset the cardiovascular effects caused by other PB parameters.

The overarching aim was to propose a visual mapping of the interplay between PB (mainly SB, LIPA, and $_{10}$ MVPA) and cardiovascular parameters, so that the end-user (i.e. health workers, carers, older persons) may have an easy to understand evidence-based point of reference.

Based on the evidence from Chapter 03 Part 1 and Part 2, it was hypothesised that the way in which one engages in one PB or another, would be evident on the resultant cardiovascular profile markers. In addition, it was anticipated that the combination of SB, LIPA, and $_{10}$ MVPA would show the strongest (physiologically explainable) relationship with cardiovascular variables. Indeed, previous analysis using combinations that included sMVPA, and total week $_{10}$ MVPA ($\text{hrs}\cdot\text{week}^{-1}$) did not show strong patterns with cardiovascular health (Appendix Chapter 03 figure A3.3.1 - 28).

Methods

The methods of data collection follow that of Chapter 03 Part 1 ($n=93$, 73.8 ± 6.2 years [yrs], female 54%). Sedentary behaviour, LIPA, and $_{10}$ MVPA were used as measures of PB (table 3.3.1). Of the 19 cardiovascular parameters assessed in Chapter 03 Part 1 and 2, 11 (including body mass index [BMI]) were selected for 3-dimensional heat mapping analyses. Participants were grouped for each cardiovascular parameter based on government or peer-reviewed research recommended threshold health values (table 3.3.2). If no threshold recommendation was available, then the quartile thresholds were used for the given cardiovascular parameters.

Table 3.3.1. Physical behaviour parameters and definitions.

Terminology	Unit	Definition
Sedentary Behaviour (SB)	hrs·day ⁻¹	The mean amount of SB, of any length, that is accumulated in a 24hr day. Any waking behaviour characterised by a seated or reclined posture (Tremblay et al., 2010).
Light Intensity Physical Activity (LIPA)	hrs·day ⁻¹	The mean amount of LIPA, of any length, that is accumulated in a 24hr day. Any standing posture that elicits 1.50 - <3.00 METs (Tremblay et al., 2010).
≥10 Minute Moderate to Vigorous Physical Activity (₁₀ MVPA)	hrs·day ⁻¹	The mean amount of MVPA that is accumulated in bouts ≥10.0 mins in a 24hr day (Department of Health Social Services and Public Safety, 2011). Any standing posture that elicits ≥3.00 METs (Tremblay et al., 2010).

Table 3.3.2. Thresholds for cardiovascular parameters and the distribution of participants between the thresholds.

Cardiovascular Parameter	Thresholds	n
BMI (kg·m ²) (World Health Organization, 2015a)	Underweight: <18.5	0
	Healthy: 18.5-24.9	27
	Overweight: 25.0-29.9	40
	Obese I: 30.0-34.9	17
	Obese II: 35.0-39.9	6
Systolic BP (mmHg) (Mancia et al., 2007)	Obese III: ≥40.0	3
	Healthy: ≤120	9
	High Healthy: 121-139	46
	High I: 140-159	26
Diastolic BP (mmHg) (Mancia et al., 2007)	High II: ≥160	12
	Healthy: ≤80	75
	High Healthy: 81-89	14
Pulse Pressure (mmHg) (Glynn et al., 2000)	High I: 90-99	3
	High II: ≥100	1
	Healthy: <53	13
Carotid AL Artery Diameter (mm) (Van Dijk et al., 2001)	High Healthy: 53-62	27
	High I: 63-76	36
	High II: ≥77	17
Carotid Far Wall IMT (mm) (Bots et al., 1997)	Healthy: ≤6.92	31
	Moderate: 6.93-7.56	25
	High: ≥7.57	34
Resting Heart Rate (bpm) (Li, 2015)	Healthy: <0.75	41
	High Healthy: 0.75-0.821	24
	High I: 0.822-0.907	10
	High II: ≥0.908	15
Brachial Artery Diameter (mm)	Healthy: <50	7
	Moderate Healthy: 50-60	28
	High Healthy: 61-70	41
	Moderate: 71-80	15
	High: >80	2
Brachial Far Wall IMT (mm)	Q1: <3.60	23
	Q2: 3.60-4.08	23
	Q3: 4.09-4.58	23
	Q4: ≥4.59	23
Popliteal Artery Diameter (mm)	Q1: <0.42	22
	Q2: 0.42-0.51	25
	Q3: 0.52-0.59	16
	Q4: ≥0.60	30
Popliteal Far Wall IMT (mm)	Q1: <5.42	11
	Q2: 5.42-6.29	13
	Q3: 6.30-7.21	10
	Q4: ≥7.22	11
Popliteal Far Wall IMT (mm)	Q1: <0.697	11
	Q2: 0.697-0.755	11
	Q3: 0.756-0.845	11
	Q4: ≥0.846	11

Q Represents quartile thresholds. BMI Body mass index. BP Blood pressure. AL Anterior longitudinal plane. IMT Intima-media thickness.

Graphical Mapping

XLSTAT (Addinsoft, New York, USA) add-in for Excel 2013 (Microsoft Inc., Washington, USA) was used to create 3-dimensional surface heat maps. The various colours represent different health groupings in terms of the magnitude of specific cardiovascular parameters, with red, orange, yellow and green generally indicating stepwise improvements in health prognosis.

Demographics

The average engagement in PB intensities and pooled cardiovascular parameters can be found in Chapter 03 Part 1, table 3.1.4 and 3.1.5.

Body Mass Index

The majority of participants were classified as overweight (40 out of 93) or healthy (27 out of 93). Engagement in low levels (below average engagement in LIPA, ${}_{10}\text{MVPA} < 0.5 \text{ hrs}\cdot\text{day}^{-1}$) of LIPA ($< 0.85 \text{ hrs}\cdot\text{day}^{-1}$), ${}_{10}\text{MVPA} (< 0.20 \text{ hrs}\cdot\text{day}^{-1})$ and a range of SB from 2.50 – 12.5 $\text{hrs}\cdot\text{day}^{-1}$ characterised participants as obese III. As engagement in LIPA increased there was a trend for SB to decrease, when ${}_{10}\text{MVPA}$ remained at $< 0.05 \text{ hrs}\cdot\text{day}^{-1}$, which led to a decrease in participant's BMI into the obese I/overweight categories. Increasing ${}_{10}\text{MVPA} (> 0.20 \text{ hrs}\cdot\text{day}^{-1})$ whilst LIPA ($1.31 \text{ hrs}\cdot\text{day}^{-1}$) and SB ($11.4 \text{ hrs}\cdot\text{day}^{-1}$) were held constant led to a movement from obese III into the overweight category. However, to be categorised as healthy BMI, participants also had to engage in $> 2.40 \text{ hrs}\cdot\text{day}^{-1}$ of LIPA, whilst ${}_{10}\text{MVPA}$ was $> 0.20 \text{ hrs}\cdot\text{day}^{-1}$ and SB was $< 7.5 \text{ hrs}\cdot\text{day}^{-1}$. Overall, to be classed as a healthy BMI, it appears that targeting an increase in LIPA, as it tends to mirror a decrease in SB, may be the best intervention as long as participants engage in $> 0.20 \text{ hrs}\cdot\text{day}^{-1}$ of ${}_{10}\text{MVPA}$ (figure 3.3.1).

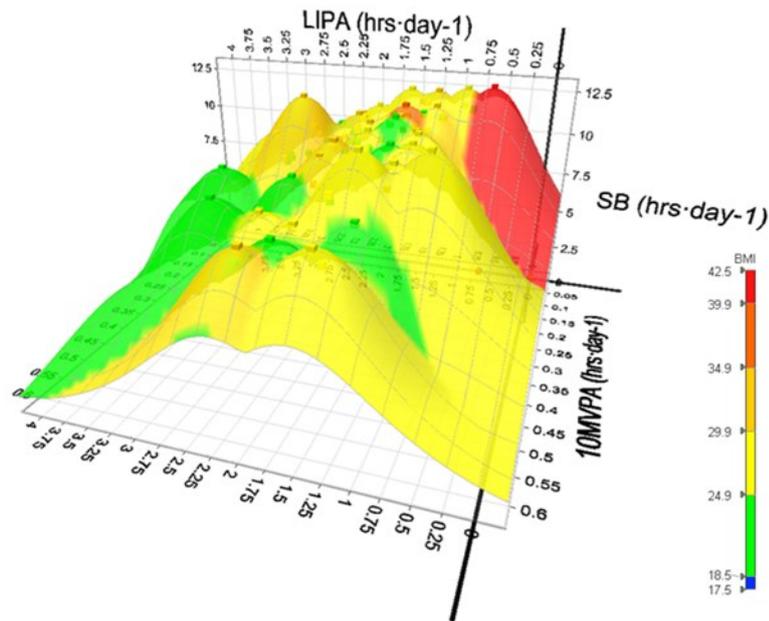


Figure 3.3.1. The combined influence of SB, LIPA, and ¹⁰MVPA on BMI in older adults. Colours represent thresholds of BMI category (kg·m²).

Systolic Blood Pressure

Over 50% of the participants were classified as having a (high) healthy systolic blood pressure (BP). However, there was no discernible influence of the combined PB, or changing PB, on systolic BP (figure 3.3.2).

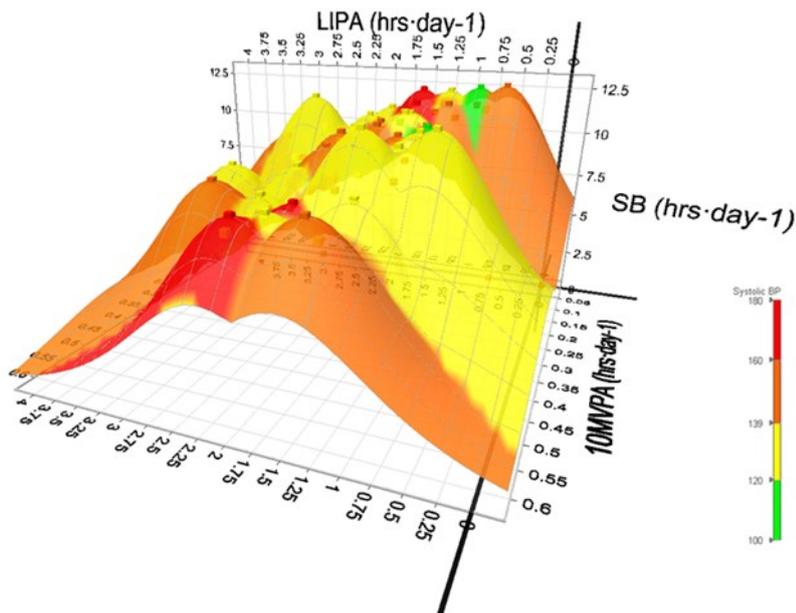


Figure 3.3.2. The combined influence of SB, LIPA, and ¹⁰MVPA on Systolic BP in older adults. Colours represent thresholds of Systolic BP (mmHg).

Diastolic Blood Pressure

Engagement in low levels of LIPA ($<0.85 \text{ hrs}\cdot\text{day}^{-1}$), $_{10}\text{MVPA}$ ($<0.20 \text{ hrs}\cdot\text{day}^{-1}$) and a range of SB from $2.50 - 12.5 \text{ hrs}\cdot\text{day}^{-1}$ characterised participants with pre-hypertensive diastolic BP. An increase in both LIPA and/or $_{10}\text{MVPA}$ from these low levels was characterised with participants displaying a healthy diastolic BP ($\leq 80.0 \text{ mmHg}$) (figure 3.3.3), which additionally, was present in more than three quarters of participants.

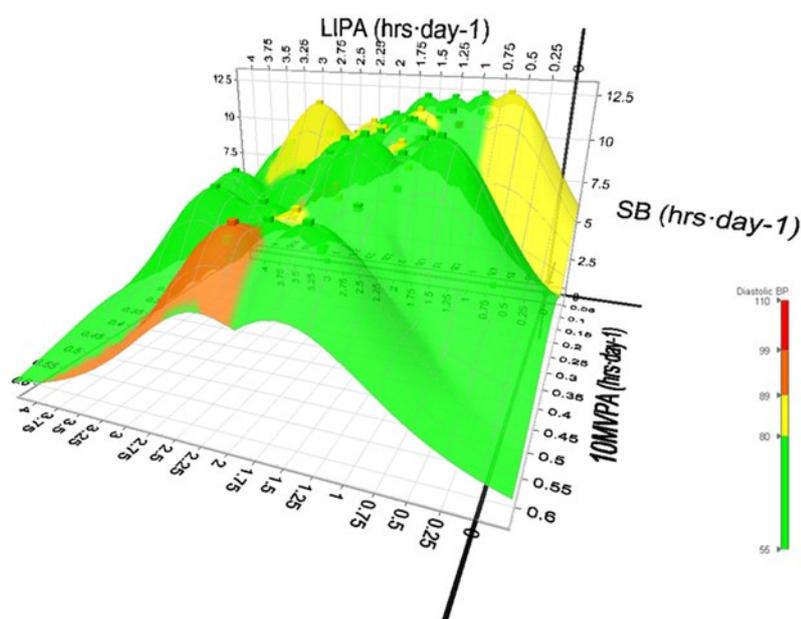


Figure 3.3.3. The combined influence of SB, LIPA, and $_{10}\text{MVPA}$ on Diastolic BP in older adults. Colours represent thresholds of Diastolic BP (mmHg).

Pulse Pressure

There was no clear association between engagement in SB, LIPA, $_{10}\text{MVPA}$, and pulse pressure (figure 3.3.4). A high pulse (63 – 76 mmHg) pressure was the most prevalent category (36 out of 93) in the older adult population (table 3.3.2).

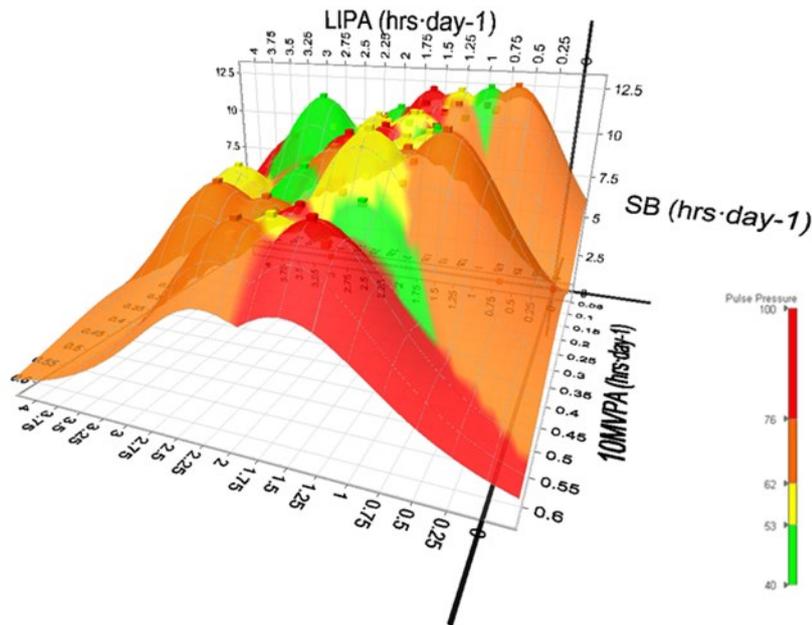


Figure 3.3.4. The combined influence of SB, LIPA, and $_{10}MVPA$ on pulse pressure in older adults. Colours represent thresholds of pulse pressure (mmHg).

Carotid Artery Diameter

The distribution of participants across the three categories of carotid AL artery diameter was fairly even (Table 3.3.2). High SB ($>11.5 \text{ hrs}\cdot\text{day}^{-1}$) and low $_{10}MVPA$ ($<0.2 \text{ hrs}\cdot\text{day}^{-1}$) characterized participants with large carotid AL artery diameter (7.57 – 11.0 mm). Artery diameter remained large as LIPA increased until participants accumulated $>2.0 \text{ hrs}\cdot\text{day}^{-1}$ of LIPA, after which participants were characterized with a healthy artery diameter ($<6.92 \text{ mm}$). In participants completing $<2.0 \text{ hrs}\cdot\text{day}^{-1}$ of LIPA, increasing $_{10}MVPA$ reduced artery diameter to a moderate level, regardless of SB engagement. Low SB ($<8.00 \text{ hrs}\cdot\text{day}^{-1}$), high $_{10}MVPA$ ($>0.5 \text{ hrs}\cdot\text{day}^{-1}$), and high LIPA ($>2.0 \text{ hrs}\cdot\text{day}^{-1}$) had a trend for participants to display a healthy artery diameter (figure 3.3.5).

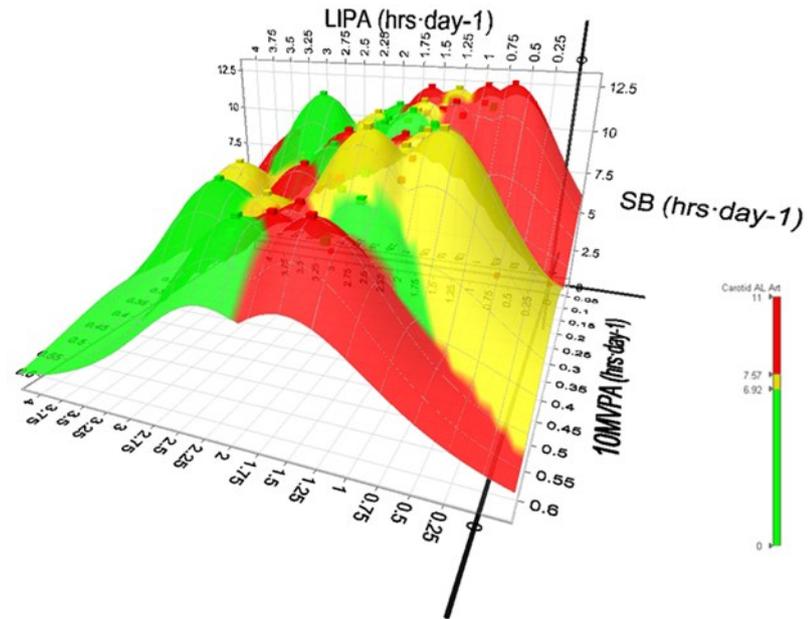


Figure 3.3.5. The combined influence of SB, LIPA, and $_{10}MVPA$ on carotid AL artery diameter in older adults. Colours represent thresholds of carotid AL artery diameter (mm).

Carotid Intima-Media Thickness

Low $_{10}MVPA$ (<0.2 hrs·day $^{-1}$) and high SB (>10.0 hrs·day $^{-1}$) was characterised with moderately sized IMT (0.75 – 0.821 mm), even as LIPA increased. Increasing $_{10}MVPA$ above 0.20 hrs·day $^{-1}$, independent of change in SB or LIPA was generally characterized with a healthy IMT (figure 3.3.6), which was the most prevalent category (41 out of 90) for the older adult's IMT.

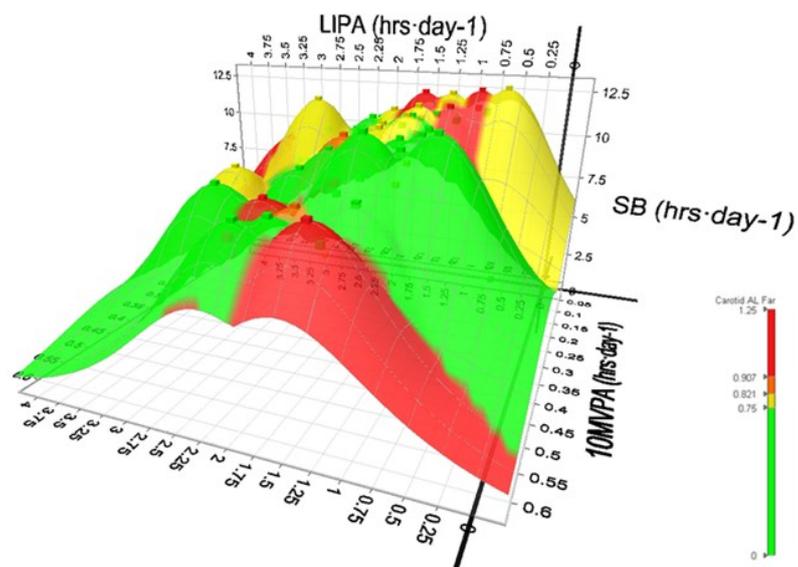


Figure 3.3.6. The combined influence of SB, LIPA, and $_{10}MVPA$ on carotid AL far wall IMT in older adults. Colours represent thresholds of carotid AL far wall IMT (mm).

Resting Heart Rate

All participants fell within the normal resting heart rate range (40 – 100 bpm). There appeared to be a relationship between LIPA and $_{10}$ MVPA time trade-off, and participant's heart rate falling above and below the 60 bpm 'ideal' threshold. If participants engaged in no $_{10}$ MVPA, they needed to engage in >2.00 hrs·day⁻¹ of LIPA to have a resting heart rate <60 bpm. However, as $_{10}$ MVPA engagement increased, less LIPA engagement was required to have a heart rate below 60 bpm (e.g. $_{10}$ MVPA: 0.44 hrs·day⁻¹, LIPA: 1.39 hrs·day⁻¹, heart rate: 57 bpm). SB appeared to have little to no influence on resting heart rate, as participants either side of this LIPA- $_{10}$ MVPA threshold line had similar SB engagement (figure 3.3.7).

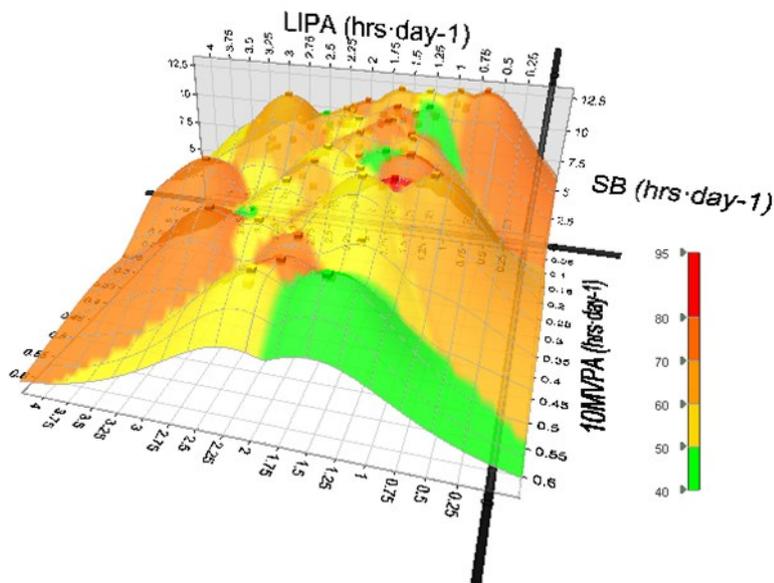


Figure 3.3.7. The combined influence of SB, LIPA, and $_{10}$ MVPA on resting heart rate in older adults. Colours represent boundaries of resting heart rate (bpm).

Brachial Artery Diameter

Those with low $_{10}$ MVPA (<0.20 hrs·day⁻¹) tended to exhibit a brachial artery diameter in the upper two quartiles, even with increasing LIPA. Those with the highest levels of SB (>11.0 hrs·day⁻¹) tended to exhibit an artery diameter in the upper quartile. Individuals with moderate engagement in $_{10}$ MVPA ($0.20 - 0.50$ hrs·day⁻¹) commonly had an artery diameter within the lowest quartile. However, occasions where SB engagement was >11.0 hrs·day⁻¹, participants displayed an

increased artery diameter. Interestingly, physically active participants ($_{10}MVPA$: >0.50 hrs·day⁻¹) also had a trend to have an increase artery diameter (figure 3.3.8).

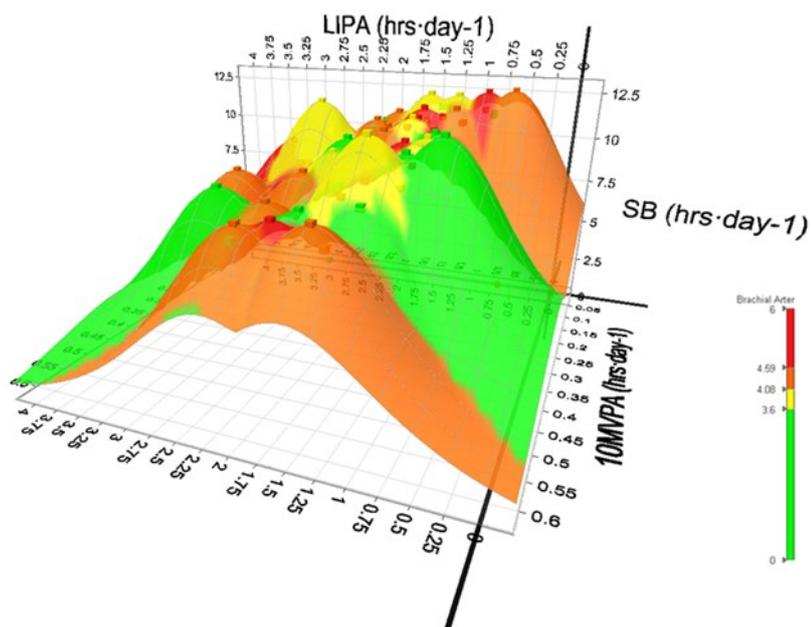


Figure 3.3.8. The combined influence of SB, LIPA, and $_{10}MVPA$ on brachial artery diameter in older adults. Colours represent quartiles of brachial artery diameter (mm).

Brachial Intima-Media Thickness

There appeared to be an optimal range for LIPA (1.00 – 2.50 hrs·day⁻¹), $_{10}MVPA$ (0.10 – 0.50 hrs·day⁻¹, and SB (<11.0 hrs·day⁻¹) engagements for participants to fall within the lowest quartile of IMT. Interestingly, high LIPA (>3.50 hrs·day⁻¹) and $_{10}MVPA$ (>0.50 hrs·day⁻¹) was characterized with IMT in the upper two quartiles (figure 3.3.9).

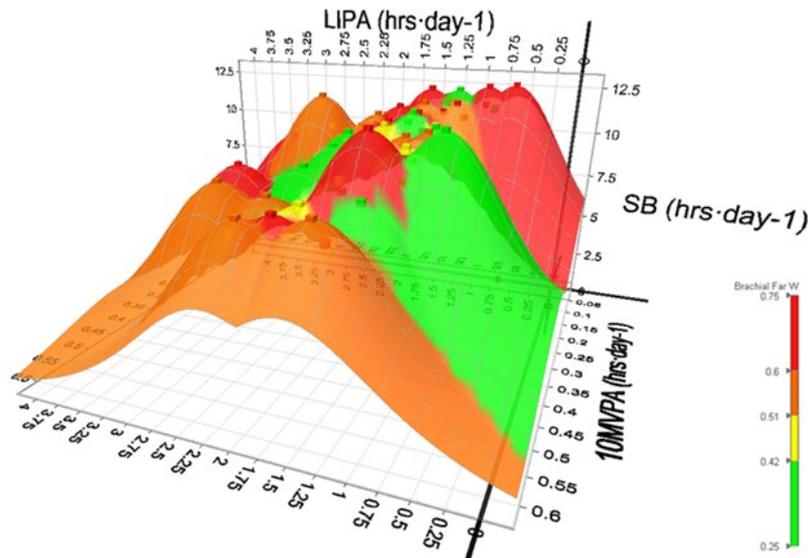


Figure 3.3.9. The combined influence of SB, LIPA, and $_{10}MVPA$ on brachial far wall IMT in older adults. Colours represent quartiles of brachial far wall IMT (mm).

Popliteal Artery Diameter

Participants with <2.00 hrs·day⁻¹ of LIPA, high SB (>9.00 hrs·day⁻¹), and were physically inactive ($_{10}MVPA$: <0.50 hrs·day⁻¹) were characterized with an artery diameter in the upper two quartiles. However, those with high LIPA (>3.50 hrs·day⁻¹) or were physically active ($_{10}MVPA$: >0.50 hrs·day⁻¹) fell within the lowest quartile of artery diameter (figure 3.3.10).

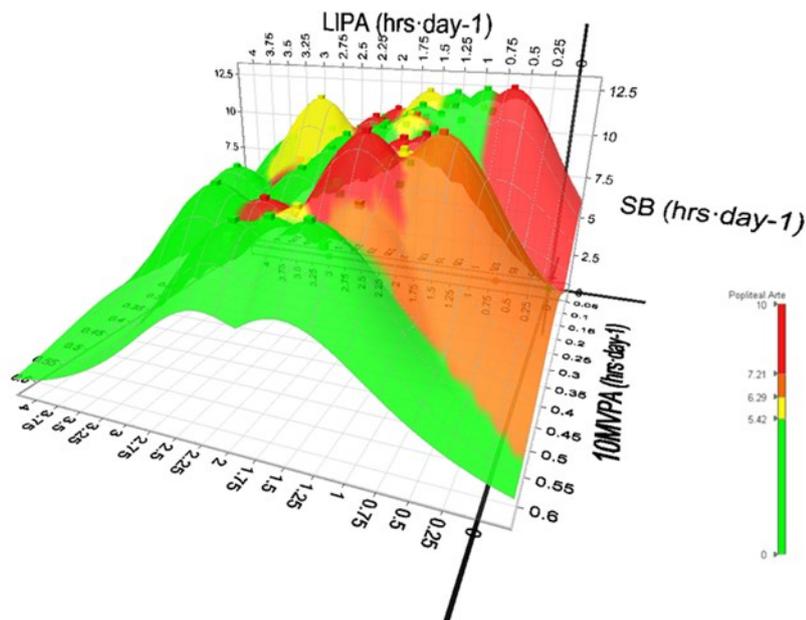


Figure 3.3.10. The combined influence of SB, LIPA, and $_{10}MVPA$ on popliteal artery diameter in older adults. Colours represent quartiles of popliteal artery diameter (mm).

Popliteal Intima-Media Thickness

Participants with LIPA engagement $>2.00 \text{ hrs}\cdot\text{day}^{-1}$ were characterized within the lowest quartile of IMT. There was a trend for those with low LIPA ($<2.00 \text{ hrs}\cdot\text{day}^{-1}$) and high SB ($>9.00 \text{ hrs}\cdot\text{day}^{-1}$) to have a greater IMT, if their $_{10}\text{MVPA}$ was $<0.50 \text{ hrs}\cdot\text{day}^{-1}$. Participants who were physically active ($_{10}\text{MVPA}: \geq 0.50 \text{ hrs}\cdot\text{day}^{-1}$) fell within the lowest quartile of IMT, regardless of LIPA and SB engagement (figure 3.3.11).

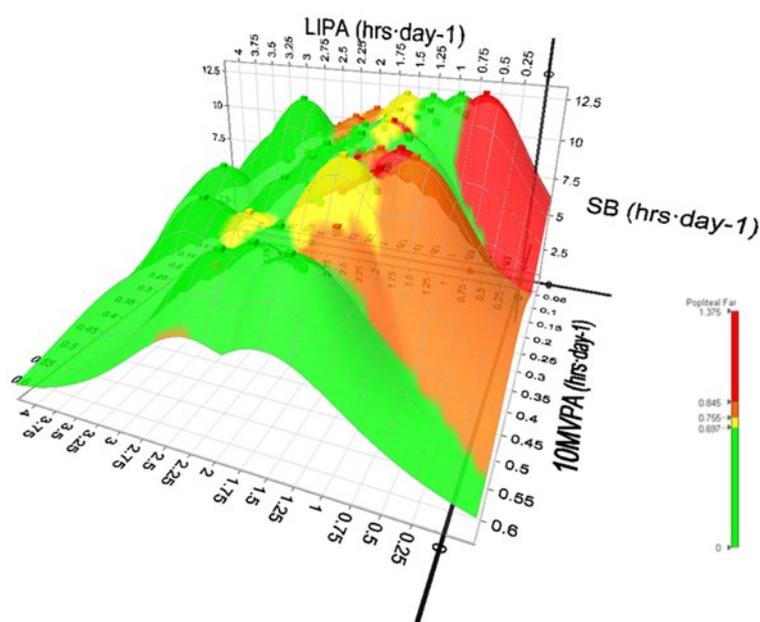


Figure 3.3.11. The combined influence of SB, LIPA, and $_{10}\text{MVPA}$ on popliteal far wall IMT in older adults. Colours represent quartiles of popliteal far wall IMT (mm).

Discussion

The objectives of the study were: 1) to illustrate any SB effect on older adults' cardiovascular health after accounting for LIPA and $_{10}\text{MVPA}$, 2) to illustrate whether there is consistently one or several PB profiles that display unfavourable measures of cardiovascular parameters, 3) to illustrate whether a greater engagement in one PB can offset the cardiovascular effects caused by other PB parameters.

The overarching aim was to propose an end-user (i.e. health workers, carers, older persons) friendly visual representation of the interaction between absolute

values of cardiovascular parameters and PB including mainly SB, LIPA, and $_{10}$ MVPA.

Body Composition

Within older adults, BMI follows a U-shaped relationship with mortality risk (Winter et al., 2014). Thus, overweight older adults have a lower hazard ratio risk of all-cause mortality than those with a healthy BMI ($23.5 \text{ kg}\cdot\text{m}^2$) (Winter et al., 2014). Increased risk of death does not occur with increasing BMI until $33.5 \text{ kg}\cdot\text{m}^2$ (Winter et al., 2014), which is Obese I within the current study. Based on being classified as obese I, the present findings suggest that leading a high sedentary ($>12.0 \text{ hrs}\cdot\text{day}^{-1}$), low physical activity (low LIPA [$<1.0 \text{ hrs}\cdot\text{day}^{-1}$], low $_{10}$ MVPA [$<0.1 \text{ hrs}\cdot\text{day}^{-1}$]) lifestyle may be associated with an increased risk of death. In order to improve BMI status to a healthy classification, it is recommend based on our data that older adults should reduce SB ($<7.5 \text{ hrs}\cdot\text{day}^{-1}$) and increase LIPA ($>3.5 \text{ hrs}\cdot\text{day}^{-1}$) without needing to increase $_{10}$ MVPA. This may be a more palatable message for high sedentary, low MVPA older adults to engage with, as most LIPA, as with SB, can be performed in conjunction with other activities, such as playing cards, bingo, and social group meetings and therefore, may not require routine adjustment, which is likely to occur when asked to increase $_{10}$ MVPA engagement. Within the UK, a lack of provision for non-sedentary behaviour and an expectation that older adults must/want to sit is highlighted as a determinant for prolonged sitting within community groups (Chastin et al., 2014).

Cardiovascular Profile

The risk of CVD death increases 6% per 10 bpm increase in resting heart rate (Li, 2015). The results of the current study suggested two possible targets for intervention, increasing LIPA or $_{10}$ MVPA. For participants to have a heart rate below 60 bpm, they either engaged in no $_{10}$ MVPA and $>2.0 \text{ hrs}\cdot\text{day}^{-1}$ of LIPA or they engaged in $>0.44 \text{ hrs}\cdot\text{day}^{-1}$ of $_{10}$ MVPA and $>1.39 \text{ hrs}\cdot\text{day}^{-1}$ of LIPA. Participants who were below these LIPA and $_{10}$ MVPA thresholds tended to display a resting heart

rate within the 61-70 or 71-80 bpm groupings suggesting an increased risk of CVD death. Interestingly, the LIPA and $_{10}$ MVPA showed no associations with resting heart rate in Chapter 03 Part 1 and Part 2 which, is probably a result of the statistics not being suitable to extract this complex trade-off between LIPA and $_{10}$ MVPA thus warranting the need to also visualise data where possible.

A 0.78 mm increase in carotid artery diameter is associated with a 2.0 (95%CI 1.3, 3.3) hazard risk of mortality over a 6.6 year follow up in older adults (Van Dijk et al., 2001). The average artery diameter of those who died during follow-up in the aforementioned study of Van Dijk et al. (2001) was 7.57 ± 0.91 mm. The current study suggested that those who are highly sedentary and engage in little PA (LIPA and $_{10}$ MVPA) will tend to have a carotid artery diameter above 7.57 mm. There was a trend for artery diameter to reduce as LIPA and $_{10}$ MVPA increased and SB decreased. This is consistent with isotemporal substitution models from Chapter 03 Part 2, which found carotid artery diameter, would decrease by 0.54 mm with the replacement of one hour of SB with LIPA. It appeared that older adults needed to engage in $_{10}$ MVPA for at least $0.4 \text{ hrs}\cdot\text{day}^{-1}$ and $4.0 \text{ hrs}\cdot\text{day}^{-1}$ of LIPA with $<8.0 \text{ hrs}\cdot\text{day}^{-1}$ of SB to have the best chance of being characterised with a healthy carotid artery diameter, as other accumulation patterns were sporadic in artery diameter.

Participants who engaged in $<0.2 \text{ hrs}\cdot\text{day}^{-1}$ of $_{10}$ MVPA tended to be characterised with a carotid IMT between 0.75 – 0.821 mm or >0.907 mm, which are suggested to carry a 2.28 and 4.21 increased odds ratio for stroke events, respectively, compared with those with an IMT <0.75 mm (Bots et al., 1997). For participants to likely be classified with an IMT <0.75 mm within the current study, the data suggested that older adults needed to engage in at least $0.2 \text{ hrs}\cdot\text{day}^{-1}$ of $_{10}$ MVPA, which equates to one 10-minute bout of MVPA per day. However, this bout of MVPA may need to be of a vigorous intensity as previous evidence has shown reductions in IMT with vigorous intensity PA (Kozàková et al., 2010) but not moderate intensity PA (Tanaka et al., 2002; Rakobowchuk et al., 2008).

There is a trend for brachial artery diameter to increase with age (Green et al., 2010). The current findings suggested that low levels of $_{10}$ MVPA resulted in participants displaying a brachial artery diameter >3.6 mm. As $_{10}$ MVPA increased above $0.2 \text{ hrs}\cdot\text{day}^{-1}$, an artery diameter <3.6 mm was most prevalent. Interestingly, high engagement in SB appeared to attenuate this observation. Suggesting that SB may influence artery diameter independent of $_{10}$ MVPA. Furthermore, those

participants who were physically active (>0.5 hrs·day⁻¹ of $_{10}$ MVPA) had larger brachial artery diameters. This may suggest a U-shaped continuum for brachial artery diameter and $_{10}$ MVPA. As gradual increases in $_{10}$ MVPA may attenuate age related increases in brachial artery diameter however, as $_{10}$ MVPA engagement exceeds 0.5 hrs·day⁻¹, it becomes sufficient to stimulate healthy arterial remodelling (Green et al., 2010). In fact, further analysis supported this hypothesis as brachial artery diameter tended to be smaller in participants that engaged in $0.21 - 0.49$ hrs·day⁻¹ of $_{10}$ MVPA compared to participants with ≤ 0.2 hrs·day⁻¹ of $_{10}$ MVPA engagement. Meanwhile, those who were physically active (≥ 0.5 hrs·day⁻¹ of $_{10}$ MVPA) had the largest brachial artery diameter (Appendix Chapter 03 figure A3.3.29).

Interestingly, the current study suggested that engaging in the upper levels of PB (SB: ≥ 11 hrs·day⁻¹, LIPA: >2.5 hrs·day⁻¹, $_{10}$ MVPA: ≥ 0.5 hrs·day⁻¹) characterised participants with a brachial IMT in the upper two quartiles (>0.51 mm). This does not support previous findings that suggested older adult's brachial IMT (baseline: 525 ± 18.7 μ m) reduces after 12 weeks of LIPA (488 ± 17.9 μ m) and then further reduces after an additional 12 weeks of $_{10}$ MVPA (454 ± 15.5 μ m) (Green et al., 2010). In addition, neither Chapter 03 Part 1 nor Part 2 showed any associations between PB and brachial IMT, therefore the visual pattern in the current study would need further research to try to explain the patterns described.

Similar to the brachial artery, popliteal artery diameter increases with age (Green et al., 2010). An enlarged popliteal artery in older adults can be a sign of an impending aneurysm, which is particularly important to diagnose, as approximately one-third of patients will have an abdominal aortic aneurysm (Dawson et al., 1997), which carries an estimated in-hospital mortality rate of 65.9% in the United Kingdom, if rupture occurs (Karthikesalingam et al., 2014). The current study suggested that those who engaged in >2 hrs·day⁻¹ of LIPA and <9 hrs·day⁻¹ of SB would likely be characterised with a popliteal artery diameter in the lowest quartile. In addition, physically active ($_{10}$ MVPA: ≥ 0.5 hrs·day⁻¹) participants also had popliteal artery diameters that were in the lowest quartile of size, regardless of SB engagement. It seemed that these levels of LIPA and $_{10}$ MVPA might be sufficient to negate the effects of SB, which appeared to push participants into the upper two quartile of artery diameter if they engaged in >12 hrs·day⁻¹ of SB. In contrast, Chapter 03 Part

1 and Part 2 displayed no association between SB, LIPA, $_{10}MVPA$ and popliteal artery diameter.

Popliteal IMT increases with age (Green et al., 2010) and is a common site for atherosclerotic disease. The current study suggested that participants who are not physically active ($_{10}MVPA$: $<0.5 \text{ hrs}\cdot\text{day}^{-1}$) need to engage in $>2 \text{ hrs}\cdot\text{day}^{-1}$ of LIPA and $<12 \text{ hrs}\cdot\text{day}^{-1}$ of SB to fall within the lowest IMT quartile. High engagement in SB appeared to not effect popliteal IMT if participants were physically active ($_{10}MVPA$: $\geq 0.5 \text{ hrs}\cdot\text{day}^{-1}$), suggesting older adults could offset the impact of prolonged SB. It was highly apparent however, that a highly sedentary, physically inactive, and low LIPA lifestyle carries a high likelihood of an enlarged popliteal IMT. This observation is in-line with Chapter 03 Part 1, which also highlighted that increasing LIPA would decrease popliteal IMT as well as a trend for popliteal IMT to decrease and increase with an increase in $_{10}MVPA$ and SB, respectively.

Conclusion

It is apparent that there are complex interactions between PB intensities and their subsequent effect on cardiovascular health markers. The improvement in some cardiovascular variables with increasing LIPA is a promising observation from the current study. This suggests that older adults may be able to improve their health through a greater engagement in activities that are within their physical capability and can be completed with limited logistical requirements. In addition, an engagement in $>2.0 \text{ hrs}\cdot\text{day}^{-1}$ of LIPA appeared to improve cardiovascular parameters and to be associated with a reduction in SB, which is also an important PB to limit prolonged engagement in, as the current study suggested SB above 8 – 11 $\text{hrs}\cdot\text{day}^{-1}$ could negatively affect cardiovascular profile. Finally, this study added further support to maintaining a physically active lifestyle, if capable, as there was a clear improvement in profile for some cardiovascular markers when $_{10}MVPA$ exceeded $0.5 \text{ hrs}\cdot\text{day}^{-1}$.

Overall, the current study suggests that older adults should aim to be physically active and reduce SB engagement. If a physically active lifestyle is not possible, or physically inactive older adults aim to improve their health, then increasing engagement in LIPA may be a useful starting point (especially where cardiovascular health is concerned).

Chapter 04:

Further ageing.

'Physical behaviour impact on cardiovascular further ageing.'

Physical behaviour impact on cardiovascular further ageing.

Introduction

As life expectancy increases, the 60 year-old and above age group are predicted to account for 25.0% of the UK population by the year 2035 (Office for National Statistics, 2012a). This may cause a socio-economic imbalance, particularly in the National Health Service (NHS), as average annual inpatient healthcare spending per person increases exponentially after the age of 50 years (50 year-old male: £605, 70 year-old male: £1,834, 89 year-old male: £5,198) (E. Kelly et al., 2016). With adults over the age of 65 years only expected to spend 55.0% of their remaining years of life in 'good' health (Office for National Statistics, 2016) the question becomes: can changes in older adults' lifestyle offset the natural ageing process and subsequently increase healthy life expectancy? Cardiovascular disease (CVD) is the leading causes of death in older adults after cancer (Townsend et al., 2015) but its mortality risk can be adjusted through changes in physical behaviour (PB) (Bussmann and van den Berg-Emons, 2013). Physically active adults (150 mins·wk⁻¹ of moderate-vigorous intensity physical activity [MVPA], accumulated in bouts ≥10.0 mins [₁₀MVPA] (Department of Health Social Services and Public Safety, 2011)) are predicted to reduce their risk of CVD mortality by 35.0% (95% confidence interval [CI] 30.0, 40.0) (Nocon et al., 2008) compared to the physically inactive whereas, sedentary behaviour (SB) can increase CVD mortality risk by 90.0% (95%CI 36.0, 166) (Wilmot et al., 2012). Although life expectancy at age 65 years (yrs) has increased year on year in the UK (1.43 yrs increase between 2001 – 2008 (Office for National Statistics, 2013)), healthy life expectancy has increased at a much slower rate (0.65 yrs increase between 2001 – 2008 (Office for National Statistics, 2012b)). Therefore, to inform how the gap between life expectancy and healthy life expectancy can be reduced, PB research should further investigate the impact on morbidity using CVD markers, such as common carotid artery diameter, which increases with age ($r^2=0.14$, $p<0.001$, age: 73.3±5.8 yrs) (Polak et al., 1996) and is positively associated with multiple CVD risk factors in adults aged 55 years (Jensen-Urstad et al., 1999). Chapter 03 has shown

that isothermoral substitution modelling suggest that older adults' carotid artery diameter can be reduced by 0.54 (95%CI 1.00, 0.07) mm with the replacement of one hour of SB with light intensity PA (LIPA). As well as total PB time, patterns of PB are also predictors of cardiovascular health status (Healy et al., 2008b; O'Donovan et al., 2017; Chastin et al., 2015a). An increase in accumulated sedentary time using bouts ≥ 10 mins is associated with an increase in carotid intima-media thickness (IMT) (García-Hermoso et al., 2015), which is a predictor of stroke in old age (72.5 \pm 5.5 yrs) (O'Leary et al., 1999) (1.49 [95%CI 1.37, 1.62] relative risk per 0.20 mm carotid IMT increase) and also increases with age (10 – 69 years) (Farro et al., 2012), suggesting SB engagement could accelerate the ageing process.

Although the distribution of the population is shifting to an increased older adult population and life expectancy has increased, the majority of ageing research has investigated the transition between young (18 – 30 yrs) to middle (40 – 60 yrs) or middle to old (60 – 80 yrs) age groups (Celermajer et al., 1994; Black et al., 2009; Dinunno et al., 1999; Dinunno et al., 2000; Eigenbrodt et al., 2006; Green et al., 2010; Moreau et al., 2006; Pierce et al., 2011; Tanaka et al., 2002; Van den Munckhof et al., 2012; van der Heijden-Spek et al., 2000). Few studies have investigated the transition from old age to older (80+ yrs) age populations (Ferrara et al., 1997; Fritze et al., 2012) i.e. further ageing. Therefore, the aims of this study are to determine which cardiovascular profile markers are affected by further ageing in older adults aged ≥ 60 yrs and to recommend what and/or how PB profile can be optimized to offset these further ageing effects. The objectives are to highlight changes in cardiovascular profile with further ageing with and without adjustment for total PB time and patterns of PB. It is hypothesised that physical activities may attenuate the effect of further ageing on cardiovascular profile (Skoumas et al., 2003) whereas SB may accelerate the further ageing process (García-Hermoso et al., 2015). In addition, as patterns of PB appear to be stronger predictors of cardiovascular profile (Chapter 03), they may also be the strongest adjusters of further ageing. Using a cross-sectional study design, participants will be grouped per lustrum of older age in order to capture greater details of the further ageing related sensitivity to PB.

Methods

Ninety-three older adults were recruited and grouped based on their age (60 – 65 yrs: $n=10$, 66 – 71 yrs: $n=21$, 72 – 77 yrs: $n=35$, 78 – 83 yrs: $n=20$, 84+ yrs: $n=7$). Participant recruitment and data collection follows that of the methods of Chapter 03. A brief methods is outlined below.

First Laboratory Visit

Participants were fitted with a thigh mounted triaxial accelerometer (GENEA, GENEActiv Original, Activinsights Ltd, Kimbolton, UK) on the dominant leg (anterior aspect, at 50% of greater trochanter to femoral condyle distance) using a waterproof adhesive patch (3M Tegaderm Film, North Ryde, Australia), for seven consecutive free-living days. Leg dominance was determined from leg preference for a single leg balance exercise. GENEActiv Residual G PB intensity cut-off points were developed against concurrent expired gas analysis (Metabolic Equivalent Tasks [METs]) of a sub-population of 20 participants during ten different PB(s) ($r^2=0.89$, $p<0.001$). SB-LIPA (1.50 METs) cut-off point was 0.057 Residual G, while the LIPA-MVPA (3.00 METs) cut-off point was 0.216 Residual G. SB and Standing posture allocation was determined using methods similar to that of the '*Sedentary Sphere*' (Rowlands et al., 2014). Intensity was classed as Standing if posture was classed as standing but Residual G was below the SB-LIPA cut-off point. This analysis used 10.0 second (s) epoch GENEActiv data (60.0 Hertz [Hz]) and was labelled as The Cheshire Algorithm for Sedentarism (CAS). Sleeping hours data was triangulated using a self-reported Sleep Diary (wake-up time, lights-off go to sleep time, naps not included) throughout the monitoring week. In brief, Total PB variables are displayed as the average daily engagement over seven days of monitoring. Patterns of PB variables are displayed as the average daily amount of bouts or average duration of bouts. The more complex calculations of patterns of PB variables are outlined in table 4.1. A complete list of definitions for PB variables within the current study can be found in Chapter 02 Part 1. Height, mass, body mass index (BMI), and medication use was also collected during this visit.

Table 4.1. Brief definitions of GENE variables.

GENEA Variable	Definition
Alpha ($\alpha \cdot \text{day}^{-1}$)	How steeply the number of SB bouts decreases with increasing SB bout duration in a power-law distribution. $= 1 + n(1 \div \sum \ln(\text{SB bout length} \div \text{smallest SB bout length}))$ (Chastin and Granat, 2010).
W50% (mins $\cdot\text{day}^{-1}$)	50% of SB time in a 24hr day is accumulated by SB bouts of this specific length or shorter. $= (\sum \text{SB bout lengths} > \text{median SB bout length}) \div \text{total SB engagement}$. (Chastin and Granat, 2010) NB. Median SB bout length criteria can be changed (increase or decrease SB bout length) until the equation equals 0.5 (50%). This criterion SB bout length is the W50%.
True Mean SB Bout (mins $\cdot\text{day}^{-1}$)	SB time (≥ 1.00 mins) between PA bouts of ≥ 2.00 mins are LOG transformed to normally distribute the data and then anti-logged to find the “True Mean” (Chastin et al., 2015c).
True Mean PA Bout (mins $\cdot\text{day}^{-1}$)	PA time (≥ 2.00 mins) between SB bouts of ≥ 1.00 mins are LOG transformed to normally distribute the data and then anti-logged to find the “True Mean” (Chastin et al., 2015c).

Second Laboratory Visit

Participants arrived in an overnight fasted, hydrated state and were provided a standardised meal (49.0% carbohydrate, 38.5% protein, 12.5% fat) before the testing session started.

A three lead electrocardiogram (ECG), to measure heart rate and the diastolic phase of the cardiac cycle, was fitted to participants before resting in the supine position for 15.0 minutes (mins). Light intensity (20.0 $\text{lm}\cdot\text{ft}^2$) and room temperature (22.0 $^{\circ}\text{C}$) were kept constant throughout testing. Hydration status, as a

percentage of total body mass, was determined using bioelectrical impedance (BodyStat 1500, BodyStat, Douglas, UK).

An echo Doppler ultrasound (model AU5; Esaote, Genova, Italy) using a 7.50 MHz broadband linear array transducer was used for vascular assessment (angle of insonation: 60.0°, B gain: 75.0, Doppler gain: 49.0, CFM gain: 47.0, depth of penetration: 49.3 mm, depth of focus: 27.0 – 31.0). Live streamings were recorded on a Hewlett-Packard computer running video capture software through an analogue to digital converter (Pinnacle, Corel Inc., Ottawa, Canada) at 25 Hz. Left common carotid artery and right brachial artery assessments were performed in the supine position whilst left popliteal artery assessments were in the prone position. Ten cardiac cycles were used to assess baseline IMT and artery diameter for all three arteries. Assessments took place within a 10 mm region of interest (ROI), 10 mm distal of the carotid bulb in the anterior longitudinal (AL) plane, 65% of upper-arm length (acromion process to lateral radial head) distal of the glenohumeral joint for the brachial artery, and 10 mm distal of the superior medial genicular bifurcation of the popliteal artery (Harris et al., 2010; Altin et al., 2005; Burke et al., 1995; Touboul et al., 2012; Naqvi and Lee, 2014).

Offline analysis of artery diameter measures for all arteries was performed using Brachial Analyzer (Medical Imaging Application LLC, Iowa, USA) whilst Carotid Analyzer (Medical Imaging Application LLC, Iowa, USA) was used for IMT measures. Data was then R-gated to ensure artery diameter and IMT was measured during the diastolic phase only. R-gated frame-to-frame measurements that did not use 70.0% of the ROI and/or were more than one standard deviation (SD) from the mean artery diameter were filtered from final analysis. All automated processes were checked for error by one researcher. Inter/Intra-day coefficients for variation (CV) are mentioned previously (Chapter 03 Part 1).

Statistical Analyses

Statistical analyses were performed using SPSS Statistics version 22 (IBM, New York, USA). A 1×5 one way Analysis of Variance (ANOVA) (or Kruskal-Wallis for non-parametric data, violation of Kolmogorov-Smirnov and/or Levene's test) was used to determine whether cardiovascular profile markers changed with further

ageing. Simple contrast analysis (Mann-Whitney U for non-parametric data) was used post-hoc to determine at which age cardiovascular markers became significantly different from that of the youngest age group. Two Analysis of covariance (ANCOVA) tests were used to determine if total PB(s), or patterns of PB(s), influenced the rate of further ageing. Once again, simple contrast analysis was used post-hoc to determine whether the cardiovascular markers were significantly different from that of the youngest age group after PB engagement had been considered. PB parameters were considered covariates if they displayed predictive qualities for the aforementioned cardiovascular markers within Chapter 03 Part 1. Covariates were added into the ANCOVA model in order of highest to lowest adjusted explained variance from Chapter 03 Part 1. One non-significant covariate was removed from the model (from lowest explained variance to highest) at a time until the ANCOVA model was stable (only significant covariables included). A 1×5 one way ANOVA (or Kruskal-Wallis for non-parametric data, violation of Kolmogorov-Smirnov and/or Levene's test) was used to determine whether demographics were different between age groups. Bonferroni tests (Mann-Whitney U for non-parametric data) were used post-hoc to highlight which age group's demographics were significantly different from one another. Natural LOG transformed cardiovascular parameter data is presented if all age groups became parametric following LOG transformation of non-parametric data. Parametric data is presented as Mean ± SD and non-parametric data as Median (Interquartile Range [IR]). Level of significance was set at $p \leq 0.05$.

Results

Ninety-three community-dwelling older adults (60-89 yrs) participated in the study, their demographics and PB data are displayed in table 4.2.

Table 4.2. Participant demographics and PB profiles

Demographics	Age Group				
	60-65	66-71	72-77	78-83	84+
<i>n</i>	10	21	35	20	7
Female (%)	90	48	57	50	43
Height (m)	1.66±0.09	1.62±0.06	1.65±0.09	1.69±0.07	1.66±0.10
Mass (kg)	71.6±13.7	72.9±14.3	76.9±12.8	77.4±12.6	82.4±10.6
BMI (kg·m ²)	30.9±6.44	27.8±4.81	26.9±4.43 ^a	27.5±3.19	29.8±5.75
Daily Medication Use (%)	70	80	77	90	71
Total PB					
SB (hrs·day ⁻¹)	9.81±1.30	9.24±1.38	9.72±1.25	9.73±1.47	10.1±1.15
Standing (hrs·day ⁻¹)	1.19±0.30	1.16±0.41	1.09±0.40	1.06±0.44	0.93±0.49
LIPA (hrs·day ⁻¹)	2.11±0.49	1.93±0.61	1.99±0.60	1.98±0.73	1.80±0.87
sMVPA (hrs·day ⁻¹)	2.68±0.58	2.89±0.57	2.56±0.54	2.39±0.77 ^b	2.11±0.71 ^b
₁₀ MVPA (hrs·day ⁻¹)	0.06(0.16)	0.17(0.28)	0.06(0.17) ^b	0.09(0.26)	0.00(0.05) ^{bd}
Patterns of PB					
SB Breaks (<i>n</i> ·day ⁻¹)	21.3±2.57	23.6±2.73	21.9±3.85	21.6±4.16	22.2±3.03
<5min SB Bout (<i>n</i> ·day ⁻¹)	6.01±1.56	6.52±1.33	6.61±2.22	5.79±2.02	6.94±2.34
≥5min SB Bout (<i>n</i> ·day ⁻¹)	16.0±1.49	17.9±2.10 ^a	16.1±2.29 ^b	16.2±2.97 ^b	16.1±1.42
True Mean SB Bout (mins·day ⁻¹)	36.1(19.0)	26.2(7.00) ^a	31.7(11.2) ^b	29.9(19.4)	28.9(9.79)
Alpha (α·day ⁻¹)	1.43±0.04	1.45±0.03	1.44±0.04	1.44±0.05	1.44±0.04
W50% (mins·day ⁻¹)	57.3±12.6	46.5±11.3 ^a	55.8±13.7 ^b	55.5±18.5 ^b	61.6±9.88 ^b
PA Bouts (<i>n</i> ·day ⁻¹)	21.3±2.57	23.6±2.73	21.9±3.85	21.6±4.16	22.2±3.03
Daily Sum of PA Bout Time (hrs·day ⁻¹)	6.16±1.09	6.21±1.46	5.82±1.57	5.48±1.73	4.56±1.86 ^{ab}
True Mean PA Bout (mins·day ⁻¹)	18.0±3.23	16.4±5.14	15.9±4.61	15.7±4.22	12.1±4.02 ^{abc}
₁₀ MVPA Bouts (<i>n</i> ·day ⁻¹)	0.29(0.29)	0.57(0.86)	0.14(0.64) ^b	0.43(0.82)	0.00(0.14) ^b
Total Week ₁₀ MVPA (hrs·wk ⁻¹)	0.41(3.18)	1.05(1.54)	0.42(1.28)	0.60(1.56)	0.00(0.34) ^{ab}
SB% (%·waking hrs ⁻¹)	60.8±6.78	59.3±8.58	61.7±9.52	63.7±10.5	69.7±10.7 ^{bc}
Standing% (%·waking hrs ⁻¹)	7.36±1.83	7.05±2.10	7.01±2.51	6.85±2.73	6.00±3.06
LIPA% (%·waking hrs ⁻¹)	13.1±2.80	12.3±3.67	12.7±3.86	12.9±4.47	11.7±5.17
sMVPA% (%·waking hrs ⁻¹)	16.7±3.94	18.6±3.64	16.4±3.37	15.5±4.80 ^b	15.2±2.49
₁₀ MVPA% (%·waking hrs ⁻¹)	0.89±1.21	1.32±1.05	0.71±0.87 ^b	1.04±1.07	0.30±0.59 ^b

^a Significantly different from 60-65 age group. ^b Significantly different from 66-71 age group. ^c Significantly different from 72-77 age group. ^d Significantly different from 72-78 age group. *p*≤0.05.

Physical Behaviour Profiles

There was no consistent pattern for changes in PB with lustrum of age increase (table 4.2). With that being said, SB were higher, and PA were lower in the oldest lustrum for a number of outcome measures compared to 66-71, and 72-77 year old groups (e.g. SB%, $_{10}$ MVPA, Daily Sum of PA Bout Time, table 4.2, all $p < 0.05$). Similarly, there are a number of lower SB and higher PA measures in the 66-71 year group, compared to the other lustrum groups (e.g. W50%, SB bout duration, table 4.2, $p < 0.05$).

Cardiovascular Profile

Further ageing did not affect systolic BP (figure 4.1), diastolic BP (figure 4.2), or pulse pressure (figure 4.3) and there were no PB covariates for these parameters. Further ageing did not affect resting heart rate, even after adjustment for sMVPA and Standing, and Daily Sum PA Bout Time and SB% (figure 4.4).

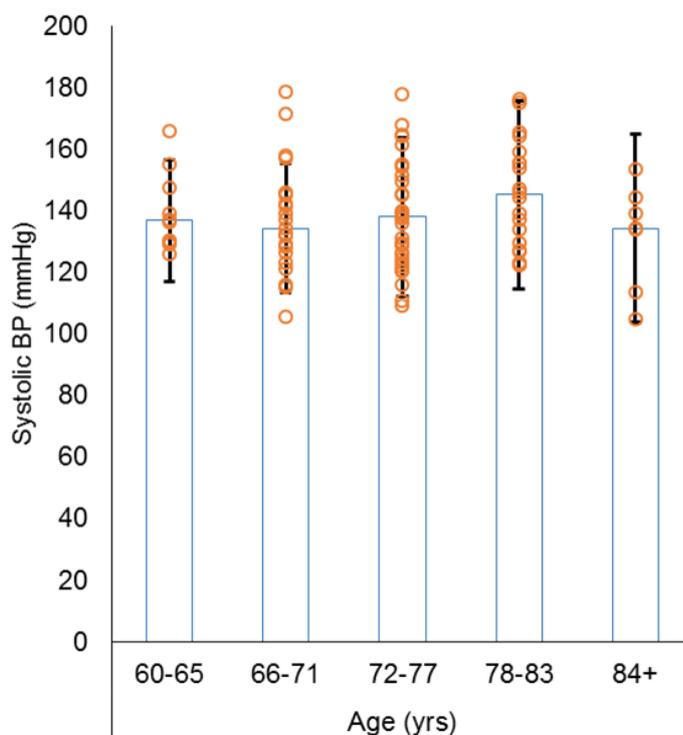


Figure 4.1. The effect of further ageing on systolic BP. Age data and error bars are presented as median and interquartile range. There were no Total PB or Patterns of PB covariates. Open circles represent individual participants' systolic blood pressure within their respective age group.

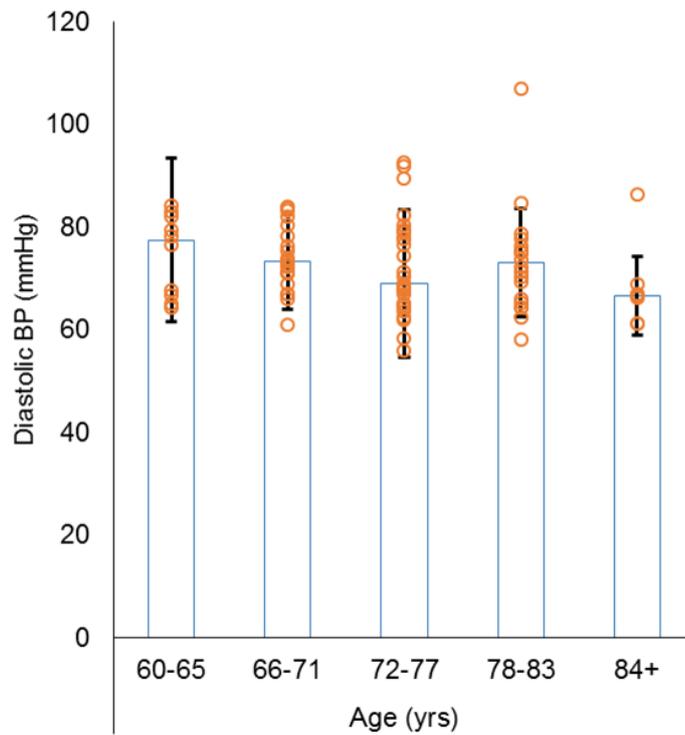


Figure 4.2. The effect of further ageing on diastolic BP. Age data and error bars are presented as median and interquartile range. There were no Total PB or Patterns of PB covariates. Open circles represent individual participants' diastolic blood pressure within their respective age group.

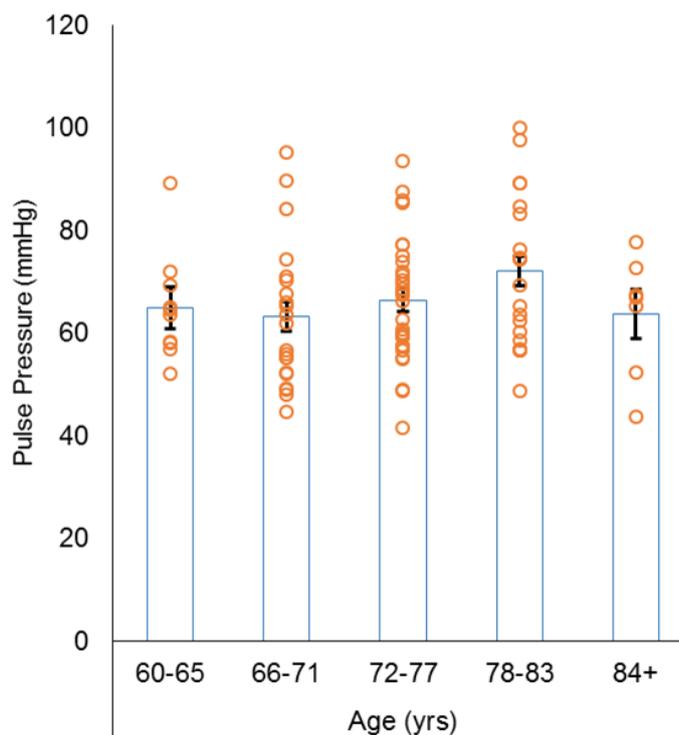


Figure 4.3. The effect of further ageing on pulse pressure. Age data and error bars are presented as mean and standard error. There were no Total PB or Patterns of PB covariates. Open circles represent individual participants' pulse pressure within their respective age group.

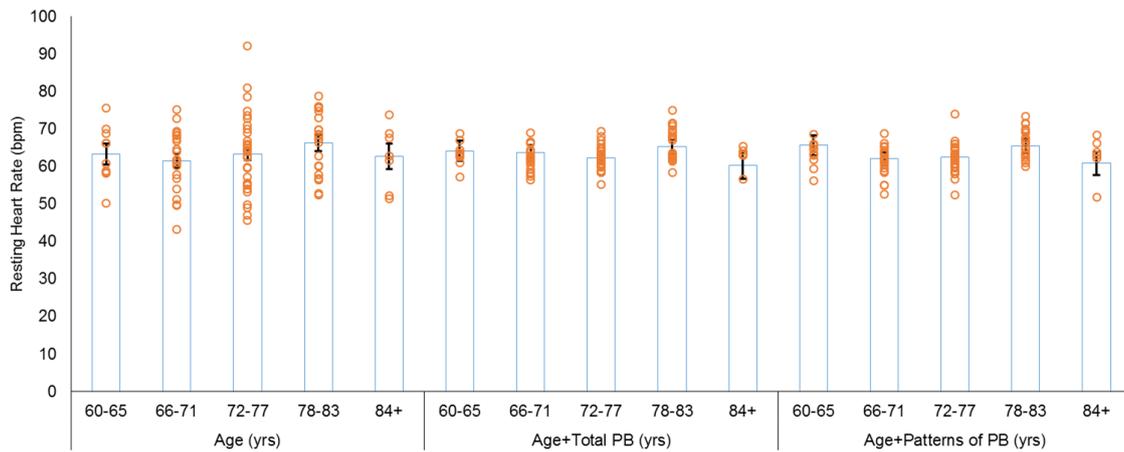


Figure 4.4. The effect of further ageing on resting heart rate. Age data and error bars are presented as mean and standard error. Age+Total PB data and error bars are presented as covariate adjusted mean and standard error. Age+Patterns of PB data and error bars are presented as covariate adjusted mean and standard error. Total PB covariates: sMVPA and Standing. Patterns of PB covariates: Daily Sum PA Bout Time and SB%. Open circles represent individual participants' pulse pressure within their respective age group.

Vascular Structure

Common carotid AL artery diameter did not increase with further ageing (figure 4.5) however, carotid AL far wall IMT was largest in the 60-65 age group, resulting in all older age groups displaying a smaller IMT compared to the former (excluding 84+ group). Interestingly, there was a trend for IMT to increase in size as age increased from 66-71 to 84+ (figure 4.6). Notably, there were no PB covariates for carotid artery diameter or IMT.

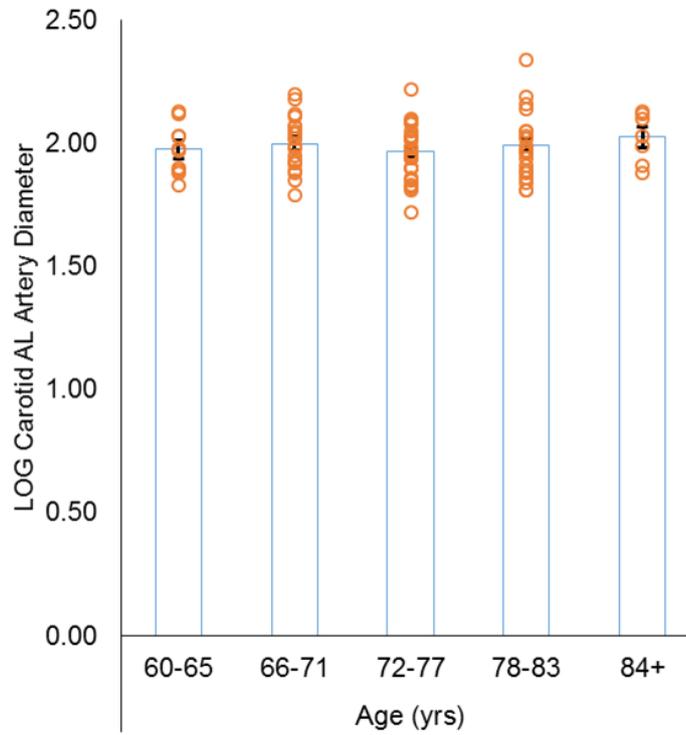


Figure 4.5. The effect of further ageing on common carotid AL artery diameter. Age data and error bars are presented as mean and standard error. There were no Total PB or Patterns of PB covariates. Open circles represent individual participants' carotid AL artery diameter within their respective age group.

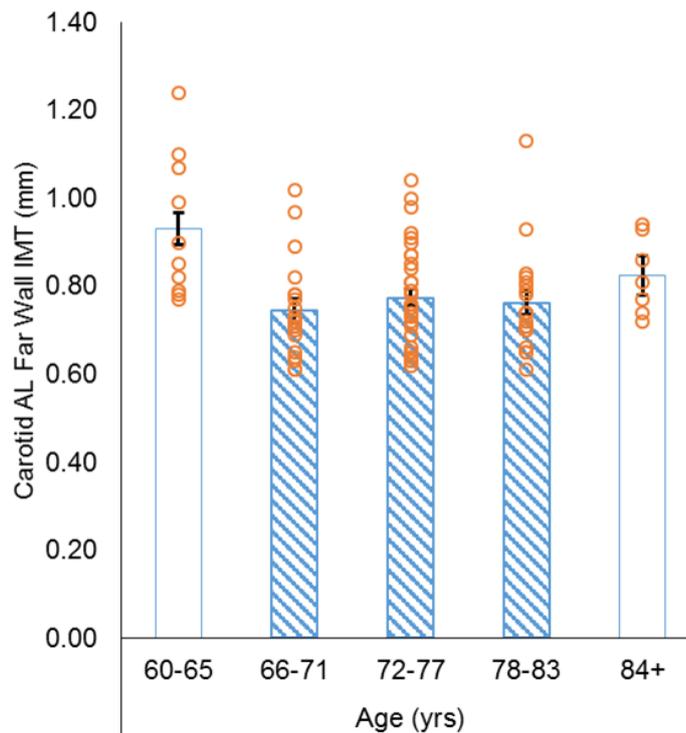


Figure 4.6. The effect of further ageing on common carotid AL far wall IMT. Age data and error bars are presented as mean and standard error. There were no Total PB or Patterns of PB covariates. Dashed series Significantly different from 60-65 year old group, $p \leq 0.05$. Open circles represent individual participants' carotid AL far wall IMT within their respective age group.

Interestingly the brachial artery diameter of participants between the age of 78 - 83 yrs was larger than that of participants aged 60-65 yrs old (figure 4.7). Meanwhile, brachial artery far wall IMT did not increase with further ageing (figure 4.8).

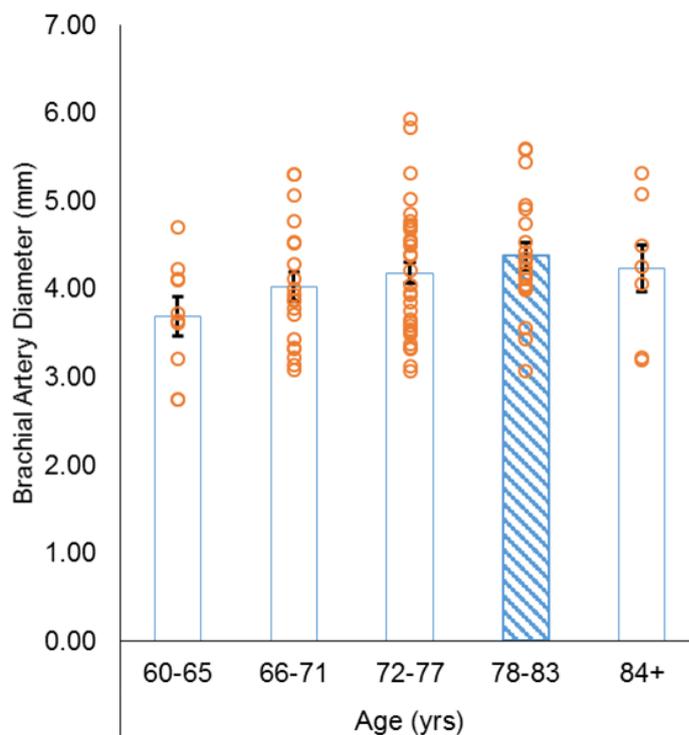


Figure 4.7. The effect of further ageing on brachial artery diameter. Age data and error bars are presented as mean and standard error. There were no Total PB or Patterns of PB covariates. Dashed series Significantly different from 60-65 year old group, $p \leq 0.05$. Open circles represent individual participants' brachial artery diameter within their respective age group.

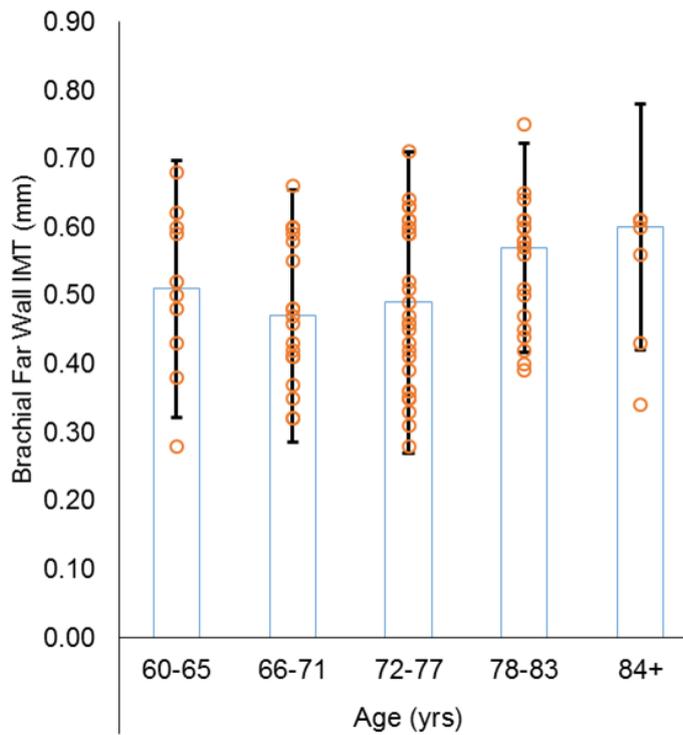


Figure 4.8. The effect of further ageing on brachial far wall IMT. Age data and error bars are presented as mean and standard error. There were no Total PB or Patterns of PB covariates. Open circles represent individual participants' brachial far wall IMT within their respective age group.

Popliteal artery diameter did not increase with further ageing even after adjustment for the Total PB covariate, Standing (figure 4.9). Popliteal IMT of participants aged 84+ years old was larger than participants aged 60-65 years old. Interestingly, this further ageing effect on popliteal IMT was somewhat attenuated when data was adjusted for LIPA and LIPA% (figure 4.10).

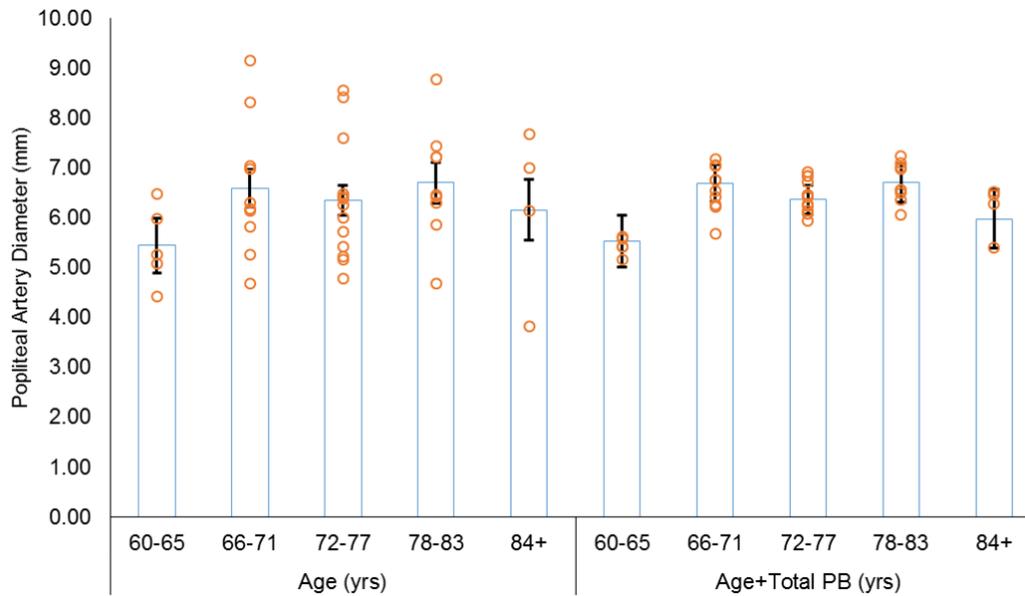


Figure 4.9. The effect of further ageing on popliteal artery diameter. Age data and error bars are presented as mean and standard error. Age+Total PB data and error bars are presented as covariate adjusted mean and standard error. Total PB covariate: Standing. Open circles represent individual participants' popliteal artery diameter within their respective age group.

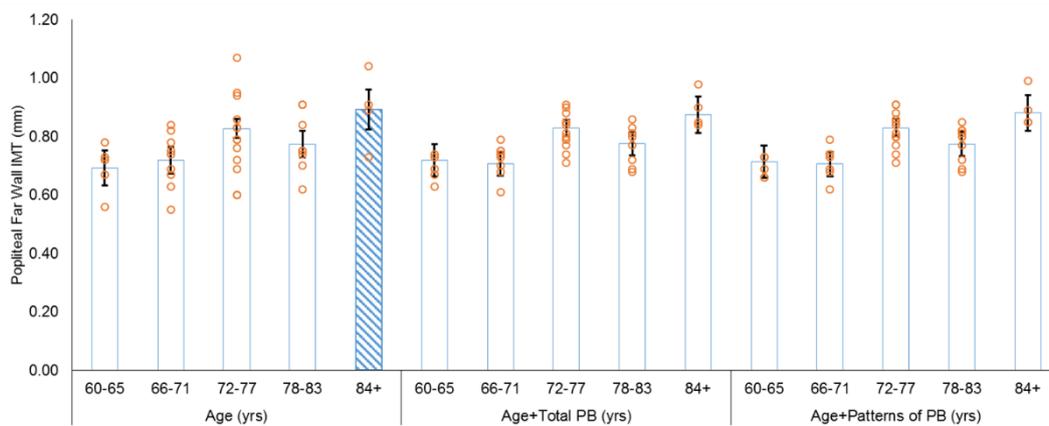


Figure 4.10. The effect of further ageing on popliteal far wall IMT. Age data and error bars are presented as mean and standard error. Age+Total PB data and error bars are presented as covariate adjusted mean and standard error. Age+Patterns of PB data and error bars are presented as covariate adjusted mean and standard error. Total PB covariate: LIPA. Patterns of PB covariates: LIPA%. Dashed series - significantly different from 60-65 year old group, $p \leq 0.05$. Open circles represent individual participants' popliteal far wall IMT within their respective age group.

Discussion

The aims of this study are to determine which cardiovascular health markers are affected by further ageing in older adults aged ≥ 60 yrs and recommend what and/or how PB profile can be optimized to offset any further ageing effects. The objectives were to highlight changes in cardiovascular health per lustrum of old age with and without adjustment for total PB time and patterns of PB.

Ageing research primarily investigates the transition between young (18 – 30 yrs) to middle (40 – 60 yrs) or middle to old (60 – 80 yrs) age groups (Celermajer et al., 1994; Black et al., 2009; Dinunno et al., 1999; Dinunno et al., 2000; Eigenbrodt et al., 2006; Green et al., 2010; Moreau et al., 2006; Pierce et al., 2011; Tanaka et al., 2002; Van den Munckhof et al., 2012; van der Heijden-Spek et al., 2000). Few studies have investigated the transition from old age to older (80+ yrs) age populations (Ferrara et al., 1997; Fritze et al., 2012). Due to the increasing percentage of older adults in the UK population (Office for National Statistics, 2012a) and the increasing life expectancy (Office for National Statistics, 2013), ‘further ageing’ research, such as the current study, is essential to ensure the disease prevention and treatment methods provided to the older old age population is appropriate to maintain quality of life.

The presence of further ageing related changes in cardiovascular parameters within the current study highlights that older adults, to a degree, are an amorphous group, which would indicate a requirement for adequately individualized treatments dependent on their age category. In this context, LIPA had the most notable effect on popliteal IMT ageing whilst other PB(s) displayed no effect on cardiovascular ageing. However, given that the greater majority of the cardiovascular parameters showed no further ageing effect would suggest that a generic lifestyle intervention would be effective across the spectrum of the 60 – 90 yr old population.

Peripheral Arteries the Target of Further Ageing

Age related increases in popliteal IMT have previously been reported in a comparison of young (26 ± 2 yrs) and older (72 ± 1 yrs) adults (0.46 ± 0.03 , 0.73 ± 0.05

mm, respectively) (Nishiyama et al., 2008). Our results add further evidence, suggesting that popliteal IMT continues to increase into older old age. However, the most notable finding from the current study was the trend for an attenuation of further ageing related increases in popliteal IMT following adjustment for LIPA and LIPA%. Although not statistically significant, participants within the 84+ age group had an average engagement in LIPA that was lower than the 60 - 65 age group (1.80 ± 0.87 , 2.11 ± 0.49 hrs·day⁻¹, respectively). This observation is supported by a 12-week LIPA (30% heart rate reserve) intervention, which reduced popliteal IMT by 5.30% in middle/older adults (59 ± 2 yrs) (Green et al., 2010). Furthermore, it supports the paradigm that LIPA can elicit health benefits, which has been a common finding in Chapter 03 Part 1 - 3 and adds to the body of evidence that LIPA should be included in the older adult's government recommendations for PA.

The current study observed a gradual increase in brachial artery diameter with further ageing, with a statistically significant difference between the 60 – 65 vs. 78 – 83 age groups. Previous findings have observed an age related change in brachial artery diameter in the comparison between young (mid 20's) and middle-older (late 50's – mid 70's) adults (Green et al., 2010; Black et al., 2009; Van den Munckhof et al., 2012). One hypothesis for the age associated increase in artery diameter is to compensate for the increase in IMT, which occurs with age and plaque formation, to maintain lumen diameter (Polak et al., 1996; Labropoulos et al., 1998). The current study does not support this hypothesis, as the increase in popliteal IMT with further ageing was not accompanied by a commensurate increase in popliteal artery diameter. Additionally, the observed further ageing effect on brachial artery diameter was not replicated for brachial IMT. Therefore, our findings would tend to support the breakdown of elastin content and recoil properties (Fritze et al., 2012; van der Heijden-Spek et al., 2000; O'rourke and Hashimoto, 2007) as the proposed mechanism for this observed increase in artery diameter with ageing. The current study demonstrates that this increase in brachial artery diameter continues into older old age and therefore, suggests that older adults should continue to take steps to attenuate this ageing effect throughout their remaining years.

A Plateau in the Impact of Ageing?

Of significant (and positive) note, is the fact that we did not observe a further ageing effect on blood vessel diameter for either the carotid or the popliteal arteries, which have previously been reported to be influenced by age (Sandgren et al., 1998; Eigenbrodt et al., 2006). It is possible that the ageing effect on carotid and popliteal artery diameter plateaus with increasing age after ~67 yrs of age, as the previous findings from Eigenbrodt et al. (2006) and Sandgren et al. (1998) were from younger populations (45 – 64, 25 – 67 yrs, respectively) compared to the current study (60 – 89 yrs). In line with this, analysis of data from Benetos et al. (1993) suggests that carotid artery diameter of normotensive participants begins to plateau at age 60 ($r^2=0.19$), following a 2nd order polynomial trend line.

The Impact of Medication on Further Ageing

A limitation of the current study is that it did not account for blood pressure lowering (60% of participants) or lipid lowering (30% of participants) medication, which would undoubtedly impact the natural ageing effect on artery diameter and thus the results of this study. However, with more than 313 million prescriptions for CVD dispensed within England in 2014 (Townsend et al., 2015), it is reasonable to say that our sample population is representative of the entire population and therefore, normalisation for medication would reduce the external validity of the study. A subsequent comparison of the effect of further ageing on cardiovascular markers between non-medicated ($n=19$) and medicated participants ($n=74$, data is based on participant self-reporting of medication) suggested that medication use influenced brachial artery diameter and IMT, as age became a significant predictor of these markers in the medicated group (b: 0.01 [95%CI 0.01, 0.06], b: 0.01 [95%CI 0.001, 0.01], respectively). In addition, the use of medication masked the effect of further ageing, as significant associations between popliteal IMT and age were attenuated in the medicated group (Appendix Chapter 04 figure A4.1 - 10). The interpretation of these pilot data needs to be done with caution as the grouping of the participants was skewed (not medicated: $n=19$, medicated: $n=74$). However, this pilot data suggests further research may be necessary to illustrate the differences

in the effect of further ageing on cardiovascular parameters between not medicated and medicated older adults.

Conclusion

The results from the current study suggest that further ageing after 60 yrs old does not affect blood pressure, resting heart rate, carotid and popliteal artery diameter, and brachial and carotid IMT. However, there is an apparent effect of further ageing on brachial artery diameter, which increased an average 0.23 ± 0.10 mm per lustrum of age (up to age 84). Notably also, popliteal IMT increased 0.08 ± 0.05 mm per lustrum increase in age however, this was attenuated when LIPA and LIPA% engagement was accounted for. This suggested that older adults may be able to combat the effect of further ageing by increasing their daily engagement in LIPA. This is an important health message in self-care for the population.

Chapter 05:

Segregating the distinct effects of sedentary behaviour and physical activity on older adults' cardio-metabolic status.

Part 1: *'Compositional data analysis approach.'*

Part 2: *'Isotemporal substitution approach.'*

Part 3: *'Z-score approach.'*

Chapter 05: Introduction and methods.

Introduction

Between 2011 and 2014, medication prescription for the prevention and treatment of circulatory diseases within an adult population increased 2.2 fold in England (Townsend et al., 2015). These prescriptions include medications such as: lipid-lowering, anticoagulant and anti-fibrinolytic drugs, with 62 – 95% of adults above the age of 55 years taking at least one prescribed drug per day for treatment or prevention of any condition (Chaplin, 2015). Critically, many of the metabolites that these drugs target are adjustable through physical activity (PA) interventions (Gennuso et al., 2013; Rosique-Esteban et al., 2017; Henson et al., 2013b). In older adult cohorts (65 – 94 years [yrs]), 3 x 60 min aerobic sessions per week, for 8 months, at 60 – 80% of heart rate reserve was sufficient to reduce total cholesterol and triglyceride concentration (Verissimo et al., 2002). Emerging-evidence also suggests that changes in sedentary behaviour (SB) could also modulate 'problematic metabolites', through mechanistic pathways, which are different from those of PA (specifically moderate to vigorous intensity PA [MVPA]) (Bey and Hamilton, 2003). In rodent modelling of SB, six hours of hind limb unloading in rats decreased oxidative skeletal muscle lipoprotein-lipase (LPL) by 50% relative to ambulatory controls, whereas, treadmill running (56 m·min⁻¹, 3.5 hr·day⁻¹) did not increase oxidative muscle LPL above that of ambulatory controls but did in glycolytic muscle (Bey and Hamilton, 2003). It is suggested that SB targets post-transcriptional modification of LPL, as its mRNA expression remained unchanged during hind limb unloading (Bey and Hamilton, 2003). Given that SB and PA (collectively known as physical behaviour [PB] (Bussmann and van den Berg-Emons, 2013)) may act through independent mechanisms in the prevention of cardio-metabolic disease, it is hypothesised that future studies should consider SB and PA together, not in isolation.

Common analysis of daily PB, uses PA as a covariate when determining the effect of SB on health parameters (Gennuso et al., 2013; Henson et al., 2013b; Horta et al., 2015; Kulinski et al., 2016; Pereira et al., 2012; Rosique-Esteban et al., 2017; Saunders et al., 2013). However, this does not take into account the finite

simplex of a day or the contributions to health status that other intensities of PB may provide. For example, SB may affect health status independent of MVPA; however, the impact of SB may be attenuated by sleep, light intensity PA (LIPA) or a combination of both. In addition, this common form of analysis does not account for the time limiting effect that engagement in one PB can have on engagement in other PB(s).

Compositional Data Analysis (CoDA) overcomes these issues by presenting the geometric mean engagement in a PB as a standardised score, relative to the geometric mean of all engaged PB(s), as a denominator of 24 hours. This allows the standardised score of a particular PB engagement of a sub-group to be presented as a log ratio, relative to the standardised score for the same PB for the sample population (log ratio = $\ln[\text{centred geometric mean of group} \div \text{centred geometric mean of population}]$) (Chastin et al., 2015b; Carson et al., 2016). CoDA is normally used to compare the composition of two or more groups, relative to the entire sample population and although a new form of analysis in PB research, CoDA has provided interesting descriptive data. CoDA of the participants from the NHANES 2005-06 cycle suggested those with a relatively low high-density lipid (HDL-C) concentration engaged in 9% less MVPA relative to the overall geometric mean composition. Whereas, those with a healthy HDL-C concentration engage in 4% more MVPA relative to the overall geometric mean composition (Chastin et al., 2015b).

Another new and pertinent form of data analysis is Isotemporal Substitution Modelling (ISM), which has previously been described in Chapter 03 Part 2. In brief, rather than analysing PB(s) as a composition, ISM illustrates how health markers may change should engagement in one PB be replaced with another PB of the same duration (Mekary et al., 2013). This is performed by removing a PB from a regression model to change the beta coefficient between the other PB(s) and the health marker, which represents the change in health status that may occur with a change in habitual PB engagement (Mekary et al., 2013; Fanning et al., 2016; Buman et al., 2013). ISM has illustrated that replacing 10 min·day⁻¹ of SB with LIPA could reduce the prevalence of metabolic syndrome (odds ratio: 0.96 [95% confidence interval (CI) 0.93, 0.98]) in healthy middle – older adults (50 - 64 yrs) (Ekblom-Bak et al., 2016). With the emergence of these new analyses, little research has yet to be conducted applying these analyses to cardio-metabolic health data of older adults (Ekblom-Bak et al., 2016; Hamer et al., 2014; Healy et al., 2015).

In addition, it has also been suggested that the patterns of PB accumulation could be just as important for the prediction of cardio-metabolic markers as total PB engagement time. The addition of 10 extra breaks in SB per day was associated with a 3.72% (95%CI 1.34, 6.13) reduction in triglyceride concentration in adults (20 – 79 yrs) (Carson et al., 2014). Therefore, it is essential to assess the effect of both total, and patterns of, PB engagement in order to capture the complete influence of PB on health status.

The **aim** of this study was therefore to illustrate which PB intensity(s) and/or pattern(s) influence cardio-metabolic parameters in older adults. The **objectives** of this study were; **Part 1**) illustrate the difference in the PB profile between older adults with 'low' and 'high' cardio-metabolic profiles using CoDA, **Part 2**) theoretically illustrate how older adults can change their cardio-metabolic profile through a change(s) in PB engagement using ISM. **Part 3**) Illustrate how the patterns of PB engagement differ between 'low' and 'high' cardio-metabolic marker groups. The **hypotheses** were: **Part 1**) that PB composition in the 'high' cardio-metabolic profile sub-groups will illustrate a greater engagement in SB and a lower engagement in PA relative to the entire sample population geometric mean. Whereas the 'low' cardio-metabolic profile sub-group will engage in more PA and less SB relative to the entire sample population geometric mean which, has been shown in previous studies (Chastin et al., 2015b; Carson et al., 2016). **Part 2**) Substitution of SB or PA with PA of higher intensity may improve cardio-metabolic profile whilst the substitution of a higher intensity PA with a lower intensity PA or SB may worsen cardio-metabolic profile, as has been illustrated previously in younger persons (Ekblom-Bak et al., 2016; Hamer et al., 2014; Healy et al., 2015). **Part 3**) The 'low' cardio-metabolic marker group will present more 'cardio-metabolic endocrine health' enhancing patterns of PB engagement than the 'high' group (Carson et al., 2014; Lord et al., 2011; Healy et al., 2011).

The statistical analyses, results and discussion of this chapter will be provided in three parts; 1) CoDA, 2) ISM, and 3) Group comparison of patterns of PB engagement z-scores.

Methods

First Laboratory Visit

The details of the first laboratory visit follows that of the Chapter 03 Part 1. Please refer to it for details regarding recruitment and accelerometer use. Absolute measures of Sleep, SB, Standing, LIPA, sporadic MVPA (sMVPA, MVPA accumulated in bouts <10 mins), and 10 mins MVPA (₁₀MVPA, MVPA accumulated in bouts ≥10 mins) were used for CoDA and ISM.

Second Laboratory Visit

Whole Blood Endocrine Analysis

Participants ($n=93$) arrived to the laboratory in an overnight (>10 hours) fasted, hydrated state. Where appropriate, participants were asked to refrain from taking medication until testing had been completed. All participants refrained from taking medication prior to the completion of the laboratory tests and all provided a 10 mL venous blood sample. Whole blood analyses of fasting plasma glucose, total cholesterol, and triglycerides were performed immediately using an Accutrend Plus (Roche Diagnostics Limited, Welwyn Garden City, UK) monitoring device and Accutrend test strips (Roche Diagnostics Limited, Welwyn Garden City, UK) (Coqueiro et al., 2014). Whole blood analysis of glycated haemoglobin (HbA1c) was performed on a sub-group of participants ($n=33$) using boronate fluorescence quenching (HbA1c 501 device and test cartridges, HemoCue, Ängelholm, Sweden). HemoCue 501 has shown good reliability (Coefficient of Variation [CV] <5.0%) and validity (Bland-Altman: 4.4 [95%CI -7.3, 16.2] mmol·mol⁻¹ compared to high performance liquid chromatography ion exchange (Phillips et al., 2014)).

Remaining blood samples were stored on ice for <2 hours before centrifugation at 1687 G for five minutes (Z380, Hermle, Gosheim, Germany).

Serum was harvested and stored at -20 °C in 1.00 mL aliquots (Eppendorf Ltd, Hamburg, Germany) until further analyses.

Serum Endocrine Analyses

Commercially available enzyme-linked immunosorbent assay kits were used to determine the concentration of serum lipoprotein lipase (LPL) (Cell Biolabs Inc., California, USA), procollagen III N-terminal propeptide (PIIINP) (Biomatik, Delaware, USA), and interleukin-6 (IL-6) (high-sensitivity, Bio-Techne, Minnesota, USA) using a two-fold sample dilution. Manufacturer reported LPL intra-assay CV was 4% whereas it reached 13% in house. For PIIINP, manufacturer sample intra-assay CV was <10%, which coincided with in house data (6.5 - 9.6%). IL-6 manufacture intra-assay CV was 7.8% whereas in house it ranged from 7.4 – 9.2%. ELISA data were derived using a 96-well spectrophotometer (EL808, BioTek, Vermont, USA) connected to a computer running Gen5 v 1.11 software (BioTek, Vermont, USA).

Part 1: Compositional data analysis approach.

Statistical Analyses

Demographics

SPSS version 22 (IBM, New York, USA) was used for statistical analysis. 1×5 independent analysis of variance (ANOVA) and bonferroni correction (Kruskall-Wallis and Mann-Whitney U for non-parametric data) was used to determine whether participant demographics changed per lustrum change in age and whether CoDA needed to be performed for age grouped or pooled populations. Data are presented as mean (standard deviation [SD]) or median (interquartile range [IR]) if rules of parametricity are violated. Statistical significance was set at $p \leq 0.05$.

Compositional Data Analysis

Participants were grouped into 'low' or 'high' endocrine concentration groups for each cardio-metabolic parameter based on whether they were less than or equal to, or above the recognised threshold concentration for the respective cardio-metabolic marker (table 5.5.1). Where threshold concentrations from previous research could not be applied, median (PIINP) and mean (HbA1c) were used as the threshold. The HbA1c threshold of 6.5% (World Health Organization, 2015a) could not be applied to the study population as the participants did not display diabetic symptoms and therefore every participant's HbA1c percentage fell below 6.5%. Similarly, the 4780 $\text{pg} \cdot \text{mL}^{-1}$ threshold (Agarwal et al., 2014) could not be applied as every PIINP concentration within the current study was below this threshold.

Table 5.1.1. Cardio-metabolic threshold values used to determine participant groupings into 'low' and 'high' endocrine concentration.

Cardio-metabolic Parameter	Threshold
Glucose	6.0 mmol·l ⁻¹ (World Health Organization, 2015a)
Total Cholesterol	5.0 mmol·l ⁻¹ (World Health Organization, 2015a)
Triglyceride	1.7 mmol·l ⁻¹ (World Health Organization, 2015a)
HbA1c	5.29%
LPL	63.5 pg·mL ⁻¹ (Rip et al., 2006)
IL-6	2.29 pg·mL ⁻¹ (Cesari et al., 2003)
PIIINP	229.215 pg·mL ⁻¹

Mean % HbA1c used as the threshold. Median PIIINP concentration used as the threshold.

Using Excel 2013 (Microsoft, Washington, USA), the geometric mean (hrs·day⁻¹) was calculated for each PB for the entire sample population. The grand geometric mean was further calculated for the entire PB (Sleep + SB + Standing + LIPA + sMVPA + ₁₀MVPA) data for the entire sample population. The sample population data were centred (*cen^o*) by dividing the geometric mean for each PB by the grand geometric mean and then by the available time in a day (24 hours). These steps were then performed on the cardio-metabolic parameter sub-groups (*cenⁱ*) ('low' and 'high'). The centred data for each PB for each sub-group, was compared to the centred data for the respective PB of the entire sample population as a log ratio ($\ln[\textit{cen}^i \div \textit{cen}^o]$) (figure 5.1.1). The log ratio represents the sub-group's engagement in a PB relative to the entire sample population's standardised engagement in the same PB (Chastin et al., 2015b; Carson et al., 2016).

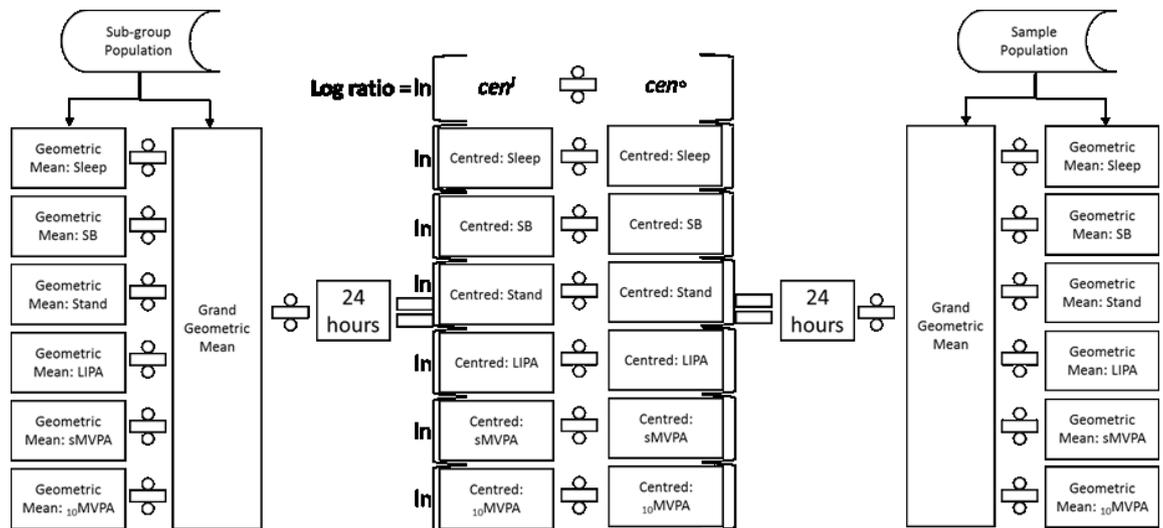


Figure 5.1.1. Flow chart of the CoDA calculations. Calculation steps starting outwards and moving towards centre.

Handling Covariates

Analysis of covariance (ANCOVA) was performed using SPSS version 22 (IBM, New York, USA) to determine whether covariates (blood pressure (BP) medication [$\text{mg}\cdot\text{day}^{-1}$], lipid-lowering medication [$\text{mg}\cdot\text{day}^{-1}$], primary CVD targeting medication [$n\cdot\text{day}^{-1}$], CVD (in)directly targeting medication [$n\cdot\text{day}^{-1}$], and inflammatory + CVD (in)directly targeting medication [$\text{mg}\cdot\text{day}^{-1}$]) influenced the concentration of the cardio-metabolic parameters within the current study (table 5.1.2) as they have previously been shown to be associated with cardio-metabolic parameters (Furberg et al., 1994; Bakris et al., 2004; McIntyre et al., 2006; Tsuboi et al., 1995). LPL was found to be influenced by inflammatory + CVD (in)directly targeting medication ($p\leq 0.05$). LPL data were adjusted for the aforementioned covariate and the participants were regrouped before CoDA was performed. No other cardio-metabolic parameters were influenced by the aforementioned covariates.

Table 5.1.2. Covariate influence of different medication variables on cardio-metabolic parameters when comparing the difference between 'low' and 'high' group's mean concentration. Data presented as significant value (*p*).

Medication	Cardio-metabolic Parameter						
	Glucose	Triglyceride	Total Cholesterol	HbA1c	LPL	IL-6	PIIINP
BP	0.71	0.93	0.57	0.53	0.14	0.95	0.70
Lipid-lowering	0.36	0.47	0.84	0.63	0.46	0.81	0.53
Primary CVD targeting	0.94	0.06	0.69	0.27	0.89	0.24	0.59
CVD (in)directly targeting	0.64	0.32	0.90	0.68	0.31	0.76	0.35
Inflammatory + CVD (in)directly targeting	0.47	0.92	0.09	0.19	0.00*	0.71	0.88

* Covariate has a significant effect on a cardio-metabolic parameter concentration ($p \leq 0.05$).

Handling 'Essential' Zeros

Within CoDA, there are rounded zeros, which represent data that could not be measured due to the sensitivity of the equipment used, and there are essential zeros, which represent real values for a parameter. In the current study, essential zeros were common in PB data, as many participants did not engage in $_{10}$ MVPA. It is not possible to log transform zeros or calculate geometric means, therefore they need to be accounted for so they still carry weight in the analyses. One method is to remove all the participants who have essential zero data, which has been performed in previous studies (Chastin et al., 2015b; Carson et al., 2016). However, given the sample size ($n=93$), removal of participants would severely reduce the power of the study and thus, is not deemed an appropriate approach. An alternative method is to add 1 to every data point, then minus 1 from the geometric mean calculations (Costa and Judge, 2013).

To determine the best method for handling zeros, the geometric mean of each PB was calculated to act as the 'gold standard'. The geometric means were

subsequently recalculated using four conditions; removing essential zeroes (Chastin et al., 2015b; Carson et al., 2016), addition and subtraction of 1 (Costa and Judge, 2013), addition and subtraction of 0.5, and addition and subtraction of 0.01. The difference between the ‘gold standard’ geometric mean and the adjusted means suggested that the addition and subtraction of 0.01 was the best method for our current data set as it displayed the smallest change in geometric mean (table 5.1.3). Therefore, this method was used during CoDA.

Table 5.1.3. Comparison between the gold standard and essential zeros adjusted methods geometric means.

Method	PB (hrs·day⁻¹)					
	Sleep	SB	Standing	LIPA	sMVPA	₁₀MVPA
Gold-standard	8.40	9.56	1.02	1.86	2.49	NA
Method	Difference from gold standard					
Remove essential zeroes	0	0	0	0	0	0.124*
Add 1, remove 1 for geometric mean	-0.004	-0.009	-0.042	-0.040	-0.029	0.129*
Add 0.5, remove 0.5 for geometric mean	-0.002	-0.005	-0.029	-0.025	-0.017	0.123*
Add 0.01, remove 0.01 for geometric mean	-4.2E-05	-9.7E-05	-0.001	-0.001	0.000	0.062*

* Geometric mean for ₁₀MVPA. The difference from gold standard cannot be displayed as the geometric mean cannot be calculated with the presence of zeros.

Physical Behaviour Co-dependence

To determine the co-dependence between PB intensities a variation matrix was used. A variation matrix displays the variance in the sample population’s log-ratios for each PB comparison (equation 5.1.1). A variance close to zero would imply the time spent in the corresponding behaviours are proportional and therefore,

suggest a change in engagement of one of those PB would likely result in a change in engagement of the corresponding PB.

Equation 5.1.1. Physical behaviour co-dependence calculation.

$$\text{Variation Matrix} = \ln(\text{PB}_1 \div \text{PB}_2)$$

Where: ln is natural LOG transformation

PB₁ is a PB

PB₂ is another PB

Results

The demographics of the 93 older adults who participated in the study (73.6 [7.17] yrs, 55% female) are displayed in table 5.1.4. Notably, there was no difference between lustrum age groups on any of the cardio-metabolic, PB, or covariate parameters. This therefore allowed follow up analyses in the pooled data.

Table 5.1.4. Participant demographics displayed per lustrum of age. Data presented as Mean(SD), Median(IR), or geometric mean.

Variable	Age Group (yrs)					
	Pooled	60 – 65	66 – 71	72 – 77	78 – 83	84+
<i>n</i>	93	10	19	31	24	9
Female (%)	55	90	47	54	54	33
Covariates						
Primary CVD Meds (<i>n</i> ·day ⁻¹)†	1.17 (1.52)	0.30 (0.48)	0.74 (1.37)	1.39 (1.61)	1.39 (1.53)	1.78 (1.92)
CVD Meds (<i>n</i> ·day ⁻¹)‡	1.62 (1.81)	0.70 (0.67)	1.05 (1.51)	1.81 (1.97)	2.04 (1.92)	2.11 (1.96)
CVI Meds (mg·day ⁻¹)§	157.86 (486.49)	293.40 (797.89)	54.71 (73.39)	93.19 (293.99)	136.67 (323.71)	494.88 (1085.38)
BP Meds (mg·day ⁻¹)	9.81 (50.79)	3.50 (6.73)	5.47 (13.29)	2.35 (4.89)	8.05 (16.71)	53.00 (154.35)
Lipid-Lowering Meds (mg·day ⁻¹)	8.12 (16.58)	11.00 (16.63)	9.41 (15.60)	3.57 (10.96)	13.33 (23.09)	4.44 (13.33)
Cardio-metabolic Parameters						
Triglyceride (mmol·l ⁻¹)	1.77 (0.81) _m	2.09 (0.86) _m	1.51 (0.84) _m	1.73 (0.68) _m	1.88 (0.56) _m	1.89 (0.57) _m
Total Cholesterol (mmol·l ⁻¹)	5.44 (1.39) _m	6.06 (0.95)	5.64 (1.08)	5.45 (1.07)	5.64 (0.89)	5.27 (0.66)
Glucose (mmol·l ⁻¹)	5.72 (1.10) _m	5.81 (0.81) _m	5.85 (1.35) _m	5.60 (1.33) _m	5.65 (1.38) _m	6.00 (1.11) _m
HbA1c (%)	5.29 (0.31)	5.18 (0.22)	5.30 (0.22)	5.34 (0.36)	5.24 (0.33)	5.38 (0.32)
LPL (pg·mL ⁻¹)	113.02 (147.80) _m	174.35 (277.28) _m	119.13 (127.16) _m	83.41 (123.97) _m	117.88 (147.07) _m	247.58 (332.56) _m
IL-6 (pg·mL ⁻¹)	2.72 (2.77) _m	3.11 (1.85) _m	2.39 (2.85) _m	2.55 (3.29) _m	2.36 (2.98) _m	4.14 (6.00) _m
PIIINP (pg·mL ⁻¹)	229.21 (247.97) _m	205.05 (239.21) _m	229.21 (208.30) _m	316.92 (228.81) _m	167.19 (248.76) _m	256.99 (406.27) _m

Table 5.1.4 continued.

Physical Behaviour Parameters						
Sleep	8.43	7.88	8.49	8.41	8.62	8.50
(hrs·day ⁻¹)	(0.77)	(0.83)	(0.53)	(0.73)	(0.79)	(1.04)
	8.40 _g	7.84 _g	8.48 _g	8.35 _g	8.58 _g	8.45 _g
SB	9.65	9.81	9.34	9.61	9.59	10.49
(hrs·day ⁻¹)	(1.33)	(1.31)	(1.38)	(1.36)	(1.29)	(1.15)
	9.56 _g	9.73 _g	9.25 _g	9.53 _g	9.51 _g	10.44 _g
Standing	1.09	1.19	1.16	1.11	1.08	0.89
(hrs·day ⁻¹)	(0.41)	(0.30)	(0.43)	(0.39)	(0.43)	(0.43)
	1.02 _g	1.14 _g	1.08 _g	1.03 _g	1.00 _g	0.81 _g
LIPA	1.97	2.11	1.86	2.06	1.99	1.73
(hrs·day ⁻¹)	(0.63)	(0.49)	(0.59)	(0.63)	(0.68)	(0.77)
	1.86 _g	2.05 _g	1.78 _g	1.95 _g	1.88 _g	1.57 _g
sMVPA	2.57	2.68	2.86	2.55	2.53	2.07
(hrs·day ⁻¹)	(0.64)	(0.58)	(0.56)	(0.59)	(0.69)	(0.67)
	2.49 _g	2.62 _g	2.80 _g	2.49 _g	2.42 _g	1.96 _g
₁₀ MVPA	0.08	0.06	0.15	0.08	0.09	0.07
(hrs·day ⁻¹)	(0.20) _m	(0.16) _m	(0.29) _m	(0.21) _m	(0.22) _m	(0.13) _m
	0.06 _g	0.07 _g	0.13 _g	0.05 _g	0.07 _g	0.03 _g

† Participants are currently prescribed an amount of medication that reduces the risk or treats CVD (i.e. statins, warfarin). ‡ Participants are currently prescribed a medication that may affect the cardiovascular system either directly or as a side effect. § Participants are currently prescribed a medication that may affect the cardiovascular system either directly or as a side effect, including inflammatory medication. _m Median (IR). _g Geometric mean.

Whole Blood Endocrine Parameters

The composition of PB for 'low' and 'high' cardio-metabolic parameter sub-groups is displayed in figure 5.1.2A-D. Triglyceride concentration appears to be highly dependent on ₁₀MVPA engagement as the 'low' triglyceride concentration group engaged in 48% more ₁₀MVPA relative to the geometric mean of the entire sample population (figure 5.1.2A). It also suggests that reducing ₁₀MVPA engagement may result in a change to a 'high' triglyceride profile more readily than

the opposite. Indeed those with a 'high' triglyceride concentration only engaged in 32% less $_{10}$ MVPA than the geometric mean of the entire sample population, whereas those with a 'low' triglyceride concentration required a 48% greater engagement in $_{10}$ MVPA compared to the entire sample population (figure 5.1.2A). It appears that differences in total cholesterol profile may be influenced by most PB intensities as those with a 'low' total cholesterol profile engaged in less sleep (3.0%), SB (2.9%), LIPA (7.1%), sMVPA (6.0%), and more standing (7.9%) and $_{10}$ MVPA (4.4%) than the entire sample population. The opposite PB profile was true for the 'high' total cholesterol concentration group (figure 5.1.2B). The composition of the 'high' glucose and HbA1c groups appeared to be similar as both engaged in more sleep and SB, and less PA (excluding sMVPA) compared to the entire sample population (figure 5.1.2C-D).

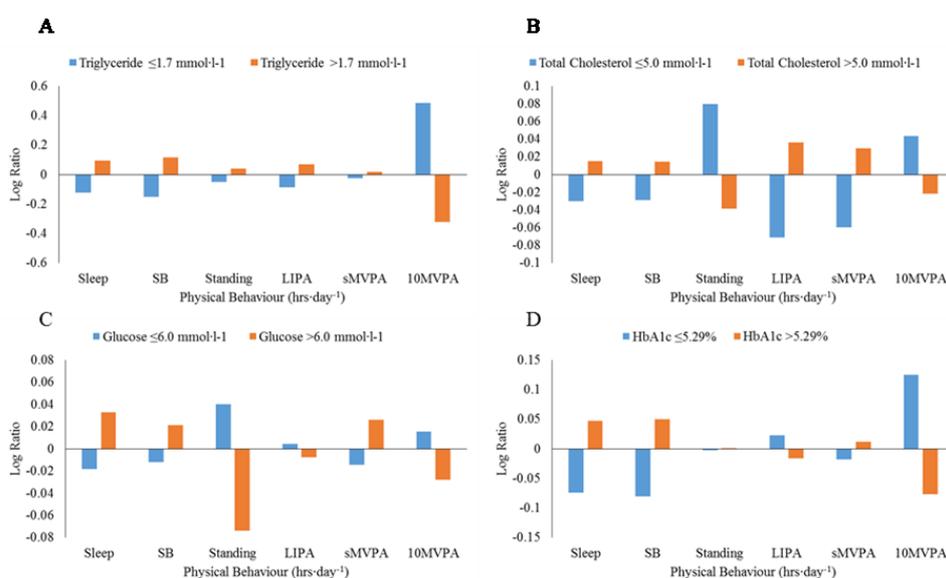


Figure 5.1.2. Compositional geometric mean bar plots displaying the difference in PB composition between 'low' and 'high' cardio-metabolic parameter sub-groups. A) triglyceride (n 'low': 39, 'high': 50) B) total cholesterol (n 'low': 30, 'high': 59), C) glucose (n 'low': 57, 'high': 32), and D) HbA1c (n 'low': 13, 'high': 20).

Serum Endocrine Parameters

Before correcting for covariate effects, LPL was heavily influenced by $_{10}$ MVPA, with a large difference in engagement compared to the entire sample population ('low' LPL: 27% less $_{10}$ MVPA, 'high' LPL: 11% more $_{10}$ MVPA) (figure

5.1.3A). Whereas the difference from the entire population for the other PB intensities did not exceed 2.2% (figure 5.1.3A). Following normalisation for inflammatory + CVD (in)directly targeting medication, the ‘low’ LPL group’s engagement in $_{10}$ MVPA was 26% less while the ‘high’ LPL group was 7.9% more than the entire population (figure 5.1.3B). Sleep appeared to be a main determinant of IL-6 concentration as those in the ‘low’ and ‘high’ IL-6 group engaged in 4.8% more and 3.1% less sleep compared to the entire sample population, respectively. Whereas the other PB intensities had a lower difference in engagement compared to the entire sample population (figure 5.1.3C). The results suggest that bouts of MVPA above 10 mins are sufficient to stimulate an inflammatory response as the ‘high’ IL-6 group engaged in 2.7% more $_{10}$ MVPA and 2.2% less sMVPA compared to the entire sample population (figure 5.1.3C). PIIINP followed a similar composition to IL-6, with sleep displaying the greatest difference from the entire sample population in both ‘low’ (6.2%) and ‘high’ (5.9%) groups compared to the other PB intensities.

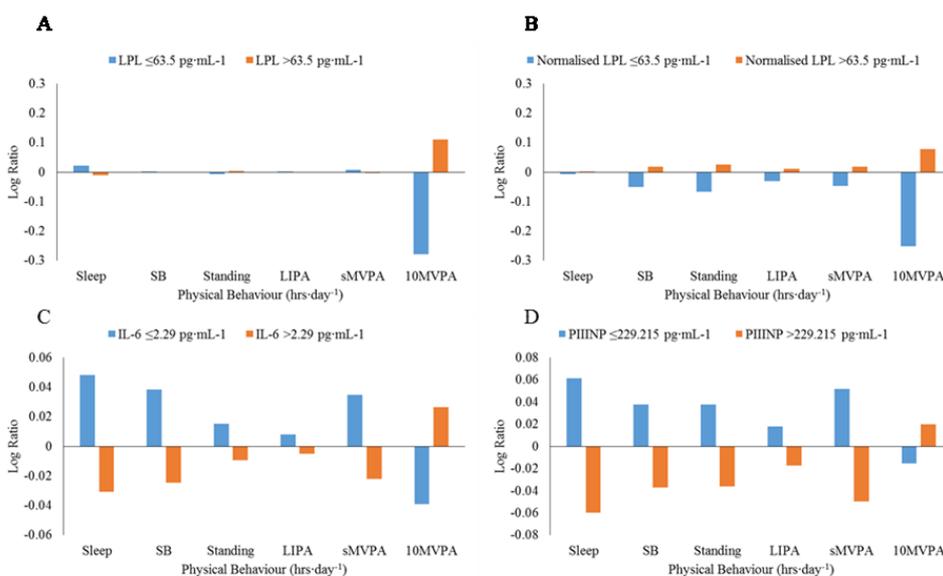


Figure 5.1.3. Compositional geometric mean bar plots displaying the difference in PB composition between ‘low’ and ‘high’ cardio-metabolic parameter sub-groups. A) LPL (n ‘low’: 26, ‘high’: 57), B) LPL normalised for inflammatory + CVD (in)directly targeting medication (n ‘low’: 21, ‘high’: 55), C) IL-6 (n ‘low’: 33, ‘high’: 52), and D) PIIINP (n ‘low’: 36, ‘high’: 37).

Physical Behaviour Co-dependence

The greatest co-dependence existed between sleep against SB, standing against LIPA, and LIPA against sMVPA, thus indicating that changes in one of these PB will likely result in a change in engagement for the corresponding PB. The lowest co-dependence existed between $_{10}$ MVPA and all other PB (table 5.1.5), indicating that changes in $_{10}$ MVPA engagement will likely not result in changes in other PB engagement.

Table 5.1.5. Variation matrix between PB(s).

	Sleep	SB	Standing	LIPA	sMVPA	$_{10}$ MVPA
Sleep		0.03	0.20	0.15	0.10	1.88
SB			0.24	0.19	0.15	1.93
Standing				0.09	0.16	1.95
LIPA					0.09	1.85
sMVPA						1.62

Discussion

The aim of this study was to illustrate which PB intensity(s) influence cardio-metabolic parameters in older adults. The objective of this chapter part (1) was to illustrate the difference in the PB profile between older adults with 'low' and 'high' cardio-metabolic profiles using CoDA. It was hypothesised that 'high' sub-group's PB composition would illustrate a greater engagement in SB and a lower engagement in PA relative to the entire sample population geometric mean. Whereas the 'low' sub-group would engage in more PA and less SB relative to the entire sample population geometric mean which, has been shown in previous studies (Chastin et al., 2015b; Carson et al., 2016). This hypothesised was confirmed throughout the cardio-metabolic markers except for IL-6 and PIIINP, where those within the 'high' sub-groups engaged in less SB and more PA.

Lipoprotein – LPL Axis

Lipoprotein lipase was one of the first identified cardio-metabolic markers to illustrate independent effects of SB and MVPA (Bey and Hamilton, 2003). It was suggested that prolonged SB targets oxidative skeletal muscle LPL activity whereas; MVPA appears to primarily target glycolytic muscle LPL activity (Bey and Hamilton, 2003). LPL is responsible for the hydrolysis of triglyceride into glycerol and fatty acid. Pre-heparin serum LPL (measured in the current study) primarily represents inactive LPL as a dimer bound to isolated remnant lipoproteins (Sato et al., 2016), which, in the presence of active LPL, augments triglyceride hydrolysis and the uptake of very low-density lipoproteins and cholesterol esters (Merkel et al., 2002). Therefore, reduced serum LPL concentration, similar to muscle LPL activity, may lead to increased circulating triglyceride concentration (Petibois et al., 2004). The comparison of PB composition between triglyceride and LPL groups supports the LPL-triglyceride-PA complex as those in the 'high' LPL concentration and 'low' triglyceride concentration groups both displayed a greater engagement in $_{10}$ MVPA (7.8% and 48.4%, respectively) compared to the entire sample population. In addition, $_{10}$ MVPA had the greatest difference from the entire sample population, compared to the other PB intensities, for both LPL and triglyceride, suggesting that these cardio-metabolic markers are influenced more by $_{10}$ MVPA compared to any other PB. This finding supports that of a previous SB break study, which found 30 mins of MVPA (which would be classified as $_{10}$ MVPA in the current study) maintained plasma triglyceride concentration (relative to baseline) following ingestion of a high fat meal (35%) (Engeroff et al., 2017). In addition, Engeroff et al. (2017) reported that short bouts of MVPA (representing sMVPA in the current study) were not sufficient to prevent an increase in triglyceride concentration following meal ingestion. This was also notable in the present study, as engagement in sMVPA in the 'low' and 'high' triglyceride groups only deviated 2.3% and 1.9% from the entire sample population, respectively, suggesting that sMVPA has little influence on circulating triglyceride levels.

The pattern of PB compositions for total cholesterol is not as clear within the current study. This is likely due to total cholesterol containing lipoproteins that have opposite responses to inactivity. Both triglyceride and low-density lipids (LDL-C) increase in concentration, whereas high-density lipids (HDL-C) decrease in

concentration, during detraining (Petibois et al., 2004). Therefore, it is difficult to ascertain whether/which PB(s) are affecting LDL-C and HDL-C profile. Future research, should conduct CoDA with total cholesterol segregated into HDL-C and LDL-C to provide a more precise understand of the effects of PB.

Overall, our results suggest that engagement in $_{10}$ MVPA influences triglyceride concentration by targeting LPL pathways. This finding advocates the need for older adults to be 'physically active' in terms of attaining sufficient $_{10}$ MVPA, as defined in the UK government PA guidelines, especially as LPL is already reduced in older adults, compared to young adults (Nikkila and Niemi, 1957).

Glucose Metabolism

The prevalence of physical inactivity and SB, even in acute episodes, has a marked influence on insulin insensitivity and subsequently on reduced glucose uptake (Stephens et al., 2011; Dunstan et al., 2012; Bailey and Locke, 2014), predominantly in skeletal muscle tissue (Stuart et al., 1988). Our results support this concept as those with a 'high' glucose concentration engaged in more sleep and SB, and less PA (excluding sMVPA), compared to the entire sample population. This increased circulating glucose concentration is thought to be due to the reduced translocation of glucose transporter type 4 (GLUT4) to the skeletal muscle cell membrane (Xu et al., 2015) and reduced expression of carbohydrate metabolism genes during bouts of reduced muscle contractile activity (Sleep and SB) such as, cytoplasmic dynein light chain 1 (*DYNLL1*) (Latouche et al., 2013) which, plays a role in GLUT4 translocation (Fletcher et al., 2000). Our results may also suggest that habitual higher SB and lower PA engagement can have a chronic effect on glucose homeostasis, as participants with a 'high' HbA1c percentage also engaged in more sleep and SB, and less $_{10}$ MVPA, compared to the entire sample population (with other PA apparently having little effect on HbA1c). HbA1c represents a 1-3 month average of blood glucose concentration (Gabbay et al., 1977) and can be used in the diagnosis of diabetes mellitus if blood tests exceed 6.5%. The results of the current study are consistent with previous findings in older English adult populations (≥ 60 yrs), which found HbA1c percentage increased as objective SB time increased from 8.45 – 9.52 hrs·day⁻¹ to >9.52 hrs·day⁻¹ (5.8 [0.8], 6.0 [0.8]%,

$p=0.01$, respectively) and reduced 0.13% (95%CI -0.24, -0.03) per 0.5 hrs-day⁻¹ increase in MVPA engagement (Stamatakis et al., 2012). Interestingly, in line with our results, Stamatakis et al. (2012) also reported that LIPA was not associated with HbA1c. Our results therefore support that PA has to be of at least moderate intensity in order to maintain increased insulin sensitivity and subsequently glucose uptake, post-exercise (Fujii et al., 2000).

Overall, the current study suggests that reduced SB and increased PA could lead to acutely reduced blood glucose concentration in older adults. However, to maintain a 'healthier' chronic glucose homeostasis, PA may have to be of a moderate-vigorous intensity. Therefore, we urge older adults to minimise SB engagement and attain a 'physically active' lifestyle to increase the likelihood of a 'healthy' glucose profile.

Inflammation and Vascular Stiffness

In older adults, IL-6 serum concentration is greater compared to young adults (Wei et al., 1992) and is associated with an increased risk of CVD (Jenny et al., 2002). The current study suggested that a greater engagement in sleep and SB could be beneficial towards the reduction in inflammation as those with a 'low' IL-6 concentration engaged in 4.8% and 3.8% more sleep and SB, respectively, compared to the entire sample population. This is in agreement with previous older adult findings, which suggested that IL-6 concentration reduced by 2.0 pg·mL⁻¹ with a >1.5 hour reduction in total awake time (Vgontzas et al., 2003). However, it was previously illustrated that an hour increase in SB would increase IL-6 by 0.24 (95%CI 0.13, 0.35) pg·mL⁻¹ in older adults (Henson et al., 2013a). The discrepancies between Henson et al. (2013a) and the current study may lie in the type of analysis. Henson et al. (2013a) used a multiple linear regression model, which does not account for the influence of other PB(s) on IL-6 in the same way CoDA does, and has been shown to under-estimate the magnitude and direction of associations, when all other PB(s) are not accounted for (Dumuid et al., 2017). It is thought that increases in serum IL-6 is a result of increased IL-6 concentration within the muscle, which occurs during repeated muscular contraction (Steensberg et al., 2000). Therefore, it is possible to postulate that an elevated amount of sleep engagement

may be necessary for older adults to manage the inflammatory response, as reduced engagement in sleep is associated with an increase in IL-6 concentration and subsequently increased pain ratings within healthy middle-aged adults (Haack et al., 2007). This relationship between pain and IL-6 may also explain why the 'low' IL-6 group engage in more SB as qualitative evidence stated that older adults' main determinant for engaging in SB is to reduce sensations of pain (Chastin et al., 2014). However, given the negative effects SB appears to have on other cardio-metabolic parameters within the current study (total cholesterol, triglyceride, glucose, HbA1c, and LPL); it would be advised that older adults engage in more sleep than SB to improve IL-6 profile.

Increased PIIINP concentration is a marker of vascular stiffness in older adults (Agarwal et al., 2014). Within PB research, there is an apparent lack of investigations into changes in PIIINP with PB (excluding resistance training). To the author's knowledge, only one study exists, which suggested 10-weeks of LIPA and MVPA were not sufficient to cause a change in middle-older adults' (51 – 71 yrs) PIIINP concentration (Cornelissen et al., 2010). Our results suggest that those with a 'low' PIIINP concentration engage in a longer duration of all PB(s) (excluding ₁₀MVPA), most noticeably sleep, compared to the sample population and *vice versa* for the 'high' PIIINP population. The current study, therefore suggests that future research should examine the associations between PB and PIIINP in older adults to confirm or refute whether PB behaviour change can help reduce vascular stiffness through PIIINP pathways.

Conclusion

The current study is the first (to the author's knowledge) to utilise CoDA to analyse the effect of PB on the cardio-metabolic profile of an older adult population. CoDA revealed that all PB intensities play a role in the maintenance of cardio-metabolic profile. For a healthy lipid profile our results suggested that older adults should attain a 'physically active' status, as ₁₀MVPA engagement was greater in the 'high' LPL concentration group and subsequently greater in the 'low' triglyceride concentration group. For glucose homeostasis, the current study recommended that older adults should reduce their engagement in SB by engaging in PA. In addition,

it was suggested that being 'physically active' may contribute to chronic glucose homeostasis, as shown by the HbA1c results. Finally, a 4.8% (approximately 25 mins·day⁻¹, based on the geometric mean of sleep for our sample population) increase in the amount of sleep engagement may be essential for older adults to reduce inflammation, especially in episodes of pain, which has been associated with increasing IL-6 concentration.

Overall, the current study recommends that older adults should aim to be 'physically active' by engaging in prolonged bouts of MVPA whilst in the remaining hours of the day, they should aim to reduce SB (in spite of the apparent benefit for IL-6), where possible (to minimise pain), by engaging in low intensity PA (standing and LIPA), as these PB are highly co-dependent.

Part 2: Isotemporal substitution approach.

Statistical Analyses

SPSS version 22 (IBM, New York, USA) was used for statistical modelling. Only total PB variables, measured in mean hrs·day⁻¹ (SB, Standing, LIPA, sMVPA, and 10MVPA) and the summation of these parameters (Total PB) were used for ISM. Pearson correlation was used to assess multicollinearity between PB parameters and total PB; no adjustment to the data was made if multicollinearity was present. The multicollinearity results are the same as those found in Chapter 03 Part 2. The largest case of collinearity was between SB and LIPA ($r^2=-0.69$), which was below the suggested limits of collinearity ($r^2>0.9$) (Mekary et al., 2013). To illustrate the effect on cardio-metabolic parameters with the replacement of one hour of a PB with another, the replaced PB parameter was removed from the linear regression model (forced entry) (i.e. replace SB model: Intercept + (β_1 x Standing) + (β_2 x LIPA) + (β_3 x sMVPA) + (β_4 x 10MVPA) + (β_5 x Total PB) + Covariates + Error) (Mekary et al., 2013). Significant predictors in the model illustrate that the replacement of a PB with the significant PB would have an effect on the cardio-metabolic parameter. To standardise the reporting the outcome of the ISM the removed PB is mentioned first. For example, *'the replacement of one hour of SB with standing reduced total cholesterol'* means that SB engagement has been reduced by one hour and replaced with one hour of standing.

ISM was performed without (Model 1) and with (Model 2) covariate adjustment (BP medication [mg·day⁻¹], lipid-lowering medication [mg·day⁻¹], primary CVD targeting medication [n ·day⁻¹], CVD (in)directly targeting medication [n ·day⁻¹], and inflammatory + CVD (in)directly targeting medication [mg·day⁻¹]). A covariate was included in the ISM if a forced entry linear regression model had highlighted an association between the covariate and the respective cardio-metabolic parameter. These covariates were chosen as they have been shown to influence the cardio-metabolic parameters assessed in the current study (Furberg et al., 1994; Bakris et al., 2004; McIntyre et al., 2006; Tsuboi et al., 1995). Cardio-metabolic data were naturally LOG transformed if they violated normal distribution (total cholesterol, triglyceride, LPL, IL-6, and PIIINP). Post-hoc power analysis was performed using

G* Power version 3.1.9.2 (Heinrich Heine University, Dusseldorf, Germany) to determine the probability of finding a 'true' effect. Data are presented as beta coefficient (95%CI) unless stated otherwise. Statistical significance was set at $p \leq 0.05$.

Results

The demographics of the participants are the same as previously mentioned in Chapter 03 Part 1 and Part 2. It is pertinent to note that for simplicity, only significant models are presented in the results section. The complete set of the results is displayed in Appendix Chapter 05 table A5.2.1-7. Significant ISM was present for total cholesterol and triglyceride (figure 5.2.1-2) i.e. two out of a possible seven cardio-metabolic markers that were monitored.

Total Cholesterol

Without covariate adjustment the replacement of one hour of SB with standing reduced total cholesterol by (anti-logged data) 1.14 (95%CI 1.28, 1.01) $\text{mmol}\cdot\text{l}^{-1}$ (figure 5.2.1). This is clinically relevant as a 0.5 $\text{mmol}\cdot\text{l}^{-1}$ decrease in total cholesterol concentration is associated with a 17% decreased risk in coronary heart disease mortality (Verschuren et al., 1995). The replacement of LIPA with SB also reduced total cholesterol (anti-logged data, 1.08 [95%CI 1.19, 1.0001] $\text{mmol}\cdot\text{l}^{-1}$). Interestingly, the replacement of LIPA with standing caused a larger reduction in total cholesterol (anti-logged data, 1.25 [95%CI 1.49, 1.04] $\text{mmol}\cdot\text{l}^{-1}$) (figure 5.2.1). Finally, the replacement of sMVPA with standing reduced total cholesterol by (anti-logged data) 1.15 (95%CI 1.32, 1.01) $\text{mmol}\cdot\text{l}^{-1}$ (figure 5.2.1). With adjustment for primary CVD targeting medication ($n\cdot\text{day}^{-1}$) and CVD (in)directly targeting medication ($n\cdot\text{day}^{-1}$), the replacement of LIPA with SB was no longer significant whilst all other models remained significant (figure 5.2.1). The power of the ISM for total cholesterol ranged between 97 – 99%.

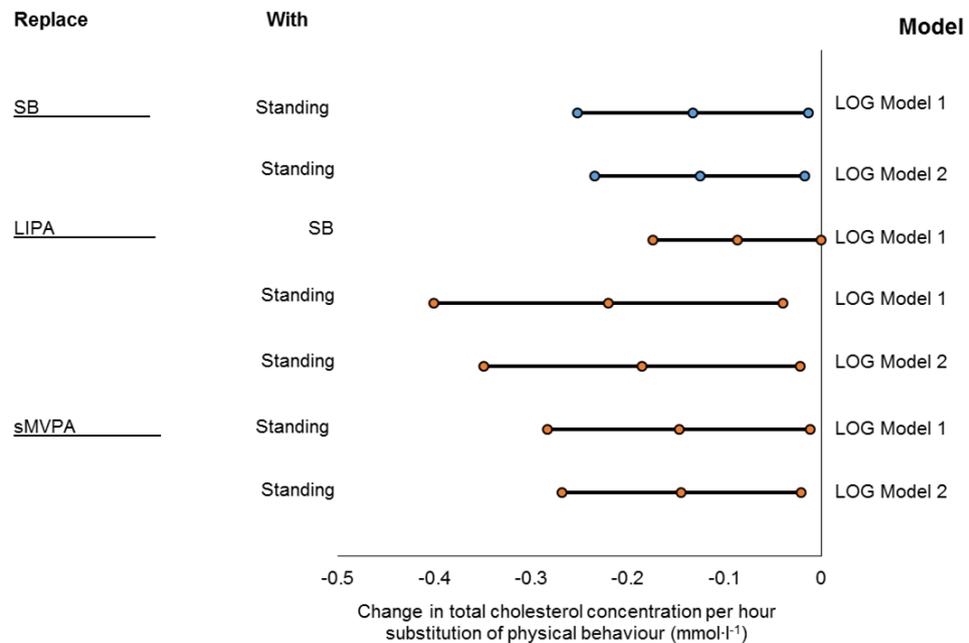


Figure 5.2.1. Significant changes in total cholesterol concentration with the replacement of one hour of PB with another. Model 1: no covariate adjustment. Model 2: adjusted for primary CVD targeting medication ($n\text{-day}^{-1}$) and CVD (in)directly targeting medication ($n\text{-day}^{-1}$). Data points represent LOG data (from left to right): -95%CI, beta coefficient, +95%CI. $p \leq 0.05$. Blue circles indicate a positive effect on cardio-metabolic status. Orange circles indicate a negative effect on cardio-metabolic status.

Triglyceride

There were no covariates for triglyceride. Most notably, the replacement of any PB with $_{10}$ MVPA decreased triglyceride concentration by similar amounts, as the change in concentration fell between (anti-logged data) 1.89 – 2.03 (95%CI range: 3.16, 1.17) $\text{mmol}\cdot\text{l}^{-1}$ (figure 5.2.2). On the other hand, the replacement of $_{10}$ MVPA with any other PB (excluding sMVPA) increased triglyceride concentration by similar amounts (anti-logged data, beta coefficient range: 1.79 – 1.86 [95%CI range: 1.17, 2.94]) $\text{mmol}\cdot\text{l}^{-1}$ (figure 5.2.2). These results are clinically relevant as a $1.0 \text{ mmol}\cdot\text{l}^{-1}$ increase in triglyceride concentration is associated with a 14% (95%CI 5, 28%, male) - 37% (95%CI 13, 66%, female) relative risk increase in CVD prevalence (Hokanson and Austin, 1996). The power of the ISM for triglyceride ranged between 93 – 98%.

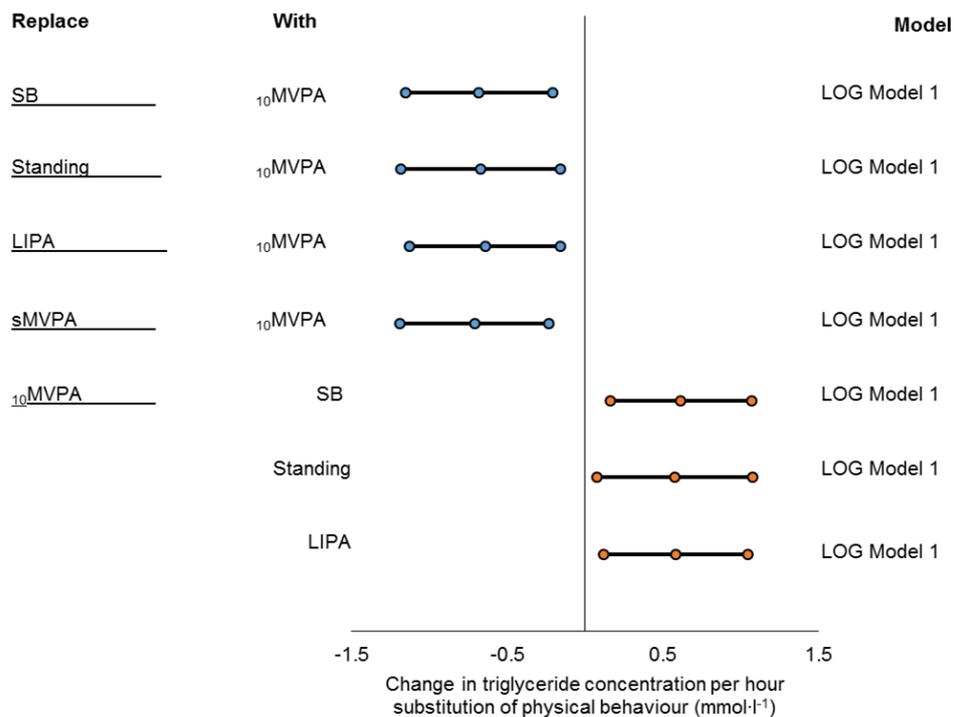


Figure 5.2.2. Significant changes in triglyceride concentration with the replacement of one hour of PB with another. Model 1: no covariate adjustment. Data points represent LOG data (from left to right): -95%CI, beta coefficient, +95%CI. $p \leq 0.05$. Blue circles indicate a positive effect on cardio-metabolic status. Orange circles indicate a negative effect on cardio-metabolic status.

Discussion

The objective of this analysis study was to theoretically illustrate how a strictly older adult population (60 – 89 yrs) can change their cardio-metabolic profile through a change(s) in PB engagement using ISM. It was hypothesised that substitution of SB or PA with PA of higher intensity may improve cardio-metabolic profile whilst the substitution of a higher intensity PA with a lower intensity PA or SB may worsen cardio-metabolic profile, as has been illustrated previously in young-middle and middle-older adult populations (Ekblom-Bak et al., 2016; Hamer et al., 2014; Healy et al., 2015). Indeed the hypotheses were confirmed as the replacement of SB with standing decreased total cholesterol concentration and the replacement of any PB with ₁₀MVPA decreased triglyceride concentration. Furthermore, the replacement of sMVPA and LIPA with standing decreased total cholesterol concentration and the replacement of ₁₀MVPA with a lower intensity PB (excluding sMVPA) increased triglyceride concentration.

Isotemporal Substitution Modelling is a relatively new form of statistical analysis within PB research, first used to predict weight change in middle-aged women (25 – 42 yrs) (Mekary et al., 2009). Since the seminal research, 38 publications have utilised ISM (PubMed, retrieved 17/07/2017). Of those, only one study investigated the effects on cardio-metabolic health in a strictly older adult population (65 – 70 yrs) (Nilsson et al., 2017). However, ISM was only used to predict the effect of PB change on clustered metabolic risk scores and waist circumference. Therefore, the current study is the first, in older adult populations, to provide a case-by-case ISM analysis for seven cardio-metabolic parameters that are associated with CVD.

The Two Sides to Standing

Total cholesterol is comprised of high-density lipid cholesterol (HDL-C), low-density lipid cholesterol (LDL-C), and triglyceride. The reduction in total cholesterol concentration, after sMVPA and LIPA were replaced with standing in the current model, may be a product of lowered HDL-C, thereby suggesting that standing negatively affects cardio-metabolic status. Indeed, one month of detraining following a LIPA intervention in older adults (mean age 75.5 ± 5.60 yrs) has been reported to decrease HDL-C concentration whilst LDL-C and triglyceride remained constant (Motoyama et al., 1995). These results are apparently trustworthy as post-hoc analysis suggests that the power of the study is 98% and therefore there was only a 2% chance of a type I error.

Not only did the replacement of sMVPA and LIPA with standing reduce total cholesterol within the current study but total cholesterol concentration also decreased when SB was replaced with standing, in the current study. It could be postulated that standing is positively affecting lipoprotein profile in this case, possibly by reducing non HDL-C lipoproteins. This postulation is in agreement with a four-day intervention study in young adults (21 ± 2 yrs), which found non-HDL-C concentration decreased from 2.94 ± 0.47 to 2.65 ± 0.48 $\text{mmol} \cdot \text{l}^{-1}$ following the replacement of six hours of SB with two hours of standing and four hours of walking, compared to a $14 \text{ hrs} \cdot \text{day}^{-1}$ of SB day (Duvivier et al., 2013). Meanwhile, there was also a non-significant trend for total cholesterol to decrease from 4.20 ± 0.67 to

3.96±0.50 mmol·l⁻¹ ($p=0.17$) (Duvivier et al., 2013). Although ISM can suggest an intervention, it cannot state how long an intervention can take to have a significant effect on health status. However, along with the results of Duvivier et al. (2013), it can be suggested that the replacement of one hour of SB with standing could reduce total cholesterol if the intervention lasts longer than four days.

Overall, these findings suggest that standing can affect circulating cholesterol, differentially, depending on which PB parameter it is displacing and thus facilitates the prediction of individualised outcomes from PB change interventions.

Benefits of Prolonged Moderate Activity

Performing longer bouts of MVPA may induce a chronic physiological effect that protects against physical inactivity, as total cholesterol concentration decreased when sMVPA but not ₁₀MVPA, was replaced with standing within the current study. In terms of the time frame for adaption in cholesterol profile with the introduction of physical inactivity, previous studies have varied considerably depending on what duration of MVPA bouts was being undertaken prior to physical inactivity. For example, the lipoprotein profile of trained endurance rowers took 29 weeks, following the cessation of exercise (performed in bouts of MVPA ≥10 mins), to become significantly different. Specifically, HDL-C decreased from 0.92±0.09 pre detraining to 0.81±0.11 mmol·l⁻¹ post detraining ($p<0.05$, a 0.004 mmol·l⁻¹ reduction per week) (Petibois et al., 2004). Whereas, it took only four weeks until HDL-C concentration was significantly reduced (from 1.42±0.05 to 1.34±0.05 mmol·l⁻¹, $p<0.05$, a 0.02 mmol·l⁻¹ reduction per week) following the cessation of a four-month interval training intervention (performed in bouts of MVPA <10 mins, 4 × 4 mins MVPA, 3 mins rest) (Mora-Rodriguez et al., 2014). Despite these previous finding being reported in middle-aged adults, there are similarities in cardio-metabolic change with a reduction in MVPA when compared with the current study. It is likely that engagement in prolonged bouts of MVPA (₁₀MVPA) would be beneficial in the preservation of long-term cholesterol profile should an older adult be temporarily reduced to a physically inactive lifestyle (i.e. due to illness or injury).

Furthermore, the decrease in fasted triglyceride concentration when any PB, including sMVPA, is replaced with ₁₀MVPA in the current study, also strengthens

the theorem that prolonged MVPA bouts would improve/maintain lipoprotein profile. In addition, the lack of effect on fasted triglyceride concentration when SB was replaced for PA suggests that a focus on reducing physical inactivity is more important than SB reduction when attempting to improve triglyceride profile. Whereas the lack of effect on fasted total cholesterol when MVPA is substituted out of the model (and improvements when SB is substituted) could suggest that SB reduction as opposed to physical inactivity reduction could be more important for total cholesterol profile improvements, possibly due to the effects on LDL-C and HDL-C pathway alluded to above. However, intervention studies would be required to confirm or deny this hypothesis.

No Effect of Physical Behaviour on Lipoprotein Lipase

Pre-heparin serum LPL concentration was measured within the current study and it is thought to represent whole-body LPL production and the systemic potential to hydrolyse triglycerides (Shirai et al., 1999; Watanabe et al., 1999). Additionally, pre-heparin serum LPL has been shown to be inversely related to the progression of coronary artery disease in young/middle – older aged populations (Hitsumoto et al. (2000): 22 – 79 yrs, Rip et al. (2006): 45 – 79 yrs), highlighting that pre-heparin serum LPL is anti-atherogenic. Furthermore, exercise has been shown to increase LPL within humans and rats in all age groups (Kantor et al., 1987; Hamilton et al., 1998; Nikkilä et al., 1978), thus suggesting that PA can protect against atherosclerosis through the mediation of the LPL pathway. Conversely, the results of the current study suggested that the modelling of replacement of any PB did not affect serum LPL concentration. A possible explanation for these results is that pre-heparin serum LPL is representative of whole-body LPL (Shirai et al., 1999; Watanabe et al., 1999), in which, the expression of LPL within certain sites can portray a pro-atherogenic effect (e.g. artery wall) (see Goldberg (1996) for a review). Therefore, the representation of both pro and anti-atherogenic LPL within pre-heparin serum LPL may cause ‘noise’ within the ISM, thus nullifying any effects on pro or anti-atherogenic LPL that may exist with PB change. Furthermore, this ‘noise’ effect is likely to be more pronounced in the older adult population, who already have an increased serum LPL concentration compared to middle aged adults (Saito et al., 1998) and are more likely to show progression of atherosclerosis, which has

been positively associated with LPL concentration within the aorta (Zilversmit, 1979).

Are These Results Generalisable to the Population?

There is a strong confidence that the results for total cholesterol are displaying a 'true' effect as post-hoc power analysis suggested that there was a 97 – 99% probability of detecting an effect of replacing a PB, if one genuinely existed, when ISM was adjusted for primary CVD targeting medication and CVD (in)directly targeting medication (Model 2). This was also the case for triglyceride, where there was a 93 – 98% probability of detecting an effect of replacing a PB, if one genuinely existed. Therefore, these predicted changes in total cholesterol and triglyceride concentration with a change in PB are generalizable to the population as a whole and likely to be observed in replicated studies.

Regarding the cardio-metabolic parameters that showed no predicted change in the ISM (glucose, HbA1c, LPL, IL-6, and PIIINP), the post-hoc power analysis suggested that the probability of detecting an effect of replacing PB ranged between 13.5% (IL-6) – 61% (LPL). This would suggest that these results would need to be replicated in larger samples in future studies in order to confirm or refute the results of the current study. Post-hoc analysis of the results of the current study would suggest a sample size between 846 (IL-6) – 139 (LPL) would be required in order to achieve a statistical power of 95%.

Conclusion

Isotemporal substitution modelling highlighted that only the lipid markers within the seven assessed cardio-metabolic markers (total cholesterol, triglyceride, LPL, plasma glucose, HbA1c, IL-6, and PIIINP) were affected by PB. Therefore, the results of the current study suggest that using PB interventions could be a useful method for the prevention and treatment of lipid disorders, such as atherosclerosis, which when it manifests in the coronary arteries, is responsible for 44.5% of CVD deaths in UK older adults (Townsend et al., 2015). The results of the current study

suggested that replacing SB with standing could decrease total cholesterol concentration, possibly through a reduction in LDL-C, although this was not measured in this study. However, the replacement of LIPA and sMVPA with standing also reduced total cholesterol concentration within the current study. It was postulated that in this instance, the decrease in total cholesterol was presenting a detraining effect, targeting HDL-C concentration, which has been shown previously in older adults (Motoyama et al., 1995).

At the other end of the PB spectrum, prolonged engagement in MVPA (bouts ≥ 10 mins) appeared to be essential to the mediation of triglyceride concentration as the replacement of any PB with ≥ 10 MVPA decreased triglyceride concentration. Overall, the current study adds further evidence to support the 10-minute bout recommendation within the MVPA guidelines of PA for older adults, as well as highlighting that quantitative guidelines are required for reducing SB engagement in older adults. Finally, the recent study has potentially shown that a PB can have both a positive and negative effect on cardio-metabolic status (though requiring further investigation), dependent on what PB it is replacing. Thus, advocating the need for individualised interventions, where possible, that account for the participant's habitual PB engagement, rather than a one size fits all approach.

Part 3: Z-score approach.

Statistical Analyses

Grouping

Excel 2013 (Microsoft, Washington, USA) was used to create radar graphs displaying the difference in patterns of PB parameters between participants with a 'low' and 'high' cardio-metabolic concentration for the parameters outlined in table 5.3.1. The thresholds provided and the handling of covariates are the same as those in Part 1 of this chapter.

Table 5.3.1. Cardio-metabolic threshold values used to determine participant groupings into 'low' and 'high' endocrine concentration.

Cardio-metabolic Parameter	Threshold
Glucose	6.0 mmol·l ⁻¹ (World Health Organization, 2015a)
Total Cholesterol	5.0 mmol·l ⁻¹ (World Health Organization, 2015a)
Triglyceride	1.7 mmol·l ⁻¹ (World Health Organization, 2015a)
HbA1c	5.29%
LPL	63.5 pg·mL ⁻¹ (Rip et al., 2006)
IL-6	2.29 pg·mL ⁻¹ (Cesari et al., 2003)
PIIINP	229.215 pg·mL ⁻¹

Mean HbA1c percentage used as the threshold. Median PIIINP concentration used as the threshold.

Correlations

SPSS version 22 (IBM, New York, USA) was used to conduct Pearson's correlations (or Spearman rho, if one variable violated normal distribution) were used to determine whether patterns of PB parameters could explain the variance in cardio-metabolic markers. Statistical significance was set at $p \leq 0.05$.

Z-scores

To improve ease of graphical representation, the mean PB pattern parameter for the 'low' and 'high' cardio-metabolic parameter concentration groups was transformed into a z-score, relative to the entire sample population (equation 5.3.1). Therefore, data within these radar graphs are dimensionless quantities with each number in effect being the number of standard deviations (SD) from the mean of the entire sample population.

Equation 5.3.1. Z-score calculation.

$$\text{Z-score} = [\text{mean of group} - \text{mean of sample population}] \div \text{standard deviation of sample population}$$

Z-score Comparison

To calculate the difference between group z-scores for a respective pattern of PB, the area (presented as a percentage within a normal distribution) left of the z-score of the 'low' and 'high' cardio-metabolic parameter groups was determined using a standard normal distribution z-score table and/or excel function =NORMSDIST(z-score). The distance (presented as a percentage of area within a normal distribution) between the calculated areas was then determined by subtracting the lowest area from the largest area.

Overall Cardio-metabolic Impact

To determine the effect patterns of PB have on overall cardio-metabolic profile ($n=7$ parameters), three methods were used as there is no single standard method to combining effects on different systems/scales. In the first, each pattern of SB and PA was ranked (highest to lowest) based on how large the distance in z-scores was between the respective 'low' and 'high' cardio-metabolic parameter groups. These rankings were then summed and ranked, from lowest to highest. This was performed separately for patterns of SB and PA.

For method 2, participants were also grouped based on how frequently they had a 'high' cardio-metabolic parameter concentration, or 'low' LPL concentration. These groups were: 0 – 3 'high' metabolites score, and 4 – 6 'high' metabolites score. This categorisation was performed using the concentrations of glucose, total cholesterol, triglyceride, LPL normalised for inflammatory + CVD (in)directly targeting medication ($\text{mg}\cdot\text{day}^{-1}$), IL-6, and PIIINP. HbA1c was excluded from this categorisation as data was only present for 33 out of 93 participants. Missing data for the other cardio-metabolic variables was considered as a 'low' cardio-metabolic concentration.

Finally, for method 3 of overall cardio-metabolic effect, participants had a z-score calculated for each cardio-metabolic parameter (e.g. $\text{z-score} = [\text{participant IL-6 concentration} - \text{study population mean IL-6 concentration}] \div \text{SD of study population IL-6 concentration}$), excluding HbA1c due to a low sample size for this variable. As a 'high' LPL concentration is associated with an improved health status, the direction of the z-scores for LPL were reversed. Therefore, a positive z-score would represent an 'unhealthy' profile for all cardio-metabolic parameters. The z-scores for the respective cardio-metabolic parameters were summed for each participant, known as a unit weighted method, to calculate a composite z-score. Participants were then grouped into a composite z-score ≤ 0 or > 0 . Participants with missing data were included in the composite z-score calculation.

Results

Demographics

The average engagement in patterns of PB are presented in table 5.3.2 and are segregated based on which cardio-metabolic marker was being assessed and whether the participant was classified into the 'low' or 'high' concentration group. A z-score of 1 in these results represented the SD of the pooled data for the respective pattern of PB and cardio-metabolic marker.

Table 5.3.2. Patterns of PB engagement segregated into pooled, 'low', and 'high cardio-metabolic marker groups. Data presented as Mean±SD.

Patterns of PB	Total Cholesterol			Triglyceride			LPL		
	Pooled	Low	High	Pooled	Low	High	Pooled	Low	High
SB% (%·waking hrs·day ⁻¹)	61.9± 9.54	61.5± 9.62	62.1± 9.58	61.9± 9.54	59.5± 8.50	63.8± 9.95	61.7± 9.34	60.4± 10.6	62.2± 8.83
SB Breaks (<i>n</i> ·day ⁻¹)	22.1± 3.51	21.4± 3.39	22.5± 3.55	22.1± 3.51	22.8± 3.73	21.6± 3.25	22.0± 3.64	21.3± 4.67	22.3± 3.17
<5min SB Bout (<i>n</i> ·day ⁻¹)	6.37± 1.94	6.27± 2.01	6.42± 1.92	6.37± 1.94	6.77± 1.88	6.07± 1.95	6.35± 1.95	5.91± 2.01	6.52± 1.92
≥5min SB Bout (<i>n</i> ·day ⁻¹)	16.4± 2.33	15.6± 2.17	16.8± 2.32	16.4± 2.33	16.8± 2.33	16.1± 2.30	16.4± 2.35	16.0± 2.94	16.6± 2.10
True Mean SB Bout (mins·day ⁻¹)	30.7± 8.96	31.3± 9.20	30.4± 8.91	30.7± 8.96	28.9± 8.60	32.2± 9.05	30.9± 9.29	32.2± 11.8	30.4± 8.15
Alpha (α·day ⁻¹)	1.44± 0.03	1.44± 0.03	1.43± 0.03	1.44± 0.03	1.44± 0.03	1.43± 0.03	1.44± 0.03	1.44± 0.03	1.44± 0.03
W50% (mins·day ⁻¹)	54.1± 14.4	56.2± 15.7	53.1± 13.8	54.1± 14.4	49.5± 13.3	57.9± 14.3	53.9± 14.0	54.5± 16.7	53.7± 13.0
Standing% (%·waking hrs·day ⁻¹)	6.97± 2.40	7.40± 2.10	6.77± 2.53	6.97± 2.40	7.13± 2.06	6.85± 2.64	7.07± 2.47	6.77± 2.54	7.18± 2.46
LIPA% (%·waking hrs·day ⁻¹)	12.6± 3.89	12± 3.75	12.9± 3.96	12.6± 3.89	12.7± 3.66	12.5± 4.10	12.7± 3.81	12.9± 4.12	12.6± 3.72
sMVPA% (%·waking hrs·day ⁻¹)	16.6± 3.92	16.4± 4.27	16.7± 3.78	16.6± 3.92	17.8± 3.55	15.7± 4.00	16.6± 3.87	16.4± 3.68	16.7± 3.97
₁₀ MVPA% (%·waking hrs·day ⁻¹)	0.92± 1.00	1.09± 1.12	0.84± 0.93	0.92± 1.00	1.23± 1.11	0.69± 0.85	0.99± 1.04	0.87± 0.92	1.03± 1.07
PA Bouts (<i>n</i> ·day ⁻¹)	22.1± 3.51	21.4± 3.39	22.5± 3.55	22.1± 3.51	22.8± 3.73	21.6± 3.25	22.0± 3.64	21.3± 4.67	22.3± 3.17
PA Bout Time (hrs·day ⁻¹)	5.79± 1.59	5.88± 1.54	5.74± 1.62	5.79± 1.59	6.21± 1.44	5.46± 1.63	5.82± 1.53	5.95± 1.75	5.77± 1.46
True Mean PA Bout (mins·day ⁻¹)	15.9± 4.57	17.2± 5.00	15.3± 4.23	15.9± 4.57	16.7± 4.28	15.3± 4.73	16.0± 4.35	16.7± 4.93	15.7± 4.14
₁₀ MVPA Bouts (<i>n</i> ·day ⁻¹)	0.46± 0.44	0.47± 0.47	0.45± 0.43	0.46± 0.44	0.58± 0.50	0.37± 0.39	0.48± 0.45	0.48± 0.46	0.48± 0.46
Total ₁₀ MVPA (hrs·week ⁻¹)	1.10± 1.56	1.30± 1.59	0.99± 1.55	1.10± 1.56	1.32± 1.43	0.94± 1.64	1.19± 1.65	1.88± 2.58	0.91± 1.00

Table 5.3.2 continued.

Patterns of PB	Glucose			HbA1c			IL-6			PIIINP		
	Pooled	Low	High	Pooled	Low	High	Pooled	Low	High	Pooled	Low	High
SB%	61.9±	61.2±	63.3±	66.7±	65.3±	67.7±	61.9±	61.8±	61.9±	62.1±	62.2±	62.0±
	9.54	9.68	9.30	9.39	11.8	7.57	9.48	10.0	9.18	9.91	11.3	8.43
SB Breaks	22.1±	22.4±	21.7±	21.4±	21.5±	21.3±	22.1±	21.4±	22.6±	22.3±	22.1±	22.5±
	3.51	3.70	3.15	3.14	3.35	3.08	3.55	3.54	3.51	3.77	3.96	3.61
<5min SB Bout	6.37±	6.49±	6.17±	5.97±	5.91±	6.00±	6.33±	5.69±	6.74±	6.37±	6.33±	6.41±
	1.94	1.98	1.89	1.90	2.00	1.88	1.96	1.87	1.93	1.97	1.75	2.18
≥5min SB Bout	16.4±	16.6±	16.1±	16.0±	16.4±	15.7±	16.5±	16.2±	16.7±	16.6±	16.6±	16.6±
	2.33	2.32	2.34	2.30	2.13	2.41	2.38	2.53	2.30	2.51	2.70	2.33
True Mean SB Bout	30.7±	31.1±	30.1±	34.1±	31.8±	35.5±	30.7±	31±	30.5±	30.7±	30.2±	31.1±
	8.96	9.93	6.91	8.55	9.26	8.00	9.05	9.92	8.57	9.28	10.4	8.12
Alpha	1.44±	1.44±	1.44±	1.43±	1.43±	1.42±	1.44±	1.45±	1.44±	1.44±	1.44±	1.43±
	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.04	0.03	0.03	0.04	0.03
W50%	54.1±	52.6±	57.0±	61.5±	59.2±	63.1±	53.9±	54.6±	53.4±	53.1±	51.7±	54.4±
	14.4	14.8	13.6	14.6	15.9	13.9	14.0	15.7	13.0	14.6	15.4	13.8
Standing%	6.97±	7.26±	6.42±	6.54±	6.54±	6.53±	7.01±	6.84±	7.11±	6.80±	6.73±	6.87±
	2.40	2.24	2.64	2.86	3.02	2.84	2.41	2.02	2.64	2.35	2.41	2.33
LIPA%	12.6±	12.8±	12.3±	11.5±	12.5±	10.9±	12.6±	12.4±	12.8±	12.3±	12.2±	12.4±
	3.89	4.14	3.44	3.83	4.64	3.18	3.85	3.97	3.81	3.87	4.23	3.54
sMVPA%	16.6±	16.7±	16.5±	14.6±	14.7±	14.6±	16.6±	16.8±	16.5±	16.7±	16.8±	16.5±
	3.92	4.27	3.29	3.60	4.13	3.35	3.90	4.64	3.40	4.04	4.42	3.70
₁₀ MVPA%	0.92±	0.97±	0.83±	0.63±	0.69±	0.60±	0.92±	0.80±	1.00±	0.96±	0.91±	1.00±
	1.00	1.05	0.91	0.84	0.88	0.83	0.97	0.79	1.08	1.02	0.97	1.08
PA Bouts	22.1±	22.4±	21.7±	21.4±	21.5±	21.3±	22.1±	21.4±	22.6±	22.3±	22.1±	22.5±
	3.51	3.70	3.15	3.14	3.35	3.08	3.55	3.54	3.51	3.77	3.96	3.61
PA Bout Time	5.79±	5.94±	5.53±	4.97±	5.28±	4.77±	5.81±	5.79±	5.82±	5.75±	5.71±	5.79±
	1.59	1.61	1.53	1.59	1.97	1.31	1.56	1.66	1.52	1.62	1.84	1.40
True Mean PA Bout	15.9±	15.9±	15.9±	14.4±	14.8±	14.2±	16.0±	16.2±	15.9±	15.7±	15.2±	16.2±
	4.57	4.21	5.22	4.96	5.28	4.85	4.50	4.27	4.67	4.67	4.85	4.51
₁₀ MVPA Bouts	0.46±	0.47±	0.43±	0.39±	0.46±	0.35±	0.45±	0.45±	0.46±	0.47±	0.46±	0.48±
	0.44	0.47	0.41	0.52	0.58	0.49	0.43	0.42	0.45	0.46	0.43	0.49
Total ₁₀ MVPA	1.10±	1.12±	1.07±	0.79±	1.08±	0.60±	1.13±	1.02±	1.21±	1.21±	1.07±	1.36±
	1.56	1.68	1.36	1.15	1.49	0.86	1.58	1.29	1.76	1.69	1.32	2.01

Which Patterns of PB Explain the Variance in Cardio-metabolic Profile?

Triglyceride concentration was associated with 11 out of 16 patterns of PB parameters. W50% and ₁₀MVPA% had the largest associations with triglyceride concentration, as they explained 12% (positive and negative association, respectively) of the variance in concentration (table 5.3.3). Glucose was associated with 4 out of 16 patterns of PB parameters. The strongest associated pattern of PB with glucose was W50%, which explained 5% (positive association) of the variation in concentration (table 5.3.3). Finally, total cholesterol was only associated with true mean PA bout, which explained 5% (negative association) of the variation in concentration (table 5.3.3). Patterns of PB did not explain any of the variance in the other cardio-metabolic endocrine profile markers.

Table 5.3.3. The percentage of the variance in cardio-metabolic parameters that is explained by patterns of PB.

Patterns of PB	Total Cholesterol	Triglyceride	LPL	Glucose	HbA1c	IL-6	PIIINP
SB%	0.00	11.0**	1.00	3.00*	1.00	0.00	0.06
SB Breaks	1.00	-2.00	2.00	-1.00	-3.00	1.00	-0.08
<5min SB Bout	0.00	-4.00*	3.00	-2.00	0.00	3.00	-0.06
≥5min SB Bout	3.00	-1.00	1.00	-0.00	-5.00	0.00	-0.70
True Mean SB Bout	0.00	6.00*	-0.00	2.00	4.00	-0.00	0.60
Alpha	-2.00	-3.00*	-0.00	-3.00*	-0.00	-1.00	-2.00
W50%	-0.00	12.0**	0.00	5.00*	1.00	0.00	2.00
Standing%	-1.0	-1.00	-0.00	-4.00*	-0.00	-0.00	0.20
LIPA%	0.00	-2.00	-1.00	-2.00	-6.00	0.00	0.60
sMVPA%	-0.00	-11.0**	-1.00	-2.00	1.00	-1.00	-0.20
₁₀ MVPA%	-1.00	-12.0**	-0.00	0.00	0.00	0.00	-0.20
PA Bouts	1.00	-2.00	2.00	-1.00	-3.00	1.00	-0.08
PA Bout Time	-0.00	-10.0**	-1.00	-3.00	-2.00	-0.00	0.00
True Mean PA Bout	-5.00*	-6.00*	-4.00	-0.00	-0.00	-1.00	1.00
₁₀ MVPA Bouts	-0.00	-8.00**	-1.00	0.00	0.00	0.00	-0.10
Total ₁₀ MVPA	-1.00	-7.00**	-3.00	1.00	-0.00	0.00	-0.10

Spearman rho correlations (one-tailed) used to examine the association between total cholesterol, triglyceride, LPL, glucose, IL-6, PIIINP, and patterns of PB parameters. Pearson correlations (one-tailed) used to examine the association between HbA1c and patterns of PB parameters, excluding W50%, ₁₀MVPA%, ₁₀MVPA Bouts, and Total ₁₀MVPA. * $p \leq 0.05$, ** $p \leq 0.01$.

Triglyceride

The group who had a 'high' triglyceride concentration had patterns of SB (figure 5.3.1A), which are thought to be unfavourable to cardio-metabolic profile (SB%, W50%, true mean SB bout), compared to the 'low' triglyceride concentration group (distance between z-scores: W50%: 22.8%, SB%: 18.0%, and true mean SB bout: 14.7%). While the 'low' triglyceride concentration group had more SB breaks (distance between z-scores: 14.5%), <5min SB bouts (14.3%), and a greater alpha value (9.26%) compared to the 'high' triglyceride group, all of which are suggested to be favourable towards cardio-metabolic profile (figure 5.3.1A). The 'low' triglyceride concentration group had a greater z-score for all of the patterns of PA parameters

compared to the 'high' triglyceride concentration group (distance between z-scores range: 2.07 [LIPA%] – 21.2% [₁₀MVPA%]) (figure 5.3.1B).

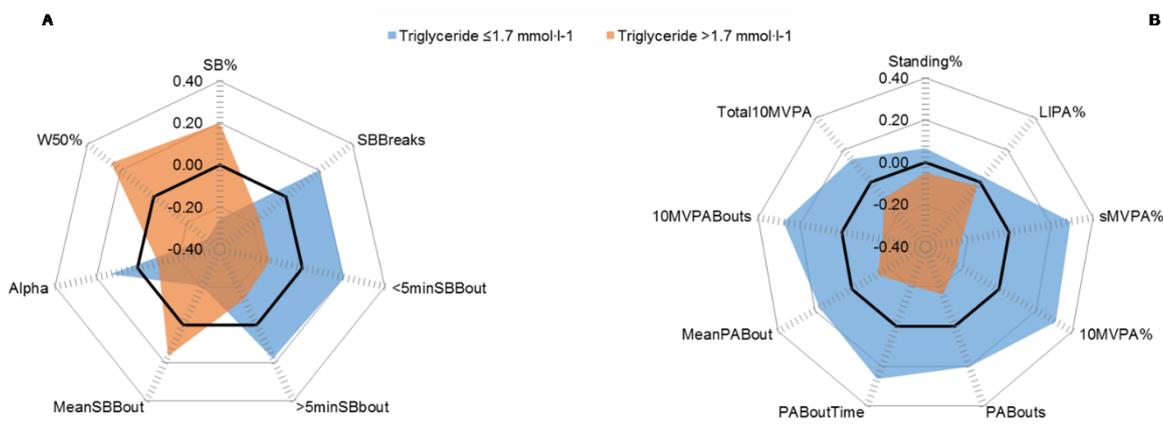


Figure 5.3.1 A) SB z-scores between 'low' (blue) and 'high' (orange) triglyceride concentration groups. B) PA z-scores between 'low' (blue) and 'high' (orange) triglyceride concentration groups. Black line indicates z-score mean (zero). 'Low' group n=40, 'high' group n=52.

Total Cholesterol

The 'low' total cholesterol group had a greater W50%, and true mean SB bout and alpha value compared to the 'high' total cholesterol group (figure 5.3.2 A, distance between z-scores: 8.59, 3.63, and 11.1%, respectively). While the 'high' total cholesterol group had a greater amount of SB breaks, <5min SB bouts, and >5min SB bouts compared to the 'low' total cholesterol group (figure 5.3.2 A, distance between z-scores: 11.6, 3.13, and 20.0%, respectively). For patterns of PA (figure 5.3.2 B), the 'low' total cholesterol group had a greater Standing%, ₁₀MVPA%, PA bout time, true mean PA bout, ₁₀MVPA bouts, and Total ₁₀MVPA, compared to the 'high' total cholesterol group (distance between z-scores range: 1.39 [₁₀MVPA bouts] – 16.9% [true mean PA bout]).

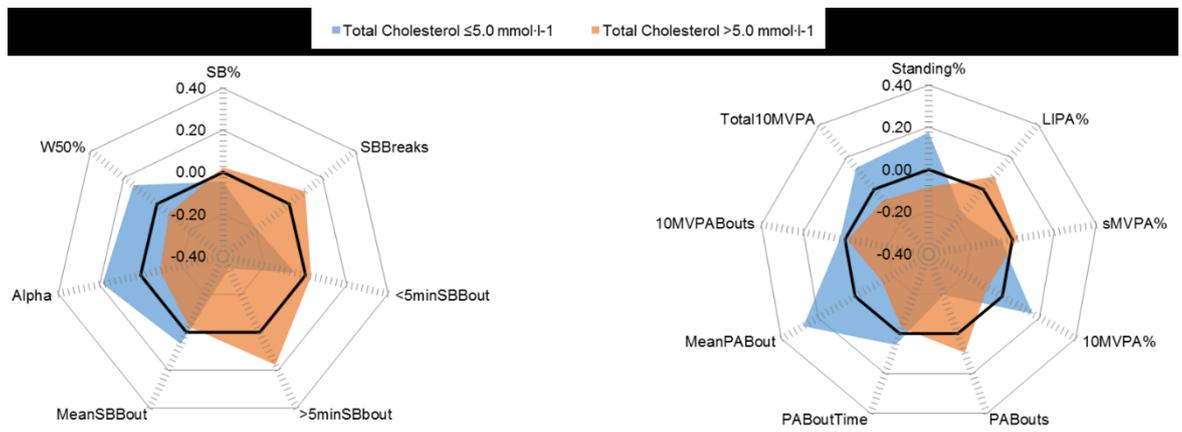


Figure 5.3.2 A) SB z-scores in 'low' (blue) and 'high' (orange) total cholesterol concentration groups. B) PA z-scores between 'low' (blue) and 'high' (orange) total cholesterol concentration groups. Black line indicates z-score mean (zero). 'Low' group n=30, 'high' group n=62.

Lipoprotein Lipase

Participants' serum LPL was normalised for inflammatory + CVD (in)directly targeting medication ($\text{mg}\cdot\text{day}^{-1}$) before being grouped. The 'low' LPL group had a greater W50%, alpha value, and true mean SB bout compared to the 'high' LPL concentration group (figure 5.3.3 A, distance between z-scores: 2.20, 1.07, and 7.56%, respectively). The 'high' LPL group a more SB%, SB breaks, <5min SB bouts, and >5min SB bouts compared to the 'low' LPL concentration group (figure 5.3.3 A, distance between z-scores: 7.77, 10.9, 12.4, and 9.25%, respectively). There was less than one percent distance in $_{10}\text{MVPA}$ bouts z-scores between the 'low' and 'high' LPL concentration groups (figure 5.3.3 B). the 'low' LPL group had a greater total $_{10}\text{MVPA}$, true mean PA bout, PA bout time, and LIPA% z-score compared to the 'high' LPL group (figure x B, distance between z-scores: 22.8, 9.29, 4.79, and 3.48%, respectively). Meanwhile, the 'high' LPL group had a greater PA bouts, Standing%, $_{10}\text{MVPA}\%$, and sMVPA z-score compared to the 'low' LPL group (figure x B, distance between z-scores: 10.9, 6.53, 6.00, and 3.35%, respectively).

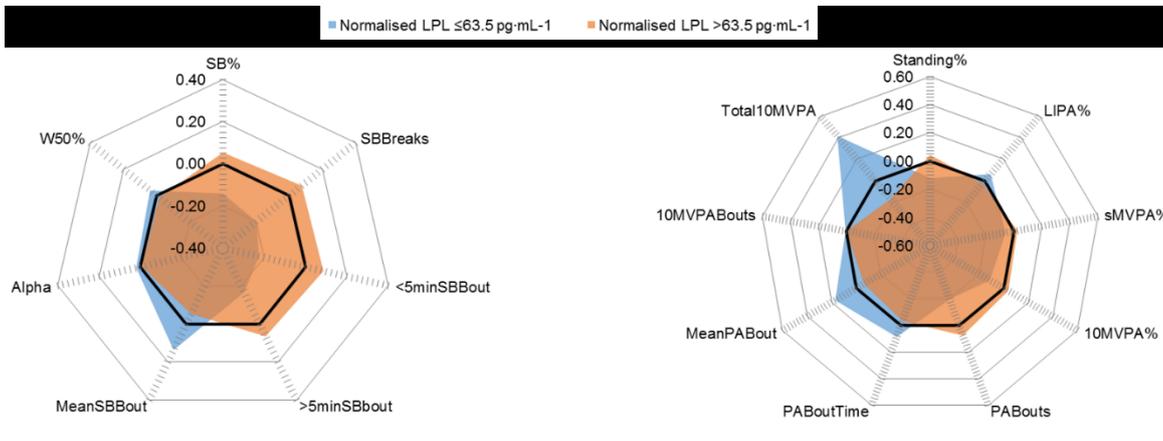


Figure 5.3.3 A) SB z-scores between 'low' (blue) and 'high' (orange) LPL concentration groups. B) PA z-scores between 'low' (blue) and 'high' (orange) LPL concentration groups. Black line indicates z-score mean (zero). 'Low' group n=22, 'high' group n=57.

Glucose

The 'high' glucose concentration group had a greater W50% (distance between z-scores: 12.0%) and SB% (distance between z-scores: 8.66%) z-score compared to the 'low' glucose group (figure 5.3.4 A). The 'low' glucose group had a greater z-score for all other patterns of SB compared to the 'high' glucose group, with the greater difference being present for >5min SB bouts (8.91%) (figure 5.3.4 A). The 'low' glucose group had greater z-scores for all patterns of PA compared to the 'high' glucose group (distance between z-scores range: 1.29 [total 10MVPA] – 13.8% [standing%]), excluding true mean PA bout, which displayed no group differences (distance between z-scores: 0.13%) (figure 5.3.4 B).

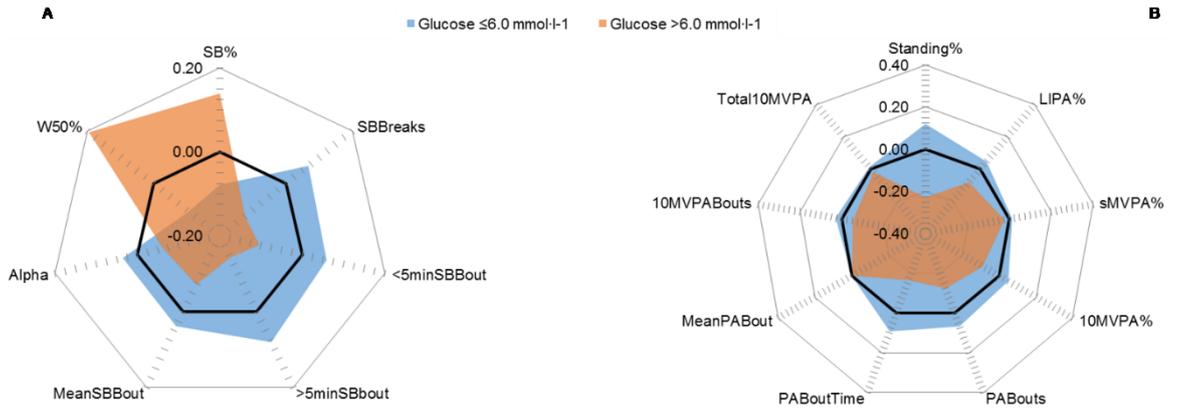


Figure 5.3.4 A) SB z-scores between 'low' (blue) and 'high' (orange) glucose concentration groups. B) PA z-scores between 'low' (blue) and 'high' (orange) glucose concentration groups. Black line indicates z-score mean (zero). 'Low' group $n=60$, 'high' group $n=32$.

Glycated Haemoglobin

The 'high' HbA1c group had a larger true mean SB bout, W50%, SB%, and <5min SB bout z-score compared to the 'low' HbA1c group (figure 5.3.5 A, distance between z-scores: 17.2, 10.4, 10.2, and 1.89%, respectively). Whilst the 'low' HbA1c group had a greater z-score for ≥ 5 min SB bouts, alpha value, and SB breaks compared to the 'high' HbA1c group (figure 5.3.5 A, distance between z-scores: 12.6, 6.57, and 3.37%, respectively). The 'low' HbA1c group had a greater z-score for all patterns of PA parameters, excluding Standing % (distance between groups: 0.19%), compared to the 'high' HbA1c group (figure 5.3.5 B, distance between z-scores range: 1.17 [sMVPA%] – 16.6% [LIPA%]).

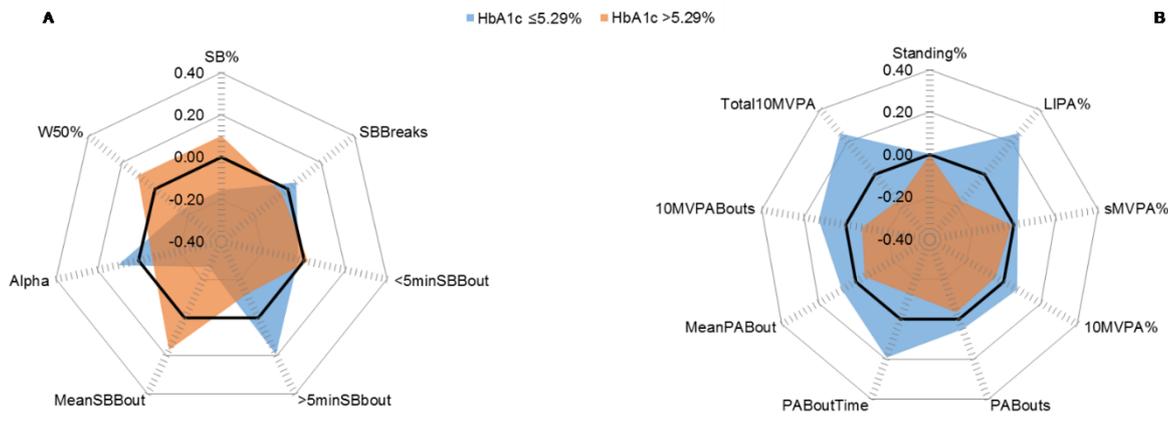


Figure 5.3.5 A) SB z-scores in 'low' (blue) and 'high' (orange) HbA1c percentage groups. B) Comparison of patterns of PA z-scores between 'low' (blue) and 'high' (orange) HbA1c percentage groups. Black line indicates z-score mean (zero). 'Low' group n=13, 'high' group n=20.

Interleukin-6

The 'low' IL-6 group had a greater z-score for alpha value, W50%, and true mean SB bout compared to the 'high' IL-6 group (figure 5.3.6 A, distance between z-scores: 10.9, 3.45, and 2.15%, respectively). The 'high' IL-6 group had a larger z-score for <5min SB bouts, SB breaks, and >5min SB bouts compared to the 'low' IL-6 group (figure 5.3.6 A, distance between z-scores: 20.9, 13.4, and 7.07%, respectively). Notably, there was no difference in z-score between groups for SB% (0.11%) (figure 5.3.6 A). There was no IL-6 group difference for ₁₀MVPA bouts or PA bout time z-scores (<1.00%) (figure 5.3.6 B). The 'high' IL-6 group had a greater z-score for PA bouts, ₁₀MVPA%, total ₁₀MVPA, Standing%, and LIPA% (figure 5.3.6 B, distance between z-scores: 13.4, 7.90, 4.77, 4.45, and 3.53%, respectively). Meanwhile the 'low' IL-6 group had a larger z-score for sMVPA% (distance between z-scores: 3.15%) and true mean PA bout (distance between z-scores: 2.75%) compared to the 'high' IL-6 group (figure 5.3.6 B).

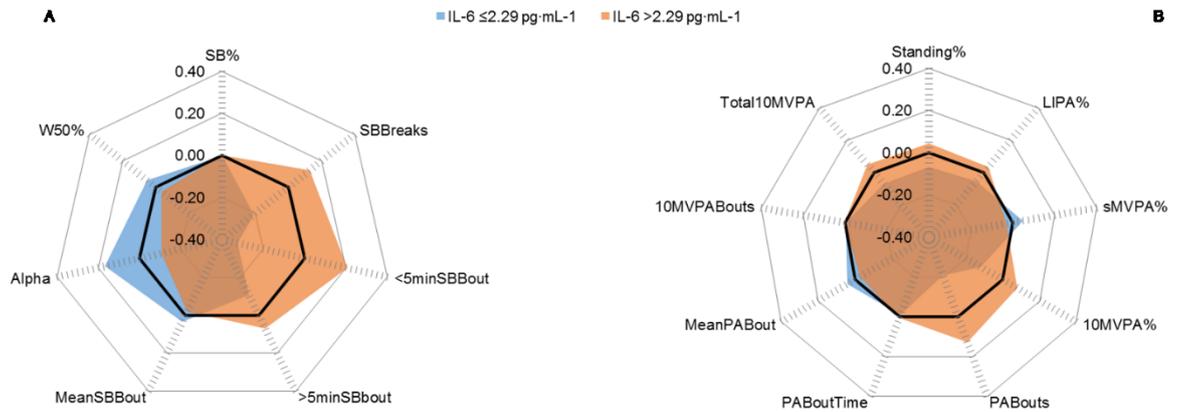


Figure 5.3.6 A) SB z-scores between 'low' (blue) and 'high' (orange) IL-6 concentration groups. B) PA z-scores between 'low' (blue) and 'high' (orange) IL-6 concentration groups. Black line indicates z-score mean (zero). 'Low' group $n=35$, 'high' group $n=53$.

Procollagen 3 N-Terminal Peptide

The 'low' PIIINP concentration group had a larger z-score for alpha value (distance between z-scores: 10.6%) (figure 5.3.7 A). There was no difference in z-score between groups for SB% (distance between z-scores: <1.00%). The 'high' PIIINP group had a greater z-score for W50%, SB breaks, true mean SB bout, <5min SB bouts, and >5min SB bouts compared to the 'low' PIIINP group (figure 5.3.7 A, distance between z-scores: 7.29, 4.14, 3.92, 1.53, and 1.38%, respectively). The 'high' PIIINP group had a greater z-score for all patterns of PA parameters, excluding sMVPA% ('low' group was greater than 'high' group), compared to the 'low' PIIINP group (figure 5.3.7 B, distance between z-scores range: 1.95 [10MVPA bouts] – 7.88% [true mean PA bout]).

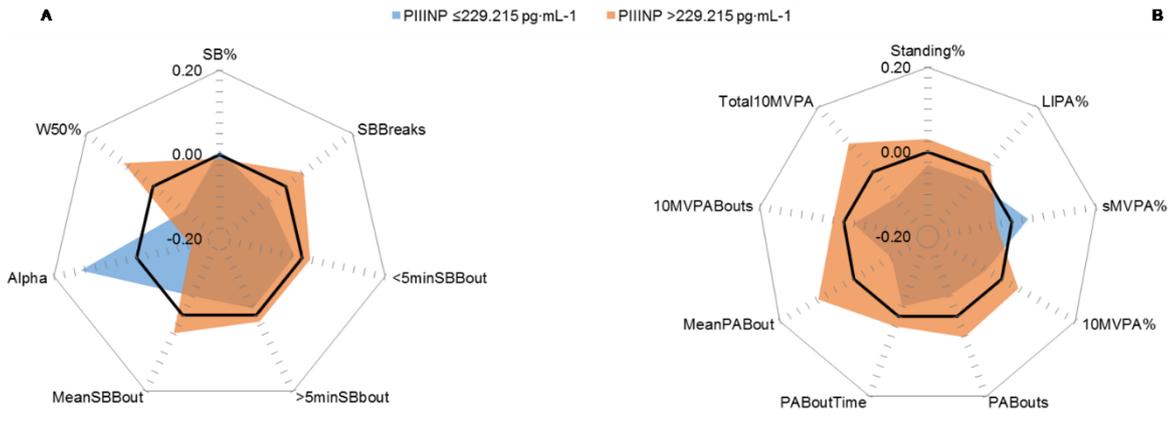


Figure 5.3.7 A) SB z-scores between 'low' (blue) and 'high' (orange) PIIINP concentration groups. B) PA z-scores between 'low' (blue) and 'high' (orange) PIIINP concentration groups. Black line indicates z-score mean (zero). 'Low' group n=38, 'high' group n=38.

Overall Cardio-metabolic Impact

Using the rank method, for patterns of SB, W50% was the best overall distinguisher between 'low' and 'high' cardio-metabolic parameter groups, as it had the lowest sum of cardio-metabolic parameter rankings (table 5.3.4). Overall, PA bouts was the best distinguisher between 'low' and 'high' cardio-metabolic parameter groups, as it had the lowest sum of cardio-metabolic parameter rankings (table 5.3.5).

Table 5.3.4. Difference between 'low' and 'high' group z-score's patterns of SB, ranked in descending order for each cardio-metabolic parameter and overall rank in ascending order based on the sum of cardio-metabolic parameter rankings.

Patterns of SB	Total Cholesterol	Triglyceride	LPL	Glucose	HbA1c	IL-6	PIIINP	Sum of Ranks	Overall Rank
SB%	7	2	4	3	4	7	7	34	7
SB Breaks	2	4	2	4	6	2	3	23	2
<5min SB Bout	6	5	1	5	7	1	5	30	4
≥5min SB Bout	1	6	3	2	2	4	6	24	3
True Mean SB Bout	5	3	5	6	1	6	4	30	4
Alpha	3	7	7	7	5	3	1	33	6
W50%	4	1	6	1	3	5	2	22	1

Table 5.3.5. Difference between 'low' and 'high' group z-score's patterns of PA, ranked in descending order for each cardio-metabolic parameter and overall rank in ascending order based on the sum of cardio-metabolic parameter rankings.

Patterns of PA	Total Cholesterol	Triglyceride	LPL	Glucose	HbA1c	IL-6	PIIINP	Sum of Ranks	Overall Rank
Standing%	3	8	4	1	9	4	6	35	5
LIPA%	5	9	7	5	1	5	7	39	7
sMVPA%	8	2	8	7	8	6	5	44	8
₁₀ MVPA%	4	1	5	4	6	2	4	26	2
PA Bouts	2	5	2	3	7	1	3	23	1
PA Bout Time	7	4	6	2	3	8	8	38	6
True Mean PA Bout	1	6	3	9	5	7	1	32	4
₁₀ MVPA Bouts	9	3	9	6	4	9	9	49	9
Total ₁₀ MVPA	6	7	1	8	2	3	2	29	3

Using method 2, for patterns of SB, the 'low' metabolite score group had a greater alpha z-score compared to the 'high' metabolite score group (distance of 14% between group z-scores) (figure 5.3.8A). The 'low' and 'high' metabolite score groups had a similar z-score for <5 min SB bout, >5 min SB bout, and true mean SB bout (figure 5.3.8A). The 'high' metabolite score group had a larger z-score for SB%, W50%, and SB breaks compared to the 'low' metabolite score group (distance

between z-scores: 14%, 10%, and 5%, respectively) (figure 5.3.8A). For patterns of PA, the 'low' metabolite score group has a greater z-score for all parameters (distance between z-score range: 23% [₁₀MVPA bouts] – 3% [LIPA%]) compared to the 'high' metabolite score group, excluding PA bouts, which was larger in the 'high' metabolite group (distance between z-scores: 5%) (figure 5.3.8B).

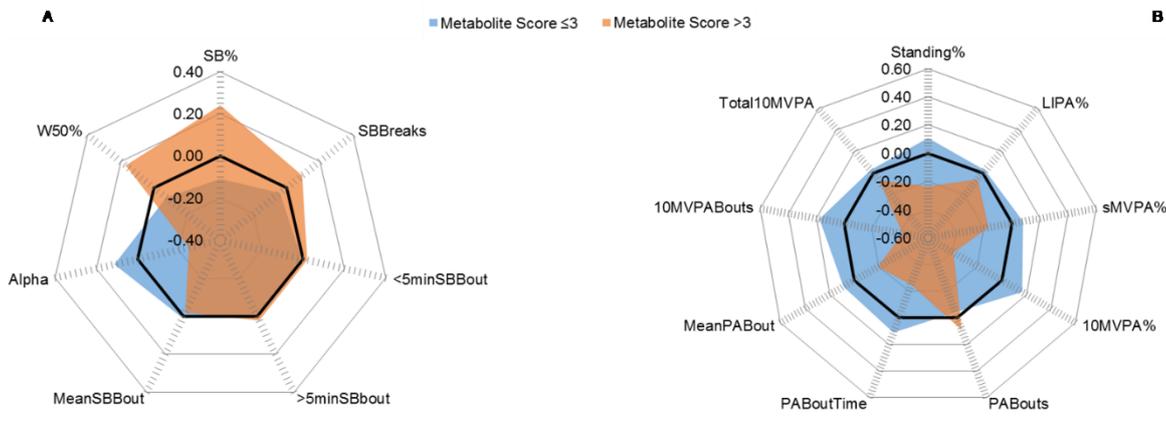


Figure 5.3.8 A) SB z-scores between 'low' (blue) and 'high' (orange) metabolite score groups. B) PA z-scores between 'low' (blue) and 'high' (orange) metabolite score groups. Black line indicates z-score mean (zero). 'Low' group n=65, 'high' group n=28.

Using method 3, for patterns of SB, alpha value displayed the greater distance (15%) between group z-scores as the 'low' composite z-score group had a greater z-score than the 'high' composite z-score group (figure 5.3.9). The 'low' composite z-score group also had a higher z-score for <5 min SB bout, >5 min SB bout, and SB breaks compared to the 'high' composite z-score group (distance between z-scores: 6%, 5%, and 3%, respectively) (figure 5.3.9). The 'high' composite z-score group had a larger true mean SB bout, W50%, and SB% (distance between z-scores: 11%, 10%, and 7%, respectively) compared to the 'low' composite z-score group (figure 5.3.9). For patterns of PA, the 'low' composite z-score group had a larger z-score for all parameters (distance between z-score range: 18% [sMVPA%] – 5% [LIPA% and PA bouts], excluding total ₁₀MVPA (figure 5.3.9), compared to the 'high' composite z-score group.

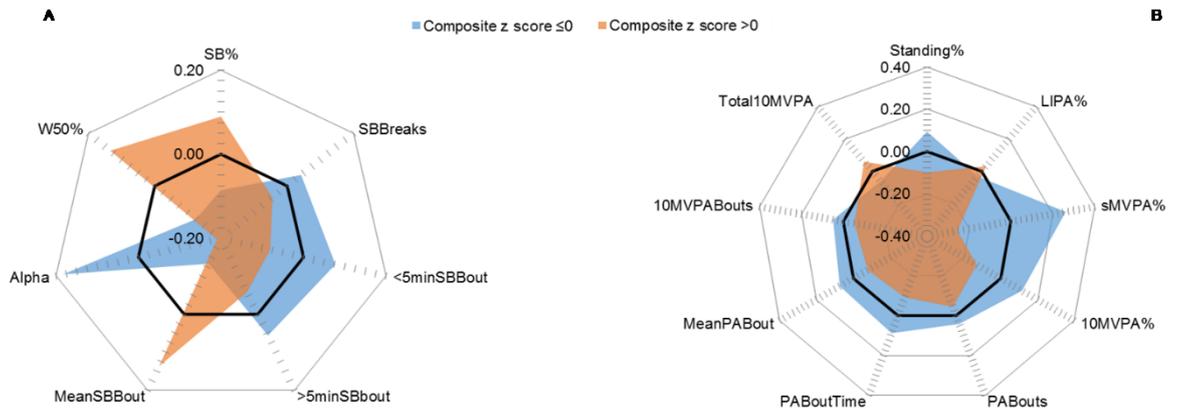


Figure 5.3.9 A) Comparison of patterns of SB z-scores between 'low' (blue) and 'high' (orange) composite z-score groups. B) Comparison of patterns of PA z-scores between 'low' (blue) and 'high' (orange) composite z-score groups. Black line indicates z-score mean (zero). 'Low' group $n=48$, 'high' group $n=45$.

Discussion

The objective of this study was to illustrate which PB intensity(s) influence cardio-metabolic parameters in older adults. For Part 3, the aim was to determine which pattern of SB and PA was most important to monitor when using future lifestyle interventions to improve the cardio-metabolic profile of older adults. The objective was to illustrate how the patterns of PB engagement differ between 'low' and 'high' cardio-metabolic marker groups. It was hypothesised that the 'low' cardio-metabolic marker group would present more favourable patterns of PB engagement than the 'high' group (Carson et al., 2014; Lord et al., 2011; Healy et al., 2011).

Z-scores are unit-less values that normalise participant data by presenting them as the number of standard deviations away from the mean, when the mean of the population is zero, assuming a normally distributed data set. Z-scores are particularly useful for comparing variables that have different units (such as SB%: $\% \cdot \text{waking hrs} \cdot \text{day}^{-1}$, and W50%: $\text{mins} \cdot \text{day}^{-1}$) as it allows the researcher to simplify the multiplicities through determining if the magnitude of difference/change in a variable is equivalent to the difference/change in another variable, which has different units. For example, is a $20\% \cdot \text{waking hrs} \cdot \text{day}^{-1}$ difference in SB% the same as a $20 \text{ mins} \cdot \text{day}^{-1}$ difference in W50%. Therefore, z-scores are useful for the current study, as it can illustrate which pattern of PB displays the largest difference in the

comparison between 'low' and 'high' cardio-metabolic parameter concentration groups and subsequently, provide a simplified indication of the overall influence of PB on all cardio-metabolic parameters of interest.

The Most Important Pattern of SB for Assessing Cardio-metabolic Profile

Using method 1, W50% displayed the largest cumulative distance between 'low' and 'high' cardio-metabolic concentration groups (table 5.3.4), however alpha had the largest distance between the 'low' and 'high' metabolite score (method 2) (figure 5.3.8A) and composite z-score (method 3) (figure 5.3.9A) groups suggesting that alpha and W50% may be the most important pattern of SB to measure within PB research. Alpha and W50% were first proposed by Chastin and Granat (2010) as a more sensitive to change measure in SB, compared to SB breaks and absolute SB engagement. Alpha is a unit-less power law scaling variable where an increase in its size would indicate that a participant would accumulate their SB time with more SB bouts of a shorter duration (Chastin and Granat, 2010). Meanwhile, W50% represents the length of an SB bout where the summation of all the SB bouts greater or equal to that length would account for 50% of absolute total SB engagement (Chastin and Granat, 2010). The recent study suggested that older adults with 'unhealthy' triglyceride and glucose concentrations would accumulate 50% of their daily SB time using longer SB bouts and that they have fewer SB bouts but of a longer duration. This was illustrated in the comparison of 'low' and 'high' groups for both triglyceride and glucose concentration, which showed that the 'high' groups had a greater W50% z-score (distance between z-scores: triglyceride, 22.8%, glucose, 12.0%) and lower alpha z-score (distance between z-scores: triglyceride, 9.3%, glucose, 3.9%) compared to the 'low' groups. Furthermore, it was suggested that W50% and alpha explained 12% and 3% of the variance in triglyceride concentration, respectively, as well as 5.0% and 3.9% of the variance in fasting glucose concentration, respectively. To the author's knowledge, no previous research has examined the relationship between alpha, W50% and cardio-metabolic health in an older adult population (60 – 89 yrs). However, the results of the current study are consistent with adult populations (58±10 yrs), where an increase in alpha value was also associated with a decrease in triglyceride and fasting plasma glucose concentration (Bellettiere et al., 2017). Conversely,

Bellettiere et al. (2017) reported a decrease in W50% (referred to as usual bout length) without observing a change in triglyceride or fasting glucose concentration. The discrepancies in W50% findings may be explained by the fact that the sample population of the current study had a mean W50% that was $27.9 \text{ min}\cdot\text{day}^{-1}$ greater than the aforementioned study (Bellettiere et al., 2017), which suggests that there may be a maximum W50% threshold that must not be exceeded to prevent W50% from influencing triglyceride and glucose concentration. As triglyceride and glucose play an important role in the pathogenesis of diabetes mellitus (Boden, 1997), the results of the current study may suggest that it is pertinent to manipulate alpha and W50% in at risk/diabetic populations. This is particularly relevant for older adults (60+ yrs), where at risk/diabetics constitute an estimated 14.8% (95% confidence interval [95%CI] 14.4, 15.2) of the population (Melzer et al., 2015).

Furthermore, participants displaying a greater PIIINP concentration, a marker of vascular stiffness (Bonapace et al., 2006), and greater IL-6 concentration, involved in the pathogenesis of atherosclerosis (Ciccione et al., 2014), had a larger and lower z-score for W50% (PIIINP only) and alpha, respectively, suggesting that extended engagement in SB bouts could increase vascular stiffness and inflammation. Vascular stiffness was not measured in the current thesis, however intima-media thickness (IMT) was, which is associated with vascular stiffness ($r^2=0.18$, $p<0.001$, $n=423$) (Shoji et al., 2010) and inflammation ($r^2=0.18$, $p<0.001$) (Ciccione et al., 2014). In Chapter 03, a 0.003 mm (95%CI -0.001, -0.00, $p=0.001$) increase in popliteal IMT was predicted with a one minute per day increase in W50%. The increase in IMT with increasing W50% may be a result of the increasing PIIINP concentration with increasing W50% as PIIINP is associated with IMT (Agarwal et al., 2014). In addition, this observed increase in PIIINP (in the current study) and IMT (Chapter 03) with increasing W50% would likely increase total peripheral resistance and thus require an increased resting heart rate (Siddiqui, 2011) to maintain the required resting cardiac output, which would explain why an increase in W50% was associated with an increase in resting heart rate (Chapter 03). Overall, the current study suggested that patterns of SB, specifically alpha and W50%, are essential to monitor in physical behaviour interventions to improve cardio-metabolic health.

The Most Important Pattern of PA for Assessing Cardio-metabolic Profile

The number of PA bouts in a day was ranked as the best determinant of cardio-metabolic markers (method 1), as the cumulative distance between 'low' and 'high' concentration groups was greatest for PA bouts compared to the other patterns of PA. The greatest distance in PA bouts occurred in the comparison between 'low' and 'high' concentration groups of lipid markers (3 out of the top 4 cardio-metabolic markers, triglyceride: 'low' 14% greater, total cholesterol: 'low' 12% greater, and LPL: 'high' 11% greater). Serum LPL is generally thought to be anti-atherogenic (Rip et al., 2006). The presence of more PA bouts in the 'high' LPL group would support this paradigm as physical activity is known to increase LPL within skeletal muscle (Bey and Hamilton, 2003), which is also anti-atherogenic (Tsutsumi, 2003). Furthermore, LPL is responsible for the hydrolysis of triglyceride (Sato et al., 2016) and therefore, the presence of more PA bouts in the 'high' LPL group and 'low' triglyceride group, compared to their respective 'low' and 'high' groups, may suggest a potential mechanistic link between increased PA engagement, increased LPL concentration, and decreased triglyceride concentration.

PA bouts was also ranked higher than patterns of PA that were specific to a PA intensity (e.g. 10MVPA bouts); it suggests that a PA bout of any intensity may be beneficial to the cardio-metabolic status of older adults. This has certainly been an ongoing theme throughout the current thesis (Chapter 03, 04, 05 Part 1 and 2) and has been illustrated in acute and chronic SB break studies that have used PA, from standing to MVPA, as a method of SB interruption (see Benatti and Ried-Larsen (2015) for a review). Benatti and Ried-Larsen (2015) suggested that as the baseline physical fitness of the participants increased, a higher intensity of PA interruption was needed to elicit health benefits; however, none of the reviewed studies included older adults so it is unknown whether this hypothesis holds true in older adult populations. Given that the majority of older adults in the UK are physically inactive (100 - 95% from Craig et al. (2009), 90.5% from the current thesis), it is likely that the physical fitness of older adults is low and therefore, it is probable that they may experience a maintenance/improvement in cardio-metabolic status in response to low intensity PA bouts. The demographic data of the 'low' cholesterol and triglyceride, and 'high' LPL groups of the current study suggests that bouts of SB

may need to be interrupted every 30.2 ± 1.21 mins with PA bouts of 16.5 ± 0.76 mins on an average 22.1 ± 0.77 times a day (which would require 17 waking hours) for older adults to have a 'healthy' lipid profile.

Interestingly, $_{10}$ MVPA bouts and sMVPA% were suggested to be the best determinants of overall cardio-metabolic profile when the metabolite score (method 2, figure 5.3.8B) and composite z-score (method 3, figure 5.3.9B) were used, respectively. This was unexpected as both of these patterns of PA were listed as the bottom two (least distance between 'low' and 'high' z-scores) when using method 1 (table 5.3.5). These three methods of collating impact are hard to compare and it is not possible to suggest which is the best method to use when assessing overall cardio-metabolic impact. Rather, when reviewing in isolation, each method tells its own story. Using the metabolic score method (method 2) would suggest that if two people engage in a similar amount of $_{10}$ MVPA over seven days, the person that accumulates this $_{10}$ MVPA time with more bouts per day and spreads their $_{10}$ MVPA engagement out across the week will have a 'healthier' cardio-metabolic profile. This was evident as there was only a 4% distance between total $_{10}$ MVPA but a 23% and 22% distance between $_{10}$ MVPA bouts (average $n \cdot \text{day}^{-1}$) and $_{10}$ MVPA% (average $\% \cdot \text{waking hrs} \cdot \text{day}^{-1}$) z-scores, respectively (figure 5.3.8B). This concept supports earlier self-reported MVPA of men from the Harvard Alumni Study (age: 66.3 years, SD not reported), which suggested that those who were 'regularly active' throughout the week have a greater reduction in mortality risk compared to those who achieve sufficient weekly MVPA targets within one-to-two days, dubbed the 'weekend warriors' (Lee et al., 2004).

The composite z-score method (method 3) suggests that those whose waking hours are made up of more sMVPA will have a healthier overall cardio-metabolic profile (figure 5.3.9B). This is a strong message for older adults who physically or mentally are unable to sustain the prolonged bouts of MVPA that government guidelines recommend, as it suggests that they would still be able to improve their cardio-metabolic profile. As mentioned earlier in the discussion, using MVPA bouts of a shorter duration than ten minutes have been shown to improve several cardio-metabolic markers (Benatti and Ried-Larsen, 2015) during phases of prolonged SB engagement. Overall, it is apparent that engaging in more bouts of PA, whether that be any PA or specifically $_{10}$ MVPA, is an important determinant of older adult's cardio-metabolic profile.

Conclusion

As PB monitoring becomes more commercially available, it is important that the patterns in which PB(s) are engaged be considered, as the current study has highlighted that these patterns, more so than total PB engagement, may influence the cardio-metabolic profile of older adults. Of note, alpha and W50% for patterns of SB and PA bouts, $_{10}$ MVPA bouts, and sMVPA% for patterns of PA had the greatest distinction in the comparison of 'low' and 'high' overall cardio-metabolic profile groups, suggesting that these patterns of PB, at least, should be monitored in older adults. Furthermore, the presence of differences in patterns of SB and PA between the 'low' and 'high' cardio-metabolic concentration groups adds further evidence to show that objective recommendations for the patterns of SB and PA (off all intensities) engagement, not just total engagement time, are required for older adults, to provide education to maintain/improve health status.

The findings of the current study illustrate that future research needs to develop laboratory based interventions to determine the magnitude to which these patterns of PB acutely influence cardio-metabolic health, specifically in older adult populations, where CVD is one of the most prevalent causes of death (Townsend et al., 2015).

Chapter 06:

Overarching discussion.

‘Summarising a 3-year research project that examined the effects of sedentary behaviour and physical activity on older adults’ cardiovascular/metabolic profiles.’

Summarising a 3-year research project that examined the effects of sedentary behaviour and physical activity on older adults' cardiovascular/metabolic profiles.

Overarching Discussion

Until recently, it was thought that being sedentary and physically inactive were interchangeable terms. However, seminal research has found that the positive effects of PA, specifically of moderate intensity, cannot always offset the negative effects of concurrent prolonged SB engagement on health status. Thus, the spectrum of PB became biaxial, segregating SB and $_{10}$ MVPA on to separate axes. Furthermore, in 2012, a letter signed by 48 researchers from around the world was written to the editor of Applied Physiology, Nutrition, and Metabolism (Barnes et al., 2012) urging that the definition of sedentary behaviour and physical inactivity be standardised to (respectively);

“Any waking behaviour characterised by an energy expenditure ≤ 1.50 METs while in a sitting or reclined posture.”

“Those who are performing insufficient amounts on moderate to vigorous physical activity (i.e., not meeting specified physical activity guidelines).”

Barnes et al. (2012)

The review of the current literature in Chapter 01 highlighted that there were methodological limitations in the monitoring of PB, especially in population specific studies, as PB intensity thresholds from middle-aged populations, along with the inability to recognise posture, were applied to children and older adult populations. This would have likely miss-estimated engagement in PB and thus any associations with health status. Furthermore, there was an apparent lack of PB epidemiological studies specific to older adults. As the demographics of the UK shifts to an increased older adult population (~ 25% of the UK population by 2035), there is a potential socio-economic burden due to the exponential annual healthcare costs within the NHS that occur with the increasing age of the patient (50 year-old male: £605, 70

year-old male: £1,834, 89 year-old male: £5,198). Although non-communicable, CVD is the leading cause of death, after cancer, in UK older adults. Becoming physically active in older age has been shown to reduce the risk of mortality by 35% (95% confidence interval [CI] 30.0, 40.0) whilst engaging in more sedentary behaviour carries a 90% (95%CI 36.0, 166) increased risk of mortality. Additionally, maintaining quality of life during their later years is also of particular importance. However, whilst life expectancy in the UK at age 65 years (yrs) increased by 1.43 years, between 2001 and 2008, healthy life expectancy only increased by 0.65 yrs, suggesting that older adults are living longer but not necessarily healthier lives.

Therefore, the aims of this thesis were 1) to illustrate how and what PB(s) older adults should engage in (increase or minimise) to improve their cardiovascular/metabolic profile and thus hopefully improve their health-related quality of later life. 2) Determine whether cardiovascular/metabolic markers are affected by increasing age after the age of 60 yrs and whether PB(s) can mediate any apparent ageing effects. To achieve these overarching aims, it was pertinent to establish the most suitable PB measurement tool. Therefore, Chapter 02 served as a development chapter to firstly determine the ability of an in-house developed accelerometer algorithm to classify PB intensity by comparing it to a criterion measure (oxygen utilisation) and secondly, compare the ability of the International Physical Activity Questionnaire (IPAQ) against the in-house developed accelerometer in the classification of PB intensity. Chapter 02 indicated that accelerometry would be the most suitable measurement tool for PB within the subsequent chapters (03 – 05). The subsequent chapters (03 – 05) answered at least one of the overarching aims however, each chapter used different statistical models that asked different questions and therefore, required more refined aims for the chapter. Table 6.1 provides an overview of the refined aims for the thesis chapters and which overarching thesis aims these refined aims contributed towards.

Table 6.1. Highlighting which thesis chapter aims supported the completion of the overarching thesis aims. Ticks indicate which overarching thesis aims would be met by achieving the thesis chapter aim.

Chapter	Chapter Aims	Overarching Thesis Aims	
		To illustrate how and what PB(s) older adults should engage in PB to improve their cardiovascular/metabolic profile.	Determine whether cardiovascular/metabolic markers are affected by increasing age after the age of 60 years and whether PB(s) can mediate any apparent ageing effects.
3.1	Provide an evidence-based recommendation for the parameter of PB that is the most prolific predictor of cardiovascular profile.		
3.2	Provide a time-constrained, alternative to bivariate/multivariate regression modelling tool, to predict how changes in PB may affect the cardiovascular profile of older adults.		
3.3	Propose a visual mapping of the interplay between PB (mainly SB, LIPA, and 10MNP/A) and cardiovascular parameters, so that the end-user (i.e. health workers, carers, older persons) may have an easy to understand evidence-based point of reference.		
4	Determine which cardiovascular profile markers are affected by further ageing in older adults aged ≥60 years and to recommend what and/or how PB profile can be optimized to offset these further ageing effects.		
5	The aim of this study was therefore to illustrate which PB intensity(s) and/or pattern(s) influence cardio-metabolic parameters in older adults.		

Total Physical Behaviour Engagement

Within the UK public media, reports often suggest that only 25% of adults (18+ yrs) are physically inactive (British Broadcasting Company, 2017; Gifford, 2017; Bates, 2017), which ranks the UK as the 35th most inactive country in the world (146 countries monitored out of a possible 195) (World Health Organization, 2015b). Self-report questionnaires often adapted to each country, such as the IPAQ are used to make these comparisons. However, as shown in Chapter 02 Part 2, self-report measures of PA are grossly overestimated leading to reports that underestimate the degree to which there is a physical inactivity crisis to contend with. Indeed from our data, over 90% of the older adult population (in Cheshire - a generally wealthy and educated population by UK standards (Fenton, 2017; Office for National Statistics, 2014)) are physically inactive (Chapter 02 Part 2). The same can be said for SB, with older adults (Chapter 02 Part 2) self-reporting 5 hrs·day⁻¹ of sitting, with the occasional participant reporting zero hours of SB, whereas accelerometer monitoring, averages these older adults at 10 hrs·day⁻¹ of SB (Chapter 02 Part 2). From Chapter 02 Part 2, it is clear that older adults know how MVPA should feel, as their accelerometer-measured sMVPA (MVPA bouts <10 mins) was similar to their self-reported ₁₀MVPA (MVPA bouts ≥10 mins). Therefore, education is not required for older adults to understand PA intensity but rather for a greater awareness of the optimal length of time they engage in PB(s) across the spectrum.

With technological advancements, epidemiological research is moving away from subjective PB monitoring towards objective monitoring, such as accelerometers. The most notable epidemiological 'big data' set is the National Health and Nutrition Examination Survey (NHANES) (Centers for Disease Control Prevention, 2009). It aimed to obtain accelerometer measured PB from 5000 US adults and has been analysed in 296 research articles (PubMed, keywords: NHANES accelerometer, search date: 14.08.2017). However, as knowledge of the level of sophistication in data analysis has improved, accelerometer data can now provide upwards of 20 PB parameters from the standard total engagement to the patterns in which they are accumulated. With this abundance of data, it is important to focus in on the essential PB parameters, which are able to predict the cardiovascular/metabolic profile of older adults.

In isolation, an hour of LIPA had a notable effect on popliteal IMT, decreasing it by 0.09 (95%CI 0.15, 0.03) mm (Chapter 03 Part 1) as well as attenuating the age related increase in popliteal IMT (Chapter 04). Meanwhile, an hour of SB would increase resting heart rate by 1.58 (95%CI 0.17, 2.99) bpm (Chapter 03 Part 1). However, in reality, the impact of a PB on health does not happen in isolation. Until recently, statistical modelling was limited to this isolative approach (Henson et al., 2013b; Bankoski et al., 2011; van der Berg et al., 2016). Seminal research, which has brought new PB modelling, namely isotemporal substitution modelling (ISM) (Mekary et al., 2009) and compositional data analysis (CoDA) (Chastin et al., 2015b). ISM predicts how health parameters may change if a PB is replaced with another of the same duration; as such, it is proposed as a useful model for practitioners who advocate behaviour change to improve health. Once again, SB and LIPA played a role in mediating cardiovascular profile of older adults, within the current thesis (Chapter 03). Modelling the replacement of one hour of SB with standing or sMVPA predicted a reduced resting heart rate by 6.20 – 3.72 bpm (Chapter 03 Part 2), which would negate the positive association observed between SB and resting heart rate in Chapter 03 Part 1. Furthermore, the replacement of one hour of SB with LIPA decreased carotid artery diameter, which is proposed to be a health benefit as increased carotid artery diameter is associated with an increased risk of stroke in older adults (Van Dijk et al., 2001). Meanwhile, $_{10}$ MVPA engagement appeared to be essential for the mediation of triglyceride concentration, as the replacement of any PB, including sMVPA, with $_{10}$ MVPA decreased triglyceride concentration (Chapter 05).

From a CoDA approach, which acknowledges that the engagement in one PB limits the time available to engage in other PB(s), $_{10}$ MVPA was again suggested to be the main mediator of triglyceride concentration. For instance, those with a low triglyceride concentration (≤ 1.7 mmol·l⁻¹) engaged in 48% more, and those with a high triglyceride concentration (> 1.7 mmol·l⁻¹) engaged in 32% less, $_{10}$ MVPA than the sample population. This interaction is probably mediated through LPL pathways, which are responsible for the hydrolysis of triglyceride (Merkel et al., 2002). Indeed participants with a high LPL (> 63.5 pg·mL⁻¹) concentration engaged in 11% more, and those with a low LPL (≤ 63.5 pg·mL⁻¹) concentration engaged in 27% less, $_{10}$ MVPA than the sample population.

Patterns of Physical Behaviour Engagement

It has been recommended that SB is monitored using the 'SITT' principle, (S) sedentary bouts of a certain duration, (I) interruptions in SB, (T) time duration of SB, and (T) type of SB (Tremblay et al., 2010). Early research that used the SITT principle, found SB breaks was a consistent predictor of health status (Saunders et al., 2013; Healy et al., 2008b; Chastin et al., 2015a). More recently, the development of W50% (Chastin and Granat, 2010), to provide a more sensitive (to change) measure of SB patterns than SB breaks, has yielded further associations with health outcomes (Bellettiere et al., 2017; Van Roekel et al., 2016). Indeed, in the current thesis, W50% was a consistent mediator of older adults' cardiovascular/metabolic profile (Chapter 03 and 05), more so than SB breaks. The most notable finding was the mediating effects W50% had on PIIINP (Chapter 05), popliteal IMT (Chapter 03), and resting heart rate (Chapter 03). It was suggested that participants with a high W50% are likely to have a high PIIINP concentration (Chapter 05), which is associated with vascular stiffness (Bonapace et al., 2006) and IMT (Agarwal et al., 2014). An increase in vascular stiffness and IMT would increase total peripheral resistance and thus, a higher resting heart rate (Siddiqui, 2011) would be required to maintain resting cardiac output. Therefore, the positive association between W50% and resting heart rate (Chapter 03) may be a result of increased IMT (Chapter 03) due to the higher concentration of PIIINP (Chapter 05) in these participants who have a longer W50%.

The current thesis proposes that engaging in PA, across the spectrum from standing to $_{10}$ MVPA, may be best for mediating older adults' cardiovascular/metabolic profile. This was due to patterns of PA parameters, such as true mean PA bout length (Chapter 03), daily sum of PA bout time (Chapter 03), and the number of PA bouts (Chapter 05) being the most frequent predictors of cardiovascular/metabolic parameters. However, when overall cardio-metabolic profile was considered (Chapter 05), it was suggested that older adults should attain a physically active lifestyle by spreading out their $_{10}$ MVPA across multiple bouts throughout the day and their weekly $_{10}$ MVPA engagement out over numerous days.

Conclusion

Throughout the thesis, it has been clear that SB and the patterns in which it is engaged is a mediator of cardiovascular/metabolic profile in older adults (figure 6.2). Therefore, there is a strong need to educate the public about the risks of prolonged SB and the methods that can be used to reduce engagement. The thesis has suggested that LIPA may be a useful substitute for SB as these two PB(s) are highly co-dependent (Chapter 05) on one another. This thesis also provided evidence to show that LIPA can mediate cardiovascular/metabolic profile (figure 6.2). This is a useful message for older adults as it shows that they could still attain health benefits, even if they are physically or mentally unable to achieve a more demanding physically active lifestyle.

However, the current thesis has also advocated that attaining a demanding physically active lifestyle (if possible) is essential for some cardiovascular/metabolic markers, in particular, reduced triglyceride concentration (figure 6.2). Therefore, the overarching conclusion of this thesis highlights that older adults need to consider both axes of the PB spectrum (figure 6.1). In other words, older persons need to ensure they attain an 'active ambulator' lifestyle (low SB, physically active) in order to attain the best chance of having a healthy cardiovascular/metabolic profile.

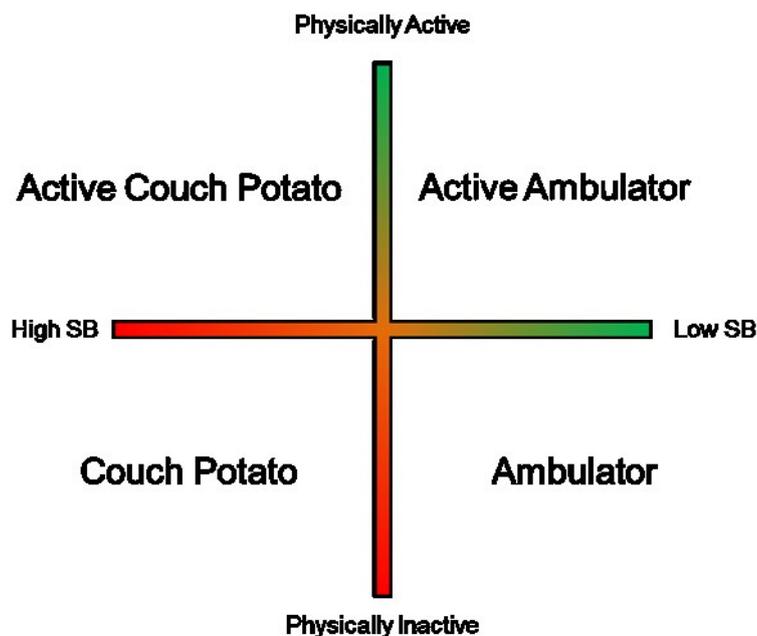


Figure 6.1. Proposed PB spectrum, which follows the recommended definition of physical inactivity (Barnes et al., 2012). Green presents the points on the spectrum that older adults should aim to place themselves, whilst red indicates the points of the spectrum that older adults should aim to avoid.

Study Limitations

Although this thesis provides a comprehensive break down of the effects PB has on cardiovascular/metabolic markers within central, upper limb, and lower limb arteries of older adults, it has some limitations. Firstly, the automated analysis of participants' flow mediated dilation data led to its omission from the thesis as it suggested a number of counter-intuitive results. A case in point is the finding that the majority of older adults had a negative response (vasoconstriction) to vascular occlusion. Therefore, it suggested that increasing PA would decrease vasodilatory response, which is not an expected association (Pierce et al., 2011; Rakobowchuk et al., 2008). In the future, reanalysis of the data will need to be conducted to confirm or refute the observed vasoconstrictive response to vascular occlusion. Secondly, energy balance was not monitored, which may have influenced some of the cardiovascular/metabolic parameters (Hjerkinn et al., 2006; Kris-Etherton et al., 1988). However, given the amount of possible dietary modulators of physical function, the sample size of the thesis would have needed to be increased substantially to retain a suitable statistic power. Finally, the older adults that were recruited for the thesis were community dwelling and living independently, mainly representing the 'healthy' proportion of the older adult population. Although, recruitment also targeted older adults living in sheltered accommodation, response rates were poor (4 older adults from 10 sheltered accommodations). Therefore, how the results of the thesis translate to a frailer, dependently living older adult is unknown and requires further investigation.

Future Directions

Although parameters of PB have shown associations with older adults' cardiovascular/metabolic profile in these cross-sectional analyses, it does not prove causation. To strengthen the evidence base of this thesis, a follow-up study of the participants' change in PB and cardiovascular/metabolic profile in the years since original data collection would provide a longitudinal data set. This would allow the determination of whether the original habitual PB engagement of the older adults

(PB data in the current thesis) could predict their cardiovascular/metabolic status three-four years later.

Furthermore, the design of both acute and chronic intervention studies would confirm or refute the changes in cardiovascular/metabolic profile that ISM predicted with hypothetical changes in PB engagement. Thus, confirming the validity of the predictability of ISM and the time course for these predicted changes in cardiovascular/metabolic profile to occur.

The ultimate goal is to further the evidence base of this thesis so that objective recommendations for total SB engagement, the patterns in which SB is engaged, and PA engagement (other than $_{10}$ MVPA) can be developed for older adults to follow so they can attain a 'healthy' cardiovascular/metabolic profile.

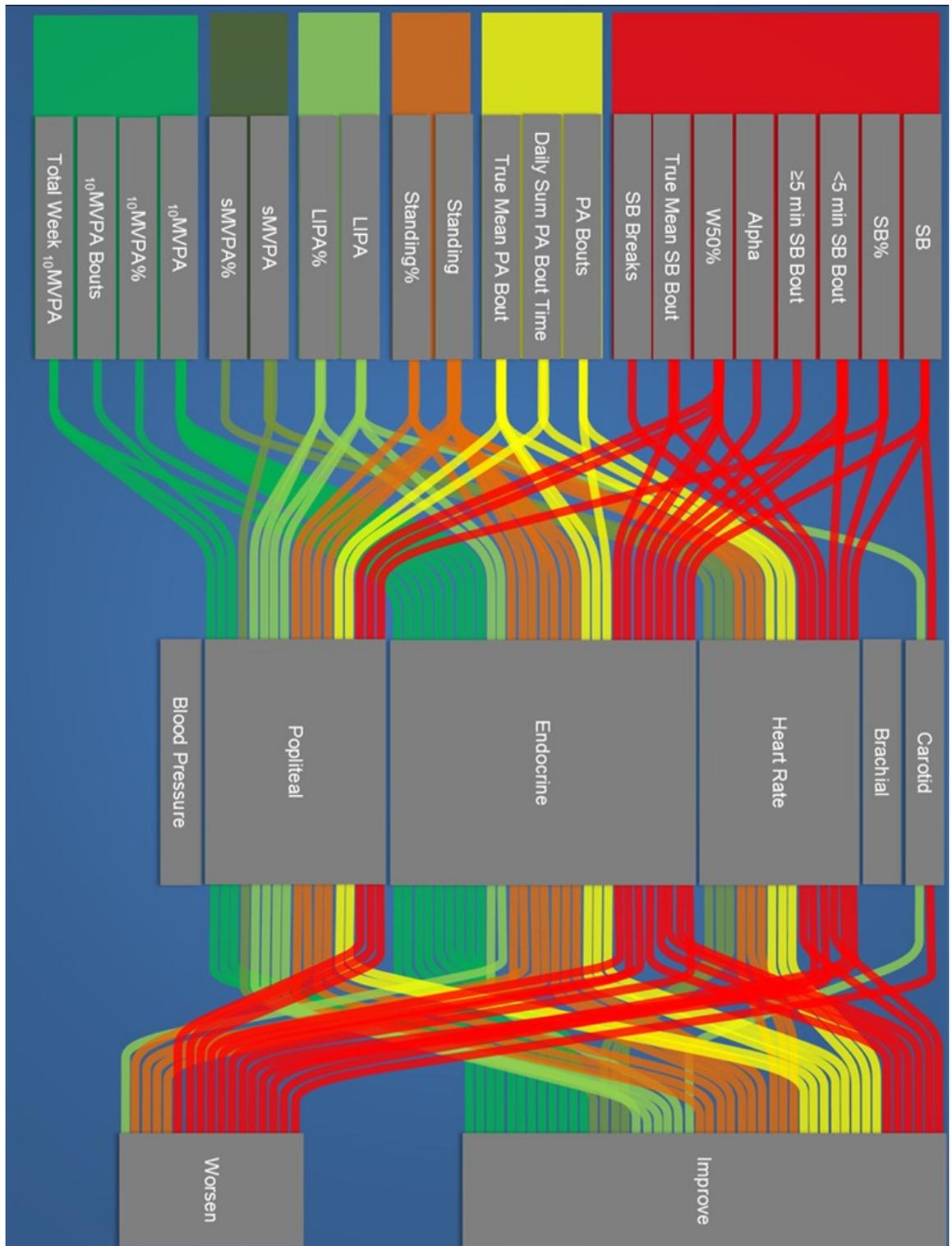


Figure 6.2. Statistically significant associations (Chapter 03 Part 1 and 2, 04, 05 Part 2) and greatest differences (Chapter 05 Part 1 and 3) between PB parameters and cardiovascular/metabolic parameters and whether the PB improved or worsened the cardiovascular/metabolic parameter. Colours indicate intensity of PB. Red – SB, Yellow – PA, Orange – Standing, Light Green – LIPA, Dark Green – SMVPA, Green – $_{10}MVPA$.

Chapter 07:

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Chapter 08:

Academic Engagement.

Part 1: 'Journal publications.'

Part 2: 'Conference proceedings.'

Part 3: 'Public engagement.'

Part 1: Journal publications.

Ryan, D. J., Stebbings, G. K. and Onambele, G. L. (2015) 'The emergence of sedentary behaviour physiology and its effects on the cardiometabolic profile in young and older adults.' *Age*, 37(5) pp. 89-100.

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Ryan, D. J., Wullems, J. A., Stebbings, G. K., Morse, C. I., Stewart, C. E. and Onambele, G. L. (2018) 'Segregating the Distinct Effects of Sedentary Behavior and Physical Activity on Older Adults' Cardiovascular Structure and Function: Part 1—Linear Regression Analysis Approach.' *Journal of Physical Activity and Health*, [In Press].

- <https://journals.humankinetics.com/doi/abs/10.1123/jpah.2017-0325>

Ryan, D. J., Wullems, J. A., Stebbings, G. K., Morse, C. I., Stewart, C. E. and Onambele, G. L. (2018) 'Segregating the Distinct Effects of Sedentary Behavior and Physical Activity on Older Adults' Cardiovascular Structure and Function: Part 2—Isotemporal Substitution Approach.' *Journal of Physical Activity and Health*, [In Press].

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Part 2: Conference proceedings.

International Conference on Movement and Nutrition in Health and Disease – Regensburg, Germany.

Utilising triaxial accelerometers and resting metabolic rate to identify an older person's physical activity vs. sedentary behaviour thresholds.

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Key words: Energy Expenditure; Indirect Calorimetry; Metabolic Equivalent Task; Posture Identification; Thigh Mounted Triaxial Accelerometer;

Background: The assessment of physical activity and sedentary behaviour levels has progressed from subjective self-reports to more objective measures using triaxial accelerometry. However, published regression equations that identify physical activity intensity from accelerometer outputs have used young populations (35 ± 11.4 yrs) and the $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1} = 1$ Metabolic Equivalent Task (MET) estimate [1]. This could cause physical activity intensities to be underestimated in older persons as 1 MET should represent resting metabolic rate (RMR), which is likely to be lower than $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$ in the elderly [2].

Objective: To determine whether using the $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1} = 1$ MET equivalent underestimates objectively measured free-living physical activity intensity and sedentary behaviour in older persons.

Methods: Five older persons (aged > 65 yrs) underwent a laboratory-based incremental physical activity protocol, which ranged from sedentary (e.g. lying, < 1.5 METs) to vigorous (≥ 6.0 METs) intensity behaviours. Participants wore a triaxial accelerometer on each thigh (50% upper limb length) while oxygen utilization was assessed using indirect calorimetry. With the accelerometer in situ, participants resumed their daily activities for 7 continuous days. Accelerometer data was subsequently analysed using physical activity intensity cut-points utilising both the $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1} = 1$ MET, and RMR = 1 MET equivalents.

Results: RMR was lower than the standard $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1} = 1$ MET equivalent ($p < 0.01$). The standard $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1} = 1$ MET equivalent tended to underestimate time spent performing moderate to vigorous intensity physical activities and overestimate time spent performing light intensity physical activity. However, it accurately estimated time spent performing sedentary behaviour, compared to RMR = 1 MET derived cut-points.

Conclusion: Using the standard $3.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1} = 1$ MET equivalent does not allow for an accurate tracking of an older person's levels of physical activity in free-living conditions. Accelerometry studies should apply RMR to 1 MET equivalents to truly reflect time spent performing physical activities of varying intensity in this segment of the population.

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Presentation type: Oral

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Healthy Ageing: From Molecule to Organisms – Hinxton, UK

Age-related differences in objectively measuring population-specific physical activity intensity and sedentary behaviour

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Introduction: Objectively measuring physical activity levels through accelerometry utilises regression equations to determine the metabolic equivalent task (METs) for a given accelerometer output. These regression equations are developed through protocols that analyse accelerometer outputs and oxygen utilisation for a given physical activity. To convert oxygen utilisation to METs, the '3.5 ml·kg·min⁻¹ = 1 MET' equivalent is used. However, 1 MET should represent resting metabolic rate (RMR). RMR declines throughout the ageing process. Therefore applying the '3.5 ml·kg·min⁻¹ = 1 MET' equivalent could indicate that a given physical activity is of the same intensity for young and ageing populations due to accelerometer outputs being similar when, in reality, the given physical activity is of a higher intensity for the older population because of a reduction in RMR. **Aim:** Therefore, the aim of this study was to examine the difference in accelerometer cut-points when applying 3.5 ml·kg·min⁻¹, and the RMR of young vs. older persons in the calculation of METs. **Methods:** Six normal weight (BMI: 18.5 – 24.9 kg·m²) participants (3 young; 20-25 yrs, 3 older; 65-70 yrs) wore a triaxial accelerometer on each thigh (50% upper limb length) while undergoing an incremental, nine stage, physical activity protocol, which ranged from sedentary behaviours (< 1.5 METs) (e.g. lying, sitting) to vigorous intensity functions (≥ 6.0 METs). Each stage was three minutes in duration, with oxygen utilisation collected during the last minute of each stage using indirect calorimetry. Oxygen utilisation was converted to METs using the '3.5 ml·kg·min⁻¹ = 1 MET' equivalent, the mean observed RMR of the young and that of the older persons. A *t*-test was used for between group RMR comparison and 2-way mixed ANOVA's were used for accelerometer output and METs for within and between group comparison. **Results:** The computation method affected the resultant MET values. The older persons exhibited markedly different RMR values compared to their younger counterparts (*p* < 0.05). There was a trend for the older persons to experience all physical activities tested to be at a higher intensity compared to the young persons (*p* < 0.05). Thus, the physical activity for a number of activities was ultimately categorised as a different physical activity intensity for the older group compared to the young group. There was a trend for accelerometer outputs to be similar across both age groups (*p* > 0.05). **Conclusion:** The development of accelerometer cut-points used to identify physical activity intensity should be based on RMR = 1 MET and should be population-specific, in order to correctly identify time spent performing free-living physical activity and sedentary behaviour, thereby allowing for adequate understanding of the impact of lifestyle on markers of healthy ageing.

The 3-Dimensional Effect of Physical Activity in Old Age – Physical, Mental, and Emotional – Kaunas, Lithuania

Is the International Physical Activity Questionnaire Long Form (IPAQ) a reliable measure of free-living sedentary behaviour and physical activity in older persons? Comparisons with accelerometer measures.

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Relevance of the research. In recent years, accurate quantification of free-living sedentary behaviour (SB) and physical activity (PA) has moved towards objective measurement methods [5]. Although, deemed a more accurate determination of lifestyle patterns, objective measures can be costly in large epidemiological studies. Self-report data collection methods are useful as they can be widely distributed, generate large data sets and are relatively more cost effective. The International Physical Activity Questionnaire (IPAQ) is used worldwide [1], however, its application in persons older than 69 years of age, is limited [4].

The **object** of the research was to compare the results of the IPAQ to accelerometer measures of free-living lifestyle patterns. The **hypothesis** was that both measures would agree in determining SB and moderate to vigorous physical activity (MVPA) in older people. The **aim** was to provide a reliable record of the degree of sedentarism and PA in older persons.

Research methods and organization. 44 older participants (74.1 ± 6.1 yrs, 57% female) wore a thigh-mounted (anterior aspect, at 50% of greater trochanter to femoral condyle distance) triaxial accelerometer (GENEActiv Original, Activinsights Ltd, Kimbolton, UK), for seven consecutive free-living days. Residual G (G), adapted from Onambele et al. [3] was the chosen accelerometer output for the study. SB was identified from the accelerometer output using 10 s epoch axis orientation and a 1.50 Metabolic Equivalent Task (MET; where 1 MET = resting metabolic rate) cut-off point (0.057 G). MVPA was identified using a 3.00 MET cut-off point (0.216 G). Sleeping time was identified using a sleep diary. After seven days, 39 participants (48% female), successfully, completed the IPAQ Long Form (English) [2]. Association between IPAQ and accelerometer measures of SB and MVPA were performed using a Spearman rho. Any sex differences were compared with independent samples *t*-tests. Significance was set at a *p* value of 0.05. Data presented as Mean ± SD.

Results and discussion. For MVPA (total hours over seven days), no association ($r = 0.07$; $p = 0.84$,) between IPAQ (45.5 ± 34.5 hrs) and accelerometer (19.3 ± 7.0 hrs) measures was present. For SB (mean hours per 24 h day), a moderate association ($r = 0.34$, $p = 0.03$) between IPAQ (5.5 ± 2.2 hrs) and accelerometer (9.2 ± 2.2 hrs) measures was found. It is notable, no sex differences were found in IPAQ or accelerometer assessed lifestyle patterns ($p > 0.05$).

Conclusions. The use of IPAQ with older participants does not appear to reflect objective measures of free-living lifestyle patterns as the IPAQ underestimates SB and overestimates MVPA. Thus, where possible, we would recommend the use of accelerometry to capture SB and/or PA.

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Comparing patterns of sedentary behaviour and physical activity accumulation between Sedentary, Ambulator, and Active Couch Potato older adults.

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Sedentary behaviour (SB) independently affects health status (Healy et al., 2008) and is prevalent in older adults (10.27 ± 0.80 hours \cdot 24 hours⁻¹, vs. young adults: 9.48 ± 0.70 hours \cdot 24 hours⁻¹) (Craig et al., 2009). However, the patterns of SB and Physical activity (PA) accumulations have not been described. The present study aimed to examine the patterns of SB and PA accumulation in a population of free-living older adults, classifying groups by mobility patterns.

A triaxial GENEActiv accelerometer (ActivInsights, Kimbolton, UK) was mounted anteriorly at 50% of femur length using 2 Tegaderm patches (3M Health Care, St. Paul, USA). Participants ($n=90$, age: 73.72 ± 6.28 years, 48 females) completed 7 days of habitual mobility before returning the accelerometer. The Cheshire Algorithm of Sedentarism (Wullems et al., 2015) was used to analyse the raw data. Participants were then grouped into Sedentary ($n=65$, 37 female) (SB: ≥ 8 hours \cdot 24 hours⁻¹, ≥ 150 min of Continuous MVPA (cMVPA): < 150 mins \cdot 7 days⁻¹), Ambulator ($n=11$, 6 female) (SB: < 8 hours \cdot 24 hours⁻¹, cMVPA: < 150 mins \cdot 7 days⁻¹), Active Couch Potato (ACP) ($n=13$, 5 female) (SB: ≥ 8 hours \cdot 24 hours⁻¹, cMVPA: ≥ 150 mins \cdot 7 days⁻¹), or Active Ambulator, 0 female) (SB: < 8 hours \cdot 24 hours⁻¹, cMVPA: ≥ 150 mins \cdot 7 days⁻¹). The single Active Ambulator participant was removed from between group comparison due to small group size.

Results are presented as Mean \pm Standard Deviation, $p = 0.05$, with groups compared by Kruskal-Wallis and Mann Whitney-U (post hoc).

Ambulators accumulated the least amount of SB (Figure 1). Number of SB breaks per 24 hours (2mins of PA following ≥ 1 min SB) was similar between groups (Sedentary: 22.0 ± 5.62 , Ambulator: 22.44 ± 4.73 , ACP: 22.36 ± 6.32). However, Ambulators accumulated 50% of their SB time (W50%) through shorter bouts (39.16 ± 13.33 mins \cdot 24 hours⁻¹, Sedentary: 58.53 ± 22.86 mins \cdot 24 hours⁻¹, ACP: 49.32 ± 20.54 mins \cdot 24 hours⁻¹). This was due to Ambulators accumulating more Standing, Light Intensity Physical Activity (LIPA), and < 10 min Continuous MVPA (sMVPA) than ACP and Sedentary populations (Figure 1). The ACP population accumulated more cMVPA than Ambulator and Sedentary populations (Figure 1) by performing more frequent cMVPA bouts per 24 hours (2.04 ± 1.93 , Ambulator: 0.42 ± 0.78 , Sedentary: 0.39 ± 0.80).

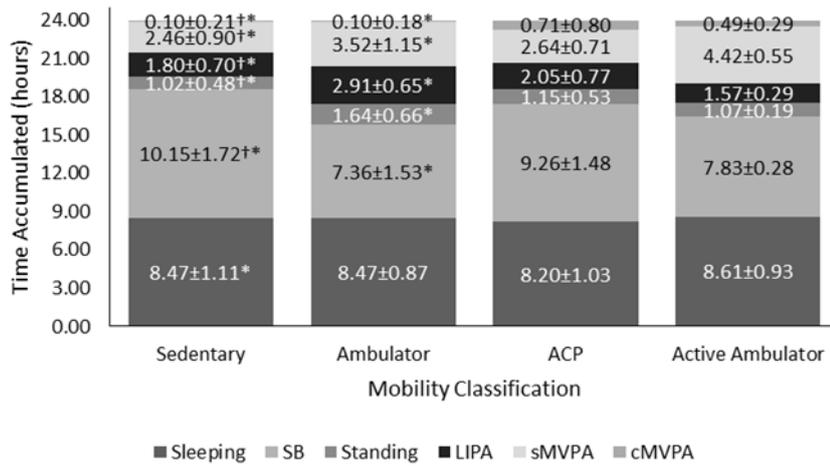


Figure 1. The distribution of an average 24-hour day across different SB and PA for Sedentary, Ambulator and ACP in a free-living older adult populations. † Significantly different from Ambulator. * Significantly different from ACP. $p \leq 0.05$.

The results suggest that older adults use different patterns of SB/PA. Thus, 1%, 12%, 14% and 72% of the study population could be categorised as Active Ambulator, Ambulator, ACP and Sedentary respectively. Those who have shorter SB bouts and accumulate the least amount of SB appear to substitute this time with Standing, LIPA and sMVPA as opposed to cMVPA. Using only SB breaks as a measure of SB patterns maybe insufficient to highlight differences between older adults. Future research should aim to examine how these multiple SB/PA patterns affect health status.

Craig, R., Mindell, J. and Hirani, V. (2009) 'Health Survey for England 2008. Volume 1: Physical Activity and Fitness.' Health Survey for England, 1 pp. 8-395.

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Modelling any impact on cardiovascular health of sedentary behaviour and physical activity lifestyle substitution in older adults.

DJ Ryan¹, JA Wullems¹, GK Stebbings¹, CI Morse¹, CE Stewart², GL Onambele-Pearson¹,

Cardiovascular disease (CVD) incidence is modifiable through lifestyle, including sedentary behaviour (SB) and physical activity (PA). Only 5-12% of older adults attain the recommended 2.5 hrs·wk⁻¹ of moderate intensity PA (MVPA), accumulated in ≥10 minute bouts (₁₀MVPA)¹² needed to minimise CVD related mortality³. Additionally, OA engage in over 9 hrs·day⁻¹ SB¹, which is an independent risk factor for CVD⁴. The aim of this study was to model the degree to which 44 key cardiometabolic parameters alter with a change in mobility using isothermal substitution modelling. Ninety-three OA (60 – 89 years) were fitted with a thigh-mounted accelerometer for seven continuous days to measure levels of SB, standing, light-intensity PA (LIPA), sporadic MVPA (sMVPA, accumulated in <10 minute bouts), and ₁₀MVPA. Replacing an hour of SB, LIPA, and sMVPA with Standing reduced LOG total cholesterol whereas LIPA would increase LOG total cholesterol when it replaced SB. Replacement of any mobility for ₁₀MVPA decreased LOG plasma triglyceride concentration and vice versa. Replacing SB with Standing and sMVPA reduced resting heart rate. Replacing SB with LIPA decreased LOG blood velocity post cuff deflation. Replacing SB with LIPA decreased carotid artery diameter (AD) and vice versa. Replacing Standing with sMVPA increased popliteal AD and vice versa. Out of 44 cardiometabolic parameters, 8 were effected by replacing SB with PA and 2 were effected by replacing 10MVPA with a lower intensity mobility. Our findings suggest that PA, other than ₁₀MVPA, may be an effective method to reduce SB and improve certain health parameters.

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Part 3: Public engagement

The Effects of Sedentary Behaviour on Older Adults – Crewe Rotary Club, Crewe, UK



The Effects of Sedentary Behaviour on Older Adults
Declan Ryan, PhD Candidate
Dr Gladys Pearson, Dr Georgina Stebbings, Dr Chris Morse, Prof Claire Stewart



<http://www.cheshire.mmu.ac.uk/heal/>

The Sitting Patterns of Crewe's Older Adults – Shavington Leisure Centre, Crewe, UK

**THE SITTING PATTERNS
OF CREWE'S OLDER
ADULTS.**

Declan Ryan – PhD Candidate,
Dr Georgina Stebbings, Dr Chris Morse, Prof Claire Stewart, Dr Gladys Pearson



Older Adults (60 – 89 years)

REPLACE SITTING

IMPROVE CARDIO-METABOLIC HEALTH

@Dekes31

R6 Declan Ryan

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Manchester Metropolitan University

Resting Heart Rate is a Predictor of Death

Resting Heart Rate \nearrow 10 bpm \equiv \nearrow 6% Risk of Cardiovascular Death

Our Findings:

Triglyceride is a Predictor of Death

Triglyceride \nearrow 1.0 mmol.l⁻¹ \equiv \nearrow 33% (men) 75% (women) Risk of Cardiovascular Disease

Our Findings:

Per Day, Replace



With



Bouts > 10 mins



Total Cholesterol is a Predictor of Death

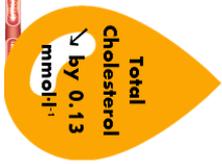
Total Cholesterol \nearrow 0.50 mmol.l⁻¹ \equiv \nearrow 17% Risk of Coronary Heart Disease Death

Our Findings:

Per Day, Replace



With



Carotid (Neck) Artery Diameter is a Predictor of Death

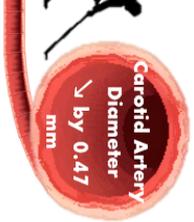
Carotid Artery Diameter \nearrow 0.78 mm \equiv \nearrow 2 X Risk of Death

Our findings:

Per Day, Replace



With



sitting

Standing

Light

Moderate

Vigorous

Running

Fast walking

Cycling

Swimming

Aerobic exercise

Strength training

Flexibility

Balance

Mental health

Social interaction

Sleep

Nutrition

Hydration

Sun protection

Safety

First aid

Emergency services

Mental health

Social interaction

Sleep

Bouts < 10 mins

Bouts > 10 mins

minute bouts

DO WE CONTINUE TO AGE AFTER 60: WHAT CAN WE DO ABOUT IT?



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YES

BRACHIAL ARTERY DIAMETER

PEOPLE AGED 78 - 83

19%

LARGER THAN PEOPLE AGED 60 - 65

POPLITEAL ARTERY WALL THICKNESS

PEOPLE OLDER THAN 84

29%

LARGER THAN PEOPLE AGED 60 - 65

NO

THESE DO NOT CHANGE WITH FURTHER AGEING:

- BLOOD PRESSURE
- RESTING HEART RATE
- CAROTID ARTERY DIAMETER
- BRACHIAL ARTERY WALL THICKNESS
- POPLITEAL ARTERY DIAMETER

PERFORM LIGHT INTENSITY ACTIVITY

(E.G. HOUSEHOLD CHORES)

WHEN ACCOUNTING FOR THESE ACTIVITIES

0%

DIFFERENCE IN POPLITEAL ARTERY WALL THICKNESS BETWEEN 60 - 65 AND 84+ AGE GROUPS

POPLITEAL ARTERY WALL THICKNESS DECREASES

0.09 mm

FOR EVERY EXTRA HOUR OF LIGHT INTENSITY ACTIVITY PER DAY

Chapter 09:

Appendices.

Chapter 03: 'Part 1: Flow mediated dilation data.'

'Part 1: Full patterns of PB results.'

'Part 2: Full ISM results.'

'Part 3: Alternative PB heat maps.'

***'Part 3: MVPA – brachial artery
diameter relationship.'***

Chapter 04: 'Effect of medication on further ageing.'

Chapter 05: 'Part 2: Full ISM results.'

Chapter 03: Part 1: Flow mediated dilation data.

Table A3.1.1. Flow mediated dilation demographics for pooled, male, and female populations.

Variable	Pooled	Male	Female
FMD			
Unscaled (%)	1.590 (6.680) _m	1.360 (5.930) _m	1.840 (8.020) _m
Scaled (%)	5.300 (1.960) _m	4.950 (1.810) _m	5.550 (2.340) _m
LOG Scaled (%)	1.672 (0.350) _m	1.793 (0.321)	1.634 (0.360)*
Artery Diameter 15 s Post Deflation (mm)	4.203 (0.758)	4.642 (0.751)	3.871 (0.578)***
Blood Velocity 15 s Post Deflation (mm·s ⁻¹)	484.000 (188.000) _m	484.000 (117.000) _m	484.000 (239.500) _m
LOG Blood Velocity 15 s Post Deflation (mm·s ⁻¹)	6.148 (0.294)	6.159 (0.235)	6.139 (0.333)
Shear Rate 15 s Post Deflation (·s ⁻¹)	905.012 (408.780) _m	821.441 (251.920) _m	982.739 (598.140) _m *
LOG Shear Rate 15 s Post Deflation (·s ⁻¹)	6.814 (0.341)	6.716 (0.244)	6.889 (0.386)*
FMD:SR (ratio)	0.006 (0.004) _m	0.006 (0.003) _m	0.006 (0.005) _m
Time to Peak Diameter (s)	54.500 (46.450) _m	49.400 (56.000) _m	56.700 (48.100) _m
LOG Time to Peak Diameter (s)	3.998 (0.760) _m	3.900 (0.840) _m	4.038 (0.860) _m

_m Median (IR). FMD:SR Unscaled FMD to shear rate 15 s post deflation ratio. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table A3.1.2. Bivariate and multivariate stepwise linear regressions between PB, covariates and FMD parameters.

Variable	Model	b	-95% CI	+95% CI	p	r ² adj.	P. Corr.
FMD							
Unscaled	SB ^a	-0.198	-1.113	0.716	0.667	-0.010	
	Standing ^b	-0.456	-3.461	2.550	0.764	-0.011	
	LIPA ^c	-0.487	-2.612	1.638	0.650	-0.009	
	sMVPA ^d	0.331	-1.373	2.035	0.700	-0.010	
	10MVPA ^e	-5.709	-10.403	-1.015	0.018	0.053	
	(in)direct	0.796	0.063	1.528	0.034	0.039	
	CVD Meds ^g	MR ^e	-5.709	-10.403	-1.015	0.018	0.053
Scaled	SB ^a	-0.066	-0.371	0.239	0.667	-0.010	
	Standing ^b	-0.153	-1.156	0.850	0.763	-0.011	
	LIPA ^c	-0.181	-0.890	-0.528	0.614	-0.009	
	sMVPA ^d	0.054	-0.515	0.623	0.851	-0.011	
	10MVPA ^e	-1.859	-3.428	-0.290	0.021	0.050	
LOG Scaled	SB ^a	0.061	0.011	0.112	0.019	0.054	
	Standing ^b	-0.129	-0.299	0.041	0.135	0.015	
	LIPA ^c	-0.134	-0.252	-0.016	0.026	0.047	
	sMVPA ^d	-0.072	-0.168	0.025	0.143	0.014	
	10MVPA ^e	0.051	0.226	0.328	0.716	-0.011	
	Primary CVD	0.047	0.001	0.092	0.045	0.035	
	Meds ^f	MR ^a	0.061	0.011	0.112	0.019	0.054
Artery Diameter 15 s Post Deflation	SB ^a	-0.034	-0.148	0.080	0.551	-0.008	
	Standing ^b	0.109	-0.258	0.476	0.557	-0.008	
	LIPA ^c	0.052	-0.213	0.316	0.699	-0.010	
	sMVPA ^d	0.103	-0.113	0.318	0.346	-0.001	
	10MVPA ^e	-0.198	-0.797	0.400	0.512	-0.007	
	Primary CVD	0.119	0.021	0.217	0.018	0.054	
	Meds ^f	Hydration ^h	0.024	0.002	0.047	0.035	0.042
	MR ^{hf}	0.028^h	0.006^h	0.050^h	0.014^h	0.110	0.273^h
		0.116^f	0.018^f	0.213^f	0.020^f		0.257^f
					0.004^{hf}		
Blood	SB ^a	13.204	-8.508	34.916	0.230	0.006	
Velocity 15 s Post Deflation	Standing ^b	-13.885	-84.446	56.677	0.696	-0.010	
	LIPA ^c	-48.257	-97.971	1.458	0.057	0.032	
	sMVPA ^d	-14.555	-55.983	26.872	0.487	-0.006	
	10MVPA ^e	-71.269	-186.497	43.958	0.222	0.006	
LOG Blood Velocity 15 s Post Deflation	SB ^a	0.025	-0.018	0.068	0.248	0.004	
	Standing ^b	-0.024	-0.164	0.115	0.731	-0.011	
	LIPA ^c	-0.089	-0.188	0.009	0.076	0.027	
	sMVPA ^d	-0.032	-0.114	0.050	0.439	-0.005	
	10MVPA ^e	0.114	-0.378	0.077	0.191	0.009	

Table A3.1.2 continued.

Shear Rate	SB ^a	36.190	-18.108	90.488	0.189	0.009
15 s Post	Standing ^b	-71.355	-247.599	104.889	0.423	-0.004
Deflation	LIPA ^c	-124.652	-249.023	-0.280	0.049	0.035
	sMVPA ^d	-58.270	-161.572	45.033	0.265	0.003
	₁₀ MVPA ^e	-102.979	-393.459	187.502	0.483	-0.006
LOG Shear	SB ^a	0.033	-0.018	0.083	0.200	0.008
Rate 15 s	Standing ^b	-0.053	-0.217	0.112	0.527	-0.007
Post	LIPA ^c	-0.192	-0.220	0.013	0.081	0.025
Deflation	sMVPA ^d	-0.055	-0.151	0.041	0.257	0.004
	₁₀ MVPA ^e	-0.107	-0.377	0.164	0.434	-0.005
FMD:SR	SB ^a	0.000	-0.001	0.000	0.269	0.003
	Standing ^b	8.1E-05	-0.001	0.002	0.917	-0.012
	LIPA ^c	0.000	-0.001	0.002	0.481	-0.006
	sMVPA ^d	0.000	0.000	0.001	0.325	0.000
	₁₀ MVPA ^e	-0.002	-0.004	0.001	0.236	0.005
Time to	SB ^a	2.225	-1.787	6.237	0.273	0.003
Peak	Standing ^b	-8.183	-21.342	4.976	0.220	0.006
Diameter	LIPA ^c	-8.222	-17.447	1.004	0.080	0.024
	sMVPA ^d	-5.447	-12.886	1.992	0.149	0.013
	₁₀ MVPA ^e	2.235	-19.184	23.653	0.836	-0.011
LOG Time to	SB ^a	0.036	-0.030	0.102	0.281	0.002
Peak	Standing ^b	-0.138	-0.355	0.079	0.209	0.007
Diameter	LIPA ^c	-0.139	-0.291	0.013	0.073	-0.026
	sMVPA ^d	-0.091	-0.214	0.031	0.142	0.014
	₁₀ MVPA ^e	0.083	-0.270	0.436	0.642	-0.009

FMD:SR Unscaled FMD to shear rate 15 s post deflation ratio. **Bold** font highlights significant ($p \leq 0.05$) bivariate and multivariate stepwise linear regression models. Hydration Change per percent increase in total body water. Primary CVD Meds Change per one unit increase in the number of medications directly targeting CVD risk. (in)direct CVD Meds Change per one unit increase in the number of medications (in)directly targeting CVD risk. MR Multivariate stepwise linear regression model. Superscript letters represent which, and what order PB variables are included in the multivariate model. b Change in cardiovascular variable per unit increase in GENE A variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. p Significance value. r^2 adj. Adjusted explained variance. P. Corr. Partial Correlation.

Table A3.1.3. Change in bivariate and multivariate stepwise linear FMD regressions models following the removal of PB outliers.

Variable	Model	b	-95% CI	+95% CI	p	r ² adj.	P. Corr.
FMD							
Unscaled	SB ^a	-0.198	-1.113	0.716	0.667	-0.010	
	Standing ^b	-0.456	-3.461	2.550	0.764	-0.011	
	LIPA ^c	-0.487	-2.612	1.638	0.650	-0.009	
	sMVPA ^d	0.331	-1.373	2.035	0.700	-0.010	
	₁₀ MVPA ^e	-5.709	-10.40	-1.015	0.018	0.053	
	(in)direct CVD Meds ^g	0.796	0.063	1.528	0.034	0.039	
Unscaled	SB ^a	-0.118	-0.907	1.143	0.819	-0.011	
	Standing ^b	-0.435	-3.783	2.912	0.797	-0.011	
	LIPA ^c	-0.487	-2.612	1.655	0.650	-0.010	
	sMVPA ^d	0.491	-2.638	2.035	0.700	-0.010	
	₁₀ MVPA ^e	-5.615	-14.41	3.185	0.208	0.008	
	(in)direct CVD Meds ^g	0.796	0.063	1.528	0.034	0.039	
Scaled	SB ^a	-0.066	-0.371	0.239	0.667	-0.010	
	Standing ^b	-0.153	-1.156	0.850	0.763	-0.011	
	LIPA ^c	-0.181	-0.890	-0.528	0.614	-0.009	
	sMVPA ^d	0.054	-0.515	0.623	0.851	-0.011	
	₁₀ MVPA ^e	-1.859	-3.428	-0.290	0.021	0.050	
	Scaled	SB ^a	0.040	-0.302	0.381	0.818	-0.011
Standing ^b		-0.084	-1.201	1.032	0.881	-0.012	
LIPA ^c		-0.181	-0.890	-0.528	0.614	-0.009	
sMVPA ^d		-0.247	-0.962	0.469	0.494	-0.006	
₁₀ MVPA ^e		-2.027	-4.972	0.918	0.175	0.011	
LOG Scaled		SB ^a	0.061	0.011	0.112	0.019	0.054
	Standing ^b	-0.129	-0.299	0.041	0.135	0.015	
	LIPA ^c	-0.134	-0.252	-0.016	0.026	0.047	
	sMVPA ^d	-0.072	-0.168	0.025	0.143	0.014	
	₁₀ MVPA ^e	0.051	0.226	0.328	0.716	-0.011	
	Primary CVD Meds ^f	0.047	0.001	0.092	0.045	0.035	
LOG Scaled	SB ^a	0.061	0.011	0.112	0.019	0.054	
	MR ^g	0.061	0.011	0.112	0.019	0.054	
	SB ^a	0.055	-0.002	0.112	0.059	0.032	
	Standing ^b	-0.130	-0.320	0.060	0.176	0.010	
	LIPA ^c	-0.134	-0.252	-0.016	0.026	0.047	
	sMVPA ^d	-0.071	-0.189	0.048	0.241	0.005	
LOG Scaled	₁₀ MVPA ^e	-0.006	-0.554	0.543	0.984	-0.013	
	Primary CVD Meds ^f	0.053	0.005	0.100	0.032	0.042	
	MR ^g	0.065	0.016	0.114	0.009	0.077	0.300
	Excluded ^a	0.213			0.064		0.218

FMD:SR Unscaled FMD to shear rate 15 s post deflation ratio. **Bold** font highlights significant ($p \leq 0.05$) bivariate and multivariate stepwise linear regression models. Orange shading highlights regression models following outlier removal. Hydration Change per percent increase in total body water. Primary CVD Meds Change per one unit increase in the number of medications directly targeting CVD risk. (in)direct CVD Meds Change per one unit increase in the number of medications (in)directly targeting CVD risk. MR Multivariate stepwise linear regression. Superscript letters represent which, and what order PB variables are included in the multivariate model. b Change in cardiovascular variable per unit increase in GENEVA variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. p Significance value. r² adj. Adjusted explained variance. P. Corr. Partial Correlation.

Table A3.1.4. Bivariate linear regressions models between patterns of PB and FMD parameters.

Variable	Model	b	-95% CI	+95% CI	p	r ²	r ² adj.	
FMD								
Unscaled	SB Breaks	-0.249	-0.612	0.114	0.176	0.021	0.010	
	<5min SB Bout	-0.143	-0.827	0.540	0.678	0.002	-0.010	
	≥5min SB Bout	-0.457	-1.017	0.103	0.108	0.030	0.019	
	True Mean SB Bout	0.022	-0.101	0.146	0.718	0.002	-0.010	
	Alpha	0.318	-36.544	37.180	0.986	0.000	-0.012	
	W50%	0.061	-0.028	0.150	0.176	0.021	0.010	
	PA Bouts	-0.249	-0.612	0.114	0.176	0.021	0.010	
	Daily Sum of PA Bout Time	-0.004	-0.018	0.010	0.595	0.003	-0.008	
	True Mean PA Bout	0.051	-0.223	0.324	0.714	0.002	-0.010	
	10MVPA Bouts	-2.051	-3.773	-0.328	0.020	0.062	0.051	
	Total Week 10MVPA	-0.827	-1.497	-0.156	0.016	0.066	0.055	
	SB%	0.015	-0.125	0.156	0.827	0.001	-0.011	
	Standing%	-0.030	-0.505	0.444	0.899	0.000	-0.012	
	LIPA%	-0.041	-0.386	0.304	0.813	0.001	-0.011	
	sMVPA%	0.084	-0.182	0.349	0.533	0.005	-0.007	
	10MVPA%	-0.937	-1.702	-0.171	0.017	0.065	0.054	
	Scaled	SB Breaks	-0.084	-0.205	0.037	0.173	0.022	0.010
		<5min SB Bout	-0.064	-0.292	0.164	0.581	0.004	-0.008
		≥5min SB Bout	-0.144	-0.331	0.044	0.131	0.027	0.015
		True Mean SB Bout	0.007	-0.034	0.048	0.743	0.001	-0.010
Alpha		0.533	-11.769	12.835	0.932	0.000	-0.012	
W50%		0.021	-0.009	0.051	0.160	0.023	0.012	
PA Bouts		-0.084	-0.205	0.037	0.173	0.022	0.010	
Daily Sum of PA Bout Time		-0.002	-0.006	0.003	0.519	0.005	-0.007	
True Mean PA Bout		0.013	-0.079	0.104	0.781	0.001	-0.011	
10MVPA Bouts		-0.692	-1.266	-0.177	0.019	0.063	0.052	
Total Week 10MVPA		-0.268	-0.492	-0.044	0.020	0.062	0.051	
SB%		0.007	-0.040	0.054	0.756	0.001	-0.011	
Standing%		-0.009	-0.168	0.149	0.909	0.000	-0.012	
LIPA%		-0.015	-0.130	0.100	0.795	0.001	-0.011	

Table A3.1.4 continued.

Scaled	sMVPA%	0.020	-0.069	0.109	0.657	0.002	-0.009	
	₁₀ MVPA%	-0.306	-0.562	-0.050	0.020	0.062	0.051	
LOG Scaled Diameter 15 s Post Deflation	SB Breaks	-0.016	-0.037	0.005	0.139	0.026	0.015	
	<5min SB Bout	-0.033	-0.072	0.006	0.098	0.033	0.021	
	≥5min SB Bout	-0.014	-0.046	0.019	0.405	0.008	-0.004	
	True Mean SB Bout	0.010	0.003	0.017	0.008	0.083	0.072	
	Alpha	-1.788	-3.773	0.196	0.077	0.038	0.026	
	W50%	0.006	0.001	0.011	0.027	0.058	0.047	
	PA Bouts	-0.016	-0.037	0.005	0.139	0.026	0.015	
	Daily Sum of PA Bout Time	-0.001	-0.002	0.000	0.047	0.047	0.036	
	True Mean PA Bout	-0.009	-0.024	0.007	0.264	0.015	0.003	
	₁₀ MVPA Bouts	0.005	-0.099	0.108	0.930	0.000	-0.012	
	Total Week ₁₀ MVPA	0.008	-0.032	0.047	0.707	0.002	-0.010	
	SB%	0.009	0.001	0.016	0.029	0.057	0.045	
	Standing%	-0.022	-0.049	0.005	0.107	0.031	0.019	
	LIPA%	-0.023	-0.042	-0.004	0.018	0.066	0.054	
	sMVPA%	-0.012	-0.027	0.003	0.128	0.028	0.016	
	₁₀ MVPA%	0.008	-0.038	0.053	0.742	0.001	-0.011	
	Artery	SB Breaks	0.011	-0.034	0.057	0.630	0.003	-0.009
	Diameter 15 s Post Deflation	<5min SB Bout	0.042	-0.041	0.126	0.319	0.012	0.000
		≥5min SB Bout	-0.004	-0.076	0.068	0.916	0.000	-0.012
		True Mean SB Bout	-0.002	-0.017	0.014	0.825	0.001	-0.012
Alpha		-0.209	-4.821	4.403	0.928	0.000	-0.012	
W50%		-0.007	-0.018	0.004	0.206	0.019	0.007	
PA Bouts		0.011	-0.034	0.057	0.630	0.003	-0.009	
Daily Sum of PA Bout Time		0.001	-0.001	0.002	0.441	0.007	-0.005	
True Mean PA Bout		0.002	-0.032	0.036	0.908	0.000	-0.012	
₁₀ MVPA Bouts		-0.061	-0.286	0.163	0.587	0.004	-0.009	
Total Week ₁₀ MVPA		-0.031	-0.117	0.054	0.472	0.006	-0.006	
SB%		-0.006	-0.023	0.011	0.497	0.006	-0.006	

Table A3.1.4 continued.

Artery	Standing%	0.017	-0.041	0.075	0.561	0.004	-0.008
Diameter 15 s	LIPA%	0.008	-0.035	0.050	0.723	0.002	-0.011
Post Deflation	sMVPA%	0.015	-0.019	0.049	0.374	0.010	-0.002
	₁₀ MVPA%	-0.030	-0.128	0.068	0.541	0.005	-0.008
Blood Velocity	SB Breaks	0.841	-7.947	9.628	0.849	0.000	-0.012
15 s Post	<5min SB	1.912	-14.504	18.329	0.817	0.001	-0.012
Deflation	Bout						
	≥5min SB	1.049	-12.736	14.834	0.880	0.000	-0.012
	Bout						
	True Mean	0.260	-2.672	3.191	0.861	0.000	-0.012
	SB Bout						
	Alpha	-310.353	-1192.640	571.935	0.486	0.006	-0.006
	W50%	0.942	-1.185	3.068	0.381	0.009	-0.003
	PA Bouts	0.841	-7.947	9.628	0.849	0.000	-0.012
	Daily Sum of	-0.255	-0.587	0.077	0.131	0.028	0.016
	PA Bout Time						
	True Mean	-5.205	-11.659	1.249	0.112	0.031	0.019
	PA Bout						
	₁₀ MVPA	-22.487	-66.426	21.452	0.312	0.013	0.000
	Bouts						
	Total Week	-11.150	-27.603	5.302	0.181	0.022	0.010
	₁₀ MVPA						
	SB%	2.367	-0.947	5.681	0.159	0.024	0.012
	Standing%	-1.887	-13.027	9.254	0.737	0.001	-0.011
	LIPA%	-7.670	-15.722	0.382	0.062	0.042	0.031
	sMVPA%	-2.028	-8.503	4.447	0.535	0.005	-0.008
	₁₀ MVPA%	-11.887	-30.722	6.949	0.213	0.019	0.007
LOG Blood	SB Breaks	0.002	-0.016	0.019	0.849	0.000	-0.012
Velocity 15 s	<5min SB	0.006	-0.027	0.038	0.737	0.001	-0.011
Post Deflation	Bout						
	≥5min SB	0.001	-0.027	0.028	0.964	0.000	-0.012
	Bout						
	True Mean	0.001	-0.005	0.007	0.763	0.001	-0.011
	SB Bout						
	Alpha	-0.612	-2.357	1.133	0.487	0.006	-0.006
	W50%	0.002	-0.002	0.006	0.418	0.008	-0.004
	PA Bouts	0.002	-0.016	0.019	0.849	0.000	-0.012
	Daily Sum of	0.000	-0.001	0.000	0.136	0.027	0.015
	PA Bout Time						
	True Mean	-0.010	-0.023	0.003	0.130	0.028	0.016
	PA Bout						
	₁₀ MVPA	-0.055	-0.142	0.031	0.208	0.019	0.007
	Bouts						

Table A3.1.4 continued.

LOG Blood Velocity 15 s Post Deflation	Total Week	-0.023	-0.056	0.009	0.161	0.024	0.012
	₁₀ MVPA						
	SB%	0.005	-0.002	0.011	0.163	0.024	0.012
	Standing%	-0.003	-0.025	0.019	0.772	0.001	-0.011
	LIPA%	-0.014	-0.030	0.002	0.083	0.037	0.025
	sMVPA%	-0.004	-0.017	0.008	0.490	0.006	-0.006
	₁₀ MVPA%	-0.025	-0.063	0.012	0.177	0.022	0.010
Shear Rate 15 s Post Deflation	SB Breaks	1.005	-21.015	23.025	0.928	0.000	-0.012
	<5min SB Bout	-3.548	-44.684	37.587	0.864	0.000	-0.012
	≥5min SB Bout	5.834	-28.683	40.351	0.738	0.001	-0.011
	True Mean	0.409	-6.937	7.755	0.912	0.000	-0.012
	SB Bout						
	Alpha	-587.413	-2800.721	1625.896	0.599	0.003	-0.009
	W50%	3.618	-1.676	8.912	0.178	0.022	0.010
	PA Bouts	1.005	-21.015	23.025	0.928	0.000	-0.012
	Daily Sum of PA Bout Time	-0.769	-1.596	0.058	0.068	0.041	0.029
	True Mean	13.971	-30.101	2.160	0.089	0.035	0.023
	PA Bout						
	₁₀ MVPA	-30.265	-140.848	80.318	0.588	0.004	-0.009
	Bouts						
	Total Week	-15.518	-57.055	26.019	0.459	0.007	-0.005
	₁₀ MVPA						
	SB%	6.874	-1.394	15.142	0.102	0.003	0.021
	Standing%	-10.351	-38.187	17.486	0.462	0.007	-0.006
	LIPA%	-19.438	-39.601	0.725	0.059	0.043	0.032
	sMVPA%	-7.980	-24.146	8.186	0.329	0.012	0.000
	₁₀ MVPA%	-17.278	-64.772	30.215	0.471	0.006	-0.006
LOG Shear Rate 15 s Post Deflation	SB Breaks	-0.001	-0.021	0.020	0.938	0.000	-0.012
	<5min SB Bout	-0.004	-0.042	0.034	0.834	0.001	-0.012
	≥5min SB Bout	0.002	-0.031	0.034	0.926	0.000	-0.012
	True Mean	0.001	-0.006	0.008	0.735	0.001	-0.011
	SB Bout						
	Alpha	-0.547	-2.608	1.514	0.599	0.003	-0.009
	W50%	0.003	-0.002	0.008	0.174	0.023	0.011
	PA Bouts	-0.001	-0.021	0.020	0.938	0.000	-0.012
	Daily Sum of PA Bout Time	-0.001	-0.001	0.000	0.090	0.035	0.023
	True Mean	-0.011	-0.026	0.005	0.166	0.024	0.011
	PA Bout						
	₁₀ MVPA	-0.040	-0.143	0.062	0.436	0.008	-0.005
	Bouts						

Table A3.1.4 continued.

LOG Shear	Total Week	-0.016	-0.055	0.022	0.407	0.009	-0.004
Rate 15 s	₁₀ MVPA						
Post Deflation	SB%	0.006	-0.002	0.014	0.121	0.029	0.017
	Standing%	-0.008	-0.034	0.018	0.561	0.004	-0.008
	LIPA%	-0.016	-0.035	0.003	0.092	0.035	0.023
	sMVPA%	-0.008	-0.023	0.007	0.305	0.013	0.001
	₁₀ MVPA%	-0.019	-0.063	0.026	0.403	0.009	-0.004
FMD:SR	SB Breaks	-3.9E-05	0.000	0.000	0.685	0.002	-0.010
	<5min SB	2.5E-06	0.000	0.000	0.989	0.000	-0.012
	Bout						
	≥5min SB	-8.0E-05	0.000	0.000	0.599	0.003	-0.009
	Bout						
	True Mean	-1.1E-05	0.000	0.000	0.734	0.001	-0.011
	SB Bout						
	Alpha	0.005	-0.014	0.024	0.612	0.003	-0.009
	W50%	-2.7E-06	0.000	0.000	0.909	0.000	-0.012
	PA Bouts	-3.9E-05	0.000	0.000	0.685	0.002	-0.010
	Daily Sum of	2.3E-06	0.000	0.000	0.534	0.005	-0.008
	PA Bout Time						
	True Mean	5.0E-05	0.000	0.000	0.487	0.006	-0.006
	PA Bout						
	₁₀ MVPA	-0.001	-0.001	0.000	0.282	0.014	0.002
	Bouts						
	Total Week	0.000	-0.001	0.000	0.244	0.017	0.005
	₁₀ MVPA						
	SB%	-2.8E-05	0.000	0.000	0.446	0.007	-0.005
	Standing%	2.4E-05	0.000	0.000	0.845	0.000	-0.012
LIPA%	7.4E-05	0.000	0.000	0.415	0.008	-0.004	
sMVPA%	7.8E-05	0.000	0.000	0.272	0.015	0.003	
₁₀ MVPA%	0.000	-0.001	0.000	0.242	0.017	0.005	
Time to Peak	SB Breaks	-0.299	-1.918	1.319	0.714	0.002	-0.010
	Diameter						
	<5min SB	-0.914	-3.929	2.101	0.548	0.004	-0.007
	Bout						
	≥5min SB	-0.067	-2.578	2.443	0.958	0.000	-0.012
	Bout						
	True Mean	0.318	-0.223	0.860	0.246	0.016	0.004
	SB Bout						
	Alpha	17.485	-145.217	180.186	0.831	0.001	-0.011
	W50%	0.216	-0.177	0.609	0.277	0.014	0.002
	PA Bouts	-0.299	-1.918	1.319	0.714	0.002	-0.010
	Daily Sum of	-0.058	-0.119	0.003	0.064	0.040	0.029
	PA Bout Time						
	True Mean	-0.929	-2.121	0.263	0.125	0.027	0.016
	PA Bout						

Table A3.1.4 continued.

Time to Peak	₁₀ MVPA	0.661	-7.191	8.512	0.868	0.000	-0.011
Diameter	Bouts						
	Total Week	0.296	-2.767	3.359	0.848	0.000	-0.011
	₁₀ MVPA						
	SB%	0.526	-0.084	1.136	0.090	0.033	0.022
	Standing%	-1.268	-3.346	0.811	0.229	0.017	0.005
	LIPA%	-1.273	-2.772	0.227	0.095	0.032	0.021
	sMVPA%	-0.786	-1.949	0.376	0.182	0.021	0.009
	₁₀ MVPA%	0.349	-3.146	3.844	0.843	0.000	-0.011
LOG Time to	SB Breaks	-0.004	-0.031	0.023	0.778	0.001	-0.011
Peak	<5min SB	-0.017	-0.067	0.033	0.503	0.005	-0.006
Diameter	Bout						
	≥5min SB	0.003	-0.039	0.044	0.891	0.000	-0.012
	Bout						
	True Mean	0.005	-0.004	0.014	0.259	0.015	0.003
	SB Bout						
	Alpha	0.467	-2.216	3.150	0.730	0.001	-0.010
	W50%	0.003	-0.003	0.010	0.354	0.010	-0.002
	PA Bouts	-0.004	-0.031	0.023	0.778	0.001	-0.011
	Daily Sum of	-0.001	-0.002	0.000	0.065	0.040	0.028
	PA Bout Time						
	True Mean	-0.016	-0.035	0.004	0.112	0.029	0.018
	PA Bout						
	₁₀ MVPA	0.034	-0.095	0.164	0.598	0.003	-0.008
	Bouts						
	Total Week	0.011	-0.039	0.062	0.657	0.002	-0.009
	₁₀ MVPA						
	SB%	0.009	-0.001	0.019	0.093	0.033	0.021
	Standing%	-0.021	-0.056	0.013	0.221	0.018	0.006
	LIPA%	-0.021	-0.046	0.003	0.087	0.034	0.023
	sMVPA%	-0.013	-0.032	0.006	0.175	0.022	0.010
	₁₀ MVPA%	0.014	-0.044	0.071	0.634	0.003	-0.009

FMD:SR Unscaled FMD to shear rate 15 s post deflation ratio. **Bold** Significant bivariate linear regressions ($p \leq 0.05$). **b** Change in cardiovascular variable per unit increase in GENE variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. **p** Significance value. r^2 Explained variance. r^2 adj. Adjusted explained variance.

Table A3.1.5. Change in bivariate linear regression models between patterns of PB and FMD parameters after patterns of PB outliers were removed.

Variable	Model	b	-95% CI	+95% CI	p	r ²	r ² adj.
FMD							
Unscaled	10MVPA	-2.051	-3.773	-0.328	0.020	0.062	0.051
	Bouts	-1.042	-4.190	2.107	0.512	0.006	-0.007
	Total Week	-0.827	-1.497	-0.156	0.016	0.066	0.055
	10MVPA	-0.853	-1.728	0.023	0.056	0.045	0.033
	10MVPA%	-0.937	-1.702	-0.171	0.017	0.065	0.054
			-0.870	-2.224	0.484	0.205	0.020
Scaled	10MVPA	-0.692	-1.266	-0.177	0.019	0.063	0.052
	Bouts	-0.425	-1.481	0.631	0.425	0.008	-0.005
	Total Week	-0.268	-0.492	-0.044	0.020	0.062	0.051
	10MVPA	-0.279	-0.572	0.014	0.062	0.043	0.031
	10MVPA%	-0.306	-0.562	-0.050	0.020	0.062	0.051
			-0.314	-0.767	0.140	0.172	0.023

Bold font highlights significant ($p \leq 0.05$) bivariate linear regressions. Orange shading highlights regression models following outlier removal. b Change in cardiovascular variable per unit increase in GENE variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. p Significance value. r² Explained variance. r² adj. Adjusted explained variance.

Chapter 03: Part 1: Full patterns of PB results.

Table A3.1.6. Complete Bivariate linear regressions models between patterns of PB and cardiovascular parameters.

Variable	Model	b	-95% CI	+95% CI	p	r ²	r ² adj.	
Systolic BP	SB Breaks	0.08	-0.90	1.08	0.86	0.00	-0.01	
	<5min SB Bout	-0.14	-2.00	1.71	0.87	0.00	-0.01	
	≥5min SB Bout	0.32	-1.20	1.84	0.67	0.002	-0.01	
	True Mean SB Bout	-0.08	-0.42	0.25	0.62	0.003	-0.01	
	Alpha	78.2	-15.8	172	0.10	0.03	0.01	
	W50%	-0.09	-0.33	0.15	0.46	0.01	-0.01	
	PA Bouts	0.08	-0.90	1.08	0.86	0.00	-0.01	
	Daily Sum of PA Bout Time	0.002	-0.03	0.04	0.91	0.00	-0.01	
	True Mean PA Bout	0.10	-0.62	0.83	0.77	0.001	-0.01	
	₁₀ MVPA Bouts	-0.60	-5.43	4.22	0.80	0.001	-0.01	
	Total Week ₁₀ MVPA	0.21	-1.66	2.10	0.81	0.001	-0.01	
	SB%	-0.04	-0.42	0.33	0.81	0.001	-0.01	
	Standing%	-0.13	-1.42	1.14	0.83	0.001	-0.01	
	LIPA%	-0.07	-0.99	0.85	0.87	0.00	-0.01	
	sMVPA%	0.22	-0.49	0.94	0.54	0.004	-0.01	
	₁₀ MVPA%	0.24	-1.90	2.39	0.82	0.001	-0.01	
	LOG Diastolic BP	SB Breaks	0.003	-0.004	0.01	0.35	0.01	-0.001
		<5min SB Bout	-0.002	-0.01	0.01	0.79	0.001	-0.01
		≥5min SB Bout	0.01	-0.002	0.01	0.10	0.02	0.01
		True Mean SB Bout	-0.001	-0.004	0.001	0.24	0.01	0.004
Alpha		0.73	0.10	1.37	0.02	0.05	0.04	
W50%		-0.001	-0.002	0.001	0.36	0.01	-0.002	
PA Bouts		0.003	-0.004	0.01	0.35	0.01	-0.001	
Daily Sum of PA Bout Time		-4.1E-05	0.00	0.00	0.74	0.001	-0.01	
True Mean PA Bout		-0.001	-0.01	0.004	0.73	0.001	-0.01	
₁₀ MVPA Bouts		-0.01	-0.03	0.02	0.71	0.001	-0.01	
Total Week ₁₀ MVPA		-0.002	-0.01	0.01	0.80	0.001	-0.01	
SB%		0.00	-0.002	0.003	0.89	0.00	-0.01	
Standing%		-0.01	-0.01	0.003	0.18	0.02	0.01	
LIPA%		-0.002	-0.01	0.004	0.44	0.01	-0.01	
sMVPA%		0.003	-0.002	0.01	0.25	0.02	0.004	
₁₀ MVPA%		-0.001	-0.01	0.01	0.87	0.00	-0.01	
Pulse Pressure		SB Breaks	-0.15	-0.90	0.59	0.68	0.002	-0.01
		<5min SB Bout	-0.03	-1.44	1.36	0.95	0.00	-0.01
		≥5min SB Bout	-0.31	-1.46	0.83	0.58	0.003	-0.01
		True Mean SB Bout	0.01	-0.23	0.27	0.89	0.00	-0.01
	Alpha	23.9	-47.8	95.8	0.50	0.01	-0.01	
	W50%	-0.03	-0.21	0.14	0.71	0.002	-0.01	
	PA Bouts	-0.15	-0.90	0.59	0.68	0.002	-0.01	

Table A3.1.6 continued.

Pulse Pressure	Daily Sum of PA Bout Time	0.01	-0.02	0.03	0.65	0.002	-0.01
	True Mean PA Bout	0.18	-0.36	0.74	0.50	0.01	-0.01
	₁₀ MVPA Bouts	-0.06	-3.70	3.57	0.97	0.00	-0.01
	Total Week ₁₀ MVPA	0.37	-1.04	1.79	0.60	0.003	-0.01
	SB%	-0.06	-0.35	0.21	0.64	0.002	-0.01
	Standing%	0.26	-0.69	1.23	0.58	0.003	-0.01
	LIPA%	0.12	-0.57	0.82	0.72	0.001	-0.01
	sMVPA%	0.04	-0.50	0.58	0.88	0.00	-0.01
	₁₀ MVPA%	0.37	-1.25	1.99	0.65	0.002	-0.01
Heart Rate	SB Breaks	-0.63	-1.13	-0.13	0.01	0.06	0.05
	<5min SB Bout	-1.34	-2.27	-0.40	0.01	0.08	0.07
	≥5min SB Bout	-0.57	-1.36	0.21	0.15	0.02	0.01
	True Mean SB Bout	0.17	0.01	0.35	0.04	0.04	0.03
	Alpha	-35.2	-84.6	14.2	0.16	0.02	0.01
	W50%	0.19	0.17	0.31	0.001	0.11	0.10
	PA Bouts	-0.63	-1.13	-0.13	0.01	0.06	0.05
	Daily Sum of PA Bout Time	-0.03	-0.05	-0.01	0.001	0.11	0.10
	True Mean PA Bout	-0.30	-0.68	0.07	0.11	0.02	0.01
	₁₀ MVPA Bouts	-2.27	-4.76	0.20	0.07	0.03	0.02
	Total Week ₁₀ MVPA	-0.93	-1.90	0.03	0.05	0.04	0.02
	SB%	0.27	0.08	0.46	0.01	0.08	0.07
	Standing%	-0.80	-1.45	-0.15	0.01	0.06	0.05
	LIPA%	-0.34	-0.82	0.14	0.16	0.02	0.01
	sMVPA%	-0.41	-0.78	-0.04	0.02	0.05	0.04
	₁₀ MVPA%	-1.02	-2.12	0.08	0.06	0.03	0.02
Brachial							
Artery Diameter	SB Breaks	0.01	-0.03	0.05	0.60	0.003	-0.01
	<5min SB Bout	0.02	-0.05	0.10	0.53	0.004	-0.01
	≥5min SB Bout	0.01	-0.06	0.07	0.83	0.00	-0.01
	True Mean SB Bout	7.5E-05	-0.01	0.01	0.99	0.00	-0.01
	Alpha	-1.08	-5.33	3.16	0.61	0.003	-0.01
	W50%	-0.01	-0.01	0.01	0.26	0.01	0.003
	PA Bouts	0.01	-0.03	0.05	0.60	0.003	-0.01
	Daily Sum of PA Bout Time	0.00	-0.001	0.002	0.56	0.004	-0.01
	True Mean PA Bout	-0.001	-0.03	0.03	0.95	0.00	-0.01
	₁₀ MVPA Bouts	-0.004	-0.21	0.20	0.96	0.00	-0.01
	Total Week ₁₀ MVPA	0.001	-0.08	0.08	0.99	0.00	-0.01
	SB%	-0.003	-0.01	0.01	0.74	0.001	-0.01
	Standing%	0.01	-0.04	0.06	0.71	0.002	-0.01

Table A3.1.6 continued.

Artery Diameter	LIPA%	0.00	-0.04	0.04	0.99	0.00	-0.01
	sMVPA%	0.01	-0.02	0.03	0.70	0.002	-0.01
	₁₀ MVPA%	0.01	-0.08	0.09	0.90	0.00	-0.01
LOG Blood	SB Breaks	0.01	-0.01	0.02	0.36	0.01	-0.002
Velocity	<5min SB Bout	0.02	-0.004	0.05	0.09	0.03	0.02
	≥5min SB Bout	0.001	-0.02	0.02	0.93	0.00	-0.01
	True Mean SB	-0.003	-0.01	0.003	0.34	0.01	-0.001
	Bout						
	Alpha	-0.34	-1.92	1.24	0.67	0.002	-0.01
	W50%	0.00	-0.004	0.004	0.89	0.00	-0.01
	PA Bouts	0.01	-0.01	0.02	0.36	0.01	-0.002
	Daily Sum of	8.2E-05	-0.001	0.001	0.79	0.001	-0.01
	PA Bout Time						
	True Mean PA	-0.002	-0.01	0.01	0.71	0.002	-0.01
	Bout						
	₁₀ MVPA Bouts	0.02	-0.05	0.10	0.51	0.01	-0.01
	Total Week	0.004	-0.02	0.03	0.78	0.001	-0.01
	₁₀ MVPA						
	SB%	-0.001	-0.01	0.01	0.82	0.001	-0.01
	Standing%	-0.001	-0.02	0.02	0.91	0.00	-0.01
	LIPA%	-0.01	-0.02	0.01	0.45	0.01	-0.01
	sMVPA%	0.01	-0.01	0.01	0.32	0.01	0.00
	₁₀ MVPA%	0.01	-0.03	0.04	0.78	0.001	-0.01
LOG Shear	SB Breaks	0.01	-0.01	0.02	0.52	0.01	-0.01
Rate	<5min SB Bout	0.02	-0.01	0.05	0.26	0.01	0.003
	≥5min SB Bout	0.003	-0.02	0.03	0.85	0.00	-0.01
	True Mean SB	-0.003	-0.01	0.004	0.39	0.00	-0.01
	Bout						
	Alpha	0.23	-1.68	2.15	0.80	0.001	-0.01
	W50%	0.002	-0.003	0.01	0.48	0.01	-0.01
	PA Bouts	0.01	-0.01	0.02	0.52	0.01	-0.01
	Daily Sum of	-9.7E-05	-0.001	0.001	0.79	0.001	-0.01
	PA Bout Time						
	True Mean PA	-0.004	-0.01	0.01	0.62	0.003	-0.01
	Bout						
	₁₀ MVPA Bouts	0.02	-0.07	0.11	0.65	0.002	-0.01
	Total Week	0.002	-0.03	0.03	0.91	0.00	-0.01
	₁₀ MVPA						
	SB%	0.001	-0.01	0.01	0.86	0.00	-0.01
	Standing%	-0.01	-0.03	0.01	0.62	0.003	-0.01
	LIPA%	-0.01	-0.02	0.01	0.42	0.01	-0.004
	sMVPA%	0.004	-0.01	0.01	0.56	0.004	-0.01
	₁₀ MVPA%	0.001	-0.04	0.04	0.96	0.00	-0.01
LOG IMT	SB Breaks	-0.002	-0.01	0.01	0.75	0.001	-0.01
	<5min SB Bout	-0.01	-0.03	0.02	0.67	0.002	-0.01
	≥5min SB Bout	-0.002	-0.02	0.01	0.86	0.00	-0.01
	True Mean SB	0.001	-0.003	0.01	0.55	0.004	-0.01
	Bout						
	Alpha	-0.20	-1.51	1.11	0.75	0.001	-0.01
	W50%	-0.002	-0.01	0.002	0.33	0.01	-0.001

Table A3.1.6 continued.

LOG IMT	PA Bouts	-0.002	-0.01	0.01	0.75	0.001	-0.01
	Daily Sum of PA Bout Time	0.00	0.00	0.001	0.70	0.002	-0.01
	True Mean PA Bout	0.002	-0.01	0.01	0.62	0.003	-0.01
	₁₀ MVPA Bouts	-0.02	-0.09	0.04	0.46	0.01	-0.01
	Total Week ₁₀ MVPA	-0.01	-0.03	0.01	0.45	0.01	-0.01
	SB%	0.00	-0.01	0.01	0.86	0.00	-0.01
	Standing%	0.01	-0.01	0.02	0.44	0.01	-0.01
	LIPA%	0.002	-0.01	0.01	0.78	0.001	-0.01
	sMVPA%	0.00	-0.01	0.01	0.94	0.00	-0.01
	₁₀ MVPA%	-0.01	-0.04	0.02	0.50	0.01	-0.01
Carotid							
AL Artery Diameter	SB Breaks	-0.01	-0.06	0.03	0.63	0.003	-0.01
	<5min SB Bout	-0.02	-0.11	0.07	0.65	0.002	-0.01
	≥5min SB Bout	-0.01	-0.09	0.05	0.63	0.003	-0.01
	True Mean SB Bout	0.01	-0.003	0.03	0.09	0.03	0.02
	Alpha	1.05	-3.77	5.88	0.66	0.002	-0.01
	W50%	0.001	-0.01	0.01	0.88	0.00	-0.01
	PA Bouts	-0.01	-0.06	0.03	0.63	0.003	-0.01
	Daily Sum of PA Bout Time	-0.001	-0.002	0.001	0.58	0.003	-0.01
	True Mean PA Bout	-0.002	-0.03	0.03	0.90	0.00	-0.01
	₁₀ MVPA Bouts	0.06	-0.18	0.31	0.58	0.003	-0.01
	Total Week ₁₀ MVPA	0.04	-0.05	0.14	0.35	0.01	-0.002
	SB%	0.01	-0.01	0.02	0.44	0.01	-0.01
	Standing%	-0.02	-0.09	0.04	0.44	0.01	-0.01
	LIPA%	-0.04	-0.08	0.01	0.09	0.03	0.02
	sMVPA%	-0.002	-0.03	0.03	0.92	0.00	-0.01
	₁₀ MVPA%	0.05	-0.05	0.16	0.29	0.01	0.001
LOG AL IMT	SB Breaks	-0.001	-0.01	0.01	0.90	0.00	-0.01
	<5min SB Bout	0.01	-0.01	0.02	0.59	0.003	-0.01
	≥5min SB Bout	-0.004	-0.01	0.01	0.54	0.004	-0.01
	True Mean SB Bout	0.00	-0.003	0.003	0.84	0.00	-0.01
	Alpha	0.69	-0.17	1.56	0.11	0.02	0.01
	W50%	0.00	-0.002	0.002	0.85	0.00	-0.01
	PA Bouts	-0.001	-0.01	0.01	0.90	0.00	-0.01
	Daily Sum of PA Bout Time	0.00	0.00	0.001	0.36	0.01	-0.002
	True Mean PA Bout	0.004	-0.003	0.01	0.23	0.01	0.01
	₁₀ MVPA Bouts	0.003	-0.04	0.04	0.90	0.00	-0.01
	Total Week ₁₀ MVPA	0.01	-0.01	0.02	0.31	0.01	0.00

Table A3.1.6 continued.

LOG AL IMT	SB%	-0.001	-0.01	0.002	0.54	0.004	-0.01
	Standing%	0.01	-0.01	0.01	0.25	0.01	0.004
	LIPA%	0.002	-0.01	0.01	0.66	0.002	-0.01
	sMVPA%	-0.001	-0.01	0.01	0.86	0.00	-0.01
	₁₀ MVPA%	0.010	-0.010	0.03	0.31	0.01	0.00
LOG AL Blood	SB Breaks	0.01	-0.01	0.02	0.43	0.01	-0.004
Velocity	<5min SB Bout	0.01	-0.01	0.04	0.42	0.01	-0.004
	≥5min SB Bout	0.01	-0.01	0.03	0.60	0.003	-0.01
	True Mean SB Bout	-0.004	-0.01	0.002	0.17	0.02	0.01
	Alpha	0.73	-0.80	2.27	0.34	0.01	-0.001
	W50%	-0.001	-0.01	0.003	0.52	0.01	-0.01
	PA Bouts	0.01	-0.01	0.02	0.43	0.01	-0.004
	Daily Sum of PA Bout Time	0.00	0.00	0.001	0.33	0.01	-0.001
	True Mean PA Bout	-6.5E-05	-0.01	0.01	0.99	0.00	-0.01
	₁₀ MVPA Bouts	0.04	-0.03	0.11	0.30	0.01	0.001
	Total Week ₁₀ MVPA	0.01	-0.01	0.04	0.27	0.01	0.002
	SB%	-0.003	-0.01	0.003	0.34	0.01	-0.001
	Standing%	-0.001	-0.02	0.02	0.92	0.00	-0.01
	LIPA%	0.01	-0.01	0.02	0.53	0.004	-0.01
	sMVPA%	0.01	-0.01	0.01	0.31	0.01	0.00
	₁₀ MVPA%	0.01	-0.01	0.05	0.27	0.01	0.002
AL Shear Rate	SB Breaks	2.66	-4.58	9.91	0.46	0.01	-0.01
	<5min SB Bout	4.04	-9.81	17.8	0.56	0.004	-0.01
	≥5min SB Bout	3.80	-7.34	14.9	0.49	0.01	-0.01
	True Mean SB Bout	-1.78	-4.21	0.64	0.14	0.02	0.01
	Alpha	197	-497	893	0.57	0.004	-0.01
	W50%	-0.47	-2.23	1.28	0.59	0.003	-0.01
	PA Bouts	2.66	-4.58	9.91	0.46	0.01	-0.01
	Daily Sum of PA Bout Time	0.12	-0.15	0.39	0.38	0.01	-0.003
	True Mean PA Bout	-0.01	-5.37	5.33	0.99	0.00	-0.01
	₁₀ MVPA Bouts	12.8	-23.1	48.9	0.47	0.01	-0.01
	Total Week ₁₀ MVPA	3.51	-10.3	17.3	0.61	0.003	-0.01
	SB%	-1.29	-4.03	1.45	0.35	0.01	-0.001
	Standing%	0.47	-8.89	9.84	0.91	0.00	-0.01
	LIPA%	3.44	-3.30	10.1	0.31	0.01	0.00
	sMVPA%	2.08	-3.16	7.32	0.43	0.01	-0.004
	₁₀ MVPA%	3.77	-12.0	19.6	0.63	0.003	-0.01
LOG AL RI	SB Breaks	-0.001	-0.01	0.01	0.85	0.00	-0.01
	<5min SB Bout	0.01	-0.01	0.01	0.45	0.01	-0.01
	≥5min SB Bout	-0.004	-0.01	0.01	0.44	0.01	-0.01
	True Mean SB Bout	-5.0E-05	-0.002	0.002	0.96	0.00	-0.01

Table A3.1.6 continued.

LOG AL RI	Alpha	-0.24	-0.90	0.42	0.47	0.01	-0.01	
	W50%	0.00	-0.002	0.001	0.79	0.001	-0.01	
	PA Bouts	-0.001	-0.01	0.001	0.85	0.00	-0.01	
	Daily Sum of PA Bout Time	-2.6E-05	0.00	0.00	0.84	0.00	-0.01	
	True Mean PA Bout	-0.001	-0.01	0.004	0.61	0.003	-0.01	
	₁₀ MVPA Bouts	-0.01	-0.03	0.02	0.75	0.001	-0.01	
	Total Week ₁₀ MVPA	-0.01	-0.02	0.01	0.23	0.01	0.01	
	SB%	0.00	-0.002	0.003	0.86	0.00	-0.01	
	Standing%	0.004	-0.01	0.01	0.38	0.01	-0.003	
	LIPA%	0.001	-0.01	0.01	0.69	0.002	-0.01	
	sMVPA%	-0.002	-0.01	0.003	0.45	0.01	-0.01	
	₁₀ MVPA%	-0.01	-0.02	0.01	0.26	0.01	0.003	
	PL Artery Diameter	SB Breaks	-0.05	-0.12	0.01	0.10	0.08	0.05
	<5min SB Bout	-0.09	-0.20	0.02	0.12	0.08	0.04	
	≥5min SB Bout	-0.05	-0.15	0.04	0.24	0.04	0.01	
	True Mean SB Bout	0.01	-0.001	0.03	0.06	0.11	0.07	
Alpha	-1.91	-7.82	3.99	0.51	0.01	-0.01		
W50%	0.01	-0.01	0.02	0.38	0.02	-0.01		
PA Bouts	-0.05	-0.12	0.01	0.10	0.08	0.05		
Daily Sum of PA Bout Time	-0.001	-0.003	0.001	0.28	0.04	0.01		
True Mean PA Bout	-0.01	-0.05	0.02	0.55	0.01	-0.02		
₁₀ MVPA Bouts	0.01	-0.29	0.32	0.93	0.00	-0.03		
Total Week ₁₀ MVPA	0.03	-0.11	0.18	0.61	0.01	-0.02		
SB%	0.01	-0.01	0.03	0.27	0.04	0.01		
Standing%	-0.01	-0.06	0.04	0.67	0.01	-0.02		
LIPA%	-0.02	-0.07	0.02	0.23	0.04	0.01		
sMVPA%	-0.03	-0.07	0.01	0.17	0.06	0.03		
₁₀ MVPA%	0.04	-0.11	0.21	0.57	0.01	-0.02		
PL IMT	SB Breaks	0.01	-0.01	0.02	0.38	0.02	-0.01	
<5min SB Bout	0.01	-0.01	0.04	0.17	0.06	0.03		
≥5min SB Bout	0.002	-0.02	0.02	0.89	0.001	-0.03		
True Mean SB Bout	-0.003	-0.007	0.002	0.24	0.04	0.01		
Alpha	1.60	0.29	2.90	0.01	0.17	0.14		
W50%	-0.001	-0.004	0.002	0.59	0.01	-0.02		
PA Bouts	0.01	-0.01	0.02	0.38	0.02	-0.01		
Daily Sum of PA Bout Time	0.00	0.00	0.001	0.62	0.01	-0.02		
True Mean PA Bout	0.001	-0.01	0.01	0.75	0.004	-0.03		
₁₀ MVPA Bouts	0.04	-0.03	0.11	0.27	0.04	0.01		
Total Week ₁₀ MVPA	0.01	-0.02	0.05	0.38	0.02	-0.01		

Table A3.1.6 continued.

PL IMT	SB%	-0.002	-0.01	0.003	0.50	0.01	-0.01
	Standing%	-0.001	-0.01	0.01	0.89	0.001	-0.03
	LIPA%	0.01	-0.01	0.01	0.42	0.02	-0.01
	sMVPA%	0.004	-0.01	0.01	0.49	0.01	-0.01
	₁₀ MVPA%	0.01	-0.02	0.05	0.40	0.02	-0.01
Popliteal							
Artery Diameter	SB Breaks	-0.01	-0.12	0.10	0.83	0.001	-0.02
	<5min SB Bout	-0.06	-0.26	0.13	0.52	0.01	-0.01
	≥5min SB Bout	0.02	-0.14	0.19	0.75	0.002	-0.02
	True Mean SB Bout	0.01	-0.01	0.04	0.34	0.02	-0.002
	Alpha	-5.58	-15.5	4.38	0.26	0.02	0.01
	W50%	0.01	-0.01	0.03	0.47	0.01	-0.01
	PA Bouts	-0.01	-0.12	0.10	0.83	0.001	-0.02
	Daily Sum of PA Bout Time	-0.002	-0.01	0.01	0.42	0.01	-0.01
	True Mean PA Bout	-0.03	-0.10	0.04	0.39	0.01	-0.01
	₁₀ MVPA Bouts	0.19	-0.42	0.81	0.52	0.01	-0.01
	Total Week ₁₀ MVPA	0.10	-0.20	0.40	0.51	0.01	-0.01
	SB%	0.01	-0.02	0.05	0.33	0.02	-0.001
	Standing%	-0.12	-0.22	-0.01	0.02	0.11	0.09
	LIPA%	-0.06	-0.15	0.02	0.17	0.04	0.02
	sMVPA%	0.02	-0.06	0.11	0.55	0.01	-0.01
	₁₀ MVPA%	0.14	-0.18	0.47	0.37	0.01	-0.01
IMT	SB Breaks	-0.01	-0.02	0.01	0.32	0.02	0.00
	<5min SB Bout	-0.003	-0.02	0.02	0.78	0.002	-0.02
	≥5min SB Bout	-0.01	-0.03	0.01	0.26	0.02	0.01
	True Mean SB Bout	0.003	-0.001	0.01	0.17	0.04	0.02
	Alpha	-1.03	-2.20	0.12	0.07	0.07	0.04
	W50%	0.003	0.00	0.01	0.04	0.09	0.07
	PA Bouts	-0.01	-0.02	0.01	0.32	0.02	0.00
	Daily Sum of PA Bout Time	-0.001	-0.001	0.00	0.001	0.15	0.13
	True Mean PA Bout	-0.01	-0.01	-0.001	0.02	0.11	0.09
	₁₀ MVPA Bouts	-0.02	-0.09	0.05	0.56	0.01	-0.01
	Total Week ₁₀ MVPA	-0.01	-0.04	0.02	0.56	0.01	-0.01
	SB%	0.01	0.001	0.01	0.01	0.13	0.11
	Standing%	-0.01	-0.02	0.004	0.17	0.04	0.02
	LIPA%	-0.01	-0.02	-0.004	0.01	0.15	0.13
	sMVPA%	-0.01	-0.01	0.001	0.09	0.06	0.04
	₁₀ MVPA%	-0.01	-0.04	0.02	0.61	0.01	-0.01
LOG Blood Velocity	SB Breaks	-0.02	-0.05	0.01	0.16	0.04	0.02
	<5min SB Bout	-0.01	-0.06	0.04	0.74	0.003	-0.02
	≥5min SB Bout	-0.04	-0.08	0.004	0.07	0.07	0.05

Table A3.1.6 continued.

LOG Blood Velocity	True Mean SB Bout	0.01	-0.001	0.01	0.07	-0.07	0.05
	Alpha	-0.85	-3.57	1.87	0.53	0.01	-0.01
	W50%	0.01	0.00	0.01	0.03	0.09	0.07
	PA Bouts	-0.02	-0.05	0.01	0.16	0.04	0.02
	Daily Sum of PA Bout Time	-0.001	-0.002	0.00	0.13	0.05	0.02
	True Mean PA Bout	-0.01	-0.02	0.01	0.46	0.01	-0.01
	₁₀ MVPA Bouts	-0.12	-0.28	0.04	0.14	0.05	0.02
	Total Week ₁₀ MVPA	-0.07	-0.15	0.01	0.06	0.07	0.05
	SB%	0.01	-0.003	0.01	0.17	0.04	0.01
	Standing%	-0.01	-0.04	0.01	0.20	0.03	0.01
	LIPA%	-0.01	-0.03	0.01	0.50	0.01	-0.01
	sMVPA%	-0.01	-0.03	0.01	0.34	0.02	-0.002
	₁₀ MVPA%	-0.07	-0.16	0.01	0.07	0.07	0.04
LOG Shear Rate	SB Breaks	-0.01	-0.05	0.01	0.31	0.02	0.001
	<5min SB Bout	0.01	-0.06	0.06	0.88	0.00	-0.02
	≥5min SB Bout	-0.04	-0.09	0.01	0.10	0.06	0.03
	True Mean SB Bout	0.01	-0.01	0.01	0.34	0.02	-0.002
	Alpha	0.08	-3.10	3.27	0.95	0.00	-0.02
	W50%	0.01	-0.003	0.01	0.19	0.03	0.01
	PA Bouts	-0.01	-0.05	0.01	0.31	0.02	0.001
	Daily Sum of PA Bout Time	0.00	-0.002	0.001	0.43	0.01	-0.01
	True Mean PA Bout	-0.002	-0.02	0.02	0.86	0.001	-0.02
	₁₀ MVPA Bouts	-0.15	-0.34	0.03	0.10	0.05	0.03
	Total Week ₁₀ MVPA	-0.09	-0.18	0.001	0.05	0.08	0.06
	SB%	0.004	-0.01	0.01	0.56	0.01	-0.01
	Standing%	0.002	-0.03	0.03	0.91	0.00	-0.02
	LIPA%	0.002	-0.02	0.03	0.86	0.001	-0.02
	sMVPA%	-0.01	-0.04	0.01	0.28	0.02	0.004
	₁₀ MVPA%	-0.10	-0.20	-0.001	0.04	0.08	0.06

Bold font highlights significant ($p \leq 0.05$) bivariate linear regressions. b Change in cardiovascular variable per unit increase in GENE variable. -95%CI Negative 95% confidence interval. +95%CI Positive 95% confidence interval. p Significance value. r^2 Explained variance. r^2 adj. Adjusted explained variance.

Chapter 03: Part 2: Full ISM results.

Table A3.2.1 Effect of PB on systolic blood pressure according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		¹⁰ MVPA		Total PB		Covariates				
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b ^h	95% CI			
Model 1	Substituted		2.36	-10.3	15.0	-3.78	-13.0	5.47	2.15	-4.82	9.12	0.40	-26.3	27.1	-0.66	-6.47	5.15
Model 2	Substituted		1.01	-11.4	13.4	-3.68	-12.7	5.40	3.43	-3.49	10.3	3.01	-23.2	29.2	-1.17	-6.93	4.59
Model 1	-4.29	-15.9	7.33	Substituted		-9.85	-26.5	6.81	-2.04	-16.0	11.9	-3.44	-32.6	25.7	4.49	-6.50	15.4
Model 2	-2.75	-14.2	8.73	Substituted		-8.07	-24.4	8.33	0.79	-13.1	14.7	0.69	-28.0	29.3	2.38	-8.49	13.2
Model 1	3.80	-5.47	13.0	6.17	-13.0	25.4	Substituted		5.94	-7.36	19.2	4.18	-23.3	31.6	-4.46	-14.4	5.49
Model 2	3.70	-5.40	12.8	4.72	-14.1	23.6	Substituted		7.13	-5.94	20.1	6.69	-20.2	33.6	-4.87	-14.6	4.88
Model 1	-1.91	-8.06	4.23	0.15	-13.8	14.1	-5.15	-16.6	6.31	Substituted		-1.60	-28.4	25.2	1.12	-4.58	6.83
Model 2	-3.01	-9.08	3.07	-2.23	-16.0	11.6	-5.95	-17.1	5.26	Substituted		0.31	-25.9	26.5	1.51	-4.10	7.13
Model 1	-0.65	-27.0	25.7	2.98	-26.4	32.3	-2.62	-29.5	24.3	0.54	-28.2	-29.3	Substituted		-0.89	-27.9	26.1
Model 2	-3.68	-29.5	22.1	-1.48	-30.3	27.3	-5.49	-31.8	20.8	-0.98	-29.0	27.1	Substituted		1.41	-25.0	27.8
Partition																	
Model 1	-0.66	-6.46	5.15	1.70	-11.4	14.8	-4.44	-14.3	5.49	1.49	-7.22	10.2	-0.26	-27.7	27.1		
Model 2	-1.17	-6.93	4.60	-0.16	-13.0	12.7	-4.84	-14.5	4.89	2.26	-6.39	10.9	1.85	-25.0	28.7		
Model 1 No covariates included; Model 2 Covariates included - Hydration ^h .																	
b Change in cardiovascular marker per one hour substitution of PB.																	
95% CI Positive and negative 95% confidence interval.																	

Table A3.2.2. Effect of PB on LOG diastolic blood pressure according to isotemporal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		¹⁰ MVPA		Total PB		Covariates								
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b ^h	95% CI							
Model 1																					
	Substituted		-0.02	-0.11	0.06	-0.03	-0.09	0.03	0.03	-0.02	0.08	0.02	-0.16	0.20	0.00	-0.04	0.04				
Model 2			-0.03	-0.12	0.05	-0.03	-0.09	0.03	0.04	0.09	0.03	0.03	-0.15	0.21	0.00	-0.04	0.04				
Model 1	0.00	-0.08	0.08			-0.06	-0.17	0.06	0.03	0.12	0.02	-0.18	0.22	0.02	-0.06	0.09					
Model 2	0.01	-0.07	0.09	Substituted		-0.04	-0.15	0.07	0.05	0.15	0.05	-0.15	0.25	0.00	-0.07	0.08	0.00	-0.01	0.00		
Model 1	0.03	-0.03	0.09	0.01	-0.12	0.14			0.06	0.15	0.04	-0.14	0.23	-0.03	-0.09	0.04					
Model 2	0.03	-0.03	0.09	-0.01	-0.13	0.12	Substituted		0.07	0.16	0.06	-0.12	0.24	-0.02	-0.09	0.04	0.00	-0.01	0.00		
Model 1	-0.02	-0.07	0.02	-0.04	-0.14	0.05	-0.05	-0.12	0.03		0.01	-0.18	0.19	0.02	-0.02	0.06					
Model 2	-0.03	-0.07	0.01	-0.06	-0.16	0.03	-0.05	-0.13	0.03	Substituted		0.02	-0.16	0.20	0.02	-0.01	0.06	0.00	-0.01	0.00	
Model 1	-0.03	-0.21	0.15	-0.04	-0.24	0.15	-0.05	-0.23	0.13	0.13	-0.01	-0.20	0.19		0.03	-0.15	0.21				
Model 2	-0.05	-0.22	0.12	-0.08	-0.27	0.12	-0.07	-0.25	0.11	-0.01	-0.20	0.17	Substituted		0.05	-0.13	0.23	0.00	-0.01	0.00	
Partition																					
Model 1	0.00	-0.04	0.04	-0.02	-0.11	0.07	-0.02	-0.09	0.04	0.03	0.03	-0.03	0.09	0.02	-0.17	0.21					
Model 2	0.00	-0.04	0.04	-0.03	-0.12	0.06	-0.02	-0.09	0.04	0.04	0.04	-0.02	0.10	0.04	-0.15	0.22			0.00	-0.01	0.00

Model 1 No covariates included. Model 2 Covariates included - Hydration^h.^h Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A3.2.3. Effect of PB on pulse pressure according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10MVPA		Total PB		Covariates		
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b ^h	95% CI	
Model 1															
	Substituted														
Model 2	3.84	-5.86	13.5	-1.60	-8.68	5.48	0.13	-5.20	5.46	-0.58	-21.0	19.8	-0.68	-5.12	3.77
Model 1															
	Substituted														
Model 2	3.34	-6.34	13.0	-1.53	-8.58	5.52	0.69	-4.69	6.06	0.84	-19.5	21.2	-1.19	-5.66	3.29
Model 1															
	Substituted														
Model 2	-4.05	-12.9	4.80	-5.83	-18.5	6.86	-3.91	-14.5	6.76	-4.57	-26.7	17.6	3.46	-4.91	11.8
Model 1															
	Substituted														
Model 2	-3.53	-12.4	5.35	-5.21	-17.8	7.47	-2.83	-13.6	7.98	-2.63	-24.8	19.5	2.41	-6.00	10.8
Model 1															
	Substituted														
Model 2	1.58	-5.51	8.67	-9.32	20.1		1.71	-8.47	11.8	1.01	-20.0	22.0	-2.26	-9.87	5.34
Model 1															
	Substituted														
Model 2	1.52	-5.55	8.58	-9.82	19.5		2.20	-7.94	12.3	2.36	-18.5	23.2	-2.71	-10.2	4.86
Model 1															
	Substituted														
Model 2	-0.21	-4.91	4.49	-7.44	13.9	-1.60	-10.3	7.17		-1.51	-22.0	19.0	-0.28	-4.65	4.08
Model 1															
	Substituted														
Model 2	-0.78	-5.50	3.94	-8.56	12.9	-2.10	-10.8	6.61		-0.67	-21.0	19.7	-0.22	-4.58	4.14
Model 1															
	Substituted														
Model 2	1.43	-18.5	21.4	-16.3	28.1	1.15	-19.2	21.5	1.01	-20.8	22.8		-2.82	-23.3	17.6
Model 1															
	Substituted														
Model 2	-0.22	-20.1	19.6	-18.5	25.8	-0.41	-20.6	19.8	0.05	-21.5	21.6		-1.79	-22.1	18.5
Model 1															
	Substituted														
Model 2	-0.68	-5.12	3.76	-6.88	13.1	-2.28	-9.87	5.31	-0.55	-7.21	6.11	-1.26	-22.2	19.7	
Model 1															
	Substituted														
Model 2	-1.19	-5.67	3.28	-7.87	12.1	-2.72	-10.2	4.83	-0.50	-7.22	6.21	-0.34	-21.2	20.5	
Model 1															
	Substituted														
Model 2	-1.19	-5.67	3.28	-7.87	12.1	-2.72	-10.2	4.83	-0.50	-7.22	6.21	-0.34	-21.2	20.5	

Model 1 No covariates included Model 2 Covariates included - Hydration^h.

b Change in cardiovascular marker per one-hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A3.2.4. Effect of PB on heart rate according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10 th MVPA		Total PB								
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI							
Model 1																			
Model 2	Substituted																		
Model 1	4.36	-1.18	9.91				4.81	-3.13	12.7	0.73	-5.95	7.41	2.51	-11.3	16.4	-5.46	-10.7	-0.22	
Model 2	Substituted																		
Model 1	-2.14	-6.50	2.23	-8.35	-17.4	0.73													
Model 2	Substituted																		
Model 1	1.64	-1.41	4.69	-4.87	-11.8	2.08	1.38	-4.32	7.07				-4.76	-18.1	8.58	-1.40	-4.24	1.44	
Model 2	Substituted																		
Model 1	-1.12	-13.6	11.4	-6.82	-20.7	7.05	1.23	-11.5	13.9	-5.02	-18.6	8.59							
Model 2	Substituted																		
Partition																			
Model 1	-1.88	-4.62	0.86	-8.08	-14.2	-1.90	0.24	-4.44	4.92	-5.60	-9.70	-1.49	-4.11	-17.0	8.83				
Model 2	Substituted																		

Model 1 No covariates included. Model 2 Covariates included – NA.

b Change in cardiovascular marker per one-hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Bold indicates significant model ($p \leq 0.05$).

Table A3.2.5. Effect of PB on brachial artery diameter according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sIMVA		¹⁰ sIMVA		Total PB		Covariates							
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b ^a	95% CI						
Model 1	Substituted		-0.18	-0.73	0.36	-0.10	-0.50	0.30	0.20	-0.10	0.50	-0.44	-1.59	0.71	0.16	-0.09	0.41			
Model 2			-0.15	-0.69	0.39	-0.14	-0.53	0.25	0.15	-0.15	0.45	-0.54	-1.68	0.59	0.18	-0.07	0.43	0.02	0.00	0.05
Model 1	0.35	-0.16	0.85	Substituted		0.40	-0.32	1.12	0.54	-0.08	1.15	-0.13	-1.39	1.13	-0.26	-0.74	0.21			
Model 2	0.30	-0.20	0.81			0.31	-0.41	1.02	0.44	-0.17	1.06	-0.28	-1.53	0.97	-0.20	-0.67	0.28	0.03	0.00	0.05
Model 1	0.10	-0.30	0.49	-0.09	-0.91	0.74	Substituted		0.30	-0.27	0.87	-0.34	-1.52	0.84	0.06	-0.37	0.48			
Model 2	0.14	-0.25	0.53	-0.01	-0.82	0.81			0.29	-0.27	0.85	-0.40	-1.57	0.76	0.04	-0.38	0.45	0.02	0.00	0.05
Model 1	-0.10	-0.37	0.17	-0.22	-0.83	0.40	-0.12	-0.61	0.38	Substituted		-0.25	-1.42	0.92	0.13	-0.11	0.38			
Model 2	-0.06	-0.32	0.21	-0.14	-0.74	0.47	-0.12	-0.61	0.37			-0.34	-1.50	0.81	0.12	-0.13	0.37	0.03	0.00	0.05
Model 1	0.46	-0.66	1.58	0.31	-0.93	1.55	0.38	-0.76	1.52	0.65	-0.58	1.87	Substituted		-0.35	-1.49	0.80			
Model 2	0.57	-0.53	1.68	0.47	-0.76	1.69	0.46	-0.67	1.58	0.71	-0.49	1.91			-0.44	-1.56	0.69	0.02	0.00	0.05
Partition																				
Model 1	0.16	-0.09	0.41	-0.02	-0.59	0.54	0.06	-0.37	0.48	0.36	-0.02	0.73	-0.28	-1.46	0.89					
Model 2	0.17	-0.07	0.42	0.03	-0.53	0.58	0.03	-0.38	0.45	0.33	-0.05	0.70	-0.37	-1.53	0.79			0.02	0.00	0.05

Model 1: No covariates included; Model 2: Covariates included - Hydration^b.^b Change in cardiovascular marker per one hour substitution of PB.

95% CI: Positive and negative 95% confidence interval.

Bold indicates significant model ($p \leq 0.05$).

Table A3.2.6. Effect of PB on LOG brachial baseline blood velocity according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10MVPA		Total PB		Covariates							
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b ^h	95% CI						
Model 1																				
	Substituted		0.06	-0.15	0.27	-0.11	-0.26	0.05	0.09	-0.03	0.20	-0.12	-0.57	0.33	-0.01	-0.11	0.09			
Model 2			0.05	-0.17	0.26	-0.11	-0.27	0.05	0.09	-0.03	0.21	-0.13	-0.58	0.33	0.00	-0.10	0.10	0.00	-0.01	0.01
Model 1	-0.05	-0.25	0.14			-0.15	-0.43	0.13	0.03	-0.20	0.27	-0.17	-0.66	0.31	0.04	-0.15	0.22			
Model 2	-0.04	-0.24	0.16			-0.14	-0.42	0.14	0.05	-0.19	0.29	-0.17	-0.66	0.33	0.04	-0.15	0.22	0.00	-0.01	0.01
Model 1	0.10	-0.05	0.26	0.16	-0.16	0.49			0.19	-0.03	0.41	-0.02	-0.48	0.45	-0.11	-0.28	0.05			
Model 2	0.11	-0.05	0.27	0.16	-0.17	0.48			0.20	-0.03	0.43	-0.02	-0.48	0.45	-0.11	-0.27	0.06	0.00	-0.01	0.01
Model 1	-0.05	-0.16	0.05	0.02	-0.22	0.25	-0.12	-0.31	0.08			-0.09	-0.55	0.36	0.00	-0.09	0.10			
Model 2	-0.05	-0.16	0.06	0.01	-0.23	0.25	-0.12	-0.31	0.08			-0.09	-0.55	0.37	0.01	-0.09	0.11	0.00	-0.01	0.01
Model 1	0.10	-0.34	0.53	0.15	-0.34	0.63	0.01	-0.44	0.45	0.19	-0.29	0.66			-0.11	-0.55	0.34			
Model 2	0.10	-0.34	0.54	0.14	-0.35	0.63	0.01	-0.44	0.45	0.20	-0.28	0.67			-0.10	-0.54	0.35	0.00	-0.01	0.01
Partition																				
Model 1	-0.01	-0.11	0.09	0.05	-0.17	0.27	-0.12	-0.28	0.05	0.08	-0.07	0.22	-0.13	-0.59	0.33					
Model 2	0.00	-0.10	0.10	0.05	-0.17	0.27	-0.11	-0.28	0.06	0.09	-0.06	0.24	-0.12	-0.59	0.34			0.00	-0.01	0.01

Model 1 No covariates included. Model 2 Covariates included - Hydration^h.^b Change in cardiovascular marker per one hour substitution of PB.^h 95% CI Positive and negative 95% confidence interval.

Table A3.2.7. Effect of PB on LOG brachial baseline shear rate according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		10MVPA		Total PB					
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI				
Model 1																
Model 2																
Model 1	-0.11	-0.34	0.12		-0.23	-0.55	0.10	-0.06	-0.34	0.21	-0.16	-0.72	0.41	0.08	-0.13	0.29
Model 2																
Model 1	0.09	-0.09	0.27	0.17	-0.21	0.54		0.13	-0.12	0.39	0.03	-0.50	0.57	-0.13	-0.32	0.06
Model 2																
Model 1	-0.03	-0.15	0.09	0.04	-0.23	0.32	-0.10	-0.32	0.12		-0.07	-0.59	0.45	-0.02	-0.13	0.09
Model 2																
Model 1	0.02	-0.49	0.54	0.08	-0.49	0.65	-0.06	-0.58	0.47	0.07	-0.49	0.63		-0.05	-0.58	0.47
Model 2																
Partition																
Model 1	-0.04	-0.16	0.07	0.04	-0.22	0.29	-0.13	-0.33	0.06	0.00	-0.17	0.17	-0.10	-0.63	0.44	
Model 2																

Model 1 No covariates included. Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A3.2.8. Effect of PB on LOG brachial IMT according to isolemporal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10MVPA		Total PB					
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI				
Model 1																
Model 2																
Model 1	-0.03	0.14			-0.02	-0.25	0.21	-0.05	-0.24	0.15	-0.08	-0.48	0.33	0.07	-0.09	0.22
Model 2																
Model 1	0.01	0.14	0.06	-0.21	0.33			-0.01	-0.19	0.18	-0.03	-0.42	0.35	0.04	-0.10	0.18
Model 2																
Model 1	0.05	0.13	0.11	-0.09	0.30	0.06	-0.10	0.22			0.07	-0.31	0.45	-0.03	-0.11	0.05
Model 2																
Model 1	0.02	0.38	0.08	-0.31	0.48	0.01	-0.36	0.37	-0.01	-0.40	0.38			0.03	-0.34	0.40
Model 2																
Partition																
Model 1	0.05	-0.03	0.13	0.10	-0.09	0.28	0.04	-0.10	0.18	0.03	-0.09	0.15	0.01	-0.38	0.39	
Model 2																

Model 1 No covariates included. Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A.3.2.9. Effect of PB on carotid AL artery diameter according to isotemporal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10M ^a VPA		Total PB		Covariates							
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b ^c	95% CI						
Model 1	Substituted		0.24	-0.39	0.87	-0.54	-1.00	-0.07	0.10	-0.25	0.44	0.46	-0.94	1.87	0.19	-0.10	0.48			
Model 2			0.23	-0.39	0.85	-0.47	-0.93	-0.01	0.11	-0.23	0.45	0.52	-0.86	1.90	0.20	-0.09	0.49	0.12	-0.01	0.25
Model 1	-0.13	-0.71	0.44				Substituted													
Model 2																				
Model 1	-0.14	-0.71	0.43				Substituted													
Model 2																				
Model 1	0.54	0.08	1.00					Substituted												
Model 2																				
Model 1	0.47	0.01	0.93																	
Model 2																				
Model 1	0.02	-0.29	0.34	0.24	0.24	-0.48	0.95	-0.34	-0.93	0.25										
Model 2	0.01	-0.30	0.32	0.23	0.23	-0.48	0.93	-0.29	-0.87	0.30										
Model 1	-0.29	-1.66	1.08	-0.03	-1.55	1.49	-0.74	-2.12	0.64	-0.21	-1.71	1.28								
Model 2	-0.31	-1.68	1.06	-0.07	-1.58	1.45	-0.69	-2.07	0.69	-0.22	-1.71	1.27								
Partition																				
Model 1	0.19	-0.10	0.49	0.43	-0.22	1.09	-0.34	-0.84	0.16	0.29	-0.15	0.73	0.66	-0.78	2.09					
Model 2	0.20	-0.08	0.49	0.43	-0.21	1.07	-0.26	-0.76	0.23	0.31	-0.12	0.74	0.72	-0.69	2.14					

Model 1 No covariates included. Model 2 Covariates included - Primary CVD Meds^d.^b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Bold indicates significant model ($p \leq 0.05$).

Table A3.2.11. Effect of PB on LOG carotid AL blood velocity according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10MVPA		Total PB						
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI					
Model 1	Substituted		-0.05	-0.26	0.15	0.02	-0.13	0.17	0.04	-0.07	0.15	-0.03	-0.46	0.40	-0.02	-0.11	0.07
Model 2	Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		
Model 1	0.05	-0.14	0.23	Substituted		0.06	-0.20	0.33	0.08	-0.14	0.31	0.02	-0.45	0.48	-0.06	-0.24	0.11
Model 2	Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		
Model 1	-0.02	-0.17	0.13	-0.07	-0.38	0.23	Substituted		0.01	-0.20	0.23	-0.05	-0.49	0.39	0.00	-0.16	0.16
Model 2	Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		
Model 1	-0.01	-0.11	0.09	-0.07	-0.30	0.15	0.05	-0.14	0.24	Substituted		-0.01	-0.45	0.42	-0.03	-0.12	0.06
Model 2	Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		
Model 1	0.06	-0.35	0.48	-0.01	-0.47	0.45	0.10	-0.32	0.52	0.09	-0.36	0.55	Substituted		-0.09	-0.51	0.34
Model 2	Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		
Partition	Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		
Model 1	-0.02	-0.11	0.07	-0.07	-0.28	0.14	0.00	-0.16	0.16	0.02	-0.12	0.16	-0.05	-0.49	0.39	Substituted	
Model 2	Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		Substituted		

Model 1 No covariates included. Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A3.2.12: Effect of PB on carotid AL shear rate according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		10MVPA		Total PB						
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI					
Model 1																	
Model 2	Substituted		-29.7	-123	63.9	32.4	-36.1	100	7.54	-44.1	59.2	-16.8	-225	191	-16.6	-60.0	26.8
Model 1	22.1	-63.4	107			48.0	-74.8	170	30.0	-73.1	133	6.30	-219	232	-35.8	-116	44.9
Model 2				Substituted													
Model 1	-32.8	-101	35.7	-62.9	-205	79.3			-25.4	-124	73.4	-49.5	-261	162	16.2	-58.4	90.9
Model 2							Substituted										
Model 1	-3.39	-49.0	42.2	-37.4	-141	66.4	38.0	-47.5	123			-19.2	-227	189	-16.0	-58.3	26.2
Model 2								Substituted									
Model 1	17.5	-181	216	-20.9	-241	199	51.3	-148	251	23.8	-192	239			-32.2	-235	170
Model 2								Substituted									
Partition																	
Model 1	-16.7	-60.2	26.6	-46.4	-143	50.3	15.7	-58.8	90.2	-9.20	-73.9	55.5	-33.5	-246	179		
Model 2																	

Model 1 No covariates included. Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A3.2.13. Effect of PB on LOG carotid AL RI according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10M ¹⁰ VPA		Total PB						
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI					
Model 1			0.03	-0.06	0.11	0.02	-0.04	0.09	-0.02	-0.07	0.03	0.08	-0.10	0.27	-0.01	-0.05	0.03
Model 2	Substituted																
Model 1	-0.03	-0.11	0.05			0.00	-0.11	0.11	-0.05	-0.14	0.05	0.06	-0.14	0.26	0.02	-0.06	0.09
Model 2				Substituted													
Model 1	-0.03	-0.09	0.04	0.00	-0.13	0.13			-0.05	-0.14	0.05	0.06	-0.13	0.25	0.02	-0.05	0.08
Model 2							Substituted										
Model 1	0.01	-0.03	0.05	0.03	-0.07	0.13	0.02	-0.06	0.10			0.06	-0.13	0.25	0.00	-0.04	0.04
Model 2										Substituted							
Model 1	-0.10	-0.29	0.08	-0.07	-0.28	0.13	-0.10	-0.28	0.09	-0.13	-0.33	0.07			0.10	-0.08	0.29
Model 2													Substituted				
Partition																	
Model 1	-0.01	-0.05	0.03	0.02	-0.07	0.11	0.02	-0.05	0.08	-0.03	-0.09	0.03	0.07	-0.12			0.26
Model 2																	

Model 1 No covariates included. Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A.3.2.14. Effect of PB on carotid PL artery diameter according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10MVPA		Total PB							
	b	95% CI																
Model 1	Substituted		0.13	-0.51	0.76	-0.18	-0.77	0.40	-0.06	-0.52	0.40	0.16	-1.26	1.57	-0.06	-0.43	0.30	
Model 2	Substituted		Substituted		Substituted		Substituted											
Model 1	-0.10	-0.60	0.40			-0.25	-1.05	0.55	-0.16	-0.80	0.49	0.07	-1.41	1.54	0.02	-0.45	0.49	
Model 2																		
Model 1	0.19	-0.40	0.78	0.32	-0.80	1.43		0.13	-0.73	0.99	0.34	-1.11	1.79	-0.25	-0.89	0.39		
Model 2																		
Model 1	0.06	-0.40	0.52	0.19	-0.58	0.96	-0.12	-0.98	0.73		0.22	-1.38	1.82	-0.12	-0.61	0.36		
Model 2																		
Model 1	0.01	-1.42	1.44	0.18	-1.41	1.77	-0.15	-1.61	1.30	-0.07	-1.69	1.54			-0.05	-1.47	1.37	
Model 2																		
Partition																		
Model 1	-0.06	-0.43	0.30	0.07	-0.67	0.81	-0.24	-0.88	0.39	-0.12	-0.60	0.36	0.10	-1.30	1.50			
Model 2																		

Model 1 No covariates included. Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A3.2.15. Effect of PB on carotid PL IMT according to isothermal substitution of one hour of SB or PA.

Substitution	b	SB		b	Standing		b	LIPA		b	SMVPA		b	10M ¹⁰ VPA		b	Total PB	
		b	95% CI		b	95% CI		b	95% CI									
Model 1		Substituted		-0.06	-0.21	0.09	0.08	-0.06	0.22	0.00	-0.11	0.11	0.12	-0.22	0.46	-0.07	-0.15	0.02
Model 2		Substituted																
Model 1	0.00	-0.13	0.12		Substituted		0.01	-0.20	0.21	0.00	-0.17	0.16	0.11	-0.26	0.48	-0.03	-0.15	0.09
Model 2		Substituted																
Model 1	-0.08	-0.22	0.06	-0.14	-0.41	0.12		Substituted		-0.07	-0.28	0.13	0.04	-0.30	0.39	0.01	-0.14	0.16
Model 2		Substituted																
Model 1	0.00	-0.11	0.11	-0.07	-0.25	0.12	0.07	-0.13	0.28		Substituted		0.12	-0.26	0.50	-0.06	-0.18	0.05
Model 2		Substituted																
Model 1	-0.10	-0.43	0.22	-0.16	-0.53	0.20	-0.02	-0.36	0.31	-0.10	-0.47	0.27		Substituted		0.04	-0.29	0.36
Model 2		Substituted																
Partition		Substituted																
Model 1	-0.07	-0.15	0.02	-0.13	-0.31	0.04	0.01	-0.14	0.16	-0.06	-0.18	0.05	0.05	-0.28	0.39			
Model 2		Substituted																

Model 1 No covariates included. Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Table A3.2.16. Effect of PB on popliteal artery diameter according to isomtemporal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10MVPA		Total PB					
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI				
Model 1																
Model 2																
Model 1	0.82	-0.13	1.77		0.44	-0.97	1.86	1.31	0.11	2.51	1.48	-1.32	4.28	-0.52	-1.41	0.37
Model 2																
Model 1	0.13	-0.81	1.07	-0.91	-2.72	0.89		0.61	-0.79	2.00	0.87	-1.69	3.42	0.06	-0.89	1.01
Model 2																
Model 1	-0.48	-1.21	0.25	-1.52	-2.83	-0.22	-0.61	-2.00	0.78		0.25	-2.64	3.14	0.67	-0.19	1.54
Model 2																
Model 1	-0.68	-3.21	1.86	-1.68	-4.53	1.17	-0.80	-3.31	1.70	-0.22	-3.07	2.64				
Model 2																
Partition																
Model 1	0.19	-0.34	0.72	-0.85	-2.04	0.34	0.06	-0.89	1.00	0.67	-0.19	1.53	0.92	-1.67	3.52	
Model 2																

Model 1 No covariates included. Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval.

Bold indicates significant model (p≤0.05).

Table A3.2.17. Effect of PB on popliteal IMT according to isotemporal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		¹⁰ sMVPA		Total PB	
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI
Model 1												
Model 2												
Model 1	0.06	-0.05 0.18	-0.03	-0.16 0.10	-0.08	-0.18 0.02	-0.02	-0.10 0.06	0.07	-0.21 0.35	-0.01	-0.07 0.05
Model 2												
Model 1	0.08	-0.02 0.18	0.05	-0.16 0.26	0.01	-0.15 0.18	0.04	-0.10 0.17	0.14	-0.17 0.45	-0.09	-0.19 0.02
Model 2												
Model 1	0.02	-0.06 0.10	-0.01	-0.16 0.14	-0.06	-0.21 0.10	0.05	-0.10 0.21	0.15	-0.13 0.43	-0.09	-0.20 0.02
Model 2												
Model 1	-0.09	-0.36 0.19	-0.13	-0.44 0.19	-0.17	-0.44 0.11	-0.11	-0.42 0.20	0.09	-0.22 0.41	-0.04	-0.13 0.06
Model 2												
Partition												
Model 1	-0.01	-0.07 0.05	-0.04	-0.18 0.09	-0.09	-0.20 0.01	-0.04	-0.13 0.06	0.06	-0.22 0.34	0.08	-0.20 0.36
Model 2												

Model 1 No covariates included Model 2 Covariates included - NA.

b Change in cardiovascular marker per one hour substitution of PB.

95% CI Positive and negative 95% confidence interval

Table A3.2.18. Effect of PB on LOG popliteal blood velocity according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		¹⁰ MVPA		Total PB		Covariates	
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b ^a	95% CI
Model 1														
	Substituted													
Model 2														
Model 1	0.01	-0.24	0.26											
				Substituted										
Model 2	0.06	-0.17	0.28											
Model 1	-0.11	-0.35	0.14	-0.21	-0.68	0.25								
							Substituted							
Model 2	-0.14	-0.35	0.08	-0.29	-0.70	0.12								
Model 1	-0.02	-0.21	0.17	-0.13	-0.47	0.21	0.08	-0.27	0.44					
										Substituted				
Model 2	-0.06	-0.23	0.11	-0.21	-0.52	0.09	0.08	-0.24	0.40					
Model 1	0.32	-0.35	0.98	0.19	-0.56	0.93	0.42	-0.24	1.07	0.34	-0.40	1.09		
													Substituted	
Model 2	0.10	-0.53	0.72	-0.08	-0.78	0.62	0.23	-0.38	0.84	0.16	-0.53	0.85		
Partition														
Model 1	-0.10	-0.24	0.04	-0.21	-0.52	0.10	0.01	-0.24	0.25	-0.08	-0.30	0.15	-0.45	-1.12
Model 2	-0.10	-0.22	0.02	-0.25	-0.52	0.02	0.04	-0.18	0.26	-0.04	-0.24	0.16	-0.22	-0.83
Model 1 No covariates included Model 2 Covariates included - Hydration ^b														
b Change in cardiovascular marker per one hour substitution of PB.														
95% CI Positive and negative 95% confidence interval.														
Bold indicates significant model (p≤0.05).														

Table A3.2.19. Effect of PB on LOG popliteal shear rate according to isotemporal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10MVPA		Total PB		Covariates				
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b ^h	95% CI			
Model 1																	
	Substituted		0.07	-0.26	0.41	0.13	-0.16	0.42	-0.05	-0.28	0.17	-0.47	-1.26	0.32	-0.13	-0.29	0.03
Model 2			0.04	-0.29	0.36	0.16	-0.12	0.43	-0.02	-0.23	0.20	-0.27	-1.04	0.50	-0.13	-0.29	0.02
Model 1	-0.14	-0.43	0.16			-0.08	-0.51	0.36	-0.18	-0.56	0.19	-0.63	-1.49	0.24	0.04	-0.24	0.31
Model 2	-0.09	-0.37	0.19	Substituted		0.00	-0.41	0.42	-0.10	-0.46	0.25	-0.37	-1.21	0.47	-0.01	-0.27	0.25
Model 1	-0.13	-0.42	0.16	-0.05	-0.61	0.50			-0.18	-0.61	0.25	-0.60	-1.39	0.19	0.00	-0.30	0.29
Model 2	-0.16	-0.43	0.12	-0.12	-0.65	0.41	Substituted		-0.17	-0.58	0.23	-0.42	-1.18	0.33	0.03	-0.25	0.30
Model 1	0.05	-0.17	0.28	0.13	-0.28	0.53	0.18	-0.25	0.61			-0.42	-1.31	0.48	-0.18	-0.45	0.08
Model 2	0.02	-0.20	0.23	0.05	-0.33	0.44	0.18	-0.23	0.58	Substituted		-0.25	-1.10	0.61	-0.15	-0.40	0.10
Model 1	0.43	-0.37	1.22	0.47	-0.43	1.37	0.55	-0.24	1.33	0.38	-0.52	1.28			-0.55	-1.36	0.25
Model 2	0.23	-0.56	1.03	0.24	-0.66	1.13	0.38	-0.40	1.16	0.22	-0.66	1.10	Substituted		-0.36	-1.16	0.44
Partition																	
Model 1	-0.13	-0.29	0.03	-0.06	-0.43	0.31	0.00	-0.29	0.29	-0.18	-0.45	0.08	-0.60	-1.40	0.20		
Model 2	-0.13	-0.29	0.02	-0.10	-0.45	0.25	0.03	-0.25	0.30	-0.15	-0.40	0.11	-0.40	-1.17	0.38		
Model 1 No covariates included. Model 2 Covariates included - Hydration ^a .																	
b Change in cardiovascular marker per one-hour substitution of PB.																	
95% CI Positive and negative 95% confidence interval.																	

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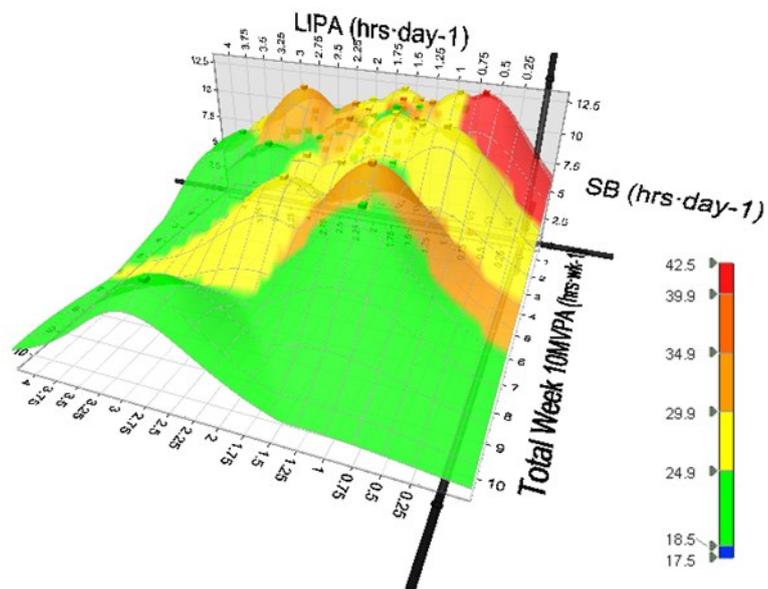


Figure A3.3.1. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on BMI in older adults. Colours represent thresholds of BMI category (kg·m²).

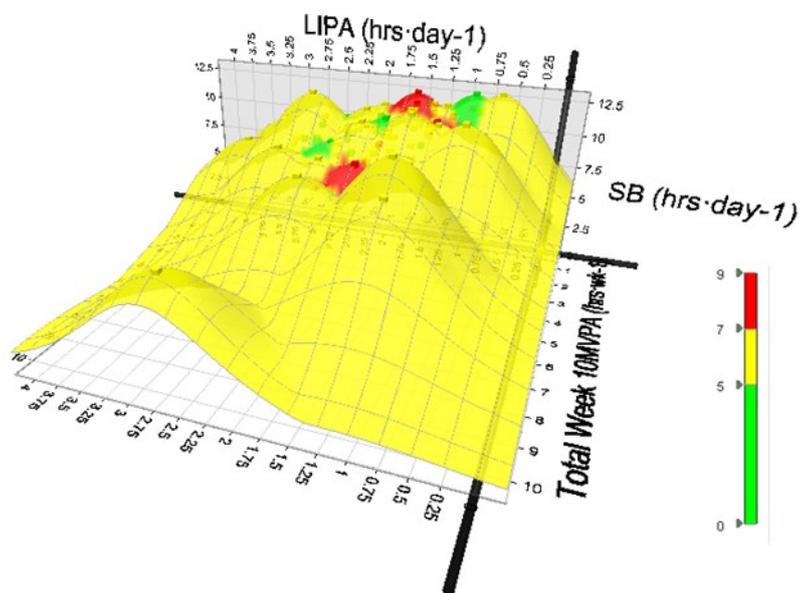


Figure A3.3.2. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on fasting blood glucose concentration in older adults. Colours represent thresholds of fasting blood glucose concentration (mmol·l⁻¹).

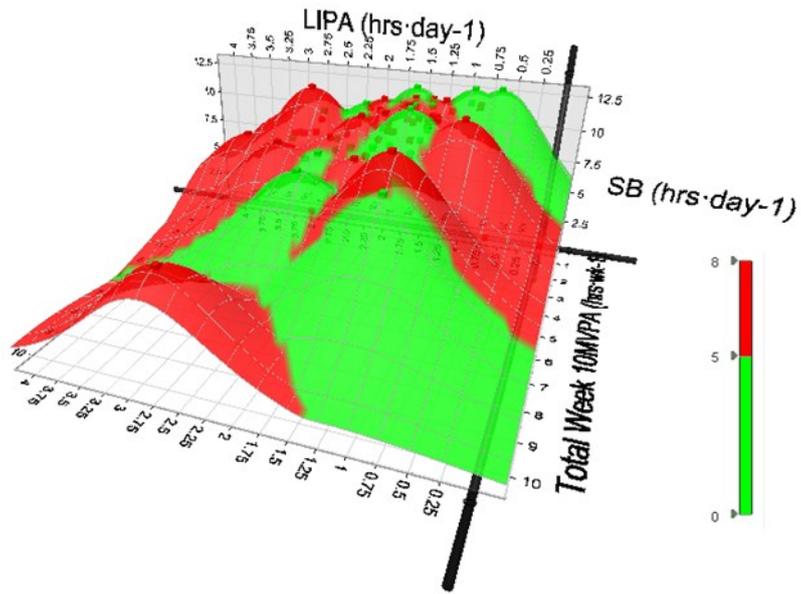


Figure A3.3.3. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on fasting blood total cholesterol concentration in older adults. Colours represent thresholds of fasting blood total cholesterol concentration (mmol·l⁻¹).

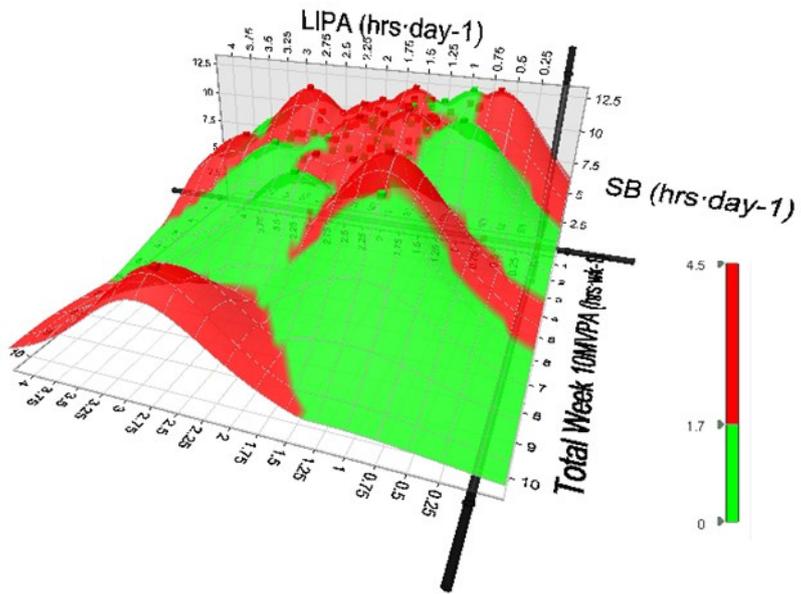


Figure A3.3.4. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on fasting blood triglyceride concentration in older adults. Colours represent thresholds of fasting blood triglyceride concentration (mmol·l⁻¹).

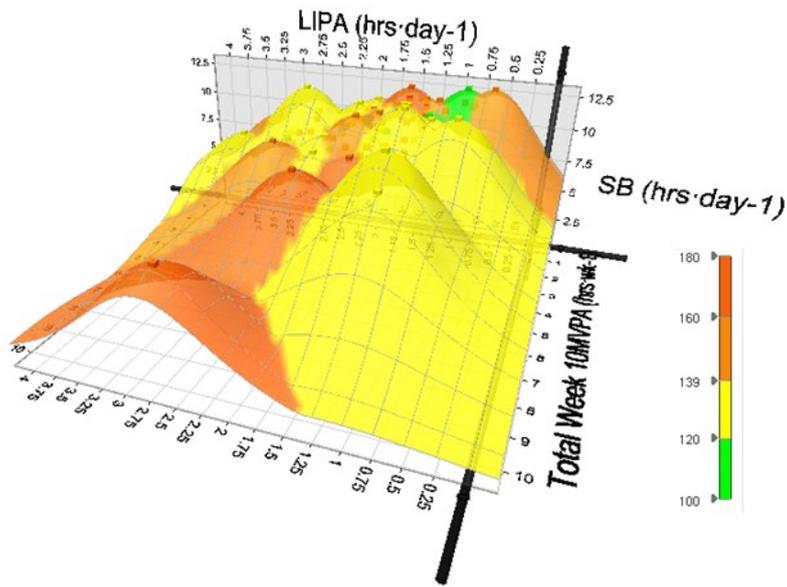


Figure A3.3.5. The combined influence of SB, LIPA, and Total Week $_{10}MVPA$ on Systolic BP in older adults. Colours represent thresholds of Systolic BP (mmHg).

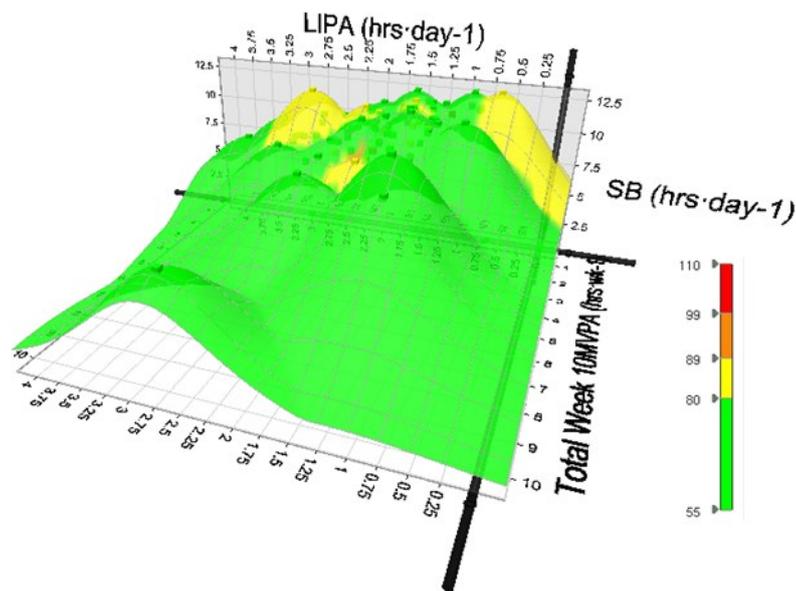


Figure A3.3.6. The combined influence of SB, LIPA, and Total Week $_{10}MVPA$ on Diastolic BP in older adults. Colours represent thresholds of Diastolic BP (mmHg).

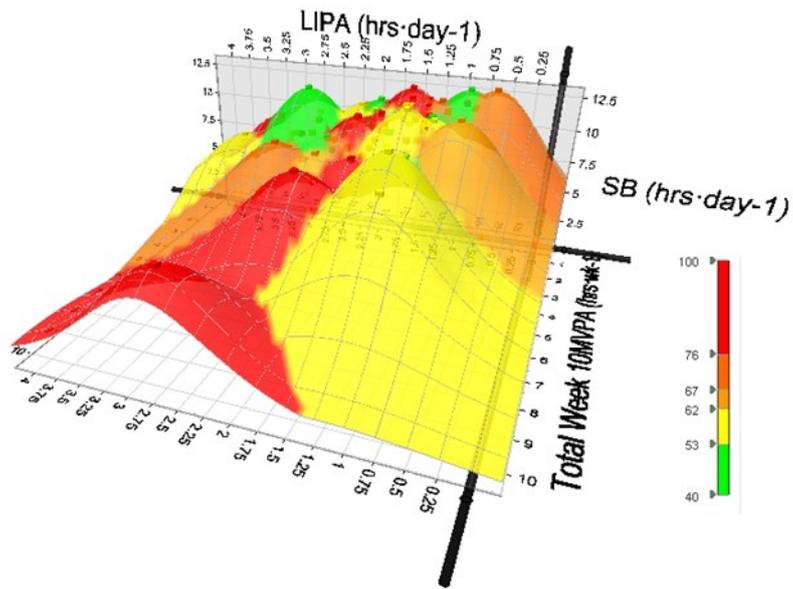


Figure A3.3.7. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on pulse pressure in older adults. Colours represent thresholds of pulse pressure (mmHg).

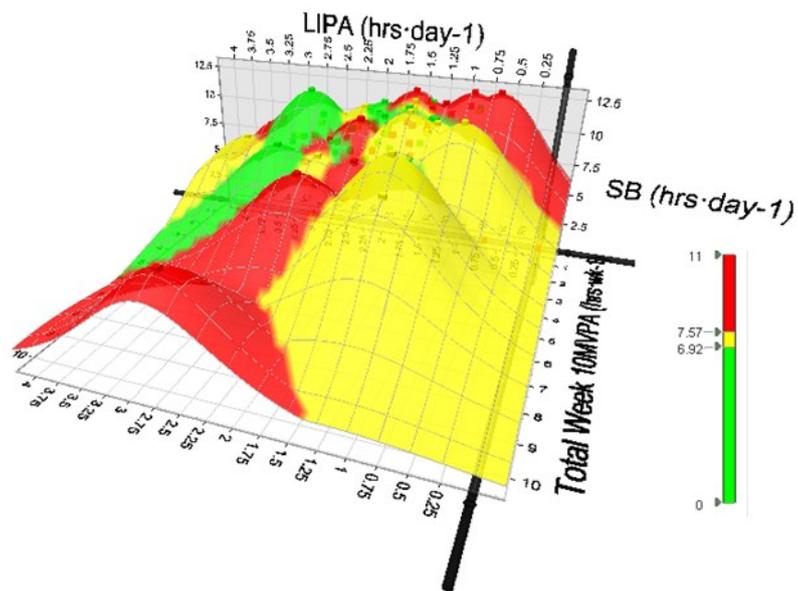


Figure A3.3.8. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on carotid AL artery diameter in older adults. Colours represent thresholds of carotid AL artery diameter (mm).

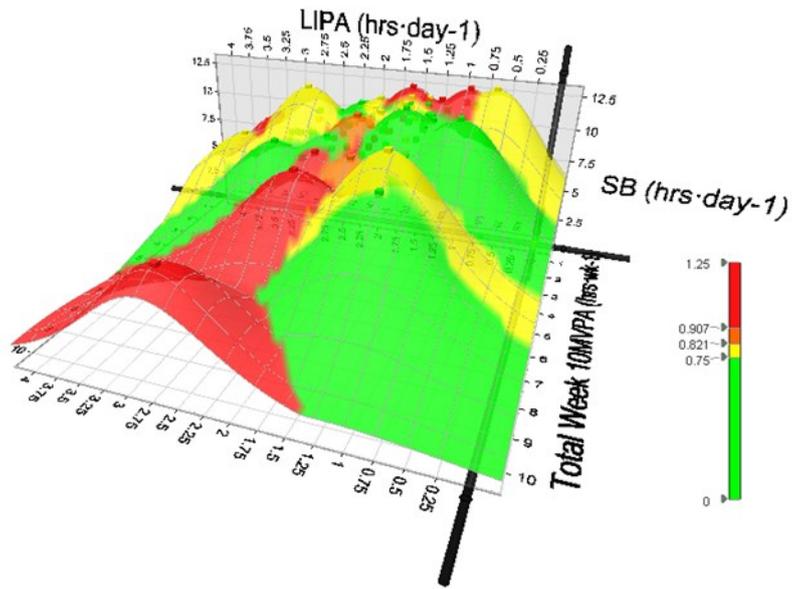


Figure A3.3.9. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on carotid AL far wall IMT in older adults. Colours represent thresholds of carotid AL far wall IMT (mm).

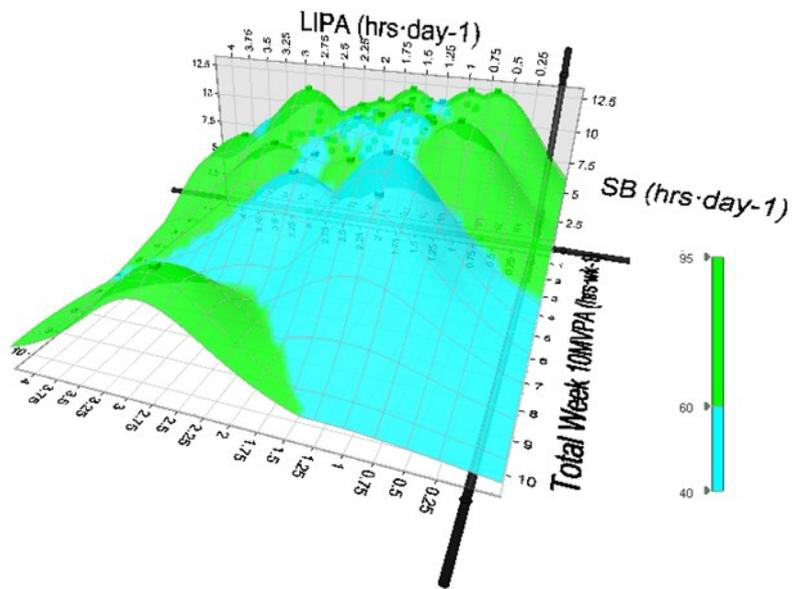


Figure A3.3.10. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on resting heart rate in older adults. Colours represent thresholds of resting heart rate (bpm).

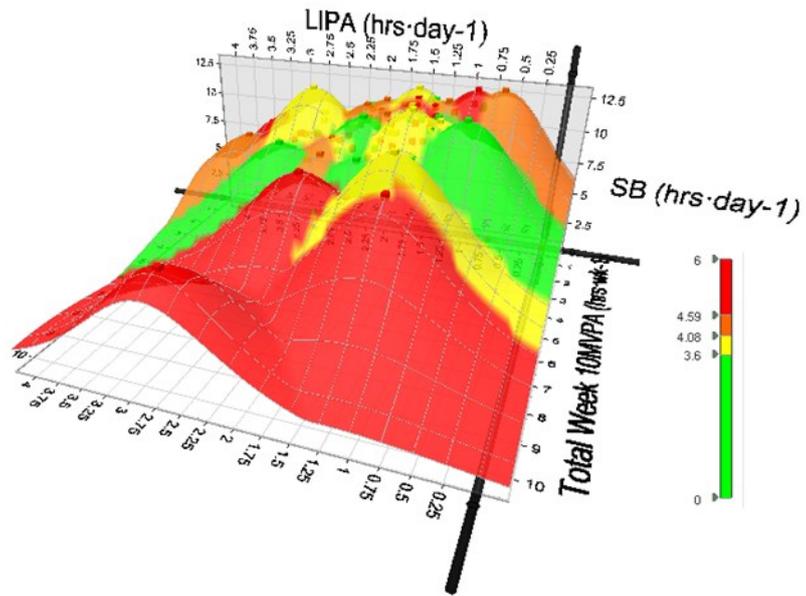


Figure A3.3.11. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on brachial artery diameter in older adults. Colours represent thresholds of brachial artery diameter (mm).

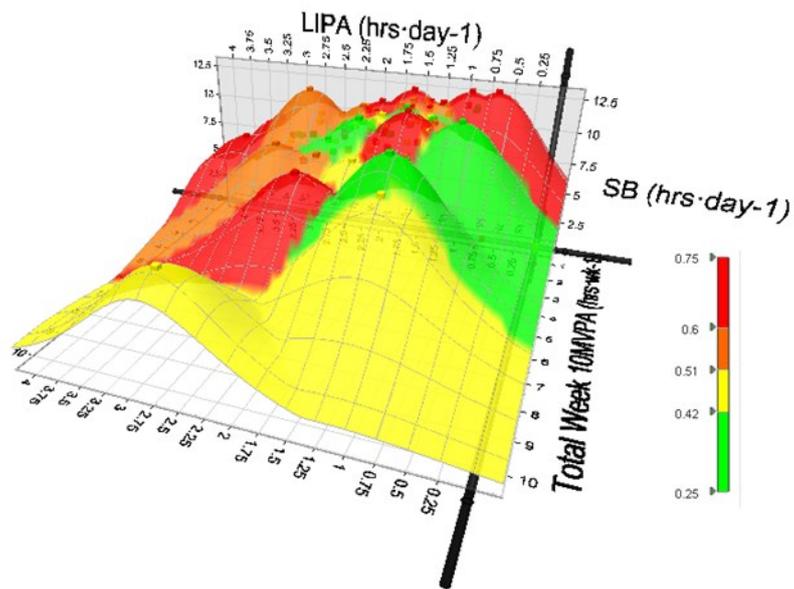


Figure A3.3.12. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on brachial artery far wall IMT in older adults. Colours represent thresholds of brachial artery far wall IMT (mm).

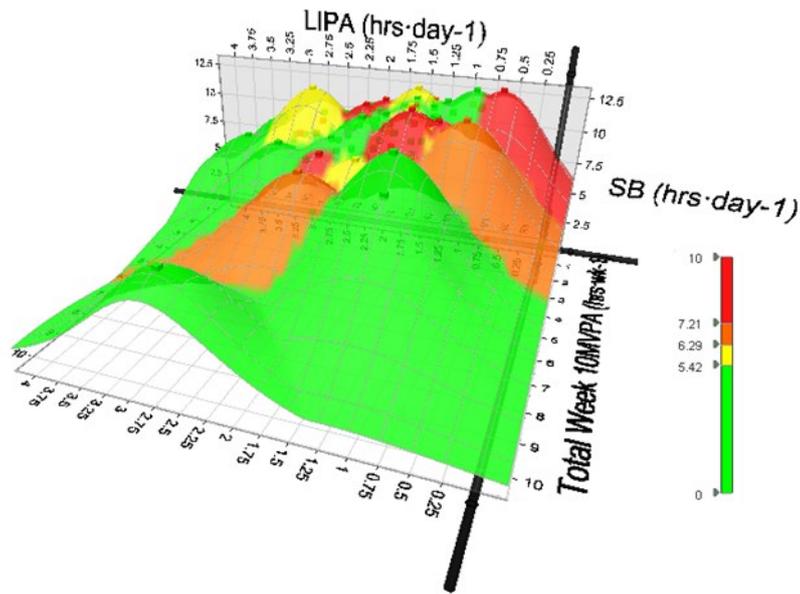


Figure A3.3.13. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on popliteal artery diameter in older adults. Colours represent thresholds of popliteal artery diameter (mm).

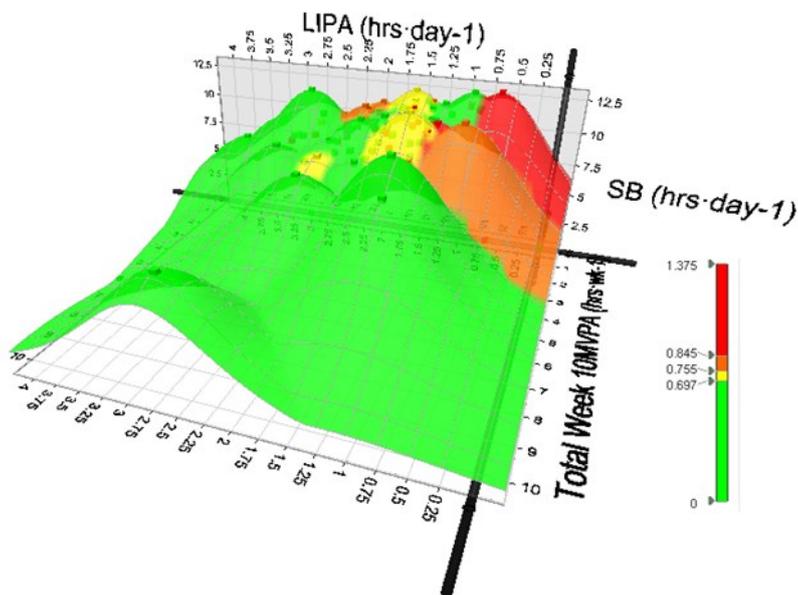


Figure A3.3.14. The combined influence of SB, LIPA, and Total Week ₁₀MVPA on popliteal artery far wall IMT in older adults. Colours represent thresholds of popliteal artery far wall IMT (mm).

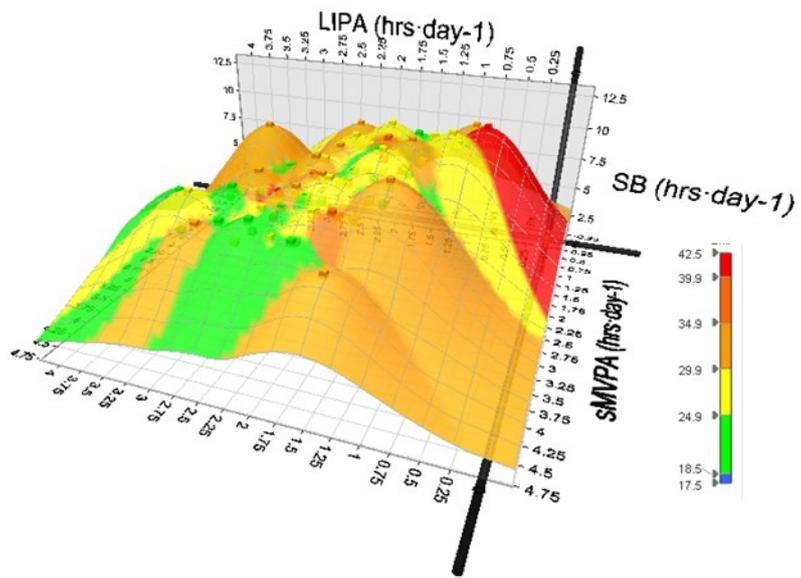


Figure A3.3.15. The combined influence of SB, LIPA, and sMVPA on BMI in older adults. Colours represent thresholds of BMI category (kg·m²).

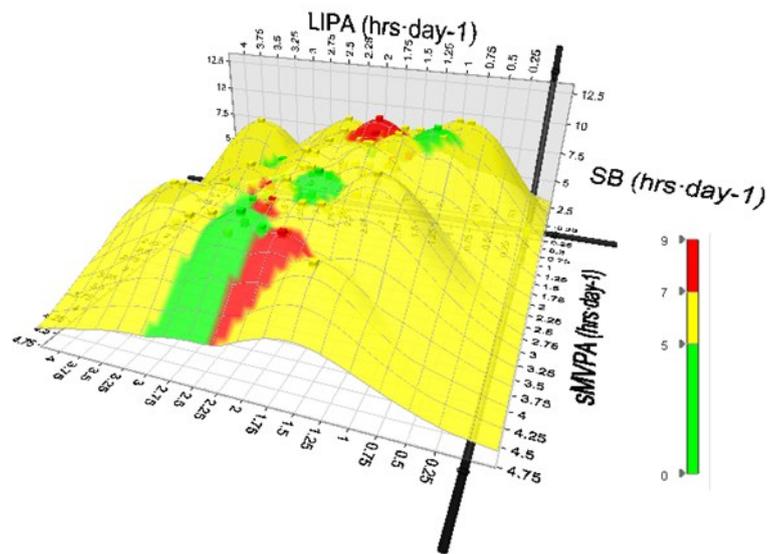


Figure A3.3.16. The combined influence of SB, LIPA, and sMVPA on fasting blood glucose concentration in older adults. Colours represent thresholds of fasting blood glucose concentration (mmol·l⁻¹).

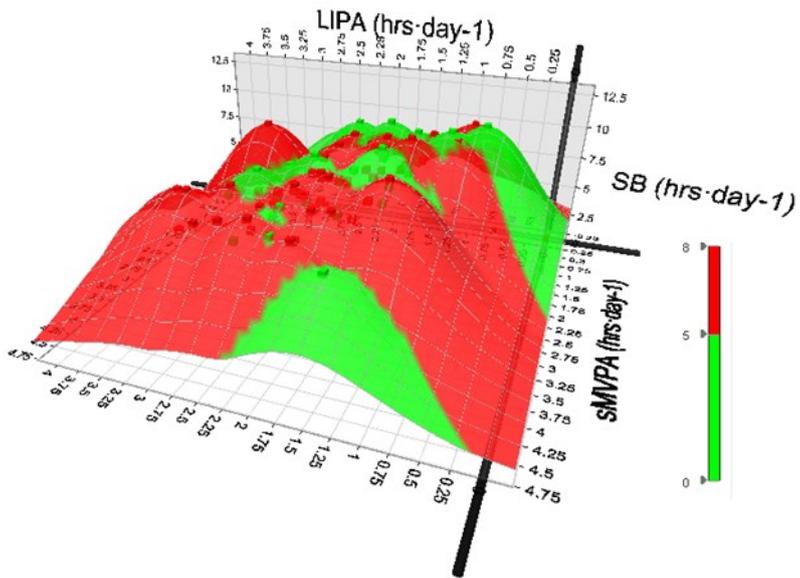


Figure A3.3.17. The combined influence of SB, LIPA, and sMVPA on fasting blood total cholesterol concentration in older adults. Colours represent thresholds of fasting blood total cholesterol concentration ($\text{mmol}\cdot\text{l}^{-1}$).

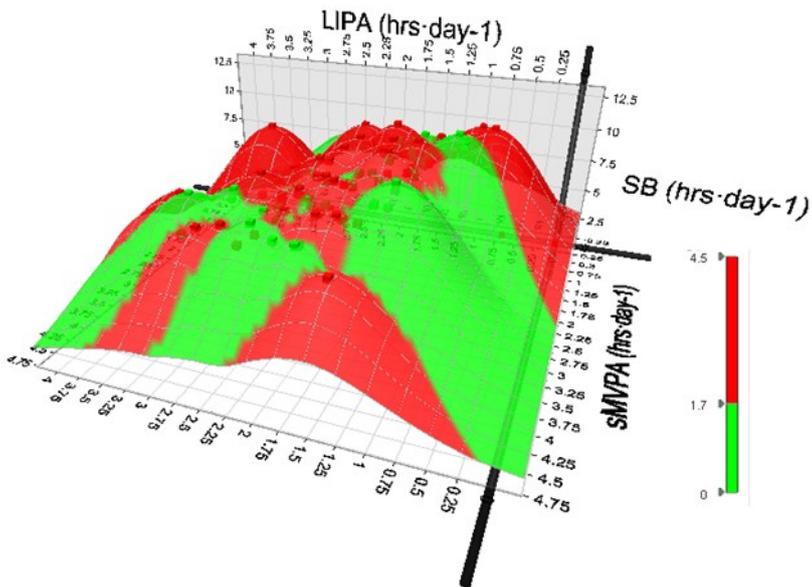


Figure A3.3.18. The combined influence of SB, LIPA, and sMVPA on fasting blood triglyceride concentration in older adults. Colours represent thresholds of fasting blood triglyceride concentration ($\text{mmol}\cdot\text{l}^{-1}$).

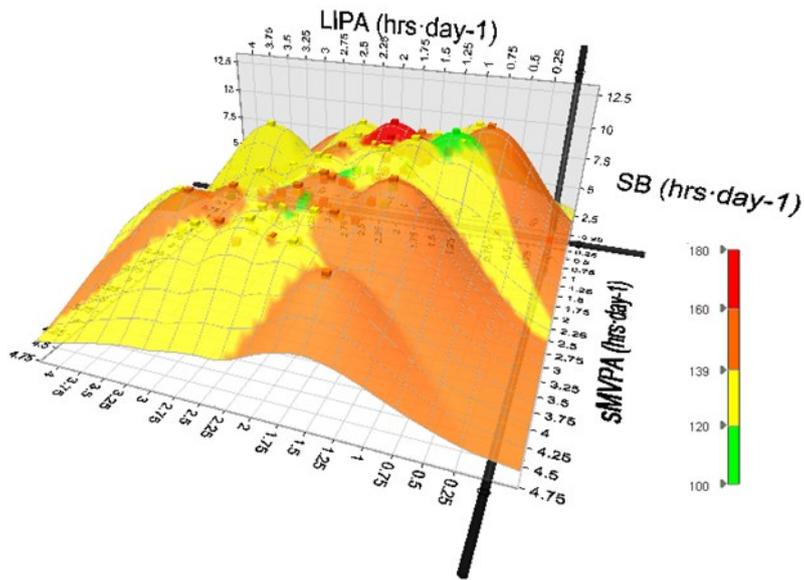


Figure A3.3.19. The combined influence of SB, LIPA, and sMVPA on Systolic BP in older adults. Colours represent thresholds of Systolic BP (mmHg).

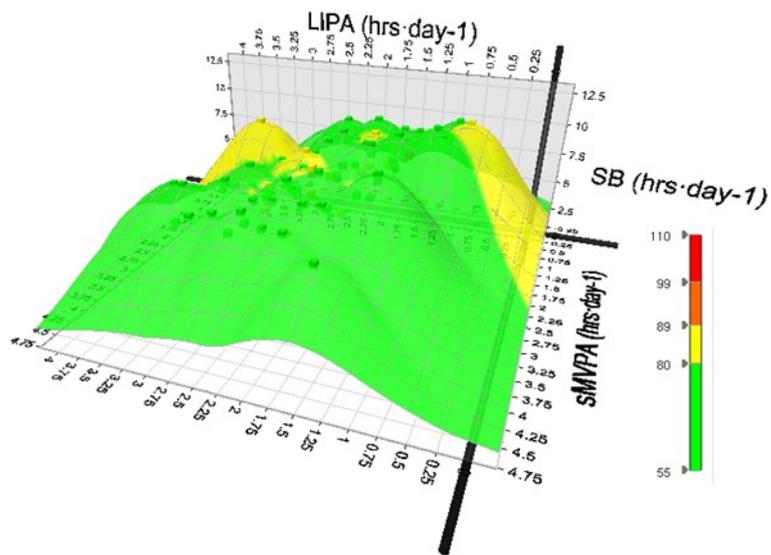


Figure A3.3.20. The combined influence of SB, LIPA, and sMVPA on Diastolic BP in older adults. Colours represent thresholds of Diastolic BP (mmHg).

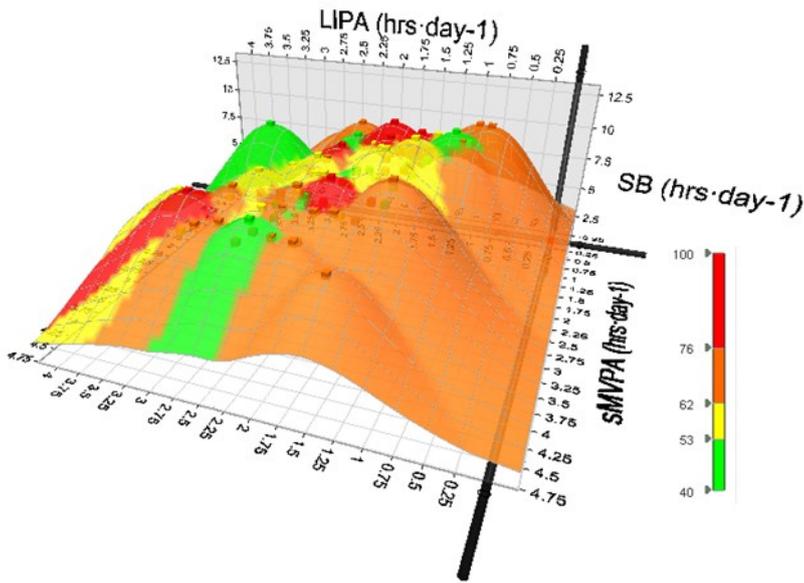


Figure A3.3.21. The combined influence of SB, LIPA, and sMVPA on pulse pressure in older adults. Colours represent thresholds of pulse pressure (mmHg).

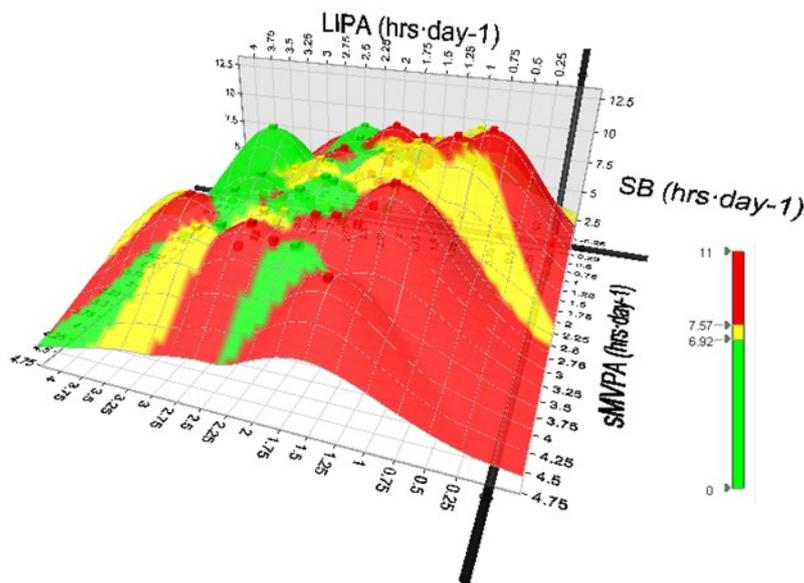


Figure A3.3.22. The combined influence of SB, LIPA, and sMVPA on carotid AL artery diameter in older adults. Colours represent thresholds of carotid AL artery diameter (mm).

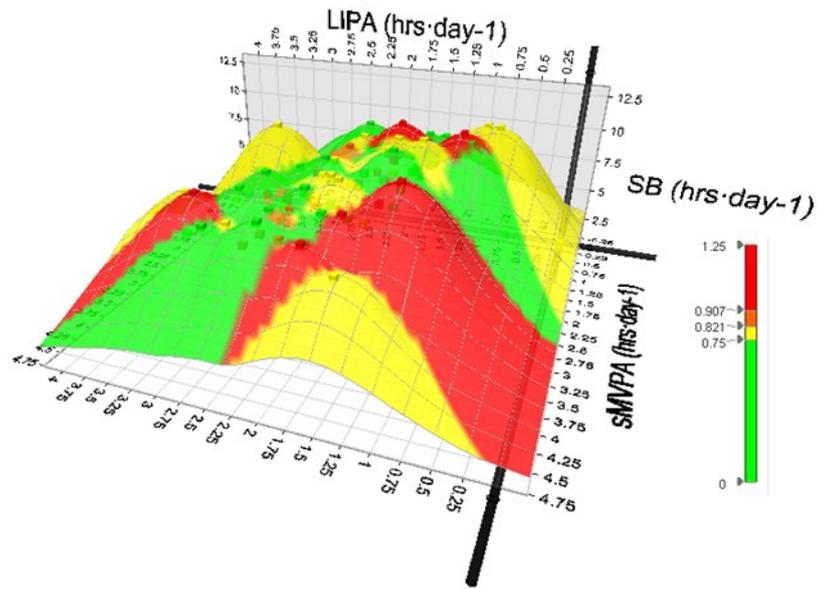


Figure A3.3.23. The combined influence of SB, LIPA, and sMVPA on carotid AL far wall IMT in older adults. Colours represent thresholds of carotid AL far wall IMT (mm).

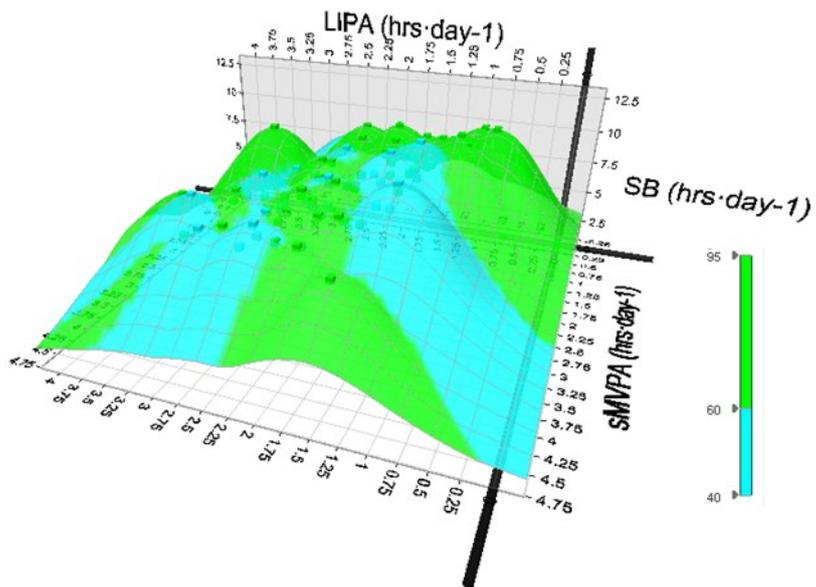


Figure A3.3.24. The combined influence of SB, LIPA, and sMVPA on resting heart rate in older adults. Colours represent thresholds of resting heart rate (bpm).

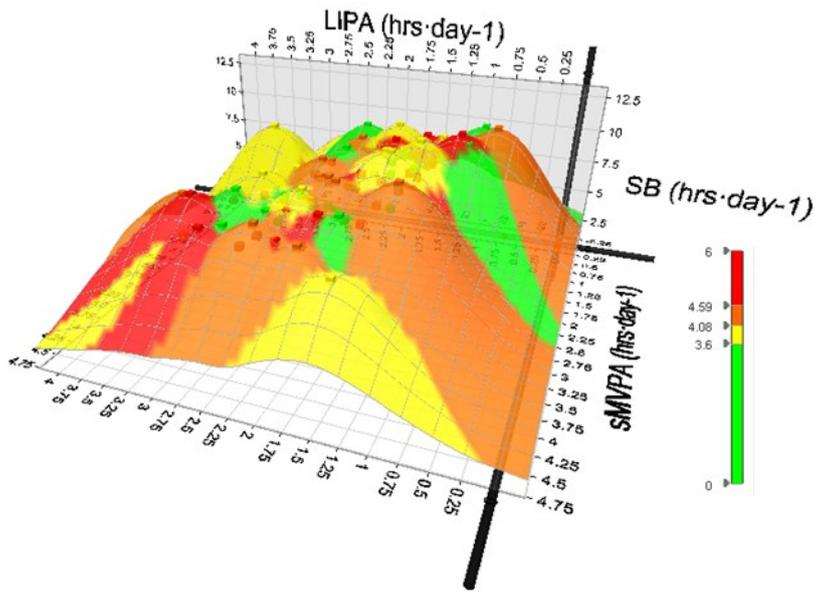


Figure A3.3.25. The combined influence of SB, LIPA, and sMVPA on brachial artery diameter in older adults. Colours represent thresholds of brachial artery diameter (mm).

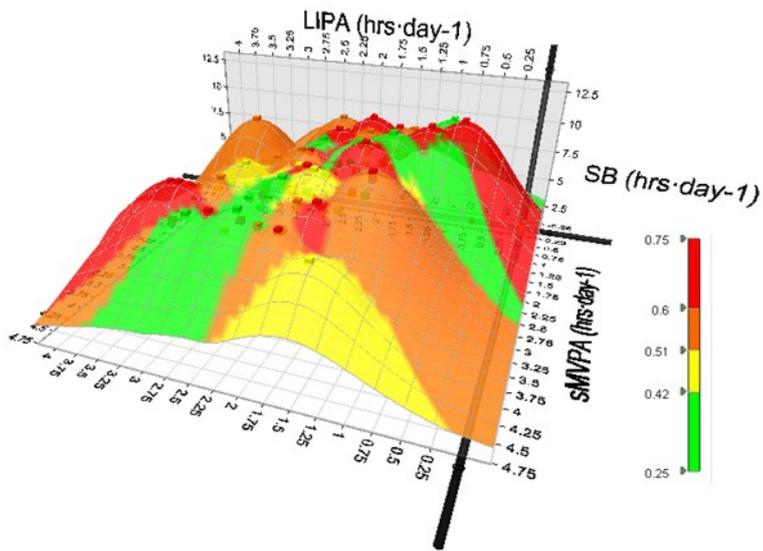


Figure A3.3.26. The combined influence of SB, LIPA, and sMVPA on brachial far wall IMT in older adults. Colours represent thresholds of brachial far wall IMT (mm).

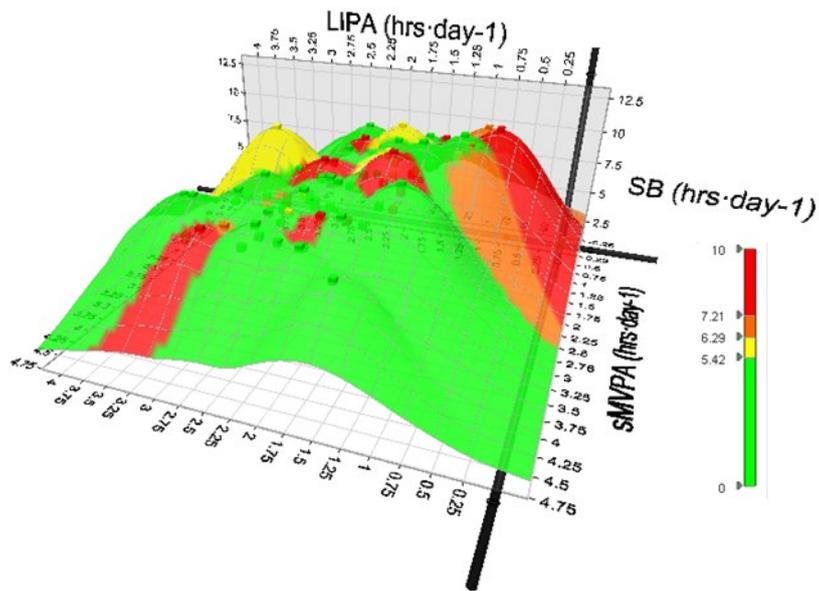


Figure A3.3.27. The combined influence of SB, LIPA, and sMVPA on popliteal artery diameter in older adults. Colours represent thresholds of popliteal artery diameter (mm).

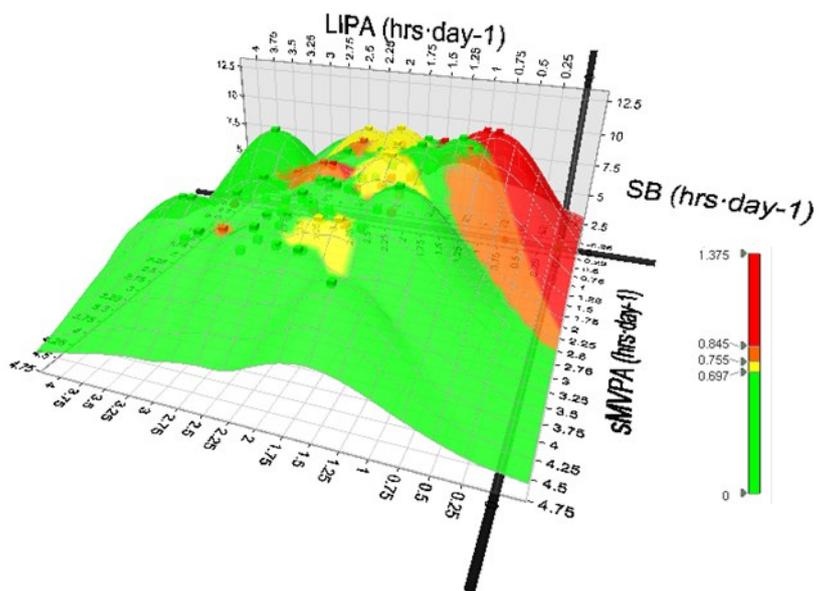


Figure A3.3.28. The combined influence of SB, LIPA, and sMVPA on popliteal artery far wall IMT in older adults. Colours represent thresholds of popliteal artery far wall IMT (mm).

Chapter 03: Part 3: MVPA – brachial artery diameter relationship.

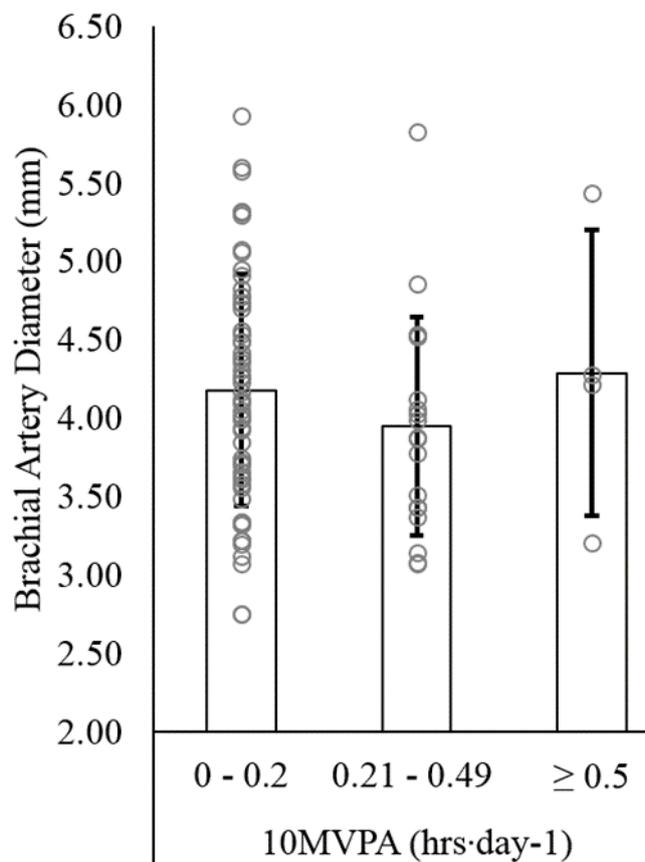


Figure A3.3.29. Change in brachial artery diameter with increasing $_{10}$ MVPA engagement.

Chapter 04: Effect of medication on further ageing.

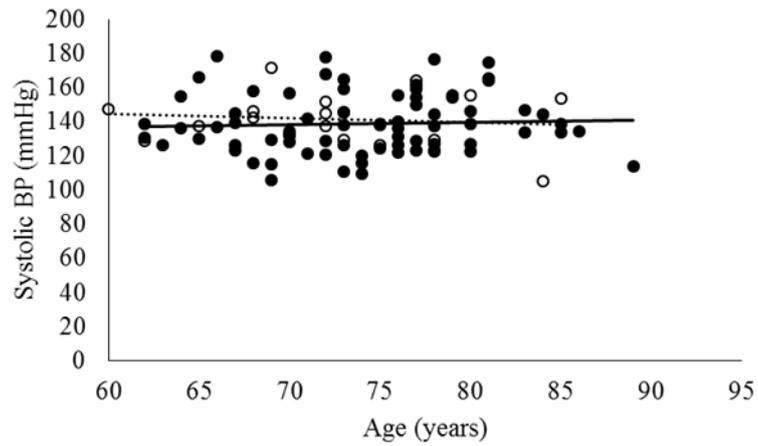


Figure A4.1. The effect of further ageing on systolic BP when participants are grouped into not medicated (\circ , dotted trend line) and medicated (\bullet , solid trend line). No significant associations.

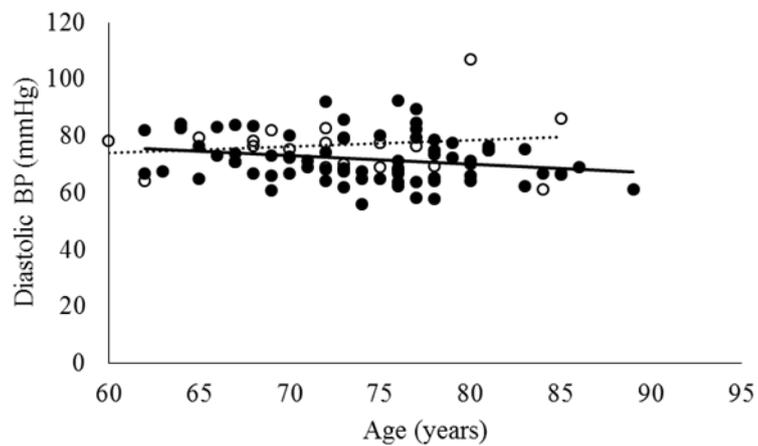


Figure A4.2. The effect of further ageing on diastolic BP when participants are grouped into not medicated (\circ , dotted trend line) and medicated (\bullet , solid trend line). No significant associations.

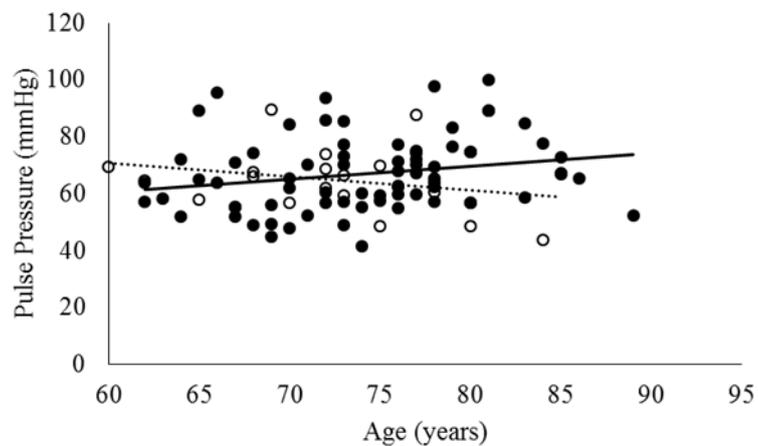


Figure A4.3. The effect of further ageing on pulse pressure when participants are grouped into not medicated (\circ , dotted trend line) and medicated (\bullet , solid trend line). No significant associations.

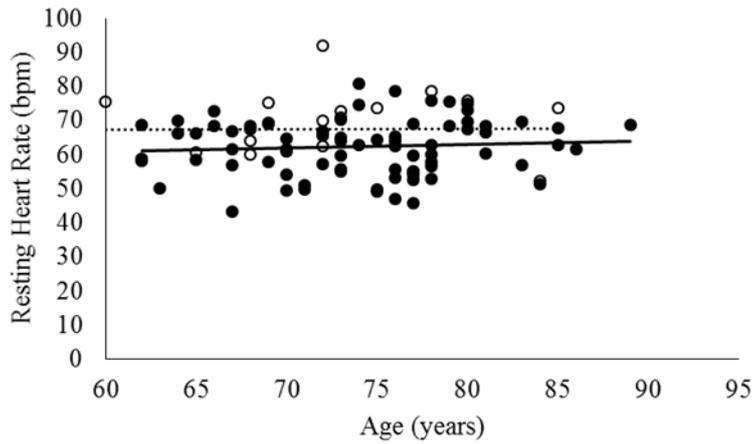


Figure A4.4. The effect of further ageing on resting heart rate when participants are grouped into not medicated (○, dotted trend line) and medicated (●, solid trend line). No significant associations.

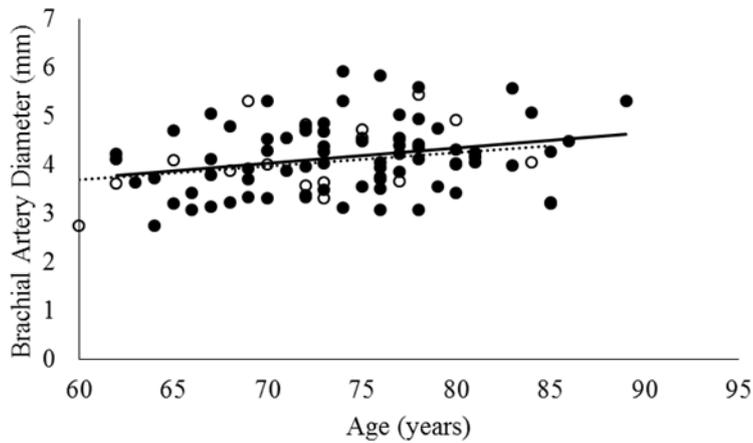


Figure A4.5. The effect of further ageing on brachial artery diameter when participants are grouped into not medicated (○, dotted trend line) and medicated (●, solid trend line). Significant association for the medicated group ($p=0.02$).

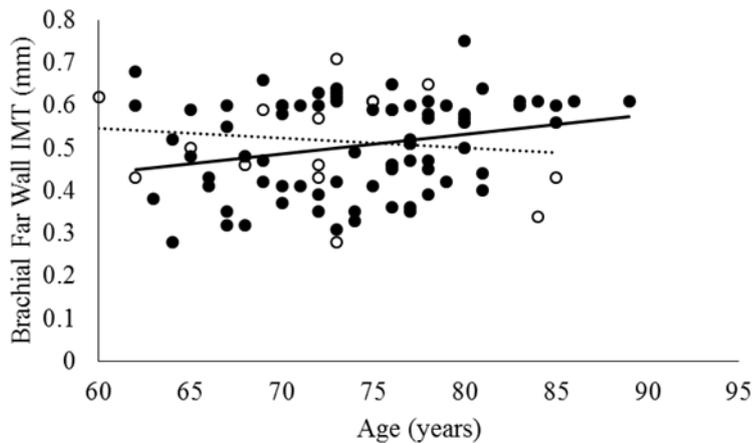


Figure A4.6. The effect of further ageing on brachial artery IMT when participants are grouped into not medicated (○, dotted trend line) and medicated (●, solid trend line). Significant association for the medicated group ($p=0.03$).

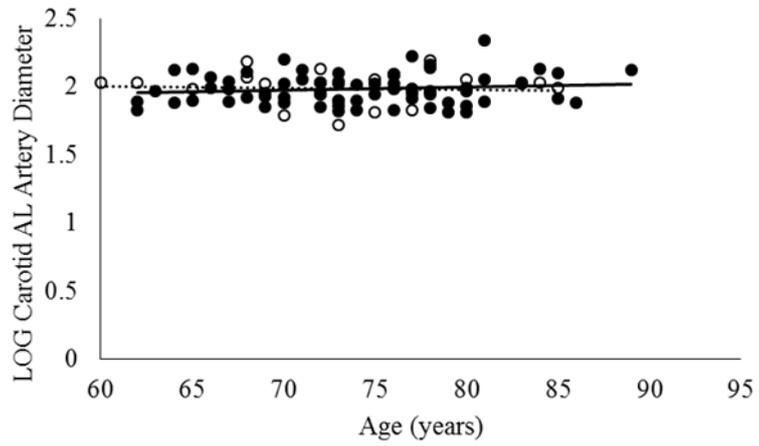


Figure A4.7. The effect of further ageing on carotid artery diameter when participants are grouped into not medicated (○, dotted trend line) and medicated (●, solid trend line). No significant associations.

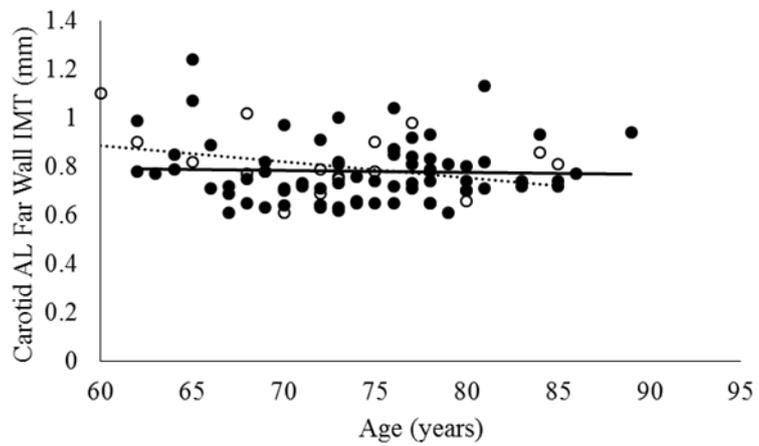


Figure A4.8. The effect of further ageing on carotid artery IMT when participants are grouped into not medicated (○, dotted trend line) and medicated (●, solid trend line). No significant associations.

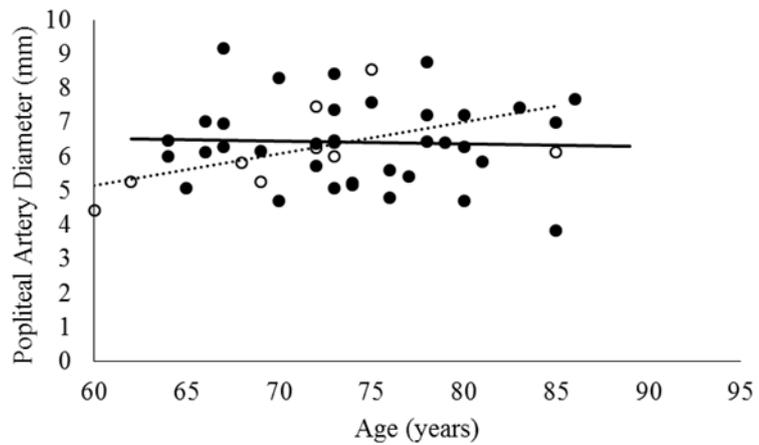


Figure A4.9. The effect of further ageing on popliteal artery diameter when participants are grouped into not medicated (○, dotted trend line) and medicated (●, solid trend line). No significant associations.

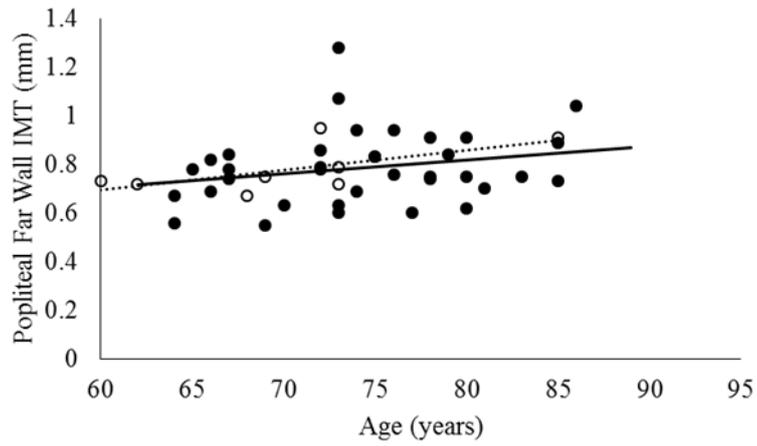


Figure A4.10. The effect of further ageing on popliteal artery IMT when participants are grouped into not medicated (○, dotted trend line) and medicated (●, solid trend line). Significant association for the not medicated group ($p=0.04$).

Chapter 05: Part 2: Full ISM results.

Table A5.2.1 Effect of PB on fasting plasma LOG cholesterol concentration according to isletemporal substitution of one hour of SB or PA

Substitution	SB		Standing		LIPA		sMMPA		¹⁰ sMMPA		Total PB		Covariates	
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI
Model 1	Substituted		-0.13	-0.25	-0.01	0.09	0.00	0.17	-0.03	-0.09	0.04	-0.06	-0.06	0.05
Model 2			-0.13	-0.23	-0.02	0.06	-0.02	0.14	-0.03	-0.09	0.02	-0.11	-0.34	0.11
Model 1	0.08	-0.03	0.20		Substituted	0.13	-0.03	0.29	0.06	-0.08	0.19	0.03	-0.25	0.31
Model 2	0.09	-0.02	0.19			0.11	-0.04	0.25	0.05	-0.07	0.18	-0.02	-0.28	0.23
Model 1	-0.09	-0.17	0.00	-0.22	-0.40	-0.04	Substituted		-0.12	-0.24	0.01	-0.15	-0.41	0.11
Model 2	-0.06	-0.14	0.02	-0.19	-0.35	-0.02			-0.09	-0.21	0.02	-0.17	-0.41	0.06
Model 1	-0.01	-0.06	0.05	-0.15	-0.28	-0.01	0.05	-0.06	0.16	Substituted		-0.14	-0.40	0.12
Model 2	0.00	-0.05	0.05	-0.14	-0.27	-0.02	0.03	-0.07	0.13			-0.18	-0.42	0.06
Model 1	0.06	-0.19	0.31	-0.08	-0.36	0.20	0.17	-0.09	0.42	0.04	0.04	-0.24	0.31	Substituted
Model 2	0.13	-0.09	0.35	-0.01	-0.26	0.24	0.19	-0.03	0.42	0.10	-0.14	0.34		Substituted
Partition														
Model 1	0.00	-0.06	0.05	-0.13	-0.26	-0.01	0.08	-0.01	0.18	-0.03	-0.11	0.05	-0.07	-0.32
Model 2	0.00	-0.05	0.05	-0.13	-0.24	-0.02	0.05	-0.03	0.14	-0.04	-0.11	0.04	-0.12	-0.35

Model 1 No covariates included. Model 2 Covariates included - Primary CVD targeting medication, CVD (in)directly targeting medication.
Bold indicates significant changes in cardio-metabolic parameter, $p \leq 0.05$.

Table A5.3.2. Effect of PB on fasting plasma triglyceride concentration according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		¹⁰ MVPA		Total PB						
	b	95% CI	b	95% CI	b	95% CI											
Model 1		Substituted	-0.04	-0.26	0.18	-0.04	-0.20	0.12	-0.12	-0.24	0.00	-0.68	-1.15	-0.21	0.05	-0.05	0.15
Model 2	0.00	-0.21	0.21	Substituted		-0.08	-0.37	0.22	-0.12	-0.36	0.13	-0.67	-1.18	-0.16	0.07	-0.12	0.26
Model 1	0.04	-0.12	0.20	0.00	-0.33	0.34	Substituted		-0.08	-0.31	0.16	-0.64	-1.12	-0.16	0.01	-0.16	0.18
Model 2	0.08	-0.03	0.19	0.02	-0.23	0.27	0.02	-0.19	0.22	Substituted		-0.71	-1.19	-0.23	0.01	-0.09	0.12
Model 1	0.62	0.16	1.07	0.58	0.08	1.08	0.58	0.12	1.05	0.49	0.00	0.99	Substituted		-0.56	-1.03	-0.10
Model 2																	
Partition																	
Model 1	0.05	-0.05	0.15	0.01	-0.22	0.24	0.01	-0.16	0.19	-0.07	-0.22	0.09	-0.63	-1.11	-0.15		
Model 2																	

Model 1 No covariates included. Model 2 Covariates included – NA.

Bold indicates significant changes in cardio-metabolic parameter. $p \leq 0.05$.

Table A5.3.3. Effect of PB on fasting serum LOG LPL concentration according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		¹⁰ MVPA		Total PB		Covariates						
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI					
Model 1	Substituted		-0.02	-0.73	0.68	-0.26	-0.76	0.25	-0.07	-0.46	0.31	0.08	-1.39	1.55	0.33	0.01	0.64		
Model 2			0.11	-0.56	0.78	-0.42	-0.93	0.09	-0.11	-0.48	0.25	-0.17	-1.57	1.23	0.41	0.11	0.72	0.00	0.00
Model 1	0.19	-0.45	0.83	Substituted		0.08	-0.84	0.99	0.11	-0.67	0.89	0.20	-1.38	1.79	0.07	-0.54	0.68		
Model 2	0.13	-0.48	0.73			-0.08	-0.96	0.80	0.02	-0.72	0.77	-0.10	-1.63	1.42	0.18	-0.40	0.77	0.00	0.00
Model 1	0.26	-0.25	0.76	0.23	-0.83	1.30	Substituted		0.18	-0.54	0.90	0.34	-1.19	1.87	0.07	-0.47	0.61		
Model 2	0.42	-0.09	0.93	0.54	-0.50	1.57			0.31	-0.38	1.00	0.26	-1.19	1.70	-0.01	-0.54	0.52	0.00	0.00
Model 1	0.28	-0.08	0.63	0.27	-0.56	1.09	0.27	-0.38	0.92	Substituted		0.72	-0.83	2.28	-0.16	-0.48	0.17		
Model 2	0.21	-0.14	0.55	0.38	-0.39	1.16	-0.16	-0.82	0.51			0.33	-1.12	1.77	0.09	-0.26	0.44	0.00	0.00
Model 1	-0.35	-1.76	1.05	-0.31	-1.84	1.22	-0.58	-2.04	0.87	-0.45	-1.98	1.08	Substituted		0.68	-0.76	2.12		
Model 2	-0.17	-1.52	1.18	0.00	-1.48	1.48	-0.54	-1.92	0.85	-0.30	-1.76	1.16			0.57	-0.80	1.94	0.00	0.00
Partition																			
Model 1	0.33	0.01	0.64	0.31	-0.43	1.04	0.07	-0.47	0.61	0.25	-0.22	0.73	0.41	-1.10	1.92				
Model 2	0.41	0.11	0.72	0.52	-0.18	1.23	-0.01	-0.54	0.52	0.30	-0.15	0.75	0.25	-1.18	1.67			0.00	0.00

Model 1 No covariates included. Model 2 Covariates included – Inflammatory + CVD (in)directly targeting medication.

Bold indicates significant changes in cardio-metabolic parameter, $p \leq 0.05$.

Table A4.3.4. Effect of PB on fasting plasma glucose concentration according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		¹⁰ MVPA		Total PB							
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI						
Model 1	Substituted		-0.02	-0.84	0.80	-0.31	-0.90	0.29	-0.07	-0.52	0.38	-0.47	-2.21	1.26	0.10	-0.28	0.48	
Model 2	0.20	-0.56	0.96	Substituted		0.07	-1.02	1.15	0.12	-0.79	1.04	-0.31	-2.20	1.58	-0.18	-0.89	0.53	
Model 1	0.31	-0.29	0.90	0.29	-0.95	1.53	Substituted		0.24	-0.62	1.10	-0.16	-1.94	1.61	-0.21	-0.85	0.43	
Model 2	0.07	-0.33	0.47	0.02	-0.89	0.92	-0.20	-0.94	0.54	Substituted		-0.46	-2.20	1.27	0.04	-0.33	0.41	
Model 1	0.38	-1.27	2.04	0.42	-1.41	2.24	0.06	-1.63	1.75	0.28	-1.52	2.09	Substituted		-0.28	-1.96	1.41	
Model 2	Partition		Partition		Partition		Partition		Partition		Partition		Partition		Partition		Partition	
Model 1	0.10	-0.28	0.48	0.08	-0.76	0.93	-0.21	-0.85	0.43	0.03	-0.53	0.60	-0.37	-2.14	1.40	Partition		
Model 2	Partition		Partition		Partition		Partition		Partition		Partition		Partition		Partition		Partition	

Model 1 No covariates included. Model 2 Covariates included - NA.

Table A4.3.5. Effect of PB on fasting plasma HbA1c according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		¹⁰ MVPA		Total PB						
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI					
Model 1	Substituted		0.18	-0.13	0.50	-0.27	-0.55	0.00	0.08	-0.14	0.29	-0.23	-1.16	0.69	-0.05	-0.20	0.10
Model 2	-0.18	-0.50	0.13	Substituted		-0.46	-0.99	0.08	-0.11	-0.50	0.29	-0.42	-1.37	0.53	0.13	-0.21	0.47
Model 1	0.27	0.00	0.55	0.46	-0.08	0.99	Substituted		0.35	-0.03	0.73	0.04	-0.92	1.00	-0.33	-0.60	-0.05
Model 2	-0.08	-0.29	0.14	0.11	-0.29	0.50	-0.35	-0.73	0.03	Substituted		-0.31	-1.33	0.71	0.02	-0.23	0.28
Model 1	0.18	-0.73	1.09	0.35	-0.59	1.28	-0.10	-1.04	0.84	0.25	-0.76	1.27	Substituted		-0.23	-1.14	0.69
Model 2	Partition												Substituted				
Model 1	-0.05	-0.20	0.10	0.13	-0.21	0.47	-0.33	-0.60	-0.05	0.02	-0.23	0.28	-0.29	-1.21	0.64		
Model 2																	

Model 1 No covariates included. Model 2 Covariates included – NA.

Table A4.3.6. Effect of PB on fasting serum LOG IL-6 concentration according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		sMVPA		10 ⁴ MVPA		Total PB						
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI					
Model 1	Substituted		0.02	-0.55	0.59	0.18	-0.24	0.60	-0.23	-0.54	0.07	0.58	-0.67	1.82	-0.02	-0.28	0.24
Model 2	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Model 1	-0.02	-0.53	0.50	Substituted		0.17	-0.57	0.91	-0.25	-0.87	0.37	0.56	-0.75	1.87	-0.01	-0.49	0.48
Model 2	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model 1	-0.18	-0.60	0.24	-0.16	-1.03	0.71	Substituted		-0.42	-1.00	0.17	0.40	-0.91	1.70	0.16	-0.28	0.60
Model 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model 1	0.15	-0.12	0.43	0.19	-0.45	0.82	0.22	-0.30	0.73	Substituted		0.62	-0.65	1.89	-0.09	-0.34	0.17
Model 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model 1	-0.52	-1.73	0.69	-0.46	-1.76	0.83	-0.27	-1.53	0.99	-0.77	-2.08	0.53	Substituted		0.49	-0.75	1.74
Model 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
Partition																	
Model 1	-0.02	-0.28	0.24	0.00	-0.60	0.60	0.16	-0.28	0.60	-0.25	-0.64	0.13	0.56	-0.73	1.84		
Model 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			

Model 1 No covariates included. Model 2 Covariates included - NA.

Table A4.3.7. Effect of PB on fasting serum LOG PIIINP concentration according to isothermal substitution of one hour of SB or PA.

Substitution	SB		Standing		LIPA		SMVPA		10 ⁶ MVPA		Total PB						
	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI	b	95% CI					
Model 1	Substituted		-0.02	-0.90	0.87	0.15	-0.50	0.79	0.04	-0.42	0.01	-1.80	1.83	0.27	-0.12	0.65	
Model 2	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Model 1	0.14	-0.65	0.93	Substituted		0.39	-0.73	1.52	0.18	-0.79	1.15	0.11	-1.84	2.05	0.08	-0.65	0.82
Model 2	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model 1	-0.15	-0.79	0.50	-0.16	-1.50	1.18	Substituted		-0.10	-1.00	0.79	-0.13	-2.03	1.77	0.41	-0.29	1.12
Model 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model 1	0.08	-0.32	0.48	0.15	-0.82	1.13	0.30	-0.48	1.08	Substituted		0.42	-1.41	2.24	0.05	-0.31	0.42
Model 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model 1	0.07	-1.63	1.78	0.03	-1.84	1.90	0.28	-1.49	2.05	0.10	-1.77	1.96	Substituted		0.21	-1.54	1.95
Model 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
Partition																	
Model 1	0.27	-0.12	0.65	0.25	-0.65	1.15	0.41	-0.29	1.12	0.31	-0.27	0.89	0.28	-1.58	2.14		
Model 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			

Model 1 No covariates included. Model 2 Covariates included - NA.

Ethics Approval

Dear Declan

Further to the amendments submitted as requested by the ESS Ethics Committee, I am writing to inform you that you have now gained ethical approval for your study.

Best wishes

Dr Islay McEwan
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Visit the Institute for Performance Research website at:
<http://www.ipr.mmu.ac.uk/groups/biomechanics/>

Before acting on this email or opening any attachments you should read the Manchester Metropolitan University's email disclaimer available on its website
<http://www.mmu.ac.uk/emaildisclaimer>

Informed Consent Form

(Both the investigator and participant should retain a copy of this form)

Name of Participant:

Supervisor/Principal Investigator: Declan Ryan BSc (Hons) (Principal Investigator),
~~Dr.~~ Georgina Stebbings (Supervisor)
Dr. Gladys Onambebe-Pearson (Director of Studies)
Project Title: Examining the link between habitual sitting and
cardiometabolic profile in older persons.
Ethics Committee Approval Number: 03.11.14(i)

Participant Statement

I have read the participant information sheet for this study and understand what is involved in taking part. Any questions I have about the study, or my participation in it, have been answered to my satisfaction. I understand that I do not have to take part and that I may decide to withdraw from the study at any point without giving a reason. Any concerns I have raised regarding this study have been answered and I understand that ~~any further concerns that arise during the time of the study will be addressed by the investigator.~~ I therefore agree to participate in the study.

It has been made clear to me that, should I feel that my rights are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform the Registrar and Clerk to the Board of Governors, Head of Governance and Secretariat Team, Manchester Metropolitan University, All Saints Building, All Saints, Manchester, M15 6BH, Tel: 0161 247 1390 who will undertake to investigate my complaint.

Signed (Participant)	<input type="text"/>	Date	<input type="text"/>
Signed (Investigator)	<input type="text"/>	Date	<input type="text"/>

This version of the form should be used from
September 2013 onwards.