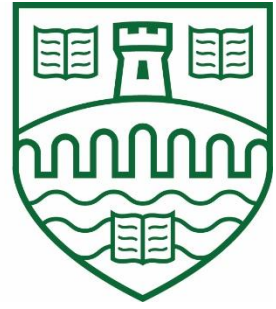


UNIVERSITY *of* STIRLING



Replacing sedentary time with physical activity: strategies to improve markers of health across the lifespan

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Declaration

I declare that this thesis was composed by myself, and all the data contained herein was collected by myself, under the current supervision of Dr Naomi Brooks and Prof Stuart Galloway, and previous supervision of Dr Ian Walshe, Dr Gemma Ryde and Dr Lee Hamilton, with the following exceptions:

- Data collected from Chapter 2 was completed alongside the wider Daily Mile research team and contributions have been acknowledged in the Chapter 2 title page, as per the CRediT author statement and in the associated publication: <https://doi.org/10.1186/s12916-018-1049-z>

Neither the thesis, nor the original work contained therein have been submitted to this or any other institution for a higher degree. This thesis does not infringe upon anyone's copyright and any material from the work of others included in this thesis is fully acknowledged in accordance with common reference practices.

Signed:

A handwritten signature in black ink, appearing to read 'Ross Chesham'.

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Abstract

Breaking up sedentary time has increasingly become an important target area for interventions aimed at improving markers of health. Many studies use various frequencies, intensities, times and types of activity to break up sitting with physical activity. However, the optimal configurations of physical activity breaks to improve markers of health across the lifespan is unclear. Many studies have assessed the effects of intermittent breaks in apparently healthy children, adults, and older adults, but fewer have assessed the response to interventions where a single bout of continuous physical activity is used to break up a sitting day. Therefore, the overall aim of this thesis is to determine the effects of continuous physical activity breaks used to break up sedentary time - in different aged populations - on markers of health.

Chapter 2 examines the responses to the Daily Mile intervention which was anecdotally reported to have several physiological benefits including increased physical activity, reduced sedentary behaviour, increased fitness and improved body composition. It determined that the Daily Mile intervention is effective at increasing levels of moderate-to-vigorous intensity physical activity (MVPA), reducing sedentary time, increasing physical fitness and improving body composition in primary school children.

Chapter 3 moves onto the adult population. Using an RCT design, it primarily examined the cardiometabolic responses to two energy matched bouts of physical activity (consisting of light-intensity physical activity (LPA) and moderate-intensity physical activity (MPA)) and a control in a sedentary inactive adult population. It primarily determined that interstitial glucose was higher during the MPA condition than during the sitting (SIT) condition, whereas an energy-matched LPA bout was not different to SIT. Chapter 4 builds on Chapter 3, utilising an RCT to explore the same LPA intensity, but focusses on determining whether a dose-response exists when comparing different lengths of LPA intensity in health inactive adults. It also secondarily explores whether responses differ depending on whether participants are in older or younger age groups. It determined that 60 minutes of LPA walking is sufficient to lower interstitial glucose in sedentary and inactive adults compared to sitting, with longer duration LPA lowering glucose to a greater extent than shorter duration LPA. It also determined that older adults did not respond to an LPA intervention in the same way as their younger counterparts.

Overall, this thesis contributes novel data to the field of breaking up sedentary behaviour by examining physical activity strategies to improve markers of health across the lifespan. It identifies that continuous physical activity breaks have the potential to influence markers of health in children and adults and that older adults may not respond in the same way as their younger counterparts.

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List of abbreviations

ALM	Appendicular lean mass
BMI	Body mass index
cGlucose	Capillary glucose
CI	Confidence Interval
DBP	Diastolic blood pressure
DEXA	Dual energy x-ray absorptiometry
FITT	Frequency, intensity, time and type
HDL	High-density lipoprotein cholesterol
IPAQ	International Physical Activity Questionnaire
I-PANAS-SF	International Positive and Negative Affect Schedule-Short Form
ISAK	International Society for the Advancement of Kinanthropometry
iFGM	Interstitial flash glucose monitor
iGlucose	Interstitial glucose
LPA	Light-intensity physical activity
LMPA	Light-to-moderate intensity physical activity
LDL	Low-density lipoprotein cholesterol
MET	Metabolic equivalent of task
MPA	Moderate-intensity physical activity
MVPA	Moderate-to-vigorous intensity physical activity
PCA	Perchloric acid
PA	Physical activity
PPHSQ	Pre-Participation Health Screening Questionnaire

PVG	Protecting Vulnerable Groups
RCT	Randomised controlled trial
RPE	Rating of perceived exertion
RMR	Resting metabolic rate
SIMD	Scottish Index of Multiple Deprivation
SWEMWBS	Short Warwick-Edinburgh Mental Wellbeing Scale
SIT	Sitting
SES	Socioeconomic status
SD	Standard deviation
SE	Standard error
SMD	Standardised mean differences
SBP	Systolic blood pressure
tAUC	Total area under the curve
TC	Total cholesterol
TRIG	Triglycerides
vGlucose	Venous glucose
vInsulin	Venous insulin
VPA	Vigorous-intensity physical activity
WHR	Waist-to-hip ratio

List of publications

Chesham, R. A., Booth, J. N., Sweeney, E. L., Ryde, G. C., Gorely, T., Brooks, N. E., & Moran, C. N. (2018). The Daily Mile makes primary school children more active, less sedentary and improves their fitness and body composition: a quasi-experimental pilot study. *BMC medicine*, 16(1), 64. <https://doi.org/10.1186/s12916-018-1049-z>

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Booth, J. N., Chesham, R. A., Brooks, N. E., Gorely, T., & Moran, C. N. (2020). A citizen science study of short physical activity breaks at school: improvements in cognition and wellbeing with self-paced activity. *BMC medicine*, 18(1), 62. <https://doi.org/10.1186/s12916-020-01539-4>

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1. Chapter One: General Introduction

1.1. Introduction

1.1.1. Why take an across the lifespan approach?

The three experimental chapters of this thesis include participants from three distinct life stages: children (Chapter Two), young to middle-aged adults (Chapter Three) and younger adults compared to older adults (Chapter Four). The World Health Organization states that sedentary behaviour is any period of low-energy expenditure while awake such as lying, reclining or sitting (2025). They also state that physical activity is any bodily movement produced by skeletal muscles that requires energy expenditure (World Health Organization 2025). Sedentary behaviours are highly prevalent across the lifespan and evidence suggests that physiological responses to replacing sedentary time with physical activity are not uniform across the lifespan. Therefore, this thesis has adopted an across the lifespan approach to examine how replacing sedentary time with physical activity may influence key physiological and health markers within these different age groups.

Additionally, these different age groups often face different physical activity barriers as they generally spend their time in different environments (e.g. school, workplace and retirement). Consequently, it is important to explore these age groups separately to better determine age-specific and environment-appropriate interventions. Moreover, the existing evidence regarding the optimal frequency, intensity, time and type (FITT) of physical activity breaks for interrupting sedentary behaviours is relatively sparse and often inconsistent across the lifespan, especially for lighter intensity physical activities, and the evidence base is even more limited in older adults. By including all three age groups, this thesis aims to contribute to age-specific recommendations, providing more nuanced and potentially more effective guidance for replacing sedentary time with physical activity.

1.1.2. Definitions: Metabolic equivalent of task, physical activity and sedentary behaviour

There are currently well recognised classifications of the intensity of physical activity, which are based on utilisation of energy in each category. The resting metabolic rate (RMR) is the amount of oxygen consumed by the body at rest (Jetté et al. 1990). Specifically, RMR is equivalent to utilising approximately 3.5 millilitres of oxygen per kilogram of body weight per minute (3.5 ml O₂/kg/min), whilst sitting quietly. The measurement term describing the

relative intensity of bodily activity, compared to rest, is metabolic equivalents (Jetté et al. 1990). A metabolic equivalent of task (MET) is the unit used to describe physical activity energy expenditure (Haskell et al. 1985). Therefore, one MET is equivalent to RMR i.e., one MET equals the oxygen consumption or energy expenditure of sitting at rest.

Light-intensity physical activity (LPA) includes activities of ≥ 1.5 METs and < 3.0 METs (World Health Organization 2020b). Moderate-intensity physical activity (MPA) includes activities ≥ 3.0 METs and < 6.0 METS that cause increased body temperature and increased breathing rate and heart rate. Whereas vigorous-intensity physical activities (VPA) are ≥ 6.0 METs and will cause a greater level of each of these responses. Moderate-to-vigorous physical activities (MVPA) include numerous activities from brisk walking to sprinting. In comparison, sedentary behaviour is described as waking behaviour characterized by an energy expenditure ≤ 1.5 METs while in a sitting or reclining posture (Viir and Veraksitš 2012). Sedentary behaviours include activities such as prolonged desk-based sitting and television viewing.

1.1.3. High sedentary time, low physical activity and the associated health risks

Conceptually, sedentary behaviour and physical activity are often viewed along the same movement-intensity continuum. However, they both exert independent and interactive influences on health. Independently, higher sedentary time increases the risk of morbidity and mortality, with higher sedentary durations associated with higher risk, especially above ~ 9.5 hours per day (Ekelund, Tarp et al. 2019). On the other hand, higher levels of total physical activity, regardless of intensity level, are associated with substantially lower premature mortality risk. The greatest risk reduction appears to occur when moving from low to moderate levels of physical activity, demonstrating a non-linear dose-response relationship (Ekelund, Tarp et al. 2019). However, although high levels of physical activity can offset much of the excess mortality risk associated with high sedentary time, evidence suggests this protection is not absolute when sedentary time is very high (Ekelund et al. 2020). For example, research has illustrated that high total physical activity is enough to attenuate most, but not all, of the cancer mortality risk associated with high sedentary time. Supporting this, one wearable device-based study observed that a median of ~ 49 minutes per day of MPA, ~ 4 minutes of VPA, or ~ 100 minutes of LPA was sufficient to attenuate most, but not all, of the cancer mortality risk associated with high sedentary time (Rezende et al. 2025). Together,

these findings indicate that both sedentary behaviour and physical activity contribute independently, but their interaction modifies overall health risk.

High sedentary time, low physical activity and the associated health risks are a widespread globally across the lifespan. According to the World Health Organization (2025) adolescents are any child aged of 10-19 and therefore children are below the age of 10. However, different cut offs are described according to different organisations. For example, the National Health Service UK (2024) states that a child is aged 4-12 years. Furthermore, research from around the world cite different cut-off for children and adolescents. This makes a consistent definition of child age difficult. In this thesis, only children of Scottish primary school age were included in the first experimental chapter and were therefore aged 12 or below, with the youngest being four years of age. Therefore, the research included in this thesis can clearly be defined as including a non-adult population in the first experimental chapter (Chapter Two). Regardless of child-to-adolescent age cut offs, high sedentary time and low MVPA is prevalent in many children and adolescents in the UK and globally.

Recent objective figures suggest that in children across the UK, ~38% of their day is spent in sedentary pursuits (~ 5.4 hours per day) (Hesketh et al. 2022). These figures are even greater in another UK study where at least half of children are estimated to be sedentary for 6.4 hours or more each day (Griffiths et al. 2013). A further study estimated children to be sedentary for > 9 hours per day (77.8%) (King et al. 2011). Time spent in MVPA is also considered to be low in the UK with one study finding less than half of children in the UK achieved the 60 minutes MVPA per day recommended for children and young people (those aged 5-18) by the UK Chief Medical Officer (UK Chief Medical Officers 2019). One UK study specified that children undertook an average of just 20-30 minutes per day of MVPA (King et al. 2011). Findings from the International children's accelerometry database utilising accelerometer data from 20 studies in 10 countries (Cooper et al. 2015), analysed the results from a cohort of individuals aged 2.8-18.4, and found that sedentary time increased with each year after five years of age, and that physical activity (especially light-intensity physical activity) also gradually lowered by 4.2% annually. Together, these studies illustrate the highly sedentary and low physical activity behaviours of many children and adolescents in the UK and globally. A high proportion of adults are also highly sedentary and physically inactive.

Adults in the UK are around 20% less active now than in the 1960s (Public Health England 2016). Over time, technological advancements and societal processes have modernised the societal environment, altered daily routines, increased leisure time and reduced the physical demands on individuals (Bailey 2021), leading to a reduction in time spent in MVPA and increasing time spent in sedentary behaviours. For example, the demands of activities of daily living such as travel and cleaning and the demands of jobs such as farming and manufacturing are now often significantly reduced. As a result, generally, a higher proportion of the world's population is more sedentary and less physically active in the modern era (Bailey 2021).

Measured objectively (using activity monitors), on average, adults in Western society spend a significant portion of their day in sedentary activities: ~ 55% of waking hours (Matthews et al. 2008). Additionally, in 2021, only 64% of adults in England aged 16 and over met the UK adult physical activity guidelines for aerobic activity with levels declining with age (Health Survey for England 2023). Therefore, only 36% of adults were achieving the physical activity guidelines set out by the UK Chief Medical Officers (2019): 150 minutes per week aerobic MPA (or 75 minutes per week of VPA), plus two sessions of resistance exercise. However, the figures from England are likely to overestimate MVPA levels as they relied on self-reported questionnaires, rather than objective measurements like accelerometers. Therefore, the evidence illustrates an adult population where the majority are highly sedentary and physically inactive. The evidence also shows that older adults are also highly sedentary and physically inactive.

According to the World Health Organisation (2016) older adults are generally considered those aged 60 and over. The figures for older adults also show high sedentary time and low physical activity. A systematic review of older people showed that older adults spend 9.4 hours per day being sedentary measured via objective measures: equating to 65–80% of their waking day (Harvey et al. 2015). In a more recent study, older adults spent as much as 65% of their waking hours in sedentary activities and as little as 2% of their time in MVPA (van Ballegooijen et al. 2019). This was supported by further recent multi-centre research across Europe illustrating older adults spent 78.8% of daily awake time in sedentary behaviours and only 2.6% of time in MVPA (Giné-Garriga et al. 2020). In the UK, older adults (which the UK Chief Medical Officers define as 65 years and older) are recommended to partake in the same volume of physical activity as adults (UK Chief Medical Officers 2019). However, the research above illustrates

that these figures are not being achieved by the majority of older adults. The impact of high sedentary time and/or low physical activity levels upon health and mortality rates is highly significant in children, adults and older adults.

In children, high sedentary time is associated with several poorer health outcomes such as decreased fitness scores (Tremblay et al. 2011) and childhood obesity has been reported to increase in a dose response manner alongside increased time spent in sedentary behaviours (Tremblay et al. 2011). High levels of childhood obesity have many negative health effects including an increased risk of being overweight in adulthood (Singh et al. 2008), and a greater risk of metabolic disease (Freedman et al. 2007).

Across the life course, regularly being in a low MET sedentary state for prolonged periods of time, just above RMR, has been highlighted as a significant cause of non-communicable disease deaths worldwide (Lee et al. 2012). Furthermore, a large study of 78,508 participants compared frail adults to adults to their robust and physically active counterparts and assessed all-cause mortality (Yang et al. 2025). The authors determined that individuals characterised by frailty - who also had the lowest levels of total physical activity, LPA, MVPA or the highest levels of sedentary time – were found to have the greatest risk of all-cause mortality.

In addition to increased mortality, when individuals are sedentary over several years, a number of negative health associations become prevalent (Bailey et al. 2019; Ekelund, Tarp et al. 2019; Ekelund, Brown et al. 2019). For example, a systematic review by Wilmot et al. (2012) examined data from nearly 800,000 participants. They observed the relative risk of diabetes development and cardiovascular events were 122% and 147% greater in highly sedentary individuals. Jingjie et al. (2022) also provide further evidence for the increased risk of cardiovascular disease morbidity in individuals with the highest total sedentary time. Their review and meta-analysis included over 1.4 million participants from a diverse range of countries. Notably, those with screen time (just one type of sedentary behaviour) of over 5-6 hours per day had similar cardiovascular disease risk to those with total sedentary time of over 10-11 hours per day. Sedentary time is also associated with an increased risk of diabetes (Wilmot et al. 2012) and persistent increased sedentary time is also associated with increased body fat mass (Macías et al. 2024), which in turn is associated with several chronic diseases. Additionally, negative health associations are also specifically reported in older adults, with

very high levels of sedentary time significantly increasing the risk of stroke in older adults (Qaisar et al. 2025). However, not all health outcomes are physical.

The WHO definition of health is “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity” (World Health Organization 2020a). In addition to increased morbidity and mortality, high sedentary time can affect mental health. Worldwide, mental health conditions also contribute to the burden of non-communicable diseases and increase economic burden on societies (Trautmann et al. 2016). The odds of developing higher levels of anxiety and depression, as well as lower well-being, and are also increased in individuals that are more highly sedentary (Allen et al. 2019; Casanova et al. 2023). Using the WHO definition of health, we can see that being highly sedentary has a detrimental effect on health when considering mortality, morbidity, and mental health.

The exact cut-off in hours, if one exists, where sedentary time becomes associated with impaired health and an increased risk of mortality is unclear (Ku et al. 2018a). However, based on Ku’s 2018 paper, evidence suggests this cut-off may be four or more hours, but the evidence is inconsistent. Considering the evidence in children, adults and older adults presented above, a large volume of individuals in the UK are therefore classed as sedentary. Nevertheless, some of the health risks that occur with high sedentary time can be reduced by participation in physical activity (Ekelund, Brown et al. 2019; Ekelund et al. 2016). Specifically, undertaking physical activity of at least a moderate-intensity for 60 - 75 minutes per day is associated with significantly decreased mortality risk (Ekelund et al. 2016). The volume of 60 - 75 minutes per day is the equivalent to 420 to 525 minutes per week of MPA. More recently, research by Lee et al. (2022) found that, over the long term, a nearly maximum association with lower mortality was achieved by performing 300-599 minutes of MPA or 150-299 minutes of VPA per week (or an equivalent combination of both). Therefore, both papers support that completing more than double the current minimal physical activity guidelines, set out by the UK Chief Medical Officers, provides the most benefit for lowering mortality risk. Together, these papers provide compelling evidence of physical activity benchmarks that can be utilised to design research studies and inform physical activity guidelines in adults and older adults.

1.1.4. Identifying suitable locations to break sedentary time and increase physical activity

There are also several barriers to physical activity in children. The primary barrier for this group is a lack of time to undertake physical activity (Zelenović et al. 2021). Additionally, many children report not engaging in physical activity due to a lack of support (Dias et al. 2015). Furthermore, research has identified a lack of motivation and a lack of energy as a significant obstruction to physical activity (Rosselli et al. 2020). Fear of injury has also been found to discourage some children from being active (Stanish et al. 2015). Furthermore, many children feel that exercise facilities are inaccessible (Butt et al. 2011). A significant barrier is also 'appearance' during exercise (Fernández et al. 2017). Other life pressures such as school obligations also were cited as barriers in some research (Youssef et al. 2013). Lastly, many children simply found that other activities were more fun than engaging in physical activity (Youssef et al. 2013). Many of these barriers can be present within school time, but several of these barriers can be overcome in a school-setting if the school environment encourages and supports students. Sedentary time is highest during classroom time in a child's school day and likely represents the largest period of sitting time throughout their entire day (Ronca et al. 2024). This time therefore presents a significant opportunity to interrupt sedentary time with physical activity breaks, which could potentially have a large impact on health outcomes. Nevertheless, any physical activity break needs to be evidence-based that can be supported and facilitated by schoolteachers and the school environment as a whole.

In adults and older adults, many of the reasons for not achieving sufficient physical activity are due to real and perceived barriers (Leese et al. 2023). Approximately sixteen hours is the typical waking time for adults. If eight of those are at work, and a further two hours are dedicated towards travel to and from work, around six hours remain. Those six hours will contain the usual activities of daily living such as washing and eating, as well as socialising and other leisure time. Evidence shows that in these non-work/travel hours, there are several barriers that prevent many individuals from participating in physical activity (Leese et al. 2023). These include people that perceive the nature of their pre-existing medical condition(s) preclude them from physical activity. Others have a lack of knowledge and understanding of the impact of physical activity on their long-term health. Many individuals also state they simply do not have enough time to perform physical activity. Others perceive a lack of social

support is a barrier, where the competing demands of leisure activities and socialising are prohibitive. Furthermore, others highlight gender based cultural barriers to activity. Additionally, major environmental barriers are prevalent including a lack of facilities and distance from available facilities. In the UK, the unpredictable nature of the weather, especially in the winter, is also cited as a significant barrier (Leese et al. 2023). Many of these barriers will be present in work and non-work environments. However, some of the barriers above are predominantly non-work time barriers, such as competing leisure activities and social interests. Therefore, as personal time can be truly limited – or perceived to be limited – and workplaces are often highly sedentary (Clemes et al. 2014), the workplace is an ideal location for physical activity interventions that also break up sedentary time. The World Health Organisation has identified the workplace environment as a key setting for noncommunicable disease prevention interventions (World Health Organization 2020b) and the highly sedentary desk-based workforce are a crucial target population. A recent review has highlighted the effect of existing workplace interventions on physical activity showing that some effectively reduce sedentary behaviour and encouraged lighter forms of physical activity. They also stated that the available evidence for MVPA interventions remains sparse (Rouyard et al. 2025).

1.1.5. Practicalities of physical activity breaks

Different break patterns can be used to interrupt sitting time, including utilising a single continuous break or using several smaller breaks. Asides from the health benefits of breaking up sedentary time discussed later (1.1.6), further advantages and no detrimental effects of breaking up sitting in a work environment are also evident. For example, when active breaks involve a change in posture a positive effect on low back pain and discomfort can be found (Waongenngarm et al. 2017). Additionally, evidence suggests these breaks may have no detrimental effect on work productivity (Waongenngarm et al. 2017). Further evidence shows that light-intensity treadmill walking breaks reduced postprandial glucose – an important marker of health - without interrupting office work (Crespo et al. 2016). Furthermore, muscle discomfort and eye strains can be significantly reduced with supplementary breaks, without impairing productivity (Galinsky et al. 2007).

Nevertheless, for certain jobs and tasks, frequently interrupting sedentary time has the potential to be disruptive to personal work and productivity, and potentially to colleagues. For example, frequently leaving the desk for walking breaks, or frequently starting and stopping a

treadmill desk workstation, or performing frequent short bouts of on-the-spot exercise, may be distracting to oneself and others. In addition, if each person in a workplace is encouraged to frequently perform short bouts of physical activity, it may prove difficult to appropriately schedule regular breaks, or to share equipment such as a treadmill desks, desk-cycles, or even corridor and room space. Furthermore, if the only option in a workplace is to exercise outside then, depending on the distance required to travel outside, regular intermittent breaks may be prohibitive due to time constraints. Additionally, if regular clothing and shoe changes are required this could further reduce time spent being productive. The same may be said for schools, particularly large schools, where the distance to suitable exercise areas may be far.

In both workplace and school environments, where many of the problems with regular intermittent break may arise, a *single* continuous bout of physical activity (such as on a treadmill desk workstation, desk-cycle or walking/running outside) may be easier to schedule and potentially less disruptive. Compared to intermittent breaks, it is also potentially easier to ensure compliance of a single continuous bout, particularly in remote or unsupervised settings. Regular, away from the desk breaks may also cause interrupted communication, especially where digital communication and collaboration tools are part of the workplace. As a result, a single continuous bout may be more appropriate in certain workplaces and school settings.

Regarding productivity, continuous breaks may be advantageous over intermittent breaks on some occasions, as should colleagues complete a single continuous bout as a couple or group, there may be a 'watercooler effect'. Here, workers may share knowledge, socialise and improve cohesion of the workforce whilst achieving their physical activity goals. In a school environment, a similar effect may occur. Some additional evidence in adults has shown that close physical proximity can improve scientific impact (Lee et al. 2010). Therefore, if breaks are performed together in research facilities/environments, this may foster improved scientific impact, whilst achieving activity goals. Furthermore, evidence shows that fostering informal conversations can help build stronger relationships between coworkers (Zhu et al. 2024). Again, performing activity breaks at the same time has the potential to fulfil a multiple benefit: improved collegiality and physical activity levels, and reducing sedentary time.

Having an activity break for a longer length of time also opens the possibility of changing into appropriate shoes and clothes and travelling to a desired location to break sedentary time,

either by foot, wheelchair or car etc. This not only could provide more mental recovery time but is also an opportunity to potentially seek out green space. This is important as green spaces have been shown to promote positive mental health and creativity (Charisi et al. 2025).

1.1.6. Breaking up school sedentary time with physical activity breaks in children: current evidence and gaps

Some evidence suggests that school-based physical activity interventions can reduce obesity in overweight children regardless of socioeconomic status, pupil age or gender (Ronca et al. 2024). Furthermore, other evidence suggests that school-based interventions can improve students' cardiorespiratory fitness, but the effects may not be consistent between certain groups (Hartwig et al. 2021). For example, different responses to interventions may occur between genders and age groups (Hartwig et al. 2021). Additionally, school-based interventions can have a positive effect on physical activity levels, but the difference may be dependent on intensity (Hartwig et al. 2021). However, a separate review concluded that the effects of active breaks on physical fitness are unclear (Reyes-Amigo et al. 2025). Evidence also suggests that active breaks can reduce sedentary behaviours and increase physical activity without negatively influencing academic achievement in school children (Larose et al. 2024). A further review concluded that school-based programs interrupting prolonged sitting can significantly increase physical activity levels (Amor-Barbosa et al. 2022). Therefore, as the evidence is not yet clear and the optimal FITT of physical activity break(s) for interrupting sedentary behaviours in children is yet unknown, more research is required to determine the impacts of different interventions on markers of health in children.

1.1.7. Breaking up workplace sedentary time with physical activity breaks in adults: current evidence and gaps

Long term interventions in adults that target a reduction on sedentary time, or that reduce sedentary time whilst increasing physical activity, have been shown to improve several cardiometabolic risk markers such as body mass, systolic blood pressure, glucose metabolism and high-density lipoproteins (Hadgraft et al. 2021). However, high quality studies lasting more than 12 months are lacking. Evidence assessing acute responses to physical activity breaks shows that breaking up sitting regularly acutely affects several markers of health in adults including glucose, insulin and blood pressure, with less consistent evidence for triglycerides (Adams et al. 2024; Loh et al. 2020; Quan et al. 2021; Saunders et al. 2018; Yin et

al. 2024). However, to improve cardiometabolic health markers and reap positive health outcomes, the optimal frequency, intensity, time and type (FITT) of physical activity break(s) for interrupting sedentary behaviours in adults is yet unknown (Benatti and Ried-Larsen 2015; Yin et al. 2024). Thus far, experimental lab trials interrupting sedentary time, with the aim of improving cardiometabolic markers of health, have consisted of various physical activity frequencies (e.g. a single continuous or frequent shorter breaks), intensities (light, moderate or vigorous), times (length of break e.g. two minutes or one hour), and types of activities (e.g. walking or cycling) (Gouldrup and Ma 2021; Hadgraft et al. 2021; Loh et al. 2020; Quan et al. 2021; Saunders et al. 2018; Yin et al. 2024).

Early research in adults showed positive glucose responses to LPA and MPA breaks (Healy et al. 2007) while later research showed further strong evidence for the benefits of intermittent breaks (Dunstan et al. 2012). Combined, evidence such as this set the scene for further research into intermittent breaks. However, to date, very few studies have been carried out assessing the same-day metabolic responses of breaking up a sedentary working day, by replacing a section with a single *continuous* bout of LPA or MPA in healthy inactive adults (Gouldrup and Ma 2021; Loh et al. 2020). Nevertheless, the limited available evidence tentatively shows that replacing sedentary time with a *single* continuous break can significantly influence glucose, insulin and triglycerides (Gouldrup and Ma 2021; Loh et al. 2020), but the evidence is not clear. For example, only two randomised controlled trials (RCTs) have compared a continuous bout of MPA to LPA whilst breaking up a full workday in healthy inactive adults (Katsanos et al. 2004; Newsom et al. 2013) with the studies showing different responses to the bouts. This leaves an available gap in the literature comparing MPA to LPA. Furthermore, only four RCTs have explored the effects of a continuous bout of LPA whilst breaking up a full workday in healthy inactive adults (Gao et al. 2024; Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Niedrist et al. 2024; Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. 2024; Katsanos et al. 2004), with only half exploring lipids (Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Niedrist et al. 2024; Katsanos et al. 2004), one exploring insulin (Katsanos et al. 2004) and the responses to glucose were not consistent (Gao et al. 2024; Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Niedrist et al. 2024; Katsanos et al. 2004). Therefore, the responses to LPA are not clear and required further

investigation. Lastly, no RCTs assess the responses to a single continuous bout of LPA breaking up a workday in healthy inactive older adults. Thus, no studies have explored different lengths of LPA in older adults compared to younger adults to determine whether a dose response exists. Therefore, an LPA dose response trial comparing older to younger healthy inactive adults is warranted. As metabolic responses can significantly differ in older compared to younger adults (Chia et al. 2018), such a trial would help to determine whether physical activity guidance for breaking sitting time should be altered according to age group. Overall, much more research is required to ascertain the optimal FITT of a single continuous physical activity break to acutely improve markers of health in different adult populations.

1.1.8. Conclusions and aims:

Globally there is evidence at the population level of increasingly sedentary and inactive behaviours over the past century. Both highly sedentary and inactive behaviours have numerous negative health associations across the lifespan. The benefits of participating in physical activity of all intensities is well-established. However, high sedentary time represents a separate problem to health, irrespective of physical activity levels. For a high proportion of society, the workplace in adults and the school classroom in children represent areas of high sedentary time and often low physical activity. Therefore, the sedentary workplace or school day represents a crucial period for targeting physical activity interventions to break up sedentary time and potentially improve markers of health in children, adults and older adults. However, the data gathered regarding the responses to such interventions is so far inconsistent in adults and children.

Evidence in children reports that when breaking up sedentary time, key markers of health including MVPA levels, sedentary levels, fitness and body composition are not consistently improved between trials. Furthermore, the optimal types of interventions to break up sitting time and improve these markers is not clear. Therefore, more research is required to increase the available evidence for what constitutes effective interventions in children. It was argued above that continuous breaks are one type of intervention that may be effective in a school environment because, in some situations, they may be easier to schedule and potentially less disruptive than intermittent breaks. This thesis will explore a long-term physical activity intervention in children - consisting of a daily continuous activity break within class time - that aimed to improve markers of health including fitness and skinfolds change. Research will

assess whether changes occurred in fitness, body composition, sedentary time and MVPA time (Chapter 2).

Additionally, data gathered by RCTs in healthy inactive adults - following acute interventions aimed at breaking up workday sitting time - have also not determined the optimum frequency, intensity time and type of activity to acutely improve markers of health. The research into the acute effects of intermittent physical activity breaks is more common and is becoming clearer, but the research into single continuous physical activity breaks is less common and the responses are even less clear. There are distinct practical advantages to performing a single continuous break over regular intermittent breaks, in the adult workplace, as previously discussed. Additionally, the acute health marker responses observed after breaking sitting with a continuous MPA break in healthy inactive adults is not well established. However, even less is known in healthy inactive adults about the acute health effects following continuous bout LPA interventions that break up a sitting day. Increasing the knowledge base regarding health markers following LPA interventions will help to inform the worthiness of such interventions, compared to the slightly more established effects of MPA continuous breaks. Furthermore, no RCTs exploring the effects of LPA have been carried out in healthy inactive adults when breaking up a sitting day and responses are therefore unclear. This thesis will explore an intervention that directly compares energy matched bouts MPA and LPA that breaks up a sitting day in adults (Chapter 3). Additionally, this thesis will explore the effects of two different lengths of LPA continuous breaks and determine whether the main study outcomes are influenced according to whether adults are younger or older (Chapter 4).

Researchers must recognise that not all individuals have the same workplace or school environments, which illustrates that one size will not fit all for interrupting sedentary time with physical activity. This thesis will explore breaking up sitting time - in children, adults and older adults – and will add to the toolkit of available evidence that will help to inform future activity recommendations across the lifespan and increase healthcare professionals' educational toolkits.

This thesis therefore has three overall aims, which align to the three experimental chapters:

1. Determine whether a physical activity intervention in children, aimed at replacing a section of sedentary time with activity, is sufficient to improve markers of health (Chapter 2).
2. Investigate whether a single bout of LPA versus an energy matched bout of MPA, used to replace a section of sedentary time with physical activity, can alter key markers of health in adults including glucose, insulin, lipids and blood pressure (Chapter 3).
3. To determine whether a dose response exists (shorter versus longer durations) when adults and older adults undertake a single continuous bout of LPA used to replace a section of sedentary time with physical activity, and whether the responses differ according to age: specifically examining the effects on glucose, lipids, blood pressure and aspects of mood (Chapter 4).

The overall hypothesis of this thesis is threefold. Firstly, participation in a school-based physical activity intervention will decrease sedentary time, increase physical activity, improve fitness and reduce levels of skinfolds in children. Secondly, both LPA and MPA will influence key markers of health, such as glucose levels, in adults. Lastly, the effects of different doses (durations) of LPA on key biomarkers will influence all adults, but younger and older adults will respond differently.

2. Chapter Two: The Daily Mile makes primary school children more active, less sedentary and improves their fitness and body composition: a quasi-experimental pilot study

Contributions

JNB, GCR, TG, NEB and CNM were involved in the design of the study and in seeking funding for it. RAC, JNB, ES, NEB and CNM were responsible for conducting the study. RAC and CNM wrote the first draft of the paper, and CNM coordinated contributions from other co-authors. CNM wrote the analysis plan used for this paper, but RAC and CNM completed all analyses. RAC, JNB, NEB and CNM managed the data collection with input from other members of the study team. All authors made critical comments on drafts of the paper. CNM was the senior author and the corresponding author.

RAC was integral to the completion of the research from day one of data collection particularly in the following areas:

- RAC, alongside CNM, led on liaising with the primary schools for organisation of equipment provision, data collection and education sessions, with RAC increasing responsibility over time.
- RAC was responsible for providing hard copy participant information, consent sheets and data collection sheets during each research phase.
- RAC was also responsible for data processing of psychology data entry (Multidimensional Students Life Satisfaction Scale), collected from participants, but that data is not discussed in this thesis.
- RAC was the individual most responsible for carrying out the logistics of accelerometer charging, setup, provision and collection, as well as all data upload and data processing throughout the research periods. RAC and CNM completed all accelerometer analyses.
- After the first days of shuttle test data collection, performed alongside CNM, RAC subsequently led the team carrying out the shuttle run testing in round one and round two of the research. RAC and CNM completed all shuttle test analyses.
- As an ISAK qualified anthropometrist, on occasions where shuttle testing was completed before anthropometry, RAC supported the primary team carrying out anthropometry. RAC and CNM completed all anthropometry analyses.

2.1. Introduction

The Daily Mile is a physical activity intervention developed at Primary School in Stirling, Scotland, in 2012 (Stirling Council 2015). The initial aim was to improve the fitness of children; although, there have since been many additional benefits anecdotally reported by children, teachers and parents (Stirling Council 2015). These include improved physical activity, sedentary time, physical fitness, body composition, sleep, diet, concentration, wellbeing and obesity levels. However, no objectively measured scientific evidence has yet been gathered on the validity of these reports.

The successful implementation at many schools, maintenance and increasing popularity of the Daily Mile seem to be a result of the simplicity of the activity, the autonomy given to classroom teachers over when they do it during the school day and the pupil determined pace. Since its development, the Daily Mile has been rolled out across the country by the Scottish Government (Scottish Government 2015) It is estimated by Education Scotland that, from local authorities who responded to their query, ~50% of primary schools in Scotland, are already doing the Daily Mile with a further 18% planning to do the Daily Mile soon (personal communication). It has also become popular in the rest of the UK with interest from the UK Government (UK Parliament 2016a; UK Parliament 2016b). Additionally, it has been introduced in The Netherlands, Belgium and parts of the USA with interest from many other countries (The Daily Mile 2016) despite the lack of rigorous evidence on the efficacy of taking part.

Globally, physical activity levels are low (Kohl et al. 2012). Furthermore, low physical activity levels in childhood are predictive of low physical activity levels in adulthood (Telama et al. 2005). The World Health Organisation (WHO) considers policies and interventions to increase physical activity levels to be important in all age groups and that any potential harm of increasing physical activity is outweighed by the associated benefits (Mendis 2014; World Health Organisation 2017). In a recent accelerometer study on children (2-11 years old) from eight European countries, the proportion of children achieving 60+ minutes per day (recommended for 5-18 year olds (Department of Health and Social Care 2011)) of moderate to vigorous intensity physical activity (MVPA) ranged from 9.5-34.1 % in boys and 2.0-14.7% girls (Konstabel et al. 2014). MVPA levels generally decline with age in both genders as sedentary time increases (Esliger and Hall 2008) and are also believed to be influenced by

socioeconomic status; however, systematic reviews are unclear on the consistency of this relationship and more evidence needs to be gathered (Biddle, Atkin et al. 2011). School based physical activity interventions like the Daily Mile are appealing because they include whole classes, therefore reaching many children regardless of socioeconomic status, physical activity level or fitness level. They also break up sedentary time as they occur during lessons and have the potential to reach much larger proportions of the population than opt-in groups like sports clubs. Therefore, understanding the impact of the Daily Mile on physical activity and sedentary behaviour levels is of key importance.

Overweight and obesity rates are of pandemic proportions and considered to be a key target for the WHO (Mendis 2014). In Scotland, 30% of children (29% for boys and 32% for girls) aged 7-11 years were overweight or obese in 2015 (Brown et al. 2016), a figure similar to that in England (Conolly 2015). At the same time, there is evidence of a decline in the performance of children in the 20-metre shuttle run (an indicator of physical endurance fitness) (Tomkinson et al. 2003). Low fitness and / or levels of physical activity in adults and children are associated with a number of risk factors for non-communicable disease and adverse health outcomes including obesity, cardiovascular disease, diabetes, some cancers, low mood and poor cognitive function (Biddle et al. 2004; Lee et al. 2012; Must and Tybor 2005; Vos and Welsh 2010; Weiss et al. 2004). Additionally, both overweight and low-fitness are also known to be related to lower socioeconomic status (Jiménez-Pavón et al. 2010; Wang and Lim 2015). Studies into the impact of the Daily Mile on fitness and body composition are important for understanding its potential to improve public health, including health inequalities, and for developing future public health policies.

The Daily Mile has the potential to impact on key areas of global public health. Whilst published evidence shows a positive health relationship between physical activity, particularly MVPA, and these outcomes (Poitras et al. 2016), it is unknown whether 15 minutes of self-paced exercise, particularly with no expectation of intensity, will provide such benefits. Given the associated loss of academic classroom time (up to 75 minutes per week), it is paramount that evidence investigating the impact of the Daily Mile on each anecdotally reported benefit is gathered to ensure that government policies are appropriate. Furthermore, any associated benefits in physiological health are likely to be small (Foulds et al. 2014) thus it is essential to use gold standard measurement techniques. Therefore, the aims of this study are to assess

the anecdotally reported physiological benefits of participation in the Daily Mile. Specifically, using a repeated measures design and gold standard measurement techniques, we will assess whether the introduction of the Daily Mile into a primary school setting leads to increased MVPA, reduced sedentary time, improved fitness and improved body composition.

2.2. Methods

2.2.1. Study design and ethics

Using the Daily Mile to turn the WHEEL (Wellbeing, Health, Exercise, Enjoyment and Learning) is a school based, quasi-experimental study designed to assess the anecdotally reported benefits of taking part in the Daily Mile. Ethical approval was obtained from the University of Stirling, School of Sport Research Ethics Committee (reference number 760). Approval was also obtained from the Director of Children, Young People and Education at Stirling Council.

2.2.2. Eligibility and recruitment

State primary schools in the Stirling Council areas were eligible for inclusion. Recruiting from only one council area reduced any potential variance in the delivery of education that may have impacted on outcome measurements. Two local primary schools were identified and approached: one that was not doing the Daily Mile but was intending to introduce it; and one that was not doing the Daily Mile and did not intend to introduce it. Both schools had expressed a desire to introduce the Daily Mile although the control school felt that it would not be possible due to the layout of their playground. Both schools had a range of levels of deprivation, although the majority of pupils were from higher socioeconomic quintiles (Figure 1). Participants were children in all years (age 4-12 years) at the time of recruitment.

Once schools had agreed to participate in the study, parents / guardians of children were sent a letter and information sheet about the study with an opt-in consent form. For children in primary 4-7 (age 7-12 years) an additional child consent form was included. Information sessions were held in both schools to allow parents to ask questions and see the study equipment. They were also given the opportunity to contact the research team by email or phone to discuss the study. Information about being able to withdraw from the study at any stage was given in the information sheet, consent form and verbally. All of the children were also asked to confirm verbally that they were happy to take part on each day of testing.

2.2.3. Intervention

The Daily Mile is a school based physical activity intervention made popular by a primary school in the Stirling Council area, Scotland (Stirling Council 2015). It involves children going outside, at a time of the classroom teacher's choosing, for ~15 minutes of exercise at a pace self-selected by each individual child. This is done during normal classroom time and is in

addition to time spent in Physical Education (P.E.) or scheduled breaks. Typically, it involves laps of a football pitch or playground area. Children often talk as they go and perform a mixture of walking and running. Those who run the whole time will complete ~1 mile in 15 minutes. Children wear their normal school clothes; most wear their normal school shoes and jackets are only worn in cold / wet weather. It is completed on most days regardless of weather conditions. A leaflet produced by the originator school, was given to the school implementing the Daily Mile. No additional instructions for initiation of the Daily Mile were given by the research team.

2.2.4. Participant Involvement

The outcome measures selected for study were chosen based on anecdotal reports of the influence of taking part in the Daily Mile. This information had been gathered from the children, their parents and their teachers by the originator school. The research question, study design and specific outcome measures were developed in part during meetings with the head teacher at the originator school who was familiar with this information. After publication, the results from this study will be disseminated to the schools involved as copies of the manuscript, infographics and during question and answer sessions in the schools.

2.2.5. Outcome measures

The primary physiology-related outcome measurements for the WHEEL project were: accelerometer assessed MVPA and sedentary time; fitness assessed using a 20 m shuttle run; and, body composition assessed using skinfolds. Additional measures of waist and hip circumference and handgrip strength, were collected for descriptive and normative comparison purposes only.

2.2.6. Participant assessments

Baseline assessments (before the intervention) were carried out in October 2015 for the intervention school and March 2016 for the control school. Outcome assessments were completed in May 2016 for the intervention school and June 2016 for the control school. Identical protocols and procedures were used at both assessments. They were undertaken by trained fieldworkers. The fieldworkers worked in groups where at least one member of each group was Disclosure checked for Protecting Vulnerable Groups (PVG) Scheme (Disclosure Scotland 2016). Children completed tasks in a random order and were given stickers for

completing each measurement type. Testing sessions lasted between 1-2 hours and were carried out over the course of two weeks depending on class size and school timetable.

ActiGraph accelerometers were used to assess physical activity and sedentary time. Five models of accelerometer were used: ActiGraph wGT3X-BT, wGT3X+, GT3X+, GT3X and GT1M. GT1M and GT3X monitors are comparable when classifying total time spent in specific intensity categories (Hänggi et al. 2013). There is also strong agreement between GT1M, GT3X and GT3X+ accelerometers making it acceptable to use them in the same study (Robusto and Trost 2012). The primary difference between wGT3X-BT and GT3X+ accelerometers is the ability of the wGT3X-BT to communicate wirelessly and all measurements should function identically (personal communication from ActiGraph). Accelerometers were worn around the waist on the left side with the same orientation to standardise position. Children were asked to wear the belt for eight consecutive days during waking hours (except when bathing or swimming). A poster and instruction sheet with visual and written prompts were provided to each child to remind them of how and when to wear the accelerometer. Upon collection by the research team, data was downloaded with the ActiLife 6 software (ActiGraph LLC, USA). Valid measurement required at least 10 hours wear for three days (Penpraze et al. 2006). A 60 second epoch was used and non-wear time was defined as strings of consecutive zeros lasting 60 minutes or more (Cooper et al. 2015). Accelerometer output is in counts per minute (cpm). Evenson cut points (Evenson et al. 2008) were used to define time spent being sedentary (≤ 100 cpm) and time spent in MVPA (≥ 2296 cpm). Accelerometer data was corrected for wear time in addition to gender and age in days on day of testing in the main analysis.

The maximal multistage 20 metre shuttle run test (Léger et al. 1988) was assessed using the Multistage Fitness Test CD (Sports Coach UK, UK) using standard procedure. Cones marked shuttle boundaries and lanes. Between four (younger) and eight (older) children completed the test at the same time depending on age group. Instructions and demonstration of the test was provided prior to each group participating and additional verbal instructions were provided during the test as necessary. The test began at 8.5 km/h and after each minute increased by 0.5 km/h. When children were unable to reach the 20-metre line prior to the bleep twice in a row, they were asked to stop and their level and shuttle score was recorded. Shuttle run tests were performed outside on tarmac. Age corrected VO_2max scores were

created according to Léger et al. (1988) for comparison with other studies (Supplementary Material Chapter 2). However, this corrects for age in years and therefore were not used for the main analysis. Instead, shuttle distance corrected for gender and age in days on day of testing was used to give improved resolution to the correction in the main analysis.

All skinfold measurements were completed with children behind a privacy screen with at least two Disclosure checked, fieldworkers present. Fieldworkers taking skinfolds measurements were trained by an International Society for the Advancement of Kinanthropometry (ISAK) Level 3 instructor prior to involvement in the study and all measurements were taken according to standard ISAK procedures (Norton et al. 2011). Triceps, biceps, iliac crest and subscapular skinfolds were measured using Harpenden skinfold callipers (Baty International, UK). All measurements were taken from the right-hand side with children standing. Where appropriate they stood on an anthropometric box. Skinfolds measurements were summed prior to analysis to give sum of skinfolds (mm). Skinfolds data were corrected for gender and age in days on day of testing in the main analysis. Hip and waist circumference (cm) were measured using Lufkin Executive Thinline Tape (Lufkin Industries, USA) as per ISAK procedures.

Height was measured, to the nearest 1 mm, without shoes using the Leicester Height Measure (Seca, UK) according to standard ISAK procedures (Norton et al. 2011). Body weight was measured without shoes in light clothing to the nearest 0.1 kg using electronic Sensa 804 scales (Seca, UK). Height (m) and weight (kg) were used to calculate BMI ($\text{weight}/\text{height}^2$). BMI z-scores relative for age were calculated using UK 1990 reference data in the LMS Growth add-in for Microsoft Excel (Pan and Cole 2011). Healthy weight was defined as BMI z-score < 1.04; overweight as BMI z-score 1.04-1.63; obesity as BMI z-score ≥ 1.64 . Handgrip strength in kilograms (kg) was measured using a handheld dynamometer (Takei Scientific Instruments Co, Ltd., Takei Digital Grip Strength Dynamometer Model T.K.K. 5001, Niigata, Japan) as per manufacturer instructions.

All anthropometric measurements were taken twice and the average taken for analyses. Where there was a substantial difference between the two measurements, a third measurement was taken and the median value was used for analyses.

2.2.7. Additional Information

Schools provided date of birth and postcodes for all consented pupils. Date of birth allowed analyses to be corrected for age on day of testing. Postcode allowed assignment of the Scottish Index of Multiple Deprivation (SIMD) (Scottish Government 2016). SIMD combines data from seven different domains of deprivation into a single score: Income, Employment, Health, Education, Access, Crime and Housing. However, it should be noted that this gives a postcode specific deprivation score which may not reflect that of an individual household.

2.2.8. Statistical analyses

Descriptive statistics, Pearson χ^2 and odds ratios were calculated in Excel 2013. Baseline group frequency comparisons were performed using a Pearson χ^2 test. Baseline group mean comparisons were performed using Student's t-test (uncorrected) or General Linear Model ANOVA (corrected for age at time of testing, gender and age*gender). The main analyses were performed in SPSS Statistics (version 21.0.0.1). General linear model regression analyses with repeated measures were used to investigate the effect of doing the Daily Mile. Analyses of all outcome measures included an adjustment for the common confounders: age at time of testing, gender and age*gender. This controls for the effects of age and gender and any different effects of age in the two genders as well as any differences in length of time in the study. Analyses for MVPA and sedentary time were additionally corrected for accelerometer wear time as a covariate. Analyses were conducted first without and then with a correction for SIMD. Standardised Mean Differences (SMD), or effect sizes, are calculated as change in intervention school relative to the control school as a proportion of the pooled standard deviation of the change. SMD of 0.2-0.5 was considered small; SMD of 0.5-0.8 was considered medium; and SMD of 0.8 or above was considered large (Cohen 1988). No correction for multiple testing was made since all four primary outcome measures were anecdotally reported to be influenced by the Daily Mile.

2.3. Results

Figure 1 shows the study profile. Overall, 77.6% of eligible pupils consented to the study (77.3% in the intervention school and 78.1% in the control school). A total of 371 (247 and 124 at the intervention and control schools respectively) provided at least one measurement in both rounds of testing. All consented children, irrespective of whether they had all measurements or not, are included in Figure 1. For each outcome measurement, the information in Table 1 and Table 2 is based only on children who had measurements at both time points.

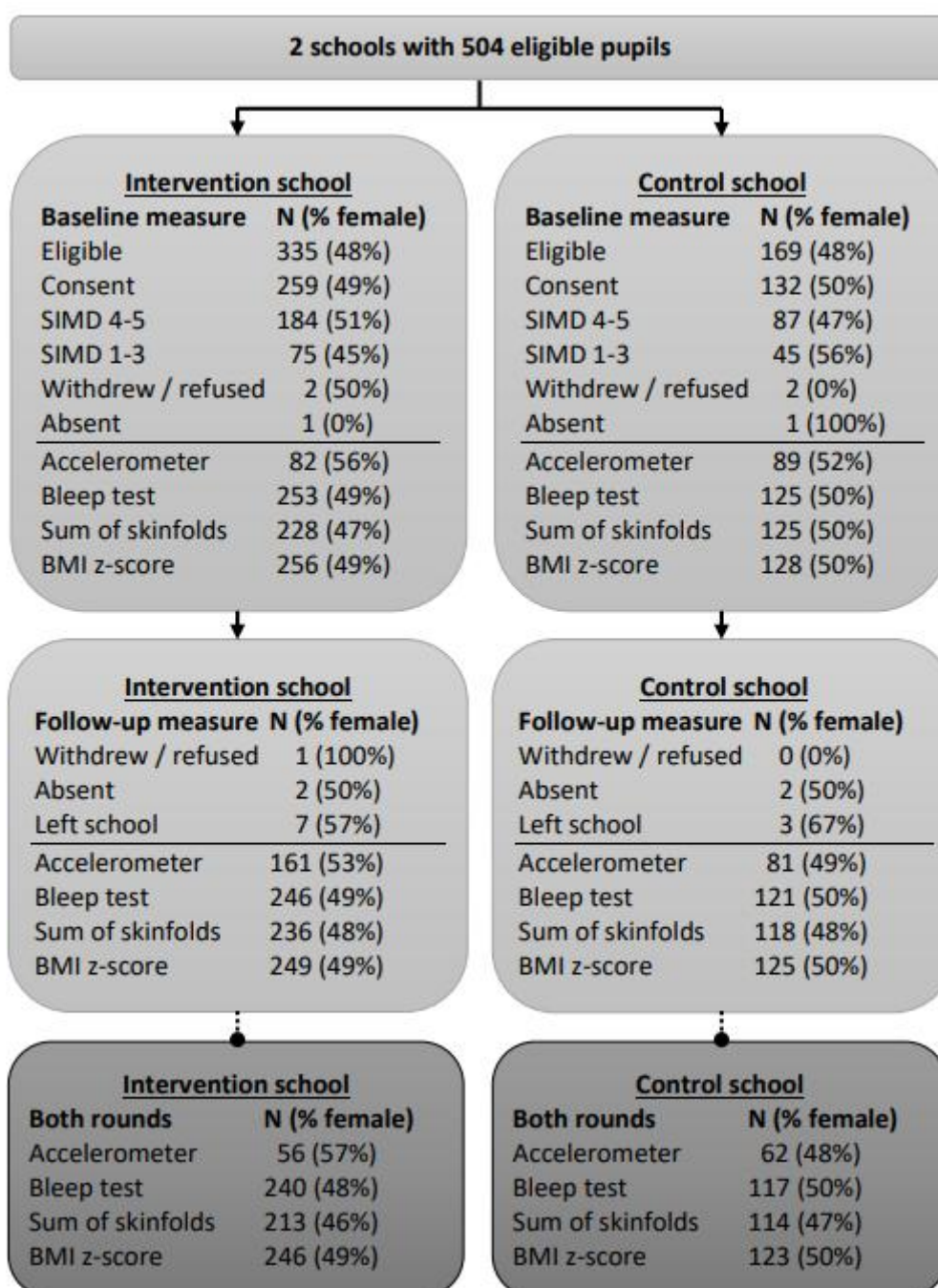


Figure 1 Trial profile. N = number of school pupil participants. The percentage of female participants with each measurement is shown in parentheses. Individual boxes show the totals for each school at baseline, or at follow-up, or the totals for participants with valid measurements in both rounds. Not all pupils had been measured at the baseline (or vice versa), since different pupils were absent at the main and follow-up assessments, some refused to complete certain measurements at each time point and some pupils left or moved between schools. BMI body mass index, SIMD Scottish Index of Multiple Deprivation.

Consent for a complete set of measurements was given in the majority of cases; although, on the day of testing, some pupils did not wish to give verbal consent for skinfolds measurements, had worn inappropriate clothing for some skinfolds measurements or were unable to complete individual tests due to pre-existing minor injuries or ailments. In total, at both baseline and follow-up between 84% and 94% of originally consented participants had data on fitness and body composition outcomes and the proportions were similar at both baseline and follow-up, in intervention and control schools and in males and females (Figure 1).

Three pupils also refused to wear accelerometers. The proportion of pupils with valid accelerometer data was lower than other measurements although the proportion of males and females was similar. Only 32% of participants in the intervention school and 67% in the control school had valid accelerometer data at baseline whilst at follow-up 65% and 64% respectively had valid accelerometer data (Figure 1). This was partially because of the requirement that wearers had at least ten hours of valid wear time on at least three days (Evenson et al. 2008). However, due to a desire to start doing the Daily Mile as soon as possible in the intervention school and a limited number of accelerometers (117) it was only possible to collect true baseline data (*i.e.* prior to beginning the Daily Mile) from a portion of the intervention school participants. These participants had been selected at random based on the availability of pupils for the other physiological tests.

*Table 1 Baseline characteristics of participants by study group and gender. Values in columns 2–4 are means $\pm$ SD (n value) or percentage (n value). Comparisons are by t test or χ^2 and odds ratio. Corrected values are from ANOVA including correction for age, gender and age*gender. Shuttle distance is given to the nearest metre. Accelerometer minutes are given to the nearest minute. It was not possible to correct percentage values for age and gender. MVPA and sedentary time were also corrected for wear time. This table includes only participants with valid measurements both before and after the intervention. MVPA moderate to vigorous intensity physical activity, OR odds ratio, SD standard deviation, SMD standardised mean difference.*

	Total		Males		Females		Intervention vs Control School (uncorrected)	Intervention vs Control School (corrected)
Age (years)	8.4 $\pm$ 2.0 (379)		8.3 $\pm$ 2.0 (192)		8.4 $\pm$ 1.9 (187)		t = 3.18	
Intervention School	8.1 $\pm$ 2.0 (252)		8.2 $\pm$ 2.0 (129)		8.0 $\pm$ 1.9 (123)		SMD = 0.346	
Control School	8.8 $\pm$ 1.8 (127)		8.6 $\pm$ 1.8 (63)		9.0 $\pm$ 1.8 (64)		p = 0.002	
Daily MVPA (mins)	55 $\pm$ 22 (118)		60 $\pm$ 20 (56)		50 $\pm$ 22 (62)		t = 0.766	F = 1.982
Intervention School	53 $\pm$ 22 (56)		53 $\pm$ 19 (24)		53 $\pm$ 24 (32)		SMD = 0.141	SMD = 0.258
Control School	56 $\pm$ 22 (62)		66 $\pm$ 19 (32)		47 $\pm$ 20 (30)		p = 0.445	p = 0.162
Daily Sedentary time (mins)	345 $\pm$ 74 (118)		338 $\pm$ 80 (56)		352 $\pm$ 67 (62)		t = 1.142	F = 7.543
Intervention School	337 $\pm$ 77 (56)		327 $\pm$ 84 (24)		344 $\pm$ 71 (32)		SMD = 0.210	SMD = 0.493
Control School	352 $\pm$ 71 (62)		346 $\pm$ 78 (32)		360 $\pm$ 63 (30)		p = 0.256	p = 0.007
Total Shuttle Distance (m)	670 $\pm$ 351 (357)		748 $\pm$ 399 (183)		589 $\pm$ 271 (174)		t = 1.944	F = 0.294
Intervention School	645 $\pm$ 351 (240)		719 $\pm$ 397 (124)		566 $\pm$ 276 (116)		SMD = 0.219	SMD = 0.061
Control School	722 $\pm$ 347 (117)		807 $\pm$ 400 (59)		635 $\pm$ 258 (58)		p = 0.053	p = 0.588
Sum of skinfolds (mm)	35.1 $\pm$ 14.7 (327)		32.6 $\pm$ 14.9 (175)		38.1 $\pm$ 13.9 (152)		t = 2.214	F = 2.117
Intervention School	33.8 $\pm$ 12.7 (213)		31.7 $\pm$ 13.5 (115)		36.4 $\pm$ 11.2 (98)		SMD = 0.257	SMD = 0.169
Control School	37.6 $\pm$ 17.6 (114)		34.4 $\pm$ 17.3 (60)		41.1 $\pm$ 17.4 (54)		p = 0.028	p = 0.147
% meeting PA guidelines	36.4% (118)		46.4% (56)		27.4% (62)		$\chi^2 = 4.29, p=0.038$	
Intervention School	26.8% (56)		25.0% (24)		28.1% (32)		OR=2.251 (1.037-4.884), p=0.040	
Control School	45.2% (62)		62.5% (32)		26.7% (30)			
% overweight or obese	16.8% (369)		18.6% (188)		14.9% (181)		$\chi^2 = 0.01, p = 0.922$	
Intervention School	16.7% (246)		19.0% (126)		14.2% (120)		OR=1.029 (0.578-1.833), p = 0.928	
Control School	17.1% (123)		17.7% (62)		16.4% (61)			
Handgrip (kg)	24.5 $\pm$ 8.7 (362)		25.9 $\pm$ 9.4 (186)		23 $\pm$ 7.8 (176)			
Intervention School	23.9 $\pm$ 8.5 (242)		25.1 $\pm$ 9 (124)		22.5 $\pm$ 7.8 (118)			
Control School	25.8 $\pm$ 9.1 (120)		27.6 $\pm$ 10 (62)		23.9 $\pm$ 7.7 (58)			
Waist circumference (cm)	58.1 $\pm$ 6.4 (361)		58.6 $\pm$ 6.3 (183)		57.6 $\pm$ 6.4 (178)			
Intervention School	57.8 $\pm$ 6 (242)		58.4 $\pm$ 6.3 (123)		57.3 $\pm$ 5.6 (119)			
Control School	58.6 $\pm$ 7 (119)		58.9 $\pm$ 6.4 (60)		58.2 $\pm$ 7.7 (59)			
Hip circumference (cm)	70 $\pm$ 7.6 (361)		70 $\pm$ 7.8 (183)		70.1 $\pm$ 7.4 (178)			
Intervention School	69.6 $\pm$ 7.3 (242)		69.7 $\pm$ 7.8 (123)		69.4 $\pm$ 6.8 (119)			
Control School	71 $\pm$ 8.1 (119)		70.6 $\pm$ 7.9 (60)		71.3 $\pm$ 8.3 (59)			

Baseline characteristics of the participants are given in Table 1. Age was significantly higher in the control school at baseline due to the difference in the time of baseline measurements meaning that the pupils were slightly further through the academic year at the control school (Table 1). Handgrip (kg), waist and hip circumference (cm) were similar between schools at baseline (Table 1). Percentage meeting the physical activity guidelines of 60+ mins MVPA per day and sum of skinfolds were both higher in the control school. However, after correction for age, gender and age*gender, only sedentary time differed significantly between schools at baseline suggesting that the correct confounders were accounted for in the analysis.

Additionally, the intra-school differences between genders and year groups were similar (Supplementary Material Chapter 2: Table S1).

Table 2 Baseline characteristics of participants by study group and SIMD. Values in columns 2–4 are means $\pm$ SD (n value) or percentage (n value). Comparisons are by t test or χ^2 and odds ratio. Shuttle distance is given to the nearest metre. Accelerometer minutes are given to the nearest minute. This table includes only participants with valid measurements both before and after the intervention. MVPA moderate to vigorous intensity physical activity, OR odds ratio, SD standard deviation, SIMD Scottish Index of Multiple Deprivation, SMD standardised mean difference

	Total	SIMD 4-5	SIMD 1-3	SIMD 1-3 vs SIMD 4-5
Age (years)	8.4 $\pm$ 2.0 (379)	8.4 $\pm$ 1.9 (264)	8.4 $\pm$ 2.0 (115)	t = 0.033
Intervention School	8.1 $\pm$ 2.0 (252)	8.1 $\pm$ 2.0 (182)	8.2 $\pm$ 2.1 (70)	SMD = 0.004
Control School	8.8 $\pm$ 1.8 (127)	8.9 $\pm$ 1.8 (82)	8.6 $\pm$ 1.8 (45)	p = 0.974
Daily MVPA (mins)	55 $\pm$ 22 (118)	56 $\pm$ 21 (92)	51 $\pm$ 25 (26)	t = 1.024
Intervention School	53 $\pm$ 22 (56)	52 $\pm$ 19 (42)	57 $\pm$ 29 (14)	SMD = 0.227
Control School	56 $\pm$ 22 (62)	60 $\pm$ 22 (50)	44 $\pm$ 17 (12)	p = 0.308
Daily Sedentary time (mins)	345 $\pm$ 74 (118)	342 $\pm$ 70 (92)	356 $\pm$ 86 (26)	t = 0.879
Intervention School	337 $\pm$ 77 (56)	337 $\pm$ 70 (42)	337 $\pm$ 97 (14)	SMD = 0.195
Control School	352 $\pm$ 71 (62)	346 $\pm$ 70 (50)	378 $\pm$ 70 (12)	p = 0.381
Total Shuttle Distance (m)	670 $\pm$ 351 (357)	702 $\pm$ 368 (252)	593 $\pm$ 294 (105)	t = 2.695
Intervention School	645 $\pm$ 351 (240)	658 $\pm$ 365 (173)	613 $\pm$ 314 (67)	SMD = 0.313
Control School	722 $\pm$ 347 (117)	800 $\pm$ 359 (79)	559 $\pm$ 255 (38)	p = 0.007
Sum of skinfolds (mm)	35.1 $\pm$ 14.7 (327)	32.9 $\pm$ 13.0 (229)	40.4 $\pm$ 16.9 (98)	t = 4.350
Intervention School	33.8 $\pm$ 12.7 (213)	32.4 $\pm$ 11.9 (154)	37.5 $\pm$ 14.0 (59)	SMD = 0.525
Control School	37.6 $\pm$ 17.6 (114)	33.8 $\pm$ 15.0 (75)	44.8 $\pm$ 20.0 (39)	p < 0.001
% meeting PA guidelines	36.4% (118)	39.1% (92)	26.9% (26)	$\chi^2 = 1.304$, p=0.253
Intervention School	26.8% (56)	26.2% (42)	28.6% (14)	OR = 0.573 (0.219-1.500), p = 0.260
Control School	45.2% (62)	50.0% (50)	25.0% (12)	
% overweight or obese	16.8% (369)	12.4% (259)	27.3% (110)	$\chi^2 = 12.291$, p < 0.001
Intervention School	16.7% (246)	12.4% (178)	27.9% (68)	OR = 2.660 (1.520 to 4.655), p < 0.001
Control School	17.1% (123)	12.3% (81)	26.2% (42)	

SIMD scores were similar across schools ($\chi^2 = 1.299$, p = 0.254): for quintiles 4-5 (least deprived), 71% versus 65% respectively for the intervention school and control school; and for quintiles 1-3 (most deprived), 29% versus 35%. These reflect lower deprivation than average across Scotland (Scottish Government 2016) where ~20% would be expected in each quintile. However, this excess of children from less deprived areas reduces the likelihood of observing an impact of the Daily Mile, rather than creating any potential artefacts, as the children are more likely to be fitter and less likely to be overweight or obese at baseline (Jiménez-Pavón et al. 2010; Wang and Lim 2015). Nonetheless, children from areas with lower socioeconomic scores (quintiles 1-3) had similar minutes of MVPA and sedentary time compared to those with higher socioeconomic scores (quintiles 4-5). They were also equally

likely to meet the physical activity guidelines. However, they had lower shuttle distance, higher sum of skinfolds and rates of overweight and obesity (Table 2). A full breakdown of baseline characteristics by socioeconomic group, school and gender is given in Supplementary Material Chapter 2: Table S2.

*Table 3 Effect of introducing the Daily Mile on outcomes assessed immediately after the end of the intervention period. SMD is calculated as the change in the intervention school relative to the control school as a proportion of the standard deviation of the change. Analyses were conducted using GLM-ANOVA corrected for age, gender, age*gender $\pm$ SIMD with repeated measures for the outcome. Sedentary time and MVPA were also corrected for accelerometer wear time. 95% CI 95% confidence interval, MVPA moderate to vigorous intensity physical activity, SIMD Scottish Index of Multiple Deprivation, SMD standardised mean difference.*

Outcome	Difference in change between schools after correction for age, gender and age*gender			Difference in change between schools after correction for age, gender, age*gender and SIMD		
	Mean (95%CI)	SMD	p-value	Mean (95%CI)	SMD	p-value
Mean MVPA per day (mins)	9.1 (5.1 to 13.2)	0.407	0.027	9.5 (5.4 to 13.5)	0.422	0.021
Mean sedentary time per day (mins)	-18.2 (-10.7 to -25.7)	0.437	0.017	-18.1 (-10.6 to -25.6)	0.435	0.018
Total Shuttle Distance (m)	39.1 (21.9 to 56.3)	0.236	0.037	37.2 (20.1 to 54.3)	0.225	0.046
Sum of skinfolds (mm)	-1.4 (-2.0 to -0.8)	0.246	0.034	-1.4 (-2.0 to -0.8)	0.258	0.026

In the main analysis, with adjustment for the common confounders of age, gender and age*gender significant improvements were observed in the intervention school relative to the control school for MVPA (+9.1 mins), sedentary time (-18.2 mins), fitness (+39.1 m) and body composition (-1.4 mm; Table 3). These relationships persisted after including a correction for SIMD (Table 3). Baseline values and change in BMI z-score and age corrected $\dot{V}O_{2max}$ scores are given in Supplementary Material Chapter 2: Tables S1-S3 only for comparison with other studies.

Change in MVPA predicted change in sedentary behaviour ($r = -0.559$, $n = 118$, $p < 0.001$; Figure 2a) but did not predict changes in other primary outcome variables. Change in shuttle distance predicted change in sum of skinfolds ($r = -0.203$, $n = 317$, $p < 0.001$; Figure 2b) but did not predict changes in other primary outcome variables. These correlations were not significantly altered by the inclusion of SIMD ($r = -0.564$ and -0.212 respectively) although change in MVPA additionally predicted change in shuttle distance ($r = 0.187$, $n = 115$, $p = 0.046$). However, the relationship between change in sum of skinfolds and change in shuttle distance differed by school (interaction $p = 0.043$). The relationship was stronger at the

intervention school than the control school ($r = -0.245$ and -0.046 respectively). The relationship between change in MVPA and change in sedentary behaviour was not different by school (interaction $p = 0.896$). However, it should be noted that the calculations for MVPA and sedentary time are linked by the finite number of minutes in a day and a change in one is likely to result in a change in the other.

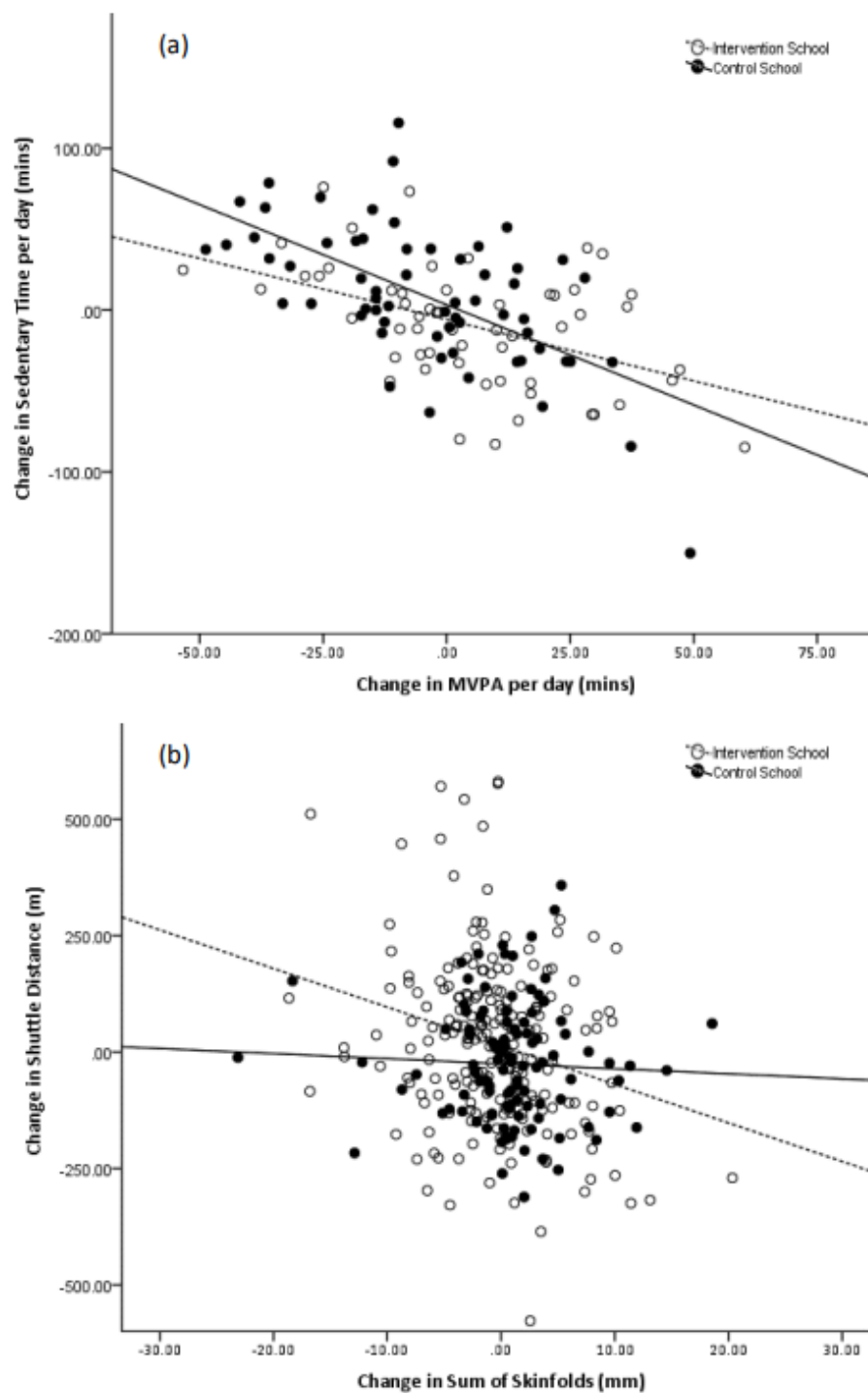


Figure 2 Relationship between change in (a) MVPA and sedentary behaviour and (b) shuttle distance and sum of skinfolds. Both graphs are drawn after correction for gender, age in days and gender*age in days. MVPA moderate to vigorous intensity physical activity.

2.4. Discussion

In this primary school based quasi-experimental pilot study which investigated the effects of taking part in the Daily Mile, we found evidence of a positive effect on our four primary outcomes – accelerometer assessed time spent in MVPA, accelerometer assessed time spent in sedentary behaviour, physical fitness and body composition – after correcting for the common confounders of age and gender with or without socioeconomic grouping.

Comparisons with other studies

Whilst no other studies have investigated the effect of taking part in the Daily Mile, some have investigated the effect of increasing physical activity throughout the school day or introducing short physical activity breaks into the school day itself (Barr-Anderson et al. 2011; Norris et al. 2015). On the whole they have had mixed results with some finding alterations in MVPA, others not. This is likely in part due to the different methods used to assess these behaviours, in part due to the different interventions involved and in part due to the different accelerometer cut-points that can be found in the literature. Additionally, some studies use self-reported physical activity measures which although easier to administer on a large scale can lead to differing estimates in comparison to accelerometry (Reilly et al. 2008). Undoubtedly, the age and demographic of the children also has an influence and an intervention that works in one setting may not work in another.

Similarly, some studies have found changes in body composition and / or fitness whilst others have not (Dobbins et al. 2021; Harris et al. 2009; Mei et al. 2016). The observed effect of the Daily Mile on fitness in the current study may be a result of the type of intervention activity involved (*i.e.* running) being similar to the fitness test. However, few studies have taken detailed physiological measurements and often only assess BMI. Changes in BMI are observed with some physical activity interventions but mostly in high-BMI groups. The decrease in the sum of skinfolds observed in this study without a concomitant change in BMI z-score is likely due to the higher resolution of skinfolds and its utility in assessing body fatness without the confounding effect of muscle mass.

The similarity in handgrip strength, waist circumference, and hip circumference between schools at baseline suggests that participants' values were comparable with regard to these functional and anthropometric characteristics, thereby supporting the internal validity of the

study. Additionally, these characteristics were consistent with normative reference data from other European research in children (McCarthy et al. 2001; McCarthy et al. 2010; Tomkinson et al. 2018).

Meaning of study findings

Scottish Government figures suggest that 73% of children in Scotland (77% of boys, 69% of girls) meet the physical activity guidelines (Brown et al. 2016). However, this figure is based on self-reported questionnaire results rather than accelerometer assessment and is likely to contain bias (Basterfield et al. 2008; Ekelund et al. 2011). Estimates of physical activity by accelerometer vary across Europe, from as low as 2% to as high as 63% of children meeting the physical activity guidelines (Griffiths et al. 2013; Konstabel et al. 2014). The children in this study fall within this range and are likely typical of Scottish and European primary school children (Inchley et al. 2007; Inchley et al. 2016). Regardless of how many meet the minimum recommended guidelines (at least 60 mins per day), higher levels of MVPA are generally considered to be better. This study shows that introducing the Daily Mile into a primary school setting does increase the MVPA of children by 9.1 minutes (SMD = 0.407). Although the Daily Mile is a 15-minute physical activity intervention, an increase of ~9 minutes is consistent with the pattern of running interspersed with periods of walking and chatting that is observed in children taking part (personal observations). Although the SMD would be considered to be small according to Cohen (Cohen 1988), small effects on a prevalent behaviour, such as physical inactivity, may have a high impact at the level of population health (Biddle, O'Connell et al. 2011). In addition, a change of this magnitude is close to the 10-minute increase in MVPA previously associated with meaningful reductions in cardiometabolic risk in children and adolescents (Ekelund et al. 2012).

Sedentary time is less well studied than physical activity. Nonetheless, the available evidence suggests that the children in this study are typical of European children (Griffiths et al. 2013; Konstabel et al. 2014). In some studies, sedentary time appears to be a predictor of chronic disease independent of physical activity levels (Young et al. 2016). Two aspects of sedentary behaviour appear to be key to this: total sedentary time and prolonged blocks of sedentary time. The Daily Mile is potentially able to address both these issues although the present analysis only investigates total sedentary time. Although children at the intervention school were less sedentary (after correction for the common confounders) at baseline, this would

make is harder to observe a reduction in sedentary behaviour rather than easier. Despite this, this study shows an ~18 minute reduction (SMD = 0.437) in average daily sedentary time with the introduction of the Daily Mile. Again, this is consistent with a target of 15 minutes of physical activity where children will at least be up from their chairs for a slightly longer period. However, if done correctly, the Daily Mile also breaks up sedentary time as it should happen in the middle of lessons when children are likely to be sitting before and after their Daily Mile. Similar to MVPA, the SMD would be considered to be small but may well have significant impacts on population health due to mass participation. Additionally, the data also show a strong correlation between increasing MVPA and reducing sedentary time. This suggests that children are not compensating for the increase in MVPA during the Daily Mile by sitting more at other times of day: they are replacing sedentary time with MVPA. However, it should be noted that the calculations for MVPA and sedentary time are linked by the finite number of minutes in a day and may be more appropriately analysed in future studies using a compositional data analysis.

The IDEFICS study (De Miguel-Etayo et al. 2014) children have median values of age corrected $VO_2\text{max}$ scores between 46.7-48.1 $\text{ml/kg}^{-1}/\text{min}^{-1}$ for boys and 45.4-47.4 $\text{ml/kg}^{-1}/\text{min}^{-1}$ for girls between the ages of 6-9 years. Relatively, the children in the current study could be considered to have high aerobic fitness (see Supplementary Material Chapter 2: Tables S1-S2 for age corrected $VO_2\text{max}$ scores). This high baseline fitness would make it less likely that a change in fitness could be observed after a small increase in physical activity. Nonetheless, an improvement in fitness, as measured by shuttle distance (39.1 m, SMD = 0.236), was observed with the introduction of the Daily Mile. $VO_2\text{max}$ is linked with cardiovascular health and all-cause mortality (Harber et al. 2017). Although the SMD would be considered to be small it may have a significant impact on a population scale. The CARDIA study in young adults suggests that having a $VO_2\text{max}$ 3.5 $\text{ml/kg}^{-1}/\text{min}^{-1}$ (~1 metabolic equivalent) higher gives a reduction in all-cause mortality of ~15% (Shah et al. 2016). Whilst we only see a relative increase of ~0.35 $\text{ml/kg}^{-1}/\text{min}^{-1}$ (Supplementary Material Chapter 2: Table S3) with the Daily Mile, this is still predictive of an ~1.5% reduction in all-cause mortality risk. Although it should be noted that the conversion from shuttle distance to $VO_2\text{max}$ includes age in years and is therefore relatively lower resolution. It has also been suggested that having a higher cardiorespiratory fitness at younger ages confers the greatest survival benefit (Harber et al.

2017). Furthermore, those with lower starting values appear likely to benefit to a greater extent (Kokkinos et al. 2010). This suggests that there are potentially useful health benefits associated with taking part in the Daily Mile.

The children at both schools in this study had lower rates of overweight and obesity than are typical of Scottish children. The Scottish Health Survey reports overweight and obesity rates in 7-11 year olds as 30% (29% for boys, 32% for girls) (Brown et al. 2016). Again, this makes it less likely that a change in adiposity could be observed after a small increase in physical activity. Still, a reduction in adiposity as measured by skinfold (1.4 mm, SMD = 0.246) was observed with the introduction of the Daily Mile. Again, although the SMD would be considered to be small, at the population level it may have significant impacts on levels of adiposity. It is also possible that the impact of the Daily Mile on body composition may be larger still in children with higher rates of overweight and obesity. This intervention may be a useful component within measures designed to help tackle the obesity pandemic (Mendis 2014). The strong correlation between those who reduced their skinfolds the most and those who gained the most fitness may indicate a common cause. Given that the pace each child completes the Daily Mile at is self-determined, it is possible that the children who gained the most benefits took a particular approach to the Daily Mile.

Evidence linking socioeconomic status to MVPA and sedentary behaviour is unclear (Biddle, O'Connell et al. 2011). This is in part due to the use of different methods of capturing these outcome measures but also due to different ways of assessing socioeconomic status in different countries. Nonetheless, these outcome measures do appear to associate with specific aspects of socioeconomic status in some studies. However, clear differences between higher and lower socioeconomic groupings could be seen in the current study for fitness and body composition: children from postcodes with higher deprivation had lower levels of fitness, higher sums of skinfolds and higher rates of overweight and obesity. This is consistent with the widely recognised health inequality gap (Truesdale and Jencks 2016). However, no differences were seen between the socioeconomic groupings in response to the introduction of the Daily Mile, suggesting that it may be beneficial to all groups regardless of background. Although it should be noted that this study was not intended to investigate this and larger more powerful studies are needed to investigate this aspect of the Daily Mile.

2.4.1. Strengths and limitations of study

This study is the first to investigate the widely publicised and adopted Daily Mile physical activity intervention. The intervention appears to be increasingly popular and has now been maintained in the originator school for more than five years. Thus, it is undoubtedly feasible to deliver and has been adopted locally in many areas. What was unknown was the efficacy for the anecdotally reported physiological benefits of taking part in the Daily Mile. Consent rates were high (>77% in both schools) as were the number of children successfully assessed at both time points for most outcome measures. We assessed MVPA and sedentary time using the gold standard accelerometer technique. Sixty second epochs were utilised to allow comparison to other major UK/European studies such as the IDEFICS study (De Miguel-Etayo et al. 2014), and matches the epoch used when Evenson cut-points were validated by Evenson et al. (2008). However, the authors recognise that this cut point would not take into account very short bursts of MVPA < 60 seconds. However, considering that minutes/day of MVPA, minutes/day of sedentary time and shuttle run performance were the outcomes of interest for the accelerometers, 60 seconds is justified.

We also assessed fitness using the 20-m shuttle run test (bleep test) which has been validated in this age group. However, several limitations must be acknowledged. Firstly, performance in the 20-m shuttle run test performance can be influenced by factors such as experience. Therefore, anyone performing the test between baseline and follow up, whether at a sports club or at school, may have improved their score irrespective any true change in aerobic fitness. Nevertheless, this limitation applies to children in both schools. Secondly, self-motivation can play a substantial role in 20-m shuttle run test performance. That is, the knowledge of being in the intervention school may have increased some children's engagement or effort beyond the 'normal' influence of partaking in a 20-m shuttle run test. This could have caused them to push to gain higher scores harder. However, considering that children were un-blinded from the start of the study, any influence is likely to have occurred at both baseline and follow-up both, reducing systemic bias. Thirdly, non-physiological reasons may alter a person's willingness to perform maximal effort in most fitness tests. As the 20-m shuttle run test relies on maximal effort to attain accurate estimates, it is possible that slight differences in weather, mood or peer influence could introduce variability in individual scores. Nevertheless, a large sample size likely reduced the influence of individual variation on the

overall findings. Lastly, the 20-m shuttle run test provides an estimate and not a direct measure of cardiorespiratory fitness. However, its criterion validity is moderate-to-high in children, when compared to direct VO_2max testing (Mayorga-Vega et al. 2015). This indicates it is an acceptable field-based measure of aerobic fitness in children and can be used where other more precise laboratory tests are not feasible.

We also assessed body composition using labour intensive skinfolds assessments rather than the more straightforward but lower resolution BMI. The technical error of measurement is a standard index of anthropometric reliability, which is calculated from repeated measurements. It is used to quantify the degree of measurement error i.e. lower TEM indicates greater precision (Ryan-Stewart et al. 2022). According to Ryan-Stewart et al. (2022) those trained to become ISAK Level 1 demonstrate a TEM of <7.5% for skinfolds and <1.5% for all other measures. Therefore, although study-specific technical error of measurement cannot be calculated retrospectively, the use of ISAK trained assessors provides confidence in the reliability of measurements collected in this study. Other research comparing skinfold thickness with DEXA in children aged 7-11 has shown that skinfolds may underestimate true body fat when compared with the gold-standard DEXA method (Noradilah et al. 2016). However, the shortcomings of skinfolds measurements were deemed acceptable as the alternative DEXA assessment was neither viable nor ethical due to the associated minimal radiation exposure and practical constraints in a large school-based field study.

We acknowledge that there was a difference in sedentary time between the schools at baseline and the socioeconomic groupings were not reflective of the whole of Scotland which are limitations of our study. However, these differences would be predicted to make any effects of the Daily Mile harder to observe, not easier. Thus, changes were observed despite these differences. It would have been preferable to assess both the intervention and control schools at the same time of year to avoid any seasonal impact on physical activity. However, we believe that October and March should be similar enough to allow comparison (Atkin et al. 2016). Additionally, it would have been better to have both schools involved in the study for the same length of time. Although, correcting for age and gender should account for this difference. It is also possible that differences in the health and wellbeing policies within the schools contributed to differences in the results. However, the schools were selected to be from the same local authority and to be of similar socioeconomic make up to minimise

potential differences. As predicted, changes in outcome variables had effect sizes at the smaller end of the distribution (0.2-0.5). However, given the involvement of whole classes, small effects could have an important impact on population health. To gain further confidence in the results, this study should be replicated in a larger number of schools. Furthermore, no monitoring of adherence, or level of adherence, to the intervention was carried out although the results suggest this was sufficient. Several aspects of this piece of research were commented upon by the authors Daly-Smith et al (2019) and a response was published by Chesham et al. (2019) addressing their comments and is attached as an appendix (Appendix 8).

2.4.2. Unanswered questions and future research

Additional anecdotally reported benefits to the Daily Mile (cognition, behaviour and wellbeing) are currently being investigated (Booth et al. 2016; Brooks et al. 2016). It is essential that the current studies are replicated in a larger number of schools and countries to ensure that the findings are both robust and repeatable in different educational contexts. Future studies should include diet and sleep quality which we are not yet investigating to explore the potential mechanisms of impact. More attention should be given to when the Daily Mile is being done during the school day and whether it is breaking up sedentary time. Additionally, future studies should investigate whether MVPA and sedentary behaviour are changing on weekdays and / or weekend days.

In 2015, the Scottish Government launched the Scottish Attainment Challenge with the aim of achieving equity in educational outcomes for all Scottish children (Scottish Government 2017). Whilst the current study found no difference in the response to the Daily Mile by socioeconomic grouping, both schools were heavily weighted towards less deprived catchment areas. Furthermore, the current study was not powered to detect such a complex interaction. The Daily Mile is a free, simple intervention that can be rolled out to schools regardless of socioeconomic status. It is necessary to conduct carefully designed studies to understand the impact of the Daily Mile in different socioeconomic settings and to understand whether it can have any impact on the attainment gap.

The sample of children participating in this study included a number with challenging behaviours including autism spectrum disorders. Nonetheless, they took part in the Daily Mile

and our investigations. Understanding the impact of the Daily Mile in children with differing learning needs should also be a future priority.

This study shows the value of introducing the Daily Mile into schools. Whilst the Daily Mile has been introduced as policy across Scotland, many schools do not have appropriate outdoor facilities to allow their children to take part. One of the challenges for policy makers and other stakeholders is to consider how to introduce the Daily Mile or alternative interventions shown to increase MVPA and fitness into such schools; or, how to adapt those schools to allow the introduction of appropriate interventions.

2.5. Conclusions

In conclusion, introducing the Daily Mile to the primary school day appears to be an effective intervention for increasing MVPA and reducing sedentary time and having measurable impacts on key aspects of metabolic health: body composition and physical fitness. This study provides the first assessment of the Daily Mile that will allow the development of evidence-based policy around introducing the Daily Mile to more schools.

3. Chapter Three: Examining the cardiometabolic effects of breaking up prolonged sitting with light versus moderate physical activity in sedentary, inactive adults.

3.1. Introduction

3.1.1. Why explore MPA and LPA breaks?

Numerous negative health effects are reported in individuals who are highly sedentary (Ekelund et al. 2016). These effects are discussed in Chapter 1. Briefly however, the risk of developing diseases such as diabetes and the risk of cardiovascular events are greatly elevated in highly sedentary individuals (Jingjie et al. 2022; Wilmot et al. 2012) and there is a significantly increased risk of mortality (Ku et al. 2018b). Based on the compelling observational study by Ekelund et al. (2016), 60 - 75 minutes of moderate-intensity physical activity (MPA) per day is a useful benchmark for the volume, intensity and frequency of physical activity required for sedentary individuals to offset several negative effects of prolonged sitting. Therefore, exploring realistic and effective strategies for breaking up sitting in an acute setting is warranted.

Despite the well-documented benefits of MPA, light-intensity physical activity (LPA) has received comparatively less examination in the context of breaking up prolonged sitting. This may be surprising given that LPA has several important practical advantages over MPA or vigorous-intensity physical activity (VPA) in sedentary, inactive, desk-based workers: who may be less able or willing to perform MVPA during work hours. For example, prescribing LPA can be particularly beneficial for deconditioned individuals. Although moderate-to-vigorous intensity physical activity (MVPA) is recommended for adults, the American College of Sports Medicine recommends lighter-intensity over higher-intensity physical activity for deconditioned persons (Garber et al. 2011). This is because, at the same absolute intensity, deconditioned individuals will be exercising relatively harder than fitter individuals. In addition, LPA can also help maintain workplace productivity.

In a desk-based workplace, a balance must be achieved between obtaining optimal health benefits whilst maintaining productivity (MacEwen et al. 2015). In this regard, a significant benefit of lighter-intensity over higher-intensity physical activity during work is that it may promote better productivity: a key employer outcome. Light-intensity physical activity (LPA) may be more conducive to productivity for reasons such as 1) higher-intensity physical activity is more likely to induce sweating and so clothes changing may be required and, in this situation, many individuals may simply not wish the interruption/hassle of changing their

clothes, 2) sweating may cause difficulties with keyboard and mouse work, and 3) others may be self-conscious about body odour and/or sweat patches – all leading to reduced productivity and/or reluctance to take part. Therefore, for sedentary inactive individuals, recommending lighter-intensity activity may be practical. Yet, the acute metabolic effects of LPA when delivered as a single continuous bout matched for energy expenditure with MPA, to replace sedentary time, remain scantily researched and poorly understood. Therefore, this chapter will focus on a direct comparison of LPA versus MPA to address the evidence gap and to determine if acute metabolic benefits can be achieved. To test this, appropriate modes of testing must be used.

In workplaces where staying at the desk is preferable to leaving the work environment altogether, treadmill desk workstations can be utilised (MacEwen et al. 2015). When using treadmill workstations, a self-selected walking pace between 1mph and 2mph may be ideal for enabling sufficient typing and mouse performance (MacEwen et al. 2015), therefore helping to maintain productivity. For most individuals, walking at this pace would be considered LPA. The trial by MacEwen et al. (2015) demonstrated MPA paced walking may not be useful for those attempting to remain productive whilst also utilising a mouse and keyboard. Nevertheless, both LPA and MVPA breaks have been shown to improve markers of health such as reductions in postprandial glucose and insulin (Chastin et al. 2015; Dong et al. 2024). However, the evidence base is still growing and the most effective optimal frequency, intensity, time and type (FITT) of physical activity breaks is still unknown (Benatti and Ried-Larsen 2015; Dong et al. 2024). Regardless of the most effective strategy to improve these markers, MVPA cannot always be used to break sitting in a workplace. Therefore, this opens the question of which variations of LPA breaks are acutely effective in eliciting observable health benefits when breaking sitting.

3.1.2. Effects of continuous bouts of physical activity on glucose and insulin

In general, the effects of breaking sitting with several physical activity breaks appears to be more effective in acutely lowering glucose and insulin than a single prolonged bout (Chastin et al. 2015; Dong et al. 2024). However, as regular breaks are not always feasible in a workplace, and MVPA is not always conducive to productivity, exploring the most effective composition of a single continuous bout of LPA is warranted. However, the availability of trials using a single continuous bout of LPA to break up a simulated working day are limited, and

their responses are also inconsistent. After a continuous LPA bout, some trials reported lower glucose levels (Gao et al. 2024; Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. 2024) whereas another reported higher glucose levels (Katsanos et al. 2004).

Additionally, even the effects of a single continuous bout of MPA to break up a simulated working day are inconsistent. Several trials report no difference in glucose levels (Benatti et al. 2017; Farah and Gill 2013; Homer et al. 2017; Katsanos et al. 2004; Maylor et al. 2018; Miyashita 2008; Miyashita et al. 2013; Miyashita et al. 2008; Miyashita et al. 2016; Newsom et al. 2013; Peddie et al. 2013) whereas others reported lower glucose levels (Bhammar et al. 2017; Kim et al. 2014; Marion-Latard et al. 2003; Miyashita et al. 2013) and others reported higher levels (Holmstrup et al. 2014; Kashiwabara et al. 2018; Wheeler et al. 2020). Typically, where same day effects on glucose and insulin were observed, no differences in insulin and glucose were observed the next day pre-prandially.

Previous dose response trials also exist that assessed a continuous bout of LPA versus MPA. However, they either only broke up a small part of the day (Tsetsonis and Hardman 1996), or measured physically active participants (Katsanos et al. 2004; Tsetsonis and Hardman 1996). The only trial to break up a full sitting day with a bout of lighter physical activity versus an MPA bout, in sedentary participants, was by Newsom et al. (2013). However, their lighter bout was 50% VO_2peak and should be considered somewhere between MPA and LPA i.e. LMPA, and they completed a mixture of walking, jogging and cycling in the lighter intensity trial arm. Therefore, it is not known if a single *continuous* bout of LPA walking, which simulates breaking up a full working day ($\geq$ seven hours) of sitting, can alter glucose handling to the same degree as an energy matched continuous bout of MPA in sedentary inactive adults.

3.1.3. Effects of continuous bouts of physical activity on lipids

Understanding how breaking up sedentary time with LPA or MPA acutely affects lipid profiles can help to inform physical activity guidelines aimed at mitigating the negative consequences of prolonged sitting. It has been shown that exercise training causes a significant but modest improvement in total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and very low-density lipoproteins (Smart et al. 2025).

Accordingly, TC, LDL, very low-density lipoproteins and triglycerides decrease and HDL increases.

Evidence is available that shows changes in LDL can occur after two hours and last as long as six hours after a meal (Jadallah and Hammad 2024). However, TC and LDL are less responsive acutely to exercise (Antunes et al. 2020). However, the pool of evidence assessing acute responses of TC and LDL following a continuous physical activity break is very limited (Loh et al. 2020), with most trials showing no changes in either marker.

Triglycerides are also influenced acutely by meals (Tiihonen et al. 2015) with peaks likely to occur between one to four hours after meals (Mikkelsen et al. 2023). Recent evidence from the trial by Antunes et al. (2020), suggests that differences in triglycerides are acutely influenced after exercise. Their trial - in a healthy male population - showed an acute bout (≤ 60 mins) of low, moderate or high intensity exercise, can lower levels of triglycerides immediately after exercise and at 60 minutes after exercise compared to a resting condition. However, a recent meta-analysis concluded that following physical activity breaks, improvements in triglycerides are not currently supported by the literature (Dong et al. 2024). Yet, the authors noted the available studies contained a wide range of prolonged sitting settings, methods of outcome measurement, and degrees of outcome focus. Therefore, the pool of evidence is still unclear regarding triglyceride responses and needs to be expanded.

Of note, a reduction in triglycerides has previously been observed soon after breaking up sitting with a continuous bout of MPA (Farah and Gill 2013; Kashiwabara et al. 2018; Katsanos et al. 2004). However, the evidence for changes after an acute bout of LPA breaking up a simulated workday is limited to one recent trial (Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. 2024), which also observed lower triglyceride values. Evidence also shows that continuous MPA and LPA breaks are impactful enough to alter triglyceride levels the following morning pre-prandially, as changes the next day have been shown elsewhere (Kim et al. 2014; Tsetsonis and Hardman 1996). However, a greater number of studies are available reporting no differences (Homer et al. 2017; Katsanos et al. 2004; Miyashita 2008; Miyashita et al. 2013; Miyashita et al. 2008). Evidence from the trial by Antunes et al. (2020), also suggests that differences in HDL are possible acutely after exercise. However, very little evidence is available for the influence of a continuous MPA or LPA break on HDL cholesterol with only one study observing same day differences in HDL after LPA

exercise (Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. 2024). Overall, the evidence of the acute effects of breaking sitting with physical activity on TC, HDL, LDL, and triglyceride levels in sedentary inactive adults is currently equivocal.

3.1.4. Effects of continuous bouts of physical activity on blood pressure

Physical activity can also acutely influence blood pressure. However, the effect of LPA and MPA walking on post exercise hypotension - for both systolic blood pressure (SBP) and diastolic blood pressure (DBP) - is milder compared to higher intensity aerobic exercise (Elizabeth Carpio-Rivera et al. 2016). To our knowledge, only two trials breaking a simulated workday have assessed blood pressure responses (Bhammar et al. 2017; Miyashita et al. 2008). Both trials saw lower SBP following a bout of MPA exercise. No trials breaking a full day of simulated workplace sitting have explored the effects of a continuous LPA bout on blood pressure responses.

3.1.5. How to compare MPA and LPA effectively?

To compare the effect of different LPA and MPA intensities, matching the energy expenditure of LPA and MPA bouts is necessary. To do this it is necessary to find the intensity of the physical activities per minute: their 'MET minute value'. Basing physical activity conditions on MET minute values is useful as they allow us to approximately match activities for energy expenditure. Using 300 MET minutes of MPA per day as a benchmark - based on previous research (Ekelund et al. 2016; Lee et al. 2022) - we can compare an energy matched bout of MPA to a bout of LPA. This can be done as follows: walking at 1.6 mph (approximately two METs) for 150 minutes would achieve 300 MET minutes (two METs * 150 minutes). Walking at 3.5 mph (approximately 4.3 METs) for 70 minutes, would also achieve ~300 MET minutes (4.3 METs * 70 minutes) (Ainsworth et al. 2011). At both speeds and times above, individuals would achieve ~4 miles walking. The researcher recognises that there are several means of completing physical activity breaks, including cycling, and bodyweight resistance. However, walking has been chosen for this trial as it provides the best balance of accessibility in real world environments and is also easily controlled in a lab environment. Additionally, walking breaks are the most common type of activity break in the literature and therefore utilising walking can be subsequently compared to available evidence.

Overall, both durations and intensities would allow researchers to compare MPA to LPA walking whilst matching energy expenditure. To our knowledge, this was the first lab trial to compare cardiometabolic responses to energy matched bouts of continuous LPA and MPA walking, versus a control, whilst breaking up a simulated workday, in apparently healthy sedentary and inactive adults.

3.1.6. Aims

In a sedentary and inactive adult population sample, this trial primarily examined the metabolic responses of interstitial glucose, venous glucose and insulin to two energy matched bouts of physical activity and a control. This randomised, controlled, three-arm crossover trial (RCT) included: 1) a continuous bout of LPA walking at 1.6 mph for 150 minutes, 2) a continuous bout of MPA walking at 3.5 mph for 70 minutes, and 3) a no-physical activity (sitting only) 'control' condition. The physical activity regimens were approximately matched for MET minutes (~ 300 METs) and distance (~ 4 miles).

Additionally, the study assessed secondary outcomes. These included ascertaining whether any changes occurred following the physical activity conditions compared to the control for 1) lipid profiles and 2) blood pressure.

3.1.7. Hypotheses

Primary hypotheses

1. Interstitial glucose will be lower, compared to a control, for both exercise conditions over 24-hours, over the lab-day, during exercise, after lunch, after snack, after dinner, and during sleep.
2. Venous glucose and insulin will be lower, compared to a control, for both exercise conditions after lunch. No difference will remain the following morning.
3. The effects of MPA will be greater than those of LPA for all biomarkers.

Secondary hypothesis

1. Triglycerides will be lower, compared to a control, for both exercise conditions after lunch and the following morning.

2. Total cholesterol (TC), low density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) will not differ between the three conditions at any time point.
3. Blood pressure will not differ between the three conditions at any time point.

3.2. Methods

3.2.1. Study design

This randomised, controlled, crossover trial took place at the University of Stirling, Scotland, and was approved by the University of Stirling NICR non-NHS projects committee paper no. 68.

3.2.2. Participants

Participants were recruited via posters, social media, University of Stirling online 'portal' and by word of mouth throughout the university and local community. Inclusion criteria: Normal, overweight and obese body mass index (BMI) (≥ 18.5 to < 40 BMI $\text{kg}\cdot\text{m}^{-2}$) men and women, aged 18 to 59, were invited to participate in the trial. Participants were required to have normal blood pressure (Systolic < 140 mm Hg or diastolic < 90 mm Hg), normal blood glucose (< 6.0 $\text{mmol}\cdot\text{L}^{-1}$ after 10 hours overnight fasting) and to sit regularly for $\geq$ five hours a day. Individuals must have done weight training $<$ two times per week and be doing < 150 minutes of moderate-to-vigorous exercise per week for a period $\geq$ three months. Only those who fully understood written and spoken English could begin. Exclusion criteria: having risk-factors, illnesses or injuries stopping safe light- or moderate exercise; having known cardiovascular or metabolic disorders that would affect the main study outcome (blood glucose); tobacco smoking; being pregnant or taking medication that lowered glucose or blood pressure. Volunteers could access these criteria and initially assess their eligibility through participant information sheets, linked via QR codes and hyperlinks to the study research page (Appendix 1: Participant Information Sheet). After initial examination of the participant information sheets, volunteers could then organise to attend an initial research meeting. This initial appointment allowed volunteers the opportunity to ask further questions, before agreeing to attend a subsequent familiarisation session.

3.2.3. Screening and familiarisation

Following initial contact and a period of $\geq$ three days, volunteers attended a screening and familiarisation session. The session began by reiterating the study details and ensuring individuals fully understood the potential risks and benefits of taking part. An opportunity for questions was then provided before participants signed an informed consent form. Copies were available to participants.

3.2.4. Screening

Subsequently, individuals filled in a Pre-Participation Health Screening Questionnaire (PPHSQ) Researchers used this to determine suitability of participants based on medical history, including any medication individuals were taking that might interfere with the main study outcome (interstitial glucose). The International Physical Activity Questionnaire–Short Form (Appendix 2: IPAQ-SF) was then used to determine physical activity (PA) and sedentary status. A PA history questionnaire was also completed by participants, to assess their PA levels in the past year and past five years. This was used to help describe the recruited sample population. After 15 minutes of seated rest, blood pressure was then measured twice using a manual sphygmomanometer (Greenlight 300, Accoson, UK) and the lowest value was used. A third measurement was taken if there had been a change > 2.0 mm Hg between the first two measurements. In this situation, the median value was taken as baseline. If blood pressure was high ($> 140/90$ mm Hg) then repeat measurements were taken on the same morning. If these remained high, then further measures would have been taken under the same conditions on a subsequent day before eligibility could be confirmed: no participants required this. Fasted blood glucose (≥ 10 hours) was then assessed using the finger prick capillary method via single use lancets (Unistik 3, Owen Mumford, UK). If values were ≥ 6.0 mmol $\cdot$ L $^{-1}$ then a second sample would have been taken to confirm this value: none of the study participants had fasted glucose values of ≥ 6.0 mmol $\cdot$ L $^{-1}$ at familiarisation.

3.2.5. Anthropometry

Participant height (cm) was measured using a Leicester Height Measure (Marsden Weighing Machine Group Ltd, UK). Body mass (kg) was measured using Seca 869 scales (Seca GmbH & Co. KB., Germany). Hip and waist girths (cm) were measured using Lufkin Executive Thinline Tape (Lufkin Industries, USA). Anthropometry was performed by the main researcher, who was also qualified to International Society for the Advancement of Kinanthropometry (ISAK) level 1, and carried this out according to protocol (ISAK, www.isak.global). All measurements were taken within two weeks of starting the first experimental period.

3.2.6. Familiarisation

To ensure participants were safe whilst exercising during the trial, they were familiarised with the two chosen treadmill speeds (1.6 mph and 3.5 mph) for a period of five minutes (HP

Cosmos Pulsar Treadmill, HP Cosmos, Germany). The use of Polar heart rate monitors (H7, Polar Electro, Finland) and the BORG 6-20 rating of perceived exertion (RPE) scale was explained at this time. The process of obtaining blood samples via venepuncture, use of FreeStyle Libre flash glucose monitoring system (Abbott Laboratories LTD, UK), application of a protective hypoallergenic translucent film (Tegaderm, 3M Company, USA) and the use of ActivPAL 4 (PAL Technologies Ltd, UK) and Actigraph (ActiGraph LLC, USA) was described to participants. Individuals then received information sheets regarding the use of this equipment. A research diary was also provided so individuals could record PA, food intake and protocol adherence.

3.2.7. Randomisation

A randomised design was utilised. The eight participants were assigned to one of six possible treatment orders, undertaking one of three exercise conditions on each occasion: 150 minutes of sitting, 150 minutes of light-intensity walking (LPA), or 80 minutes of sitting followed by 70 minutes of moderate-intensity walking (MPA). The sequence of randomisation was generated prior to participant recruitment. Once a participant had completed familiarisation, the next sequential randomisation sequence available was assigned to them.

3.2.8. Study design: restrictions

Participants were required to attend three research periods, each lasting five days (Figure 3). Individuals were given a set of instructions to follow over the five days of each research period and additional instructions were given on the lab-day. On day one, participants were asked to begin avoiding MVPA and asked to take minimally active transport options such as the train, bus or car. On day two, participants were asked to begin avoiding alcohol and to wear the monitors provided. On day three, no lab time was required, but participants were additionally asked to start avoiding caffeine consumption. They were also asked to avoid eating or drinking (except water) after 20:00 and told to get a full night's sleep. On day four, during lab time, participants were asked to remain seated/reclined and to minimise shuffling during prolonged sitting periods. They could undertake sedentary workplace activities such as computer work and television viewing during non-physical activity periods, but no standing was permitted. To minimise PA, participants were transported between laboratories and to the toilet via wheelchair. At the end of the lab-day, participants were asked to get a full night's sleep that evening/night. Restrictions were lifted at 08:15 on day five. These instructions were designed

to reduce the respective influences of these activities on the main study outcome (Bird and Hawley 2017; Elizabeth Carpio-Rivera et al. 2016; Kido et al. 2016; Reis et al. 2019; Zhu et al. 2019). Five-day research periods allowed researchers to assess the potential influence of the exercise conditions over 24-hours on IFGM data from 08:00 on day four to 08:00 on day five, which was inclusive of the highly controlled lab period. Attending the lab on day five also allowed a final blood draw and blood pressure to be taken at ~08:15 to 08:20.

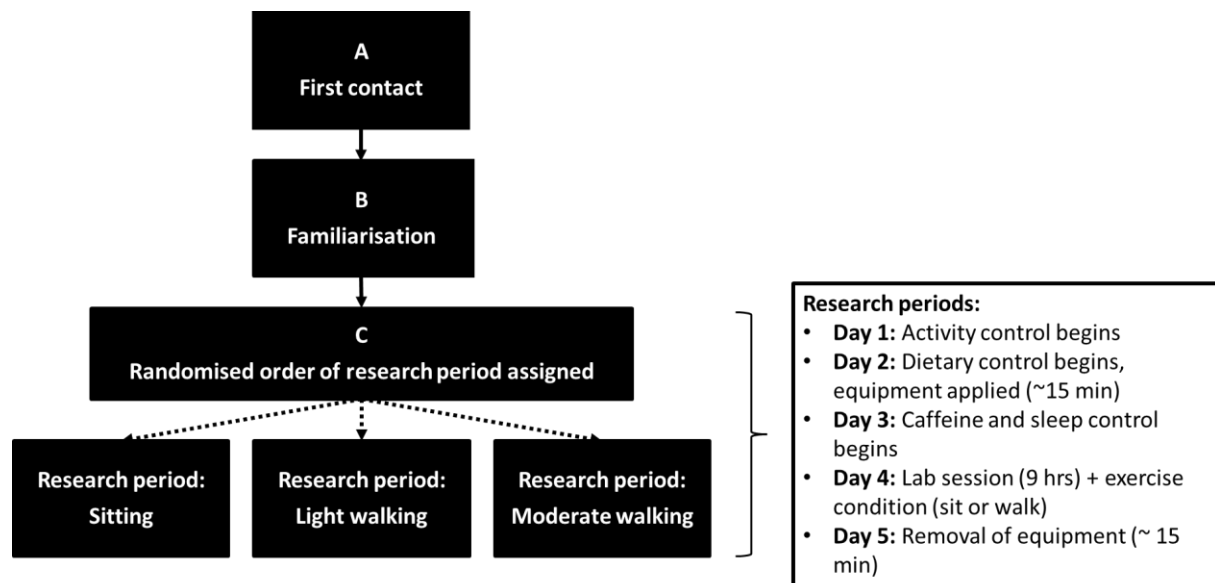


Figure 3 Flowchart of the experimental design (randomised controlled crossover trial), detailing study phases (A, B and C) and providing an overview of research periods and their associated lab-day exercise conditions.

3.2.9. Interstitial glucose monitoring

On day two, a subcutaneous IFGM sensor was applied to the participant's posterior upper arm, on their non-dominant side. Once initialised, the sensor was covered with hypoallergenic film. This reduced the chance of accidental damage to the skin, or removal of the device. The monitor was removed after 08:15 on day five. The memory capacity of the model had a maximum of eight hours. Therefore, the glucose sensor must be read with its monitor within that time to ensure no data loss. Alarms were set to remind participants to record readings. Less than 1% of all IFGM data was lost due to participant data loss, which was due to the participants failing to scan their monitor when the pre-set alarms sounded.

3.2.10. Activity monitoring

An ActiGraph GT3x (ActiGraph, FL, USA) was attached via a belt at the right anterior-superior iliac spine on day two. This activity monitor allowed researchers to validate two details: 1) adherence to PA restrictions out with lab time and 2) intensity of PA and sedentary time during lab time. Once applied, an instruction sheet was provided to encourage proper treatment of equipment. The monitor was subsequently removed after 08:15 on day five. Physical activity booklets and ActiGraph activity monitors confirmed that individuals were adhering to study protocols. This ruled out that individuals were compensating for their sedentary lab-day by performing MVPA after the lab-day.

3.2.11. Experimental (lab-day) protocol

Day four contained the main laboratory-controlled research segment, which ran for nine hours from 08:00 to 17:00 (Figure 4). When participants arrived at 08:00, they began by sitting quietly in the lab. They were asked if they had adhered to trial instructions, and their research diary was examined. Any deviation from the study protocol resulted in trial delay.

After 15 minutes (08:15), blood pressure was taken from the contralateral arm to the IFGM sensor. Immediately after, a venous blood sample was obtained from the antecubital fossa of the ipsilateral side. Participants were then transported to the nutrition lab where they were provided with breakfast at 08:30. They were asked to eat breakfast within 15 minutes. Participants were required to complete the following three conditions in random order: 1) sitting (SIT); 2) light-intensity PA walking (LPA) and 3) moderate-intensity PA walking (MPA) during the nine-hour simulated workday (lab-day).

In the SIT condition, individuals sat from the end of breakfast until 13:00. In the MPA condition, individuals sat until 11:50 when they began walking at 3.5mph for 70 minutes. In the LPA condition, individuals sat until 10:30 when they began walking at 1.6mph for 150 minutes. All walking was carried out on the same treadmill, with room temperature set at 20°C. To describe intensity, heart rate monitors were applied at 10:30 in each condition and BORG scores were recorded every 15 minutes during PA. At 13:00 heart rate monitors were removed, and individuals were transported to the nutrition lab.

Lunch started at 13:15. At 13:30 lunch ended, and participants were returned to the sitting lab where they sat until 17:00. At 17:00 participants walked a short distance to the nutrition

lab (< 10 meters) where they ate the snack provided and ended their controlled lab time. Snacks were eaten by 17:15. They were then free to return home to eat their evening meal between 19:00 and 20:00. This was followed by an overnight fast until 08:15. Participants were asked if they had complied with the protocol and their research diary was checked. At 08:15 a final set of blood pressure and blood samples were taken. By 08:30 the ActiGraph and glucose sensor were removed by the researcher, ensuring electrode removal and that sites were free from irritation or infection. Participants were then free to leave the lab, or eat a meal provided if they chose to do so.

Each of the three research period arms were separated by at least seven days, with a maximum of two weeks separating consecutive periods. The sitting condition was considered the control condition with no increase in energy expenditure or change in posture. The LPA and MPA conditions elicited a change in posture and energy expenditure, with LPA being upright for longer. However, these PA conditions differed in time spent upright as well as intensity, although total energy expenditure was estimated to be similar (~300 MET minutes) and distance the same (~4 miles).

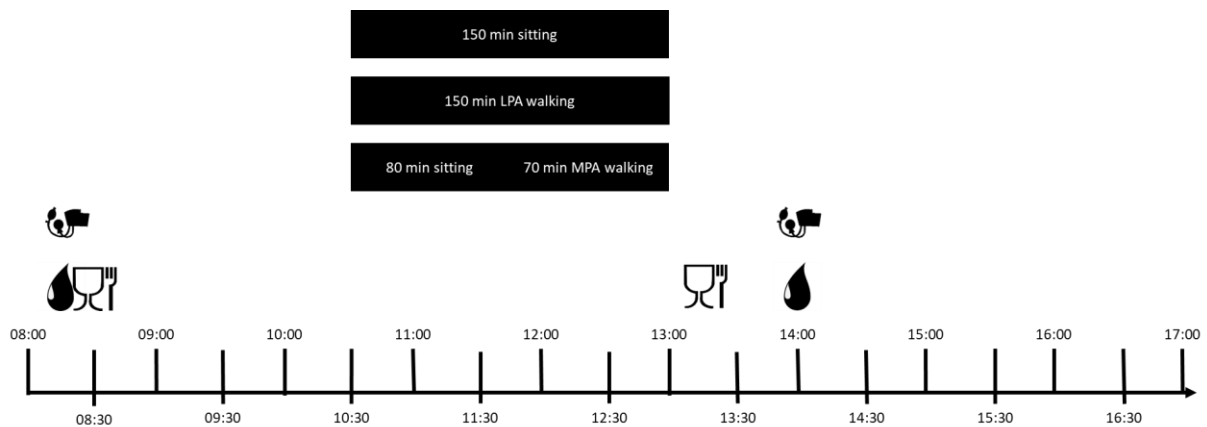


Figure 4 Timeline of the 9-hour lab visit protocol: including two venous blood draws (droplet icons) and two blood pressure measurements (cuff icons) at 08:15 and at 14:00. Participants consumed two standardized meals at 08:30 and 13:15 (cup and fork icons). Participants were assigned to one of six possible treatment orders, undertaking one of three exercise conditions on each occasion: 150 minutes of sitting, 150 minutes of light-intensity walking (LPA), or 80 minutes of sitting followed by 70 minutes of moderate-intensity walking (MPA). This protocol was designed to evaluate the modulating effect of different exercise conditions on the primary study outcomes.

3.2.12. Venous blood samples and blood pressure timing

Three venous blood samples were taken over a 24-hour period. The first sample was taken at 08:15 on day four, the second at 14:00 and the third was taken the next day at 08:15 (+ 24-hours). The post-lunch sample corresponded to 45 minutes after food intake. The timing was chosen as previous data from our lab showed IFGM values commonly peaked between 45 and 60 minutes postprandially in a similar population (data not shown). Additionally, a 2007 study in healthy subjects, using continuous glucose monitors, saw interstitial glucose peaks between 46 and 50 minutes postprandially after different meals (Freckmann et al. 2007). In addition, although plasma glucose concentrations typically peak at 60 minutes after meals (American Diabetes Association 2001), the 45-minute timing may have been able to capture possible early peaks as a result of the exercise conditions. Blood pressure sampling corresponded to the venous blood sample timing and was carried out on the contralateral arm to the IFGM.

3.2.13. Blood samples treatment

Venous blood was extracted into 10ml serum sample tubes (BD Vacutainer, Becton Dickson USA) by a trained phlebotomist. Before tube inversion, 100µl of blood was aliquoted into 1ml 0.4 M perchloric acid (PCA), vigorously shaken and placed in ice water. Subsequently, the serum tubes were gently inverted 10 times and placed on ice for 1 hour. Serum blood samples were then centrifuged (Heraeus Multifuge 3SR+, Thermo Fisher Scientific Inc, UK) at 3500rpm and 4°C for 15 minutes before serum extraction and freezing at -80°C. PCA samples were centrifuged (Heraeus Fresco 21, Thermo Scientific, UK) at 12.5G at 4°C for 5 minutes then frozen at -80°C. When ready for analysis, serum and PCA samples were defrosted and analysed using the Altair 240 Stanbio Chemistry Analyser (Aspect Scientific Ltd, UK). The Altair is advantageous as multiple blood markers can be analysed simultaneously. Glucose, TC, HDL, LDL and triglycerides were analysed. Lastly, insulin was analysed from serum samples using Insulin ELISA Kits (Biosupply UK Ltd, UK), prepared in the Thermo-Mixer HC (Star Lab Group, UK) and read on the BioTek Synergy LX Multi Mode Reader (Agilent, USA) as per equipment manufacturing protocols.

3.2.14. Diet

To control for diet induced outcome variability, food consumption was partially controlled during the research periods, and five standardised meals were provided. On day three, participants were required to eat as normal, except they were provided with their first individualised meal, which was to be eaten between 19:00 and 20:00. This was followed by a period of fasting until breakfast the next day.

The second, third and fourth meals were provided on day four and eaten in the lab at 08:30 (breakfast), 13:15 (lunch) and 17:00 (snack). All meals were prepared and cooked for participants allowing them to remain seated. Water intake was consumed ad libitum throughout the first lab-day and was replicated in subsequent trials. The fifth, and last meal provided, was an evening meal that was identical to the first and eaten between 19:00 and 20:00 on day four. Participants then fasted until 08:15 the next day (day five) when the research period arm ended.

Meal composition closely reflected the average UK adult's dietary composition: 17% protein, 36% fat and 47% carbohydrate (Whitton et al. 2011). A PA factor of 1.4 (reflecting a sedentary

lifestyle) was used to determine individualised energy requirements using Nutritics (Nutritics LTD, Ireland). The Mifflin St Jeor equation was chosen to calculate resting metabolic rate, as it is relatively accurate in healthy obese and non-obese populations compared to other commonly used equations (Frankenfield 2013). Each of the five meals contained 25% of estimated daily calorific needs. On days three to five, participants were required to record everything they ate in their research diary. They could then replicate their energy intake in each subsequent research period. At home, participants were asked to cook their evening meals in the same manner each time. Electronic food scales were provided to ensure accurate measurement of food (CS-2000B Digital Kitchen Scale, Ohaus Corporation, USA).

3.2.15. Power calculation

This pilot study was the first trial of its type: comparing cardiometabolic responses to energy matched bouts of continuous physical activity (LPA or MPA) versus a control, in sedentary and inactive adults over a full simulated workday. As an exploratory trial, it aimed to generate preliminary data to inform future research. To estimate sample size, interstitial glucose data from the trial by Crespo et al. (2016) was utilised to calculate effect size. Sample size calculation was performed in G*Power. The following parameters were entered: effect size (f) = 0.35, alpha error probability = 0.05, power (1-beta error prob) = 0.80, number of groups = 1.0, number of measurements = 3.0, correlation among measures = 0.50, and nonsphericity correction = 0.75. Therefore, this pilot trial aimed to recruit a sample size of 18. However, recruitment was challenging, and the trial was ended after 14 months, with eight participants completing the study.

3.2.16. Statistical analysis

The main outcome of interest was interstitial glucose total area under the curve (tAUC). For IFGM, several different segments were analysed over the lab-day's 24-hours: 1) tAUC over 24-hours (08:00 to 08:00); 2) tAUC during the lab-day (08:00 to 17:00); 3) tAUC within-exercise (10:30 to 13:00); 4) tAUC 3-hours post-lunch start (13:15 to 16:15); 5) tAUC 2-hours post-snack start (17:00 to 19:00); 6) tAUC 3-hours post-evening meal start (19:00 to 22:00) and 7) tAUC during sleep (23:00 to 06:30).

As the first bout of walking began at different times in each condition, data were analysed for the full 150 minutes from 10:30, which is referred to as the 'within-exercise' period. For the

snack period analysis, not enough time was available to do a full three-hour analysis between 17:00 and 20:00, as the subsequent evening meal was eaten between 19:00 and 20:00. Therefore, the analysis was curtailed to stop at 19:00. Additionally, although wake and sleep times varied somewhat per participant, alarms were set at 23:00 and 6:30am for the IFGM and so between this time was categorised as sleep. This was therefore used for the analysis sleep time window, as done in previous trials (Crespo et al. 2016)

The trapezoidal method was used to calculate tAUC (Wolever and Jenkins 1986) using 15-minute measurement windows. The FreeStyle Libre measures glucose levels every minute and automatically stores them as an average over 15 minutes (epochs), so sub-15-minute analysis segments were not possible. Although commonly used in other research, incremental area under the curve was not utilised to assess post-mealtime periods in this study. By only including the area above baseline values, iAUC may overlook important information regarding the overall response of the post-exercise period, such as significant drops below baseline. Ignoring these fluctuations may have led to only a partial representation of the physiological changes. The primary aim of this study was not to isolate the effects of lunch alone (or other meals), but to explore the modulating effect of exercise on specific periods, such as the post-lunch period. Therefore, only tAUC was included in analysis.

The main secondary outcomes of interest included venous measures of glucose, TC, HDL cholesterol, LDL cholesterol, triglycerides and insulin. Additionally, measurements of blood pressure were also performed as secondary outcome measures. These secondary outcomes were analysed as three separate times blood draws. Total AUC (rather than incremental area under the curve) was utilised during their analysis for the same reasons as the primary outcome of interest.

Linear mixed-effects models were used to analyse data as this type of model can account for each subject as a random coefficient in a cross-over trial and has been recommended elsewhere (Bhammar et al. 2017). The primary outcome of interest was interstitial glucose (iGlucose). For the analysis, the baseline value at the beginning of each lab-day was a covariate in the 24-hour iGlucose model analysis and lab-day model analysis. For the within-exercise model analysis, the immediately pre-exercise iGlucose values were used as baseline covariate. Baseline values were not utilised for lunch, snack, dinner or sleep, as this would have masked any potential changes that occurred through the effect of exercise on the main study

outcomes. Exercise condition (SIT, LPA or MPA) were fixed factors in all models. Evidence exists for potential differences in males and females after fasting and after glucose challenges as discussed in the article by (Mauvais-Jarvis 2018). Therefore, gender was always initially included in the models. However, to ensure the simplest model and to avoid over fitting, gender was removed if the fit (assessed via AIC or BIC) or the explanation of variance (via r -squared conditional) was not improved. In addition, the potential impact of treatment order and BMI were also examined. These factors and covariates were removed if they did not improve, or worsened, model fit or explanation of variance. Factors coding was set to 'dummy'. Participants were included as a cluster variable and selected as random coefficient.

The secondary outcomes of interest were venous glucose (vGlucose), TC, HDL cholesterol, LDL cholesterol, triglycerides, insulin and systolic blood pressure (SBP) and diastolic blood pressure (DBP). For the same reasons outlined in the primary outcome of interest, linear mixed-effects models were utilised. The baseline value at the beginning of each experimental lab-day was used as a covariate in their analyses. The choices of which fixed factors and covariates to utilise, as well as coding and clustering, was the same protocol as employed for iGlucose analysis.

In line with best practice guidelines for reporting linear mixed-effects models, effect sizes in this chapter are communicated through the reporting of fixed-effect parameter estimates (β coefficients) and their associated uncertainty, rather than a single standardised metric, as no universally accepted protocol exists for linear mixed-effects models. Meteyard and Davies (2020) argue that there is no single correct way to implement a linear mixed-effects model; instead, the decisions made by researchers during analysis represent one option among many possible alternatives. In this chapter, Cohen's d was not used to estimate effect sizes because standardised mean differences (such as those used in Chapter 2) do not account for clustering of repeated measurements within individuals or covariate adjustment and so are not appropriate for effect size estimation in linear mixed-effects model analysis (Lorah 2018).

Instead, fixed effect estimates are reported for individual independent variables. These fixed-effect estimates represent differences relative to the reference category for each independent variable, as they quantify the magnitude and direction of the association between each independent variable and the dependent variable. Standard errors (SE) and confidence intervals (CI) are also reported to quantify the precision and uncertainty of fixed effect

estimates and reporting these are endorsed as good practice for linear mixed-effects models (Meteyard and Davies 2020). Also recommended by Meteyard and Davies (2020), random effect variances from the final model were reported in full, including participant-level random intercept variance and residual variance.

Additionally, marginal R^2 is reported as a standardised measure of variance explained by the fixed effects in the linear mixed-effects models, representing the proportion of variance explained by the fixed effects taken together (Nakagawa et al. 2013). Conditional R^2 is also reported, as it represents the total variance explained by both fixed and random effects, including between-participant variability. Both measures are recommended and illustrated in the reporting framework proposed by Meteyard and Davies (2020).

Jamovi (Version 2.4.11.0) was utilised for all statistical analyses. Statistical significance was assumed at the level of $p < 0.05$. All p values were reported in fixed effects omnibus tests. However, p values of < 0.10 were reported in parameter estimates and post hoc tests to indicate trends and borderline significance in this explorative analysis. Additionally, if it was reported that a covariate (e.g., baseline or BMI values) was a statistically significant predictor of the dependent variable in fixed effects omnibus tests, the covariate results from the corresponding fixed effects parameter estimates were not reported: as they always followed the same pattern of significance. Post hoc testing was conducted using the Holm method to control for family-wise error rate, offering a balance between preserving statistical power and minimising Type 1 errors (Holm 1979). Assumptions of normality and linearity were assessed using tests of normality of residuals, Q-Q plots, histograms and scatterplots of residuals. No transformation of data was required.

3.3. Results

Participant characteristics are described in Table 4. Between genders, statistical differences were observed for height only ($t(6) = -5.25, p = 0.002$). All eight participants completed all three trial conditions.

Table 4 Participant characteristics split by males and females. IPAQ sedentary hours = International Physical Activity Questionnaire (number of daily self-reported sedentary hours).

	Gender	N	Mean	Median	SD	Minimum	Maximum
Age (years)	Female	5	39	38	13.4	23	59
	Male	3	22	21	3.2	20	26
Body mass (kg)	Female	5	60.6	58	7.9	53.0	70.0
	Male	3	80.0	77	21.7	60.0	103.0
Height (m)*	Female	5	1.62	1.59	0.1	1.58	1.72
	Male	3	1.83	1.82	0.0	1.80	1.86
Body Mass Index	Female	5	23.0	23	3.7	18.0	27.0
	Male	3	24.0	24	6.0	18.0	30.0
Waist (cm)	Female	5	76.2	75	9.6	67.0	90.0
	Male	3	84.0	85	13.5	70.0	97.0
Hip (cm)	Female	5	100.4	99	3.9	96.0	105.0
	Male	3	105.3	99	11.0	99.0	118.0
Waist to hip ratio	Female	5	0.78	0.8	0.1	0.70	0.90
	Male	3	0.80	0.8	0.1	0.70	0.90
Blood pressure systolic (mm Hg)	Female	5	112.6	111	9.3	100.0	124.0
	Male	3	122.0	120	14.1	109.0	137.0
Blood pressure diastolic (mm Hg)	Female	5	75.4	73	6.3	69.0	84.0
	Male	3	74.0	72	8.2	67.0	83.0
IPAQ 7 sedentary (hours)	Female	5	10.2	10	1.1	9.0	12.0
	Male	3	11.3	12	2.1	9.0	13.0
Fasted glucose (mmol/L)	Female	5	5.3	5.7	0.7	4.3	5.9
	Male	3	4.7	4.7	0.15	4.6	4.9

3.3.1. Glucose and insulin

3.3.1.1. 24-hour iGlucose

Time of day had a significant effect on iGlucose ($F(95, 2186) = 18, p < 0.001$, Figure 3). Fixed effects parameter estimates indicated that baseline iGlucose values were significantly different (all $p < 0.05$) to the periods of 09:00 to 10:45, 13:30 to 16:00 and 17:15 to 23:45. Post hoc tests were not performed.

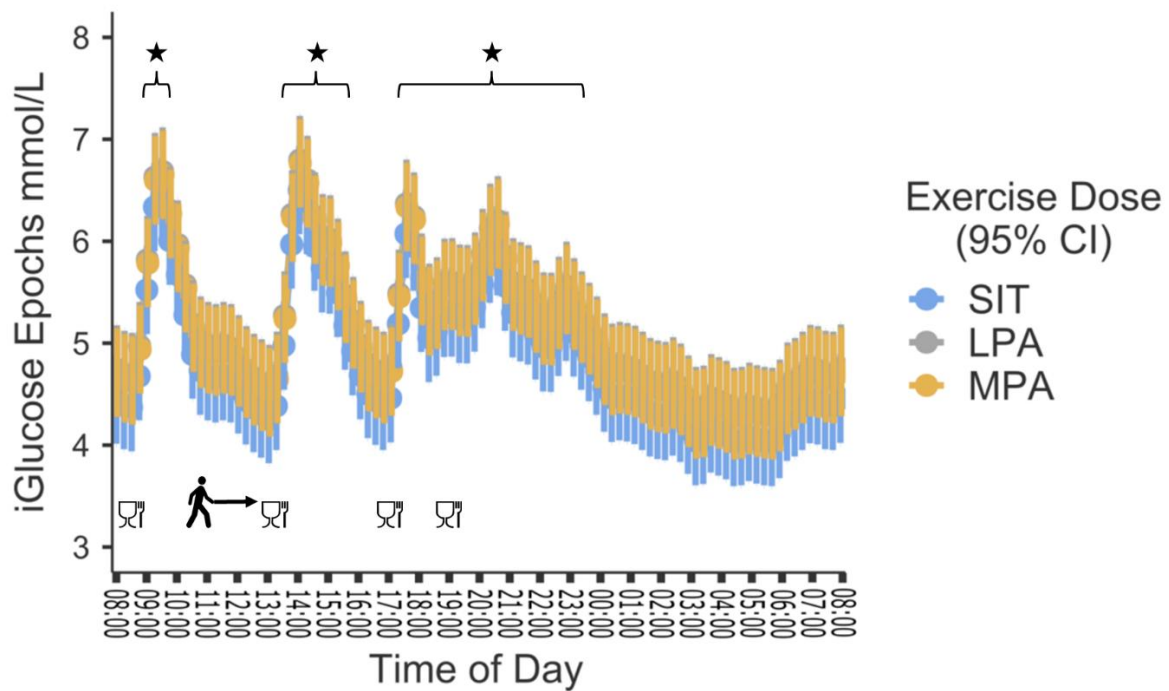


Figure 5 15-minute epoch interstitial flash glucose values (mmol/L) throughout the 24-hours (08:00-08:00) for the three exercise conditions: no walking (SIT) in blue, light-intensity walking (LPA) in grey, and moderate-intensity walking (MPA) in gold. Breakfast began at 08:30. The exercise condition period began at 10:30 and ended at 13:00. Subsequently, meals were lunch at 13:15, afternoon snack at 17:00, dinner between 19:00 and 20:00. Meals are represented by cup and fork icons: Meal 1 (breakfast), Meal 2 (lunch), Meal 3, (snack), Meal 4 (dinner). The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. * Starred areas represent statistically significantly different values from baseline values at 08:00.

3.3.1.2. 24-hour interstitial flash glucose monitor total area under the curve

Fixed effects omnibus tests indicated that fasted baseline iGlucose values at the start of the 24-hour period were a significant predictor of iGlucose tAUC throughout the 24-hours ($F(1, 18.6) = 28.49, p < 0.001$). Additionally, exercise condition was also a significant predictor of iGlucose ($F(2, 12.6) = 5.09, p = 0.024$). The marginal R^2 was 0.58, and the conditional R^2 was 0.87.

Fixed effects parameter estimates also indicated that the MPA versus SIT exercise conditions were significantly different for iGlucose tAUC (Estimate = 30.7, SE = 9.89, 95% CI [11.29, 50.1], $p = 0.009$). Furthermore, no significant differences were observed for LPA versus SIT (Estimate

= 20.5, SE = 9.44, 95% CI [2.00, 39.00], $p = 0.050$) although this was on the borderline of the threshold of significance. Random-effect variance components indicated a participant-level random intercept standard deviation of 27.3 (variance = 745) and a residual standard deviation of 18.7 (variance = 348). Post hoc comparisons indicated that SIT versus MPA was significantly different (Difference = -30.7, SE = 9.94, $p = 0.027$) with MPA being significantly higher than SIT. Figure 6 represents this relationship.

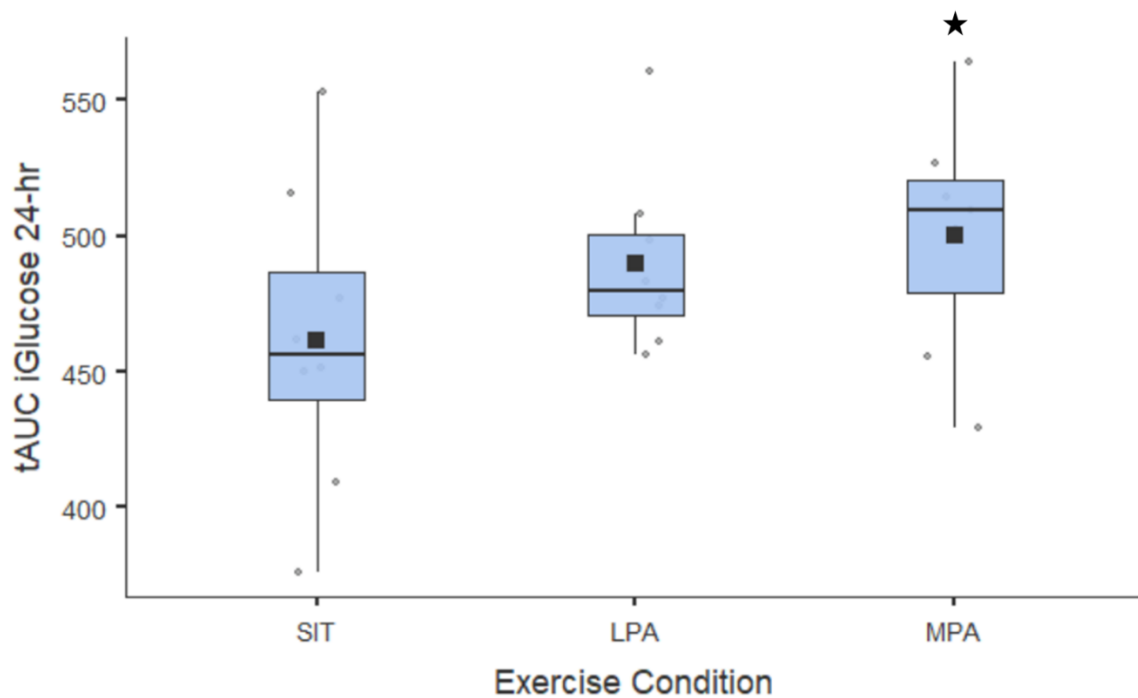


Figure 6 Box and whisker plot showing the relationship between exercise condition and interstitial glucose (iGlucose) total area under the curve (tAUC) throughout the 24-hour period for the three exercise conditions: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions. The black star * represents significantly higher values than SIT.

3.3.1.3. Venous glucose

Three venous samples were obtained at 08:15 A, 14:00 and 08:15B (24-hours after the first sample). Fixed effects omnibus tests indicated that fasted baseline vGlucose values were a significant predictor of vGlucose during the three blood draws ($F(1, 27) = 8.671$, $p = 0.007$).

Additionally, time of day was a significant predictor of vGlucose ($F(2, 53.8) = 21.064, p < 0.001$). In contrast, exercise condition was not a significant predictor ($F(2, 54.9) = 0.927, p = 0.402$), nor was the interaction between time of day and exercise condition ($F(4, 53.8) = 0.297, p = 0.879$). The marginal R^2 was 0.43, and the conditional R^2 was 0.53.

Fixed effects parameter estimates indicated that time of day was a significantly different between 14:00 and 08:15 A (Estimate = 0.74, SE = 0.30, 95% CI [0.16, 1.33], $p = 0.016$). No other parameters were significantly different (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 0.29 (variance = 0.08) and a residual standard deviation of 0.596 (variance = 0.36). Figure 7 represents the relationship between time of day, blood marker and exercise condition. Post hoc tests further indicated that 08:15 A was significantly different than 14:00 (Difference = -0.95, SE = 0.17, $p < 0.001$), and 14:00 was significantly different than 08:15 B (Difference = 1.02, SE = 0.18, $p < 0.001$), both indicating that the 14:00 time point had higher vGlucose.

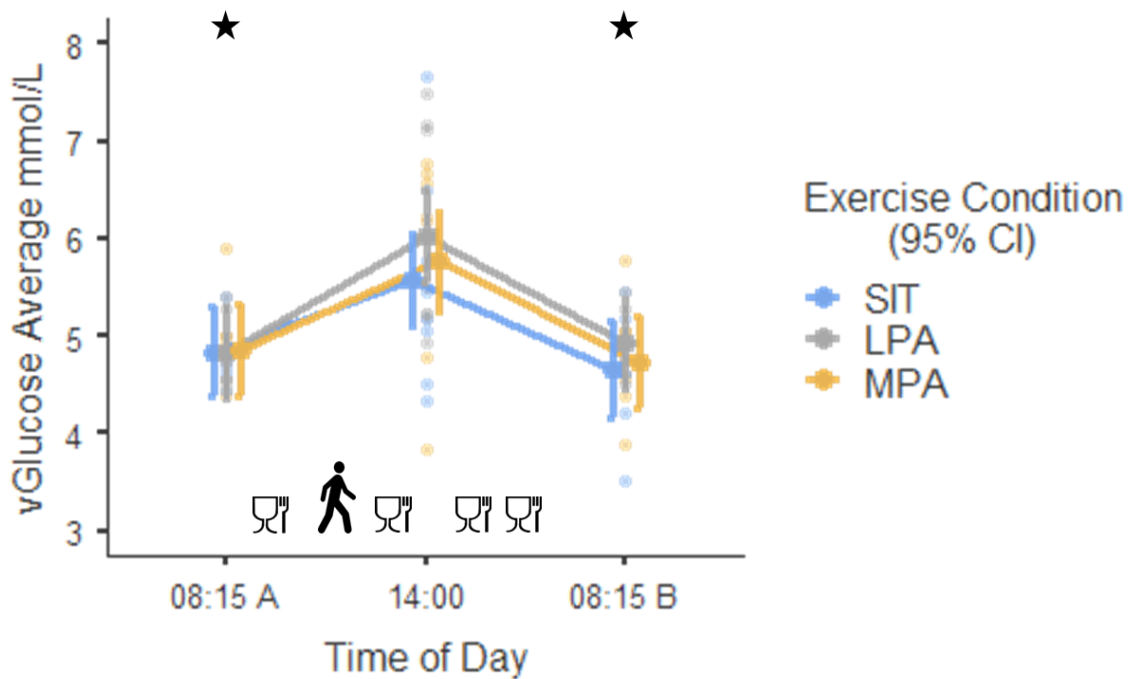


Figure 7 Average values (mmol/L) during the three blood draws for venous glucose (vGlucose). The three exercise conditions are represented: no walking (SIT) in blue, light-intensity walking (LPA) in grey, and moderate-intensity walking (MPA) in gold. The lab-day began at 08:00 and breakfast began at 08:30. The exercise condition period began at 10:30 and ended at 13:00. Subsequently, meals were lunch at 13:15, afternoon snack at 17:00, and dinner between 19:00 and 20:00. Meals are represented by cup and fork icons. Blood draws and blood pressures were first taken at 08:15 (8:15 A), then 14:00 and again at 08:15 the next day (08:15 B). The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. Individual dots represent each subject's vGlucose during the three blood draws within the respective exercise conditions. * Starred areas represent statistically significantly different values from 14:00 (post hoc).

3.3.1.4. Venous insulin

Fixed effects omnibus tests indicated that fasted baseline venous insulin values were a significant predictor of insulin during the three blood draws ($F(1, 31.2) = 4.356, p = 0.045$). Additionally, time of day was a significant predictor of insulin ($F(2, 53.5) = 116.533, p < 0.001$). In contrast, exercise condition was not a significant predictor ($F(2, 54.3) = 0.143, p = 0.867$), nor was the interaction between time of day and exercise condition ($F(4, 53.3) = 0.100, p = 0.982$). The marginal R^2 was 0.75, and the conditional R^2 was 0.78.

Fixed effects parameter estimates indicated that time of day was significantly different between 14:00 and 08:15 A (Estimate = 30.685, SE = 4.05, 95% CI [22.74, 38.63], $p < 0.001$). No other parameters were significantly different (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 2.99 (variance = 8.93) and a residual standard deviation of 8.10 (variance = 65.68). Post hoc tests further indicated that 08:15 A was significantly different than 14:00 (Difference = -31.746, SE = 2.37, $p < 0.001$), and 14:00 was significantly different than 08:15 B (Difference = 31.419, SE = 2.40, $p < 0.001$), both indicating that the 14:00 time point had higher insulin. Figure 8 represents this relationship.

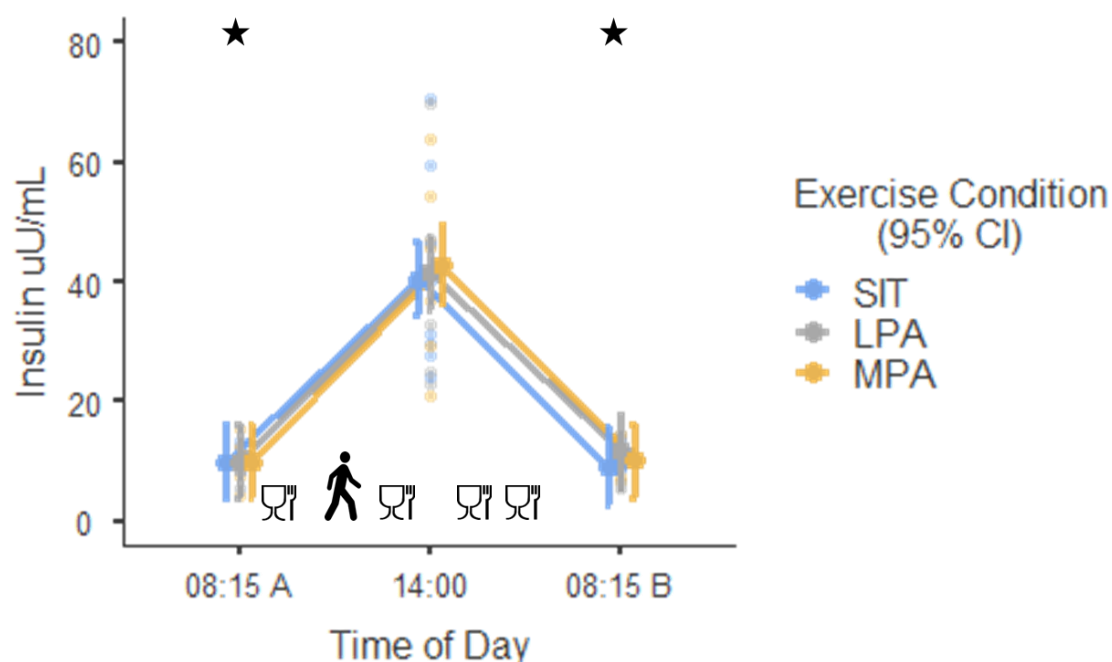


Figure 8 Average values (uU/mL) during the three blood draws for venous insulin. The three exercise conditions are represented: no walking (SIT) in blue, light-intensity walking (LPA) in grey, and moderate-intensity walking (MPA) in gold. The lab-day began at 08:00 and breakfast began at 08:30. The exercise condition period began at 10:30 and ended at 13:00. Subsequently, meals were lunch at 13:15, afternoon snack at 17:00, and dinner between 19:00 and 20:00. Meals are represented by cup and fork icons. Blood draws and blood pressures were first taken at 08:15 (8:15 A), then 14:00 and again at 08:15 the next day (08:15 B). The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. Individual dots represent each subject's insulin during the three blood draws within the respective exercise conditions. * Starred areas represent statistically significantly different values from 14:00 (post hoc).

3.3.1.5. Summary: Glucose and insulin

In Table 5, a summary is provided of the significant results for the primary glucose variables of interest which are exercise dose, and the potential interaction of exercise dose by age group. The main time of interest was the full 24-hour period. The *a priori* sub-analysis time periods are also presented to illustrate where other differences occurred. A detailed breakdown of the sub-period analysis can be found in 'Supplementary Material Chapter 3'.

Table 5 Significant results for interstitial flash glucose monitor (iFGM) total area under the curve (tAUC) over the seven time periods analysed and venous insulin (vInsulin), and venous glucose (vGlucose) significant average values over the 24-hour period analysed. The three exercise conditions are represented: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). Only the primary variable of interest is included in the table (exercise condition) to improve clarity of the summary results table.

Period/ measure	Main effects	Fixed effects parameter estimates	Post Hoc Tests
24-hours iFGM	• Exercise condition	• MPA - SIT: higher tAUC for MPA	• MPA - SIT: higher tAUC for MPA
vGlucose	• None	• None	• None
vInsulin	• None	• None	• None
Lab-Day iFGM	• None	• MPA - SIT: higher tAUC for MPA	• None
Exercise iFGM	• None	• None	• None
Lunch iFGM	• None	• None	• None
Snack iFGM	• Exercise condition	• MPA - SIT: higher tAUC for MPA • LPA – SIT: higher tAUC for LPA	• MPA - SIT: higher tAUC for MPA
Dinner iFGM	• None	• MPA - SIT: higher tAUC for MPA	• None
Sleep iFGM	• None	• None	• None

3.3.2. Lipid panel venous blood

3.3.2.1. *Venous total cholesterol*

Fixed effects omnibus tests indicated that fasted baseline venous TC values were a significant predictor of venous TC during the three blood draws ($F(1, 8.37) = 780.566, p < 0.01$). In contrast, time of day was not significant ($F(2, 53.87) = 0.599, p = 0.553$), nor was exercise condition ($F(2, 55.04) = 0.227, p = 0.798$), nor the interaction between time of day and exercise condition ($F(4, 53.86) = 0.425, p = 0.790$). The marginal R^2 was 0.96, and the conditional R^2 was 0.96. Fixed effects parameter estimates indicated no significant differences for the parameters (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 0.08 (variance = 0.01) and a residual standard deviation of 0.19 (variance = 0.04). Figure 9 represents the relationship between time of day, blood marker and exercise condition. No post hoc tests were performed.

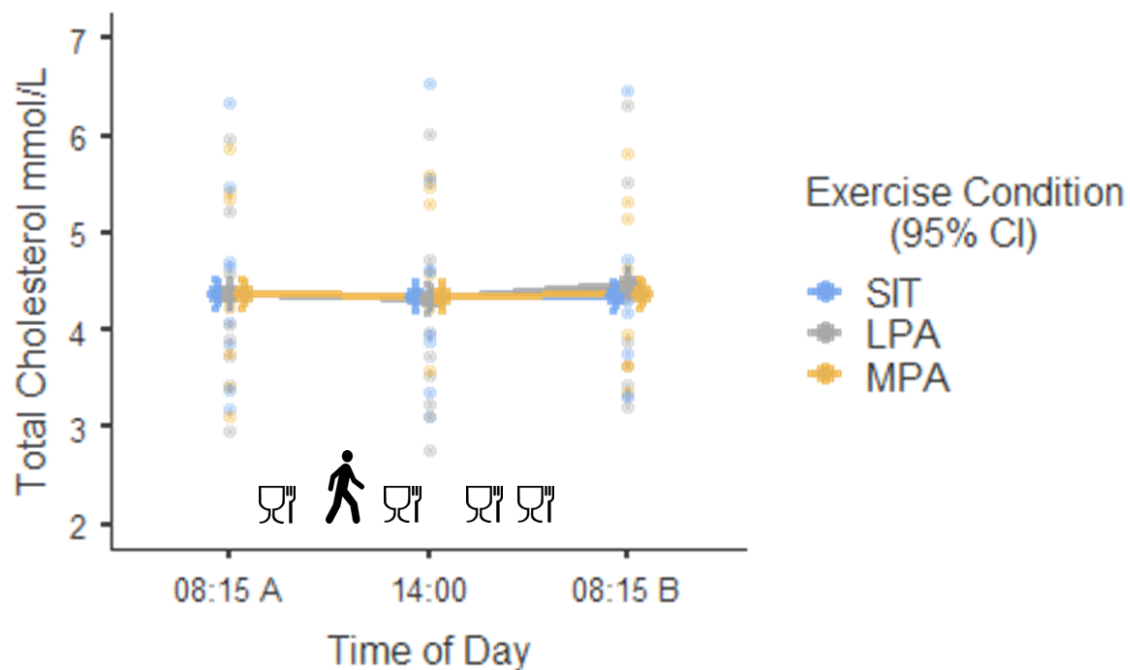


Figure 9 Average values (mmol/L) during the three blood draws for venous total cholesterol. The three exercise conditions are represented: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). The lab-day began at 08:00 and breakfast began at 08:30. The exercise condition period began at 10:30 and ended at 13:00. Subsequently, meals were lunch at 13:15, afternoon snack at 17:00, and dinner between 19:00 and 20:00. Meals are represented by cup and fork icons. Blood draws and blood pressures were first taken at 08:15 (8:15 A), then 14:00 and again at 08:15 the next day (08:15 B). The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. Individual dots represent each subject's total cholesterol during the three blood draws within the respective exercise conditions.

3.3.2.2. Venous LDL cholesterol

Fixed effects omnibus tests indicated that fasted baseline LDL values were a significant predictor of LDL during the three blood draws ($F(1, 11.30) = 459.792, p < 0.001$). Additionally, time of day was a significant predictor of LDL ($F(2, 53.60) = 4.666, p = 0.014$). In contrast, exercise condition was not a significant predictor ($F(2, 55.40) = 0.190, p = 0.408$), nor was the interaction between time of day and exercise condition ($F(4, 53.60) = 0.617, p = 0.652$). The marginal R^2 was 0.94, and the conditional R^2 was 0.95. Fixed effects parameter estimates

indicated no significant differences between any parameters (all $p > 0.05$), although 14:00 versus 08:15 A approached but did not achieve significance (Estimate = 0.13, SE = 0.07, 95% CI [-0.27, 0.01], $p = 0.074$). Figure 10 represents the relationship between time of day, blood marker and exercise condition. Random-effect variance components indicated a participant-level random intercept standard deviation of 0.07 (variance = 0.01) and a residual standard deviation of 0.14 (variance = 0.02). Post hoc tests further indicated that 08:15 A was significantly different than 14:00 (Difference = -0.100, SE = 0.04, $p = 0.040$), and 14:00 was significantly different than 08:15 B (Difference = -0.120, SE = 0.04, $p = 0.019$), both indicating that the 14:00 time point had lower LDL.

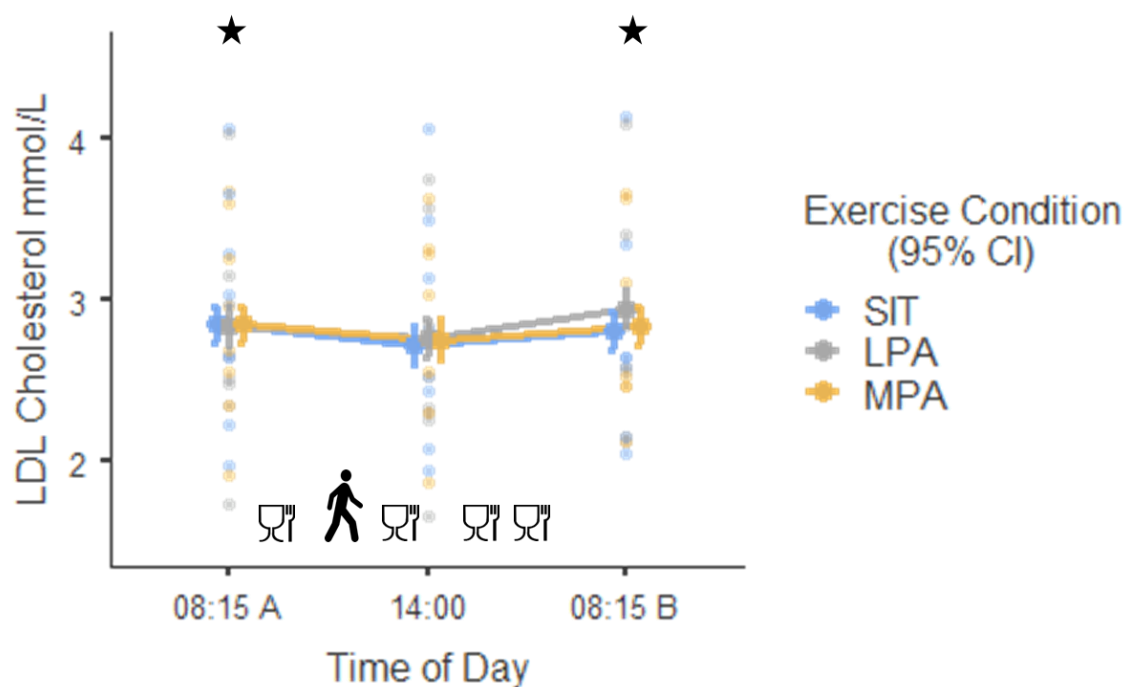


Figure 10 Average values (mmol/L) during the three blood draws for venous LDL cholesterol. The three exercise conditions are represented: no walking (SIT) in blue, light-intensity walking (LPA) in grey, and moderate-intensity walking (MPA) in gold. The lab-day began at 08:00 and breakfast began at 08:30. The exercise condition period began at 10:30 and ended at 13:00. Subsequently, meals were lunch at 13:15, afternoon snack at 17:00, and dinner between 19:00 and 20:00. Meals are represented by cup and fork icons. Blood draws and blood pressures were first taken at 08:15 (8:15 A), then 14:00 and again at 08:15 the next day (08:15 B). The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. Individual dots represent each subject's LDL during the three blood draws within the respective exercise conditions. * Starred areas represent statistically significantly different values from 14:00 (post hoc).

3.3.2.3. Venous HDL cholesterol

Fixed effects omnibus tests indicated that fasted baseline venous HDL values were a significant predictor of venous HDL during the three blood draws ($F(1, 7.21) = 510.204, p < 0.001$). In contrast, time of day was not significant ($F(2, 54.00) = 0.412, p = 0.664$), nor was exercise condition ($F(2, 55.81) = 1.002, p = 0.373$), nor the interaction between time of day and exercise condition ($F(4, 54.00) = 0.401, p = 0.807$). The marginal R^2 was 0.92, and the conditional R^2 was 0.93. Fixed effects parameter estimates indicated no significant differences

for the parameters (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 0.02 (variance = 0.0006) and a residual standard deviation of 0.09 (variance = 0.01). Figure 11 represents the relationship between time of day, blood marker and exercise condition. No post hoc tests were performed.

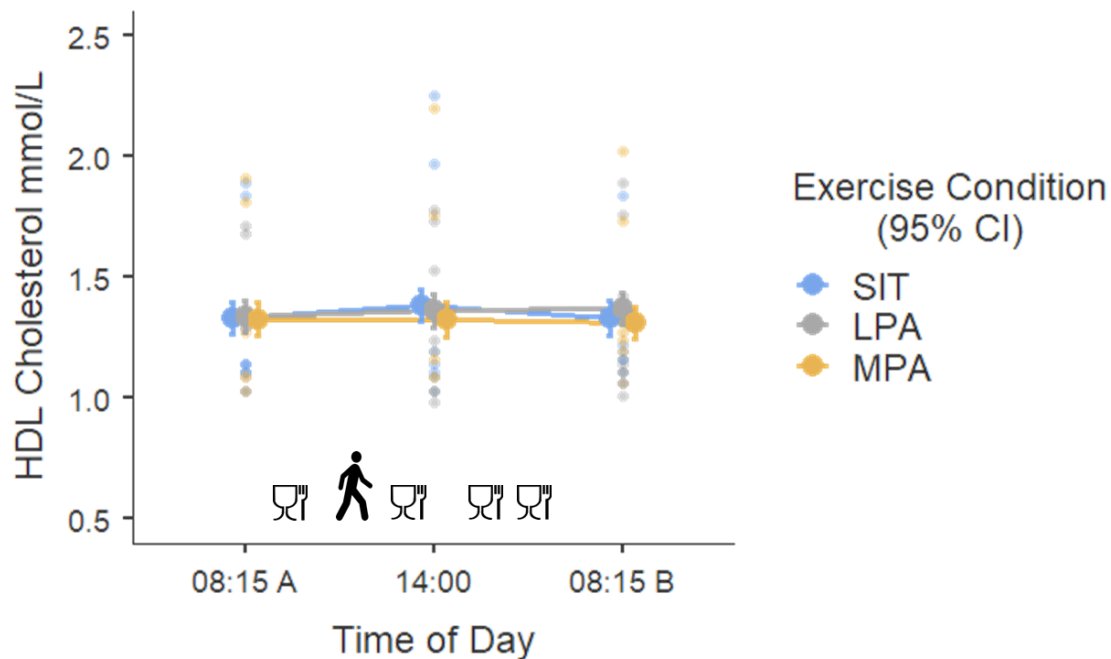


Figure 11 Average values (mmol/L) during the three blood draws for venous HDL cholesterol. The three exercise conditions are represented: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). The lab-day began at 08:00 and breakfast began at 08:30. The exercise condition period began at 10:30 and ended at 13:00. Subsequently, meals were lunch at 13:15, afternoon snack at 17:00, and dinner between 19:00 and 20:00. Meals are represented by cup and fork icons. Blood draws and blood pressures were first taken at 08:15 (8:15 A), then 14:00 and again at 08:15 the next day (08:15 B). The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. Individual dots represent each subject's HDL during the three blood draws within the respective exercise conditions.

3.3.2.4. Venous triglycerides

Fixed effects omnibus tests indicated that fasted baseline venous triglycerides values were a significant predictor of triglycerides during the three blood draws ($F(1, 89.0) = 94.347$, $p < 0.001$). Additionally, body mass index was a significant predictor of triglycerides ($F(1, 59.0) =$

8.138, $p = 0.006$) and time of day was a significant predictor of triglycerides ($F(2, 59.0) = 30.825$, $p < 0.001$). In contrast, exercise condition was not a significant predictor ($F(2, 59) = 0.688$, $p = 0.506$), nor was the interaction between time of day and exercise condition ($F(4, 59) = 1.155$, $p = 0.340$). The marginal R^2 was 0.77, and the conditional R^2 was 0.77.

Fixed effects parameter estimates indicated that time of day was a significantly different between 14:00 and 08:15 A (Estimate = 0.48, SE = 0.10, 95% CI [0.29, 0.67], $p < 0.001$). No other parameters were significantly different (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 0.00 (variance = 0.00) and a residual standard deviation of 0.19 (variance = 0.04). Post hoc tests further indicated that 08:15 A was significantly different than 14:00 (Difference = -0.400, SE = 0.06, $p < 0.001$), and 14:00 was significantly different than 08:15 B (Difference = 0.372, SE = 0.06, $p < 0.001$), both indicating that the 14:00 time point had higher triglycerides. Figure 12 represents the relationship between time of day, blood marker and exercise condition.

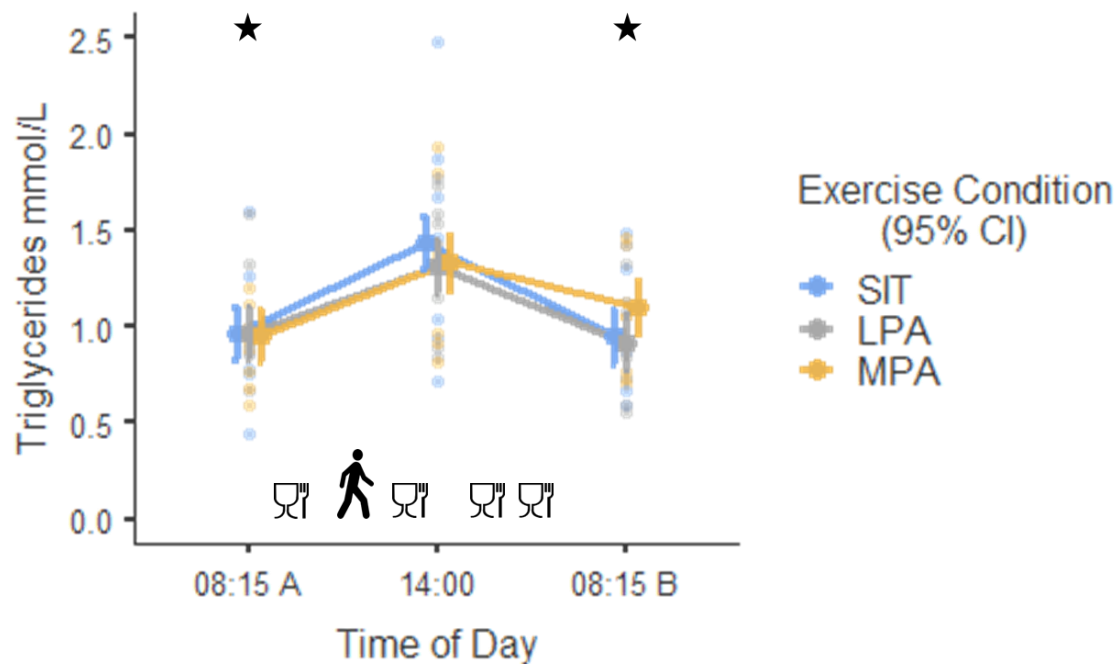


Figure 12 Average values (mmol/L) during the three blood draws for venous triglycerides. The three exercise conditions are represented: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). The lab-day began at 08:00 and breakfast began at 08:30. The exercise condition period began at 10:30 and ended at 13:00. Subsequently, meals were lunch at 13:15, afternoon snack at 17:00, and dinner between 19:00 and 20:00. Meals are represented by cup and fork icons. Blood draws and blood pressures were first taken at 08:15 (8:15 A), then 14:00 and again at 08:15 the next day (08:15 B). The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. Individual dots represent each subject's triglycerides during the three blood draws within the respective exercise conditions. * Starred areas represent statistically significantly different values from 14:00 (post hoc).

3.3.2.5. Lipid panel venous blood summary

Table 6 illustrates the results for the lipid panel variables of interest and whether any of the primary predictors of interest were significant. The main predictors were exercise dose, and the potential interaction of exercise dose by age group.

Table 6 Significant results for venous total cholesterol (TC), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides (TRIG) and insulin during the three blood draws analysed. The three exercise conditions are represented: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). Only the primary variable of interest is included in the table (exercise condition) to improve clarity of the summary results table.

Period/ measure	Main effects	Fixed effects parameter estimates	Post Hoc Tests
TC	• None	• None	• None
LDL	• None	• None	• None
HDL	• None	• None	• None
TRIG	• None	• None	• None

3.3.3. Blood pressure results

3.3.3.1. Systolic and diastolic blood pressure after exercise and lunch

In Figure 13A the DBP average values during the three samples obtained are compared between the three exercise conditions. Fixed effects omnibus tests indicated that baseline diastolic blood pressure was a significant predictor of systolic blood pressure during the three samples ($F(1, 12.2) = 55.193, p < 0.001$). Additionally, time of day was a significant predictor of diastolic blood pressure ($F(2, 53.19) = 20.627, p < 0.001$). Exercise condition was not a significant predictor of diastolic blood pressure ($F(2, 54.5) = 2.527, p = 0.089$) although it did approach the threshold of significance. The interaction of time of day and exercise dose was also not significant ($F(4, 53.9) = 0.750, p = 0.562$). The marginal R^2 was 0.70, and the

conditional R^2 was 0.71. Fixed effects parameter estimates indicated that the time point 14:00 was significantly different than 08:15 A for DBP (Estimate = -8.625, SE = 2.18, 95% CI [-12.89, -4.36], $p < 0.001$), indicating 14:00 having significantly lower DBP values. No other parameters were significant (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 2.77 (variance = 7.69) and a residual standard deviation of 4.35 (variance = 18.94). Post hoc tests were performed for time of day and indicated that 08:15 A was significantly different from 14:00 (Difference = 7.77, SE = 1.26, $p < 0.001$). Additionally, 14:00 was significantly different from 08:15 B (Difference = -5.77, SE = 1.26, $p < 0.001$). Both differences indicated that the 14:00 DBP values were lower.

In Figure 13B the SBP average values during the three samples obtained are compared between the three exercise conditions. Fixed effects omnibus tests indicated that baseline systolic blood pressure was a significant predictor of systolic blood pressure during the three samples obtained ($F(1, 8.35) = 172.996$, $p < 0.001$). However, time of day was not a significant predictor of systolic blood pressure ($F(2, 55.83) = 2.797$, $p = 0.070$) although it approached the threshold of significance. Additionally, exercise condition was not a significant predictor of systolic blood pressure ($F(2, 56.24) = 1.055$, $p = 0.355$). The interaction of time of day and exercise dose was also not significant ($F(4, 55.83) = 0.730$, $p = 0.575$). The marginal R^2 was 0.81, and the conditional R^2 was 0.83. Fixed effects parameter estimates also indicated no significant differences for the other parameters (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 1.54 (variance = 2.37) and a residual standard deviation of 4.68 (variance = 21.88). No post hoc tests were performed.

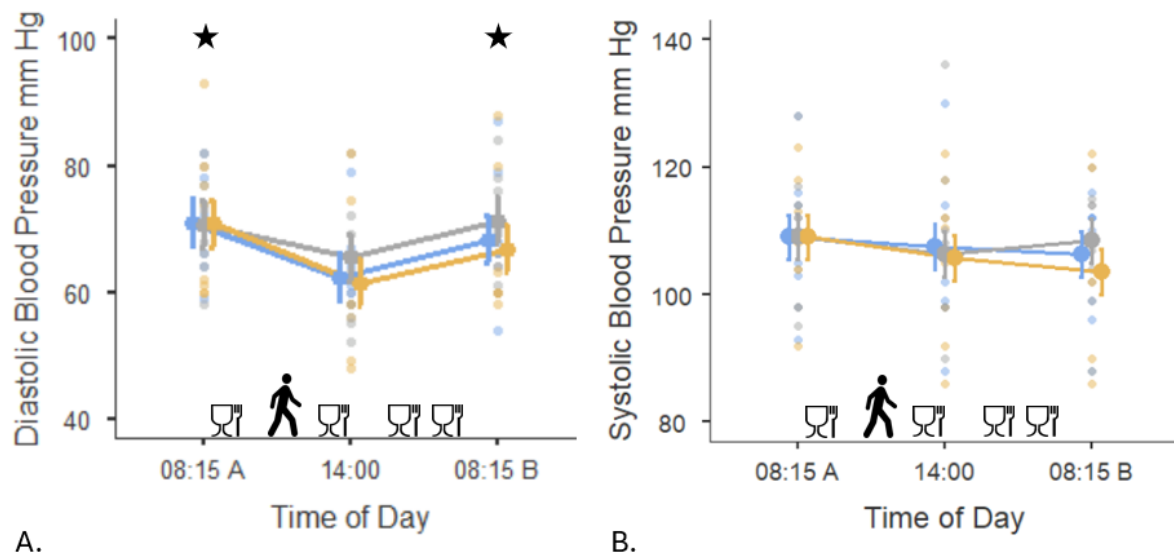


Figure 13 A-B Average values (mm Hg) during the three samples obtained for diastolic blood pressure (A) and systolic blood pressure (B). The three exercise conditions are represented: no walking (SIT) in blue, light-intensity walking (LPA) in grey, and moderate-intensity walking (MPA) in gold. The lab-day began at 08:00 and breakfast began at 08:30. The exercise condition period began at 10:30 and ended at 13:00. Subsequently, meals were lunch at 13:15, afternoon snack at 17:00, and dinner between 19:00 and 20:00. Meals are represented by cup and fork icons. Blood draws and blood pressures were first taken at 08:15 (8:15 A), then 14:00 and again at 08:15 the next day (08:15 B). The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. * Starred areas represent statistically significantly different values from 14:00.

3.4. Discussion

This study explored cardiometabolic responses to breaking up a workday in apparently healthy sedentary and inactive adults. The primary finding was that over 24-hours - during the snack period - the tAUC for iGlucose was higher during the MPA condition than during the SIT condition, whereas an energy-matched LPA bout was not different. No other sub-analysis periods were significantly different for iGlucose. Additionally, no differences between groups were observed for venous insulin or vGlucose after exercise plus lunch. These results contrasted our hypothesis that iGlucose, vGlucose and insulin would be lower compared to SIT during both the MPA and LPA conditions, with MPA being the lowest. For the venous samples, we also hypothesised that no difference would remain the following morning pre-prandially, which we confirmed was the case.

In our secondary analysis, we also found that there was no difference for triglycerides between groups after exercise plus lunch or the following pre-prandially, which contrasted with our hypothesis. However, we did confirm that there were no differences for TC, HDL or LDL between groups at any time point. Additionally, no differences for SBP or DBP between groups at any time point, which aligned with our hypothesis.

3.4.1. Glucose responses

Physiological mechanisms underlying differences in glucose responses to LPA and MPA

There are physiological reasons that may explain our glucose results. Due to the intensity and length of the MPA condition, the body will have needed to perform glycogen synthesis during exercise via glycogenolysis. It has been shown that both men and women rely greatly on carbohydrate stores to sustain moderate aerobic exercise, although the ratio of fat to carbohydrate utilisation may differ between males and females (Cano et al. 2022; Sanchez et al. 2024). Therefore, it is likely that the MPA condition will have triggered a significant utilisation of blood glucose, as well as a significant depletion of the skeletal muscles' glycogen stores, whereas LPA may not have depleted to the same extent. It is also possible that, as the MPA condition progressed, liver stores of glycogen may have been exploited to a greater extent than in the LPA condition. Therefore, to maintain homeostasis and metabolic reserves, the body will have attempted to replenish glucose via gluconeogenesis (Trefts et al. 2015). Furthermore, during the recovery phase after exercise, gluconeogenesis is likely to have

continued to replenish reserves and elicit post-exercise responses. This may help to explain why over 24-hours the levels of iGlucose were higher on the MPA condition compared to SIT, but not in the LPA condition. That is, there is a greater flux of glucose to replenish energy stores in the MPA condition. In contrast, the LPA condition may have relied on alternative energy sources more heavily.

Although MPA and LPA were approximately isoenergetic (~ 300 MET minutes), less intense exercise is known to rely more of fat oxidation (Mittendorfer and Klein 2003). So, in the LPA trial, carbohydrate oxidation may have occurred to a lesser extent due to a preference for fat oxidation, especially in the early to mid-phase of exercise. This could have proportionally reduced glucose utilisation in the LPA condition, as glycogen stores would not have been depleted to the same degree as MPA, and the need for glycogen replenishment would be less. Therefore, the *potential* mechanism of glucose stacking after snack time would have been reduced in LPA compared to MPA. This may explain the LPA condition was not statistically higher than SIT. However, without hepatic measures and tissue biopsies these theories are hard to corroborate.

Importantly, venous blood showed no differences in vGlucose or insulin between exercise conditions at 45 minutes after lunch in either MPA or LPA conditions. This supports the IFGM iGlucose results, which also showed no differences in iGlucose during the period after lunch in either MPA or LPA conditions. Although these methods measure different aspects of glucose dynamics, the direction and magnitude of change is expected to be relatively similar as IFGM measurements typically lag venous glucose by several minutes (Schmelzeisen-Redeker et al. 2015). The timing of the post-meal blood draw was timed to encompass a glucose peak that may have occurred at 45 minutes after food ingestion began (and 60 minutes after exercise ended). However, although glucose can peak as early as 45 minutes after food ingestion, this timing varies and often occurs around 60 minutes (American Diabetes Association 2001). Therefore, the true peaks for each individual and condition may have occurred at different times that were not identified with a single sample. This, therefore, highlights the importance of the regularity of the IFGM to encompass these responses.

Regardless of utilising IFGM or venous samples, it is possible the impact of exercise conditions may not have been powerful enough to override the normal physiological responses to standardised meals, at 45 minutes after lunch in either MPA or LPA conditions i.e. the acute

initial influx of glucose into the bloodstream from lunch was too high to be significantly impacted by prior exercise. Previous trials have observed the insulin lowering effect of morning exercise to be lost after lunch (Farah and Gill 2013). Other research has also shown that providing food after exercise can attenuate the insulin-sensitising effect (Gillen et al. 2021; Holtz et al. 2008). This response may be modulated by the macronutrient make-up, with an increased carbohydrate ratio potentially attenuating the insulin-sensitising effect (Gillen et al. 2021). Therefore, should the blood draws have been taken prior to lunch, or if our carbohydrate ratio had been lower, our results may have differed in MPA and/or LPA conditions.

MPA specific responses

In alignment with our results, several studies observed increases in glucose following MPA. The trial by Kashiwabara et al. (2018) assessed the response to 30 minutes of MPA walking in older females with hypertriglyceridemia and observed increased tAUC of glucose compared to sitting during the lab-day. Similarly, Wheeler et al. (2020) examined older adults following 30 minutes of MPA walking compared to a control and observed an increase in tAUC over the lab period. In a trial by Miyashita et al. (2016), researchers explored the effects of 30 minutes MPA walking in older females. They did not observe differences in glucose between MPA and sitting. However, in the same trial, glucose tAUC and iAUC values were higher following continuous walking compared to the intermittent walking arm. This suggests some evidence for a post-exercise increase in glucose following continuous MPA walking, whereas intermittent bouts were statistically lower than sitting. In the trial by Holmstrup et al. (2014) researchers studied young adults at risk of diabetes and observed an increase in glucose levels following 60 minutes of post-breakfast MPA. Analysis revealed higher glucose levels began to occur relatively soon after morning exercise and continued throughout a significant proportion (~20%) of the subsequent 12-hour measurement period. Nevertheless, several studies saw no differences between MPA and control groups.

Where our research saw an increase in glucose following a continuous bout of MPA compared to sitting, several studies did not show a difference. For example, two studies found no differences between a 30-minute continuous bout of MPA and a control during the lab-day (Maylor et al. 2018; Peddie et al. 2013). Similarly, Benatti et al. (2017) reported no difference in glucose response between sitting and 30 minutes MPA walking during the lab-day or the

next day postprandially. In a further study, researchers measured ~70 minutes of light-to-moderate-intensity physical activity (LMPA), versus ~50 minutes MPA compared to a control (Newsom et al. 2013). They saw no difference in glucose between the conditions after exercise. Additionally, Farah and Gill (2013) studied the effects of 60 minutes of MPA walking compared to a control. No differences between groups were observed. In a previous trial by Katsanos et al. (2004), researchers studied the effects of around 91 minutes of MPA walking on glucose and observed no difference in glucose compared to sitting. Furthermore, several studies examined the next-day responses to MPA.

In three studies, researchers saw no difference in glucose between 30 minutes MPA walking and sitting the next morning pre- or postprandially (Homer et al. 2017; Miyashita 2008; Miyashita et al. 2008). Further evidence from Miyashita et al. (2013) also saw no difference in glucose after 30 minutes MPA walking following morning pre-prandially. Lastly, another study examined the effects of 90 minutes of MPA walking on glucose, compared to a control, and observed no differences between groups the following day pre- or postprandially (Tsetsonis and Hardman 1996). Much like our research, these studies repeatedly show a lack of difference between activity conditions and a control in the pre-prandial period the following day after exercise.

Considering the results of studies that observed no difference between MPA and sitting, alongside those that saw higher glucose after MPA, it may be that exercise duration, age and health status of the participants are key determinants of acute glucose responses following a continuous bout. Additionally, while several studies observed no differences *during the lab-day* of the MPA interventions, many did not assess glucose levels during the evening period following exercise. Therefore, they could not rule out differences between trials that may have occurred in the evening that may have been ‘washed out’ by the next morning. Our observations suggest that the period between the end of the lab-day and the following morning is important to measure as it may contain delayed glucose responses. However, that period was not explored in several previous studies. Our study was novel as we utilised an IFGM to observe an increase in iGlucose during the post-lab to pre-breakfast period (during the snack period in our case), which would otherwise have been missed. In contrast to higher glucose levels however, a limited number of studies do report lower glucose values after a continuous MPA break, but their conclusions are nuanced.

Four trials reported lower glucose levels after a continuous MPA bout (Bhammar et al. 2017; Kim et al. 2014; Marion-Latard et al. 2003; Miyashita et al. 2013), but only two observed differences during the same day as the physical activity and did not assess the next-day postprandial period (Bhammar et al. 2017; Marion-Latard et al. 2003). The trial by Marion-Latard et al. (2003) assessed the response to 60 minutes of MPA compared to sitting. Although they reported lower glucose levels at a single time point during the MPA bout, this effect was not present in the three hours afterwards, so evidence for an overall glucose lowering effect was not robust/long lasting. They also did not assess evening responses or assess next day responses, which may have enriched their conclusions, as was shown in several other studies discussed previously. Similarly, in the trial by Bhammar et al. (2017), researchers explored the glucose responses to 30 minutes of MPA compared to a control. They found that in the 18.7 hours following the end of exercise, there was a decrease in mean glucose, but not glucose AUC, in the MPA group. Sub analysis within the 18.7 hours showed mean glucose to be lower during the lab, evening and sleep periods, but not for incremental or net incremental AUC. Of note, in the morning period before the 18.7 hours post exercise measurement period began, both the MPA group and the sitting control group were still sitting (MPA did not start until 12:00). Therefore, it is hard to interpret why the glucose excursion was so much higher in the sitting group than the walking group according to their figure: Both groups' immediately pre-exercise baseline should have been near identical up to that point as no MPA had yet occurred. In their analysis, linear mixed-effects models were utilised, but it appears they only corrected for age and sex, not immediately pre-exercise baseline glucose levels. Therefore, it is harder to be confident with the MPA results they present.

LPA specific responses

Although several MPA studies exist, research into continuous LPA bouts is scarcer. In our research, we saw no difference in glucose following a continuous bout of LPA compared to sitting over 24-hours, although an increase in glucose for LPA approached the threshold of significance during the snack period ($p = 0.050$). Currently, there is limited comparable evidence available assessing the responses of a continuous bout of LPA exercise versus a control. Furthermore, within these studies, the results do not report a consistent direction of change.

In the study by Tsetsonis and Hardman (1996), researchers assessed the responses of 180 minutes of LPA compared to a sitting control (Tsetsonis and Hardman 1996). The LPA activity was no different than sitting the next day pre- or postprandially. However, they did not break a full day of sitting which makes comparisons to our research less clear. Additionally, they did not measure intervention-day evening responses. In contrast, other trials reported higher glucose values after LPA. In the LPA walking arm of the trial by Katsanos et al. (2004), participants walked for around 240 minutes before their post-activity meal. They observed higher mean glucose levels two hours after their first post-activity meal, but not at any other time in the subsequent six hours in the lab, or the following morning, and evening responses were not assessed. This may suggest where LPA is utilised, it may need to be longer than our 150 minutes to elicit an increased glucose response soon after exercise. This idea may align with our theory, presented in the first paragraph of this section, regarding the possible importance of intensity *and* duration on post-exercise increases in glucose levels following MPA or LPA. In contrast, another trial showed lower glucose levels after continuous LPA. Gao et al. (2024) studied the effects of 30 minutes LPA walking in adult males. They reported glucose values were lower than sitting controls during the lab-day. Considering the evidence by Gao et al. (2024) and Katsanos et al. (2004), alongside our own results, this may suggest that a shorter bout of LPA may elicit an acute glucose lowering response, and that a longer bout may elicit an overall rise in glucose. Thus, the effect of LPA exercise on glucose levels may be predominantly dependent on length and timing of measurement.

However, in a recent trial by Hoffmann et al. (2024), researchers explored the effects of continuous LPA walking compared to sitting in adults. Participants appeared to walk for the full 8-hour lab period. They reported lower glucose values for LPA when compared to sitting. This somewhat contradicts the theory presented above, as their walking was considerably longer than any of the previous trials. However, their participants walked at one mph, which was the slowest speed seen in trials, and this may have influenced the substrate utilised over such a long period. That is, lipids may have been the primary energy source, with glucose in a lower proportion. This may have led to glucose being reduced somewhat over time, but without the need for significant glucose recovery from the liver to increase glucose levels observed in other long duration LPA research.

3.4.2. Insulin responses

MPA specific responses

Changes in insulin were used as biomarkers of change in several studies assessing responses to a continuous bout of MPA compared to sitting. Several trials reported no differences between groups for insulin concentrations during the intervention day (Kashiwabara et al. 2018; Maylor et al. 2018; Miyashita et al. 2016; Peddie et al. 2013). Newsom et al. (2013) also observed no differences in insulin for separate MPA and LMPA conditions compared to sitting during the intervention day. Additionally, three other trials observed no differences the next day pre-prandially (Miyashita 2008; Miyashita et al. 2013; Miyashita et al. 2008). The studies above align with our results observing no differences in insulin during the intervention day or the following morning pre-prandially. In contrast, however, some studies observed differences in insulin during the intervention day or the next day after MPA.

Newsom et al. (2013) observed that insulin sensitivity was significantly elevated (~35%) compared to the control in the LMPA activity the following morning after exercise. MPA was also 20% higher, but not statistically significant. Why the effects of a lower intensity activity appeared to show a greater beneficial effect than a higher intensity activity were not clear. However, the authors posited that it may have been due to the longer duration of LMPA versus MPA, even though energy expenditure was matched. We observed no such difference in our results. However, Newsom et al. (2013) use a euglycemic clamp procedure, whereas we used venous blood draws: outcome measures variations that may explain our differing results.

Both Farah and Gill (2013) and Wheeler et al. (2020) investigated insulin responses to MPA versus sitting. They observed lower insulin in the period before lunch (which included the exercise period) and over the whole lab-day, but not in the post-lunch period alone. We also did not observe lower insulin levels after lunch. The results from the two trials above highlight an important point regarding insulin responses and meal timing: The timing of our venous blood draws may not have been aligned to observe differences in insulin that may have arisen during the pre-lunch period. So, rather than no differences in insulin occurring, measurement timing may have been insufficient to observe possible underlying differences between trials. Furthermore, several other trials also saw a reduction in insulin levels during or soon after MPA exercise (Benatti et al. 2017; Holmstrup et al. 2014; Katsanos et al. 2004; Marion-Latard

et al. 2003), and when lunch was provided, no differences were seen in the postprandial lunch period alone. Of note, we found no RCTs that reported higher insulin levels after a continuous MPA bout compared to sitting.

LPA specific responses

The availability of studies breaking up a full working day of sitting with a continuous bout of LPA is very limited, and even fewer have assessed insulin responses. These trials have shown no difference in insulin levels compared to sitting after a continuous bout of LPA during the intervention day (Katsanos et al. 2004) or the next day (Katsanos et al. 2004; Tsetsonis and Hardman 1996). Therefore, studies align with our results showing no differences between LPA and sitting for insulin during the lab-day or fasted the next morning. Importantly, no trials showed an increase in insulin compared to sitting after LPA.

Overall, these studies provide evidence that continuous bouts of MPA have the potential to lower insulin soon after exercise, or the following day, although our research did not observe such a difference. Furthermore, we saw no differences between a continuous bout of LPA and sitting for insulin. Considering the scarce availability of literature regarding LPA insulin responses, it is hard to draw robust conclusions regarding LPA's effects.

3.4.3. Lipid panel responses

This trial was novel as it helped to clarify the exercise and postprandial responses to breaking up a simulated workday with energy-matched bouts of LPA and MPA, by specifically examining their impact on TC, HDL, LDL, and triglyceride levels in sedentary inactive adults. The trial revealed that one hour after exercise (which was 45 minutes after lunch), no differences were observed between LPA, MPA and the control group. It was also novel, as it helped to clarify whether a differing response would occur in the next day pre-prandially following the exercise conditions: no differences were observed.

The lack of differences between exercise groups and sitting may have been for several reasons. Firstly, this trial included both males and females which may have masked potential exercise response differences (Cano et al. 2022). However, this seems unlikely as gender was not significant when utilised as a covariate. Secondly, the current trial fed participants soon after exercise (15 mins), and obtained samples after a further 45 minutes, potentially influencing acute responses through meal ingestion. Thirdly, no next-day postprandial measures were

taken. These latter two points suggest that the timing of the post-exercise venous blood samples may have limited the scope of our conclusions. The available literature is nevertheless not fully aligned regarding the response of lipids following continuous MPA or LPA bouts.

MPA specific responses

The trial by Farah and Gill (2013) took five blood samples after lunch (taken at 30, 60, 120, 180 and 210 minutes) and saw a significant decrease in triglycerides after the exercise interventions (30 minutes MPA) compared to sitting. The results from their figures indicate the most apparent differences in triglycerides occurring between 60 and 120 minutes after lunch. This has implications to our trial, as if responses grew with time, it may be that the window of opportunity to observe differences in triglycerides was missed as bloods were taken solely 45 minutes after lunch on the lab-day. Additionally, the trial by Kashiwabara et al. (2018) observed a decrease in triglycerides after continuous exercise (30 minutes MPA) compared to sitting. Again, changes were observed after lunch following blood draws at 60, 180 and 300 minutes after lunch began. In a separate trial, undertaking ~91 minutes of MPA, triglyceride levels were lower four hours after the exercise condition compared to sitting, but not in hours one to three or five to eight (Katsanos et al. 2004). Similarly, tAUC for triglycerides was also significantly lower for MPA compared to the other LPA arm of the trial. These results suggested that the impact of the higher intensity, which was considerably shorter than LPA, had a strong effect on the lipid profile compared to sitting. We on the other hand saw no differences, but as mentioned previously, this lack of difference following the MPA trial is possible due to sample timing. Other trials have showed a delayed effect of MPA on lipid markers.

In the study by Kim et al. (2014), the triglyceride response to 60 minutes of MPA was assessed the following day pre-prandially. Triglycerides were significantly lower compared to the sitting trial. This contrasts with our results and may be due to the timing of exercise which was late in the day comparatively to our study. Additionally, the triglyceride values continued to be significantly lower into the seven-hour period after a high fat tolerance test. In contrast to Kim et al. (2014), but in alignment with our results, three 30-minute MPA trials (Miyashita 2008; Miyashita et al. 2013; Miyashita et al. 2008) observed no differences in triglycerides the following morning in the pre-prandial state. However, after the breakfast meal, triglyceride

values were lower in the subsequent postprandial period compared to sitting in each of the three trials.

In several studies exploring the same-day effects of MPA on lipids (Maylor et al. 2018; Miyashita et al. 2016; Peddie et al. 2013; Wheeler et al. 2020), no differences in triglycerides were observed during the same day as their 30-minute MPA conditions, which aligns with our findings. HDL cholesterol was also not different than sitting in the trial by Maylor et al. (2018), further supporting our results. Additionally, the trial by Homer et al. (2017) saw no difference compared to sitting for triglycerides in the fasted state the next day after 30 minutes MPA exercise. However, their results somewhat contrast to other literature utilising 30-minute a MPA bout, as triglycerides were not different between groups in the postprandial state the next day. This difference may have been due to the considerably different meal timing and compositions between those trials.

LPA specific responses

As with the evidence for glucose and insulin responses after continuous bouts of physical activity, the evidence base is very limited for lipid responses following a bout of LPA. One study compared ~four hours of LPA walking against sitting, and did not observe differences during the intervention day, or the next day pre-prandially, for triglycerides or HDL (Katsanos et al. 2004). In contrast, the recent study by Hoffmann et al. (2024) observed that triglycerides, HDL, LDL and TC were all lower after the lunch period. Additionally, HDL remained lower at the end of the lab-day. In the trial by Tsetsonis and Hardman (1996), same-day measures were not measured, but LPA triglyceride levels were lower both pre- and postprandially after the next day breakfast meal.

Of note, no trials reported higher lipid levels after LPA or MPA exercise. Our trial did not assess postprandial lipids the next day, so potential delayed responses to 70 minutes MPA or 150 minutes LPA could not be determined. Nevertheless, our results add to the body of evidence that differences in lipids are not present 45 minutes after a meal following a continuous bout of MPA or LPA.

3.4.4. Blood pressure responses

This trial was novel as it compared the effects of breaking up a full working day, with a single continuous bout of LPA walking on blood pressure versus an energy matched continuous bout

of MPA, in sedentary inactive adults and a control group. As per our hypothesis, blood pressure did not differ between the three conditions during either time point after physical activity.

The majority of trials breaking up sitting with a continuous bout of LPA or MPA did not measure blood pressure responses. However, two studies showed a decrease in SBP after a continuous bout of MPA (Bhammar et al. 2017; Miyashita et al. 2008). Unlike their trials, we only took one sample 60 minutes after exercise ended (45 minutes postprandially) and so our sampling may have missed potential differences before or after that time. Nevertheless, the lack of differences between SIT, LPA and MPA conditions at 08:15 A and 08:15 B shows the rigour of the blood pressure protocol, and reinforces that participants were at complete rest when undertaking these measures. In addition, the results show that any effects of exercise on blood pressure that may have appeared after 14:00, were not present by the following morning. However, our trial contained a relatively small number of participants ($n = 8$) and may be underpowered to detect changes between groups. Future trials could 1) stratify participants by age, fitness and other characteristics, and 2) take more regular blood pressure measures – potentially using an automated cuff – during and after exercise, to ensure possible deviations between conditions are not missed.

3.4.5. Strengths, limitations and future research

This study benefitted from a robust design including tight participant inclusion and exclusion characteristics such as inactivity and sedentary status. Additionally, the trial included well-evidenced participant restrictions detailed in the methods section. These include well-controlled restrictions during the within-lab and out-of-lab periods. The utilisation of the IFGM also greatly enhanced the range of observations across a long time period and enhanced the clarity of post-exercise responses. Furthermore, solid meals were utilised which replicate real life food intake in contrast with previous trials utilising liquid meals.

We chose not to obtain evening venous blood samples. Therefore, our trial could not determine if differences in any of the lipid markers occurred according to exercise condition after the main lab-day ended, and before 08:15 B. Additionally, differences in lipid results may have become more apparent the next day in the postprandial state. So, latent differences in lipid markers cannot be ruled out following the exercise conditions but also cannot be

corroborated. Additionally, for glucose and insulin, the evidence suggests that obtaining next-day postprandial measures would have enhanced our conclusions. Therefore, future research should consider regular blood markers in the evening period and the next day postprandially.

Also, while this trial did not recruit the number of participants initially intended to ensure full sample power, statistically significant differences were still observed. This suggests that the observed effects were strong enough to be detected despite the smaller sample size. However, future studies with larger sample sizes are recommended to corroborate the robustness of these findings and to ensure greater generalisability.

This study employed what could be described as a ‘sledgehammer’ approach to exercise dosing. That is, it utilised exercise doses that are not necessarily realistic for real-world conditions for replacing sedentary time with physical activity (70 minutes MPA and 150 minutes LPA). Nevertheless, this approach helped to ascertain a baseline for future trials breaking up sitting using either MPA or LPA. Future trials may choose to utilise shorter bouts of physical activity to replace sedentary time, to align with lengths that may be deemed more achievable for sedentary workers.

Additionally, an increase in glucose for 150 minutes of LPA approached the threshold of significance during the snack period, and as previously mentioned, the length of LPA bout may modulate the post-exercise response. Therefore, future exploration of the post-meal responses to shorter bouts of LPA that may respond differently than a longer bout, using dose-response methods, is warranted.

Finally, this study exclusively examined younger adults and did not include older adults. However, research comparing adults to older adults is warranted as evidence suggests individuals progressively lose the ability to regulate glucose levels compared to when they were younger, and this may peak in the seventh decade (Chia et al. 2018). Importantly, this has not been explored in sedentary, inactive populations by replacing sedentary time with a continuous bout of LPA.

These considerations regarding LPA duration, the approach to significance in the snack period, and age-specific responses directly informed the research design of Chapter Four, which adopted more pragmatic physical activity bout lengths (0, 30 and 60 minutes of LPA), ensured the snack period remained included, and explicitly compared adults to older adults.

3.5. Conclusions

This trial provides important insights into the acute cardiometabolic responses to breaking up sedentary time with a single continuous bout of MPA or an energy-matched bout of LPA versus a control. Firstly, in sedentary inactive adults, only a 70-minute bout of MPA significantly increased iGlucose compared to a control - with responses most clearly indicated in the postprandial period. In contrast, a 150-minute energy-matched bout of LPA was not statistically different than sitting. Although our results contrasted our hypothesis regarding the direction of change in glucose (with glucose increasing after MPA rather than decreasing), they are consistent with a growing body of evidence in the field. Our research was useful as it highlights that changes in glucose can occur during the same day as the exercise condition for a continuous bout of MPA. However, in the context of the broader literature, our findings underscore the importance of measurement frequency (e.g. one blood sample vs. several) and the timing of measurements (such as including evening and next day samples) as these factors are crucial for capturing comprehensive metabolic responses and ensuring more robust conclusions. Additionally, future research should explore the influence of different participant characteristics such as age. Our trial focused on younger adults, but considering the metabolic changes that occur in older adults, exploration of the effects of LPA and MPA across different age groups is warranted to better understand age-related differences in glucose regulation.

Secondly, we did not detect changes in lipid markers after either a continuous bout of MPA or LPA, which aligns with several studies. However, evidence also suggests that same-day lipid responses are possible. Overall, analysis of the different study designs and results available highlights the importance of measuring multiple time points over a wide period (>24 hours) to ensure robustness of conclusions. Additionally, the available literature illustrating the lipid responses to a continuous bout of LPA is limited and presents opportunity for future research.

4. Chapter Four: Breaking up prolonged sitting: how much light physical activity is required to improve health markers in young and older adults

4.1. Introduction

Sedentary behaviour is described as waking behaviour characterized by an energy expenditure ≤ 1.5 METs while in a sitting, lying or reclining posture (Viir and Veraksitš 2012). Over time, a multitude of technologies and processes have modernised the societal environment and decreased the physical burden of humans (Bailey 2021). As a result, generally, a higher proportion of the world's population is now sedentary (Bailey 2021). On average, adults in Western society spend a significant portion of their day in sedentary activities: ~ 55 - 65% of waking hours (Matthews et al. 2008; van Ballegooijen et al. 2019), with older adults spending around 60% of their waking time in sedentary pursuits (Matthews et al. 2008). Regularly being in this low MET sedentary state for prolonged periods of time has been highlighted as a significant cause of non-communicable disease deaths worldwide (Lee et al. 2012).

Prolonged sitting is associated with negative effects on glucose and lipid metabolism (Loh et al. 2020) as well as vascular function (Thosar et al. 2014). Therefore, intervention strategies are required to interrupt sitting and combat these negative health effects. Observational studies have demonstrated that interrupting sitting with physical activity can improve glucose metabolism (Healy et al. 2008). In a recent meta-analysis by Dong et al. (2024), the authors determined that *intermittent* moderate-intensity physical activity (MPA) or light-intensity physical activity (LPA) breaks every 20-60 minutes can lower glucose levels acutely in individuals without type II diabetes. Despite these findings, there is still limited research available to guide informed decisions regarding the effectiveness of a single *continuous* bout of MPA to break up a typical sedentary working day. Even less evidence is available on the effects of a single bout of LPA in this context. Therefore, more research is needed, particularly on the impact of a single continuous bout of LPA during a sedentary workday.

A typical working day for adults lasts at least seven hours or more (Giattino et al. 2020). Several studies have explored the effects of a single continuous bout of LPA ($< 50\%$ VO_2max) without breaking up a full working day i.e. their research lasted less than seven hours. Some of these studies reported decreased glucose levels within the first day following a continuous LPA break (Aadland and Høstmark 2008; Hashimoto et al. 2013; Høstmark et al. 2006; Lunde et al. 2012; Nygaard et al. 2009). Others reported no difference in glucose levels after a continuous LPA break (Hardman and Aldred 1995; Hashimoto et al. 2011; Takaishi et al. 2012; Tsetsonis and Hardman 1996) and no trials reported higher glucose after an LPA break. The study design

characteristics varied widely between these trials. For example, most contained only males or females, while others included both sexes (Aadland and Høstmark 2008; Hardman and Aldred 1995; Tsetsonis and Hardman 1996). In addition, the length of LPA bout ranged from 7.25 minutes (Takaishi et al. 2012) to 90 minutes (Hardman and Aldred 1995; Tsetsonis and Hardman 1996) and some studies were not randomised (Aadland and Høstmark 2008; Høstmark et al. 2006; Lunde et al. 2012; Takaishi et al. 2012). Furthermore, the details regarding the specific pre-and within-trial restrictions were limited for several studies (including a lack of detail on controlling prior MVPA, alcohol or caffeine ingestion). Therefore, to effectively study breaking up sitting time with a continuous physical activity bout, more randomised controlled trials (RCTs) with thorough study restrictions, lasting at least a full working day, are needed. Additionally, activity breaks should be a plausible duration to elicit physiological change.

Three RCTs with continuous LPA bouts ≥ 30 minutes have assessed same-day glucose responses when breaking up a full working day, with two observing lower values after LPA (Gao et al. 2024; Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. 2024) and one reporting transiently higher values after LPA (Katsanos et al. 2004). Two of these trials measured lipid responses during the LPA day with one observing lower triglyceride levels (Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. 2024) and the other seeing no differences compared to the control (Katsanos et al. 2004). However, none of these trials were in older adults.

All previous adult RCTs breaking a sedentary workday with a continuous bout of LPA included younger adults (age <60) and none contained only older adults (age >60). This represents a significant age-related gap in the literature. As older adults spend around 60% of their waking time in sedentary activities (Matthews et al. 2008), and the incidence rate of diseases such as type II diabetes typically increase with age (Nguyen et al. 2012), this population warrants further investigation. Even in non-diabetic individuals, evidence suggests some individuals progressively lose the ability to regulate glucose levels compared to when they were younger, and this may peak in the seventh decade (Chia et al. 2018). This age-related decline in insulin sensitivity is believed to be driven by multiple factors, such as reduced skeletal muscle mass, increased fat mass and altered fat distribution (Chia et al. 2018). Therefore, as insulin sensitivity is the key mediator of glucose responses to physical activity, it is possible that older

adults may not respond metabolically in the same manner to LPA compared to younger adults. Therefore, investigating differences between younger and older adults is warranted and will be investigated here. Additionally, it is not known whether glucose and lipid responses to a single LPA break differs between older and younger adults.

Furthermore, blood pressure is also a useful marker of health that is known to change with age (Singh, Nguyen et al. 2023). Younger adults often exhibit faster recovery rates post-exercise compared to older adults (Dimkpa and Ugwu 2008). However, the intensity of LPA activity may not be intense enough to elicit a change in blood pressure after a continuous LPA break. However, no RCTs have examined possible differing responses between older and younger adults. Therefore, an RCT assessing whether age moderates the effect of breaking up a workday with a single continuous bout of LPA is warranted.

Aside from affecting cardiometabolic markers of health, there is evidence that physical activity is effective for improving depression and anxiety across a very wide range of populations, although higher intensities of physical activity are associated with greater benefits (Singh, Olds et al. 2023). Other research in sedentary adults, shows that breaking up sitting with a 30-minute continuous MPA break can improve feelings of vigour, but not mood (Bergouignan et al. 2016). Furthermore, research shows that breaking up sitting with LPA breaks is associated with improvements in some aspects of cognitive performance but not on mood (Wu et al. 2023). Although, anecdotally, adults report improved mood after a continuous LPA break, it is not known if a *single* continuous bout of LPA, aimed at breaking up a workday, can alter mood compared to a sitting control.

Neither of the previous trials breaking a full workday with a continuous bout of LPA assessed whether a dose response existed for cardiometabolic markers or mood (Gao et al. 2024; Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Niedrist et al. 2024; Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. 2024). Based on the available evidence discussed, it seems plausible that a longer duration of LPA will cause a greater effect on glucose, triglycerides and potentially mood, but a dose response relationship has not been previously assessed.

In summary, no previous study has assessed the potential glucose dose response relationship to breaking a sedentary workday with a continuous bout of LPA in older versus younger adults.

Additionally, none have concurrently assessed lipid panel responses, blood pressure and mood. Such an investigation would be useful for designing future trials and developing physical activity recommendations.

4.1.1. Methodological considerations and justifications for study design change

Several methodological nuances were highlighted in the previous study chapter that have been altered in this chapter's study to improve the overall efficacy of the research. Firstly, in the previous chapter, the three venous blood samples did not appear to capture the dynamic nature of glycaemic responses, especially in the post-exercise and postprandial period, whereas the IFGM revealed more nuanced patterns that venous samples appeared to miss. Therefore, this chapter utilised regular capillary sampling in the lab-day to enable broader resolution of glycaemic responses, whilst additionally allowing more lipid markers to be assessed. Additionally, capillary sampling is less burdensome than venous sampling as it is less invasive and generally more acceptable to participants and so this method was adopted.

Secondly, the long lab-day in Chapter 3 (Nine hours) was arguably longer than a standard workday which typically lasts at least seven hours or more in adults (Giattino et al. 2020). Therefore, a shorter seven-hour lab-day was adopted. Thirdly, the effect of 150 minutes of LPA on glucose responses approached significance during the snack period, which suggests that LPA has the potential to modulate glucose responses. However, although 150-minutes of LPA was a well justified physical activity length (see Chapter 3 introduction), 150 minutes is a very long period to apply in real-world situations and therefore, more pragmatic physical activity lengths were chosen (30 and 60 minutes of LPA). It is also possible that a shorter period of LPA would modulate glucose responses differently than 150 minutes. Investigating these shorter bouts would also help to identify if there were a minimum effective dose of LPA to elicit changes. Furthermore, as described in the introduction, as older adults' glucose responses may not react in the same manner as younger adults, this provided strong reasoning why investigating both populations are warranted.

Additionally, as body composition has been identified as a key modulator of glycaemic responses (see introduction), dual energy x-ray absorptiometry (DEXA) was implemented for this trial to ascertain whether differences in composition drove any potential changes in the metabolic markers that were to be measured. Next, handgrip strength was added to baseline

measures as it provides a validated proxy for overall strength, without the need for onerous strength testing. Furthermore, the addition of walking speed measures helped to characterise the study sample for comparison to current and future research. Also, the Short Warwick-Edinburgh Mental Wellbeing Scale (SWEMWBS) was added to provide baseline mental wellbeing. This allowed the researchers to control for baseline differences in general mental wellbeing when analysing positive or negative affect mood scores during the exercise conditions. Lastly, to ensure a treadmill speed that was acceptable/feasible to both younger and older adults and ensure physical activity remained at light-intensity, MPA was removed from the study design. Together these changes represent methodological improvements and alterations that help to investigate the aims of the subsequent research.

4.1.2. Aims

In a sedentary, inactive adult population, this study primarily examined the response of interstitial glucose to two different continuous bouts of LPA walking in younger (18-40) and older adults (60+). This three-armed crossover RCT included: 1) a continuous bout of LPA walking at 1.6 mph for 30 minutes, 2) a continuous bout of LPA walking at 1.6 mph for 60 minutes, and 3) a no-physical activity (sitting only) 'control' condition.

Additionally, the study assessed secondary outcomes. These included ascertaining whether any changes occurred following the physical activity conditions compared to the control for 1) lipid profiles, 2) blood pressure, and 3) positive and negative affect (aspects of mood).

4.1.3. Hypotheses

Primary hypotheses

1. Interstitial glucose (iGlucose) and capillary glucose (cGlucose) will be lower, compared to control, for both physical activity conditions.
2. The effects of 60 minutes of LPA will be more pronounced than those of 30 minutes of LPA for glucose measures.
3. There will be an interaction effect for age group and exercise conditions for glucose.

Secondary hypothesis

1. Triglycerides will be lower, compared to control, for both exercise conditions.

2. Total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol will not differ between conditions.
3. Blood pressure will not differ between the three conditions.
4. Positive and negative affect will improve after both 30 and 60 minutes of walking compared to control.

4.2. Methods

4.2.1. Study design

This randomised, controlled, crossover trial took place at the University of Stirling, Scotland, and was approved by the NHS East of Scotland Ethics Service, reference: 19/ES/0004, and was registered with IRAS, project ID: 258371.

4.2.2. Participants

Participant recruitment criteria were similar to those discussed in Chapter 3. However, there were three differences. Firstly, two age categories were evenly recruited: 18 to 40 and ≥ 60 years of age. Secondly, male and female participants were recruited evenly between age groups. Thirdly, participants could not have self-reported memory impairment that would affect study outcomes. Volunteers could access these criteria and initially assess their eligibility through participant information sheets, linked via QR codes and hyperlinks to the study research page (Appendix 3: Participant Information Sheet). After initial examination of the participant information sheets, volunteers could then organise to attend an initial first contact research meeting (Figure 14 A). This initial appointment allowed volunteers the opportunity to ask further questions, before agreeing to attend a subsequent familiarisation session. Participants understood they would receive £100 reimbursement for participation in the trial.

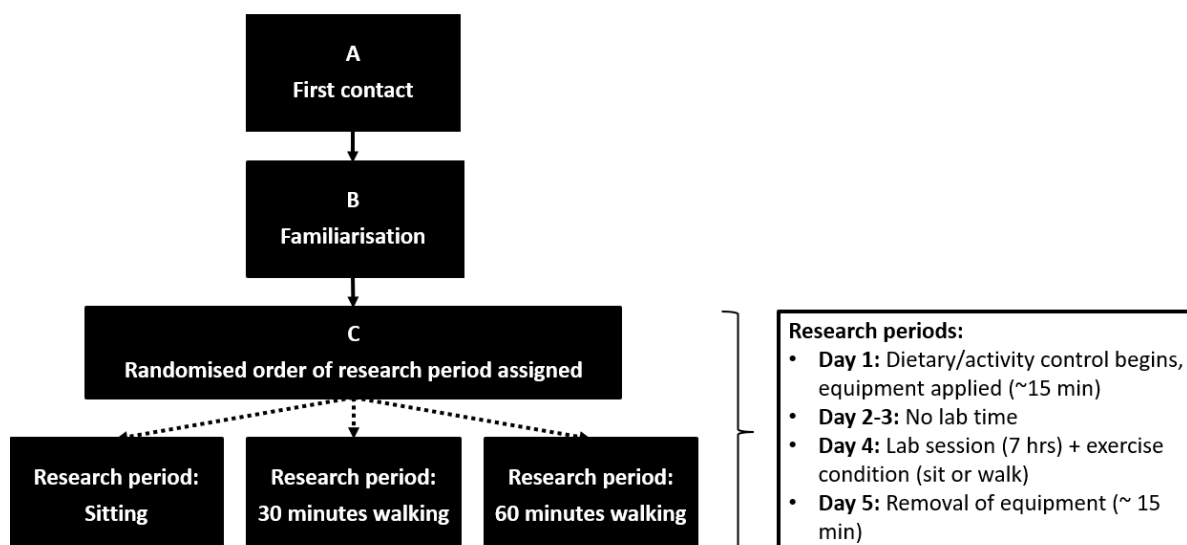


Figure 14 Flowchart of the experimental design (randomised controlled crossover trial), detailing study phases (A, B and C) and providing an overview of research periods and their associated lab-day exercise conditions.

4.2.3. Screening and familiarisation

Following initial contact and a period of $\geq$ three days, volunteers attended a screening and familiarisation session. The session began by reiterating the study details and ensuring individuals fully understood the potential risks and benefits of taking part. An opportunity for questions was then provided before participants signed an informed consent. Copies were available to participants.

4.2.4. Screening

Most participant screening processes were the same as those recruited in Chapter 3. However, there two notable differences. Firstly, the SWEMWBS was also completed to assess mental wellbeing (Appendix 4: SWEMWBS). Secondly, fasted (≥ 10 hours) blood glucose was assessed once using the finger prick capillary method via single use lancets (Unistik 3, Owen Mumford, United Kingdom). If blood glucose was high (≥ 6.0 mmol $\cdot$ L $^{-1}$) then repeat measurements were taken on the same morning. If these values remained high, then further measures were taken under the same conditions on a subsequent day before eligibility could be confirmed.

4.2.5. Anthropometry

Anthropometry was performed as detailed in the methods for Chapter 3.

4.2.6. Dual energy x-ray absorptiometry

After anthropometry, participants then filled in an additional DEXA consent form. Prior to scanning, volunteers drank 500ml of water two hours prior to attending. This was to improve accuracy of the scan (Rodriguez-Sanchez and Galloway 2015). Participants then emptied their bowel and bladder as well as possible. Females also undertook a pregnancy test. Scans were carried out on an iDXA (GE Healthcare, USA). Scans were obtained to assess the potential impact of appendicular lean mass (ALM) on primary outcomes and to help describe the recruited sample population.

4.2.7. Functional tests

Several functional tests were performed. Firstly, handgrip strength in kilograms of force (kg) was assessed using a handheld dynamometer (Takei Scientific Instruments Co, Ltd., Takei Digital Grip Strength Dynamometer Model T.K.K. 5001, Niigata, Japan). Three measures were taken, and the maximum handgrip strength was used. Additionally, volunteer walking speed was also assessed: Individuals walked for 50 meters outside, under cover, between two cones. Individuals were first asked to walk at their “usual, comfortable walking speed”. Subsequently, they were asked to walk “as briskly as possible, without running”. Time (s) was recorded on a stopwatch.

4.2.8. Safety, education and adherence

To ensure participants were safe whilst exercising during the trial, they were familiarised with the treadmill speed used in the trial conditions (1.6 mph) (HP Cosmos Pulsar Treadmill, HP Cosmos, Germany). The use of Polar heart rate monitors (H7, Polar Electro, Finland) and the BORG 6-20 rating of perceived exertion (RPE) scale was explained at this time. The process of obtaining capillary blood samples via finger prick, use of FreeStyle Libre interstitial flash glucose monitoring system (IFGM) (Abbott Laboratories LTD, UK), application of a protective hypoallergenic translucent film (Tegaderm, 3M Company, USA) and Actigraph use (ActiGraph LLC, USA) was described to participants. Individuals then received information sheets regarding the use of this equipment. A research diary was also provided so individuals could record PA, food intake and protocol adherence. All measurements were taken/provided within

two weeks of starting the first experimental period. In one case, DEXA was performed at the end of the trial, due to unforeseen weather-related circumstances preventing equipment access at familiarisation.

4.2.9. Randomisation

A randomised cross-over design was utilised. The 18 potential participants were assigned to one of six possible treatment orders, undertaking one of three conditions on each separate occasion: 60 minutes of sitting (control), 60 minutes of LPA walking, or 30 minutes of sitting followed by 30 minutes of LPA walking. The sequence of randomisation was generated prior to participant recruitment. Once a participant had completed familiarisation, the next sequential randomisation sequence available was assigned to them.

4.2.10. Study design: restrictions

Participants were required to attend three 'research periods', each lasting five days (Figure 14). A series of restrictions were imposed on participants during those times. Individuals were given a set of instructions to follow over the five days of each research period and additional instructions were given on the lab-day. On day one, participants were asked to begin avoiding alcohol and MVPA and asked to utilise minimally active transport options such as the train or bus. No new restrictions were imposed on day two. On day three, participants were asked to avoid caffeine consumption from 08:30, and to get a full night's sleep. On day four, during lab time, participants were asked to remain seated/reclined and to minimise shuffling during prolonged sitting periods. They could undertake sedentary workplace activities such as computer work and television viewing during non-physical activity periods, but no standing was permitted. To minimise PA, participants were transported between laboratories and to the toilet via wheelchair. At the end of the lab-day, participants were asked to get a full night's sleep that evening/night. Restrictions were lifted at 08:30 on day five. These instructions were designed to reduce the respective influences of these activities on the main study outcome (Bird and Hawley 2017; Elizabeth Carpio-Rivera et al. 2016; Kido et al. 2016; Reis et al. 2019; Zhu et al. 2019). Five-day research periods allowed researchers to assess 24-hours of data from 08:30 on day four to 08:30 on day five, which was inclusive of the highly controlled lab period.

4.2.11. Interstitial glucose and activity monitoring

The IFGM sensors and ActiGraph monitors were applied as per the methods in Chapter 3. However, in this trial, the sensors and monitors were removed after 08:30 on day five, rather than 08:15. Only 1.2% of all IFGM data was lost due to participant data loss, which was due to the participants failing to scan their monitor when the pre-set alarms sounded.

4.2.12. Experimental (lab-day) Protocol

Day four contained the main laboratory-controlled research segment, which ran for seven hours from 08:30 to 15:30 (Figure 2). When participants arrived at 08:30, they began by sitting quietly in the lab for 15 minutes. They were asked if they had adhered to trial instructions, and their research diary was examined. At this time, they filled in a sleep diary that contained the Karolinska sleepiness scale (Appendix 5: Sleep diary) and the International Positive and Negative Affect Schedule-Short Form (I-PANAS-SF) to assess mood (Appendix 6: I-PANAS-SF). Accounting for sleep isolates the effect of the intervention on mood and improves the validity of the measure.

At 08:45, blood pressure was taken from the contralateral arm to the IFGM. Immediately after, a finger capillary blood sample was obtained from the opposite side. Participants were then transported to the nutrition lab where they were provided with breakfast at 09:00. All lab meals were eaten within 15 minutes. Later that morning, individuals were required to complete one of the following three conditions in random order: 1) sitting (SIT); 2) 30 minutes light-intensity PA walking (30WALK @1.6mph) and 3) 60 minutes light-intensity PA walking (60WALK @1.6mph) during the workday (lab-day).

In the SIT condition, individuals sat from the end of breakfast at 09:15 until 11:45 (Figure 2). In the 30WALK condition, individuals sat until 11:15 when they began walking for 30 minutes. In the 60WALK condition, individuals sat until 10:45 when they began walking for 60 minutes. All walking was performed on the same treadmill, with room temperature set at 20°C. To describe intensity, heart rate monitors were applied before 10:45 in each condition and BORG scores were recorded every 15 minutes during PA. At 11:45 heart rate monitors were removed, and individuals filled in the I-PANAS-SF for the second time. They were then transported to the nutrition lab.

Lunch started at 12:00. At 12:15 lunch ended, and participants were returned to the sitting lab where they sat until 15:30. At this time participants walked a short distance to the nutrition lab (< 10 meters) where they ate a snack provided and ended their controlled lab time. Snacks were eaten by 15:45. Participants were then free to return home to eat their evening meal between 19:30 and 20:30. This was followed by an overnight fast until 08:30. Participants were subsequently asked if they had continued to comply with the final hours of the protocol and their research diary was checked. The ActiGraph and glucose sensor were then removed by the researcher, ensuring electrode removal and that skin sites were free from irritation or infection. Participants also filled in a second sleep diary (overnight day four until day five), and this ended the respective research period arm.

Each of the three research period arms were separated by at least seven days, with a maximum of two weeks separating consecutive periods. The sitting condition was considered the control condition with no increase in energy expenditure or change in posture. The 30WALK and 60WALK conditions elicited a change in posture and energy expenditure, with 60WALK being upright for longer, walking further and expending more energy than 30WALK.

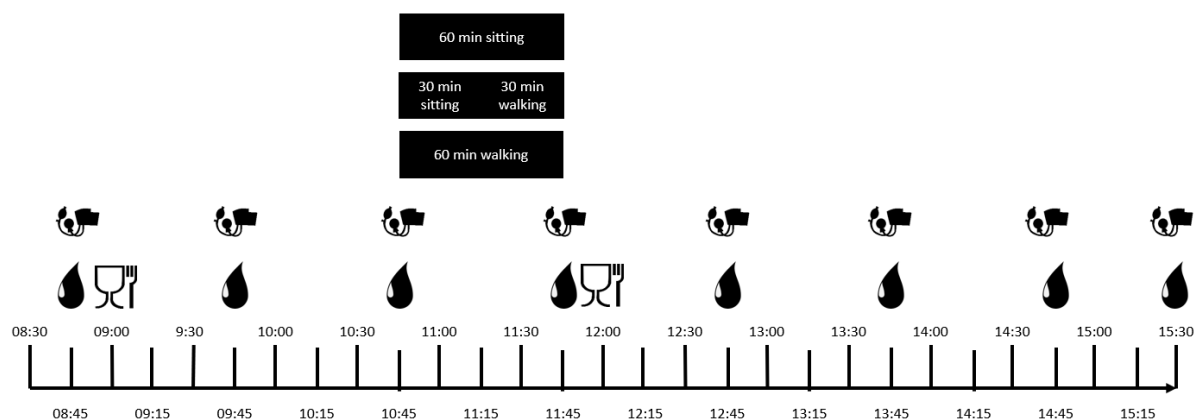


Figure 15 Timeline of the 7-hour lab visit protocol: including eight capillary blood draws (droplet icons) and eight blood pressure measurements (cuff icons) starting at 08:45 and ending at 15:30. Participants consumed two standardised meals at 09:00 and 12:00 (cup and fork icons). Participants were assigned to one of six possible treatment orders, undertaking one of three exercise conditions on each occasion: 60 minutes of sitting, 30 minutes of sitting followed by 30 minutes of walking, and 60 minutes of walking. This protocol was designed to evaluate the modulating effect of different exercise conditions on the primary study outcomes.

4.2.13. Capillary blood samples and blood pressure

Eight capillary blood samples were taken during the lab-day (Figure 15). The first sample was taken at 08:45 and were then taken hourly until 14:45. A final eighth sample was taken at 15:30. The post-lunch sample corresponded to 45 minutes after food intake and timing was chosen according to previous data (see Chapter 3). Immediately after each blood sample was obtained, samples were inserted into a Cholestech LDX cassette (Cholestech Corporation. Cholestech LDX System. Hayward, CA). This cassette was then placed in the Cholestech LDX. The machine uses enzymatic reactions and reflectance photometry to obtain values for total TC, HDL cholesterol, triglycerides and glucose. LDL cholesterol was estimated by the machine using the TC/HDL ratio. This machine has been shown to correlate highly significantly to hospital lab results when performed appropriately (Carey et al. 2006). The Cholestech LDX was not utilised out with the lab environment. Blood pressure sampling corresponded to the capillary blood sample timing and was carried out on the contralateral arm to the IFGM.

4.2.14. Diet

Diet control was similar to the trial in Chapter 3, but subtle differences occurred. Namely, on day three, participants were required to eat as normal, except they were provided with their first individualised meal, which was to be eaten between 19:30 and 20:30. This was followed by a period of fasting until breakfast the next day. The second, third and fourth meals were provided on day four and eaten in the lab at 09:00 (breakfast), 12:00 (lunch) and 15:30 (snack). The fifth, and last meal provided, was an evening meal that was identical to the first and eaten between 19:30 and 20:30 on day four. Participants then fasted until 08:30 the next day when the research period arm ended. Meal composition closely reflected the average UK adult's dietary composition: 17% protein, 36% fat and 47% carbohydrate (Whitton et al. 2011). A physical activity factor of 1.4 (reflecting a sedentary lifestyle) was used to determine individualised energy requirements using Nutritics (Nutritics LTD, Ireland). The Mifflin St Jeor equation was chosen to calculate resting metabolic rate, as it is relatively accurate in healthy obese and non-obese populations compared to other commonly used equations (Frankenfield 2013). Each of the five meals contained 25% of estimated daily calorific needs. On days three to five, participants were required to record everything they ate in their research diary. They could then replicate their energy intake in each subsequent research period. At home, participants were asked to cook their evening meals in the same manner each time. Electronic

food scales were provided to ensure accurate measurement of food (CS-2000B Digital Kitchen Scale, Ohaus Corporation, USA).

4.2.15. Power calculation

The power calculation was performed in the same manner as in Chapter 3. Therefore, researchers aimed to recruit $n=18$ participants. However, due to UK-wide coronavirus lockdown in March 2020 lab activities ceased and recruitment was finalised at $n=17$.

4.2.16. Data processing and statistical analysis

The main outcome of interest was interstitial glucose total area under the curve (tAUC). For IFGM, several different segments were analysed over the 24-hours: 1) tAUC over 24-hours; 2) tAUC during the lab-day; 3) tAUC within-exercise (10:45-11:45); 4) tAUC 3-hours post-lunch start; 5) tAUC 3-hours post-snack start; 6) tAUC 3-hours post-evening meal start and 7) tAUC during sleep. As the first bout of walking began at different times in each condition, data were analysed for the full hour from 10:45, which is referred to as the 'within-exercise' period. Although wake and sleep times varied somewhat per participant, average reported sleep times were between 22:30 and 06:30. This was therefore used for the analysis sleep time window. Interstitial glucose tAUC was calculated according to methods in Chapter 3.

The main secondary outcomes of interest (measured by the Cholestech LDX) included capillary measures of glucose, total cholesterol, HDL cholesterol, LDL cholesterol (estimated) and triglycerides. Additionally, measurements of blood pressure were also performed as secondary outcome measures. These secondary outcomes were analysed over the lab-day, during the within-exercise period and after lunch. Total AUC was calculated according to methods in Chapter 3.

Linear mixed-effects model analyses were conducted following the same approach as described in Chapter 3. Specifically, baseline values, covariates, factors coding, cluster variables, gender, treatment order and BMI were incorporated in the same way. Exercise dose (SIT, 30WALK or 60WALK) and age group ('Older' or 'Younger') were fixed factors in all models. Additionally, however, appendicular lean mass to height ratio and appendicular lean mass to BMI ratio were individually entered as covariates, but removed if fit, and explanation of variance was not improved. In addition to physiological measures of health, an additional psychological outcome measure was completed by participants: the I-PANAS-SF. The delta

change was used to assess changes in positive and negative affect scores following the three 'intervention' types. For the reasons outlined in the physiological measurements, mixed-effects models were utilised to analyse the I-PANAS-SF scores. Exercise dose and age group were fixed factors in all models. The potential influence of gender, BMI, and treatment order were also explored as fixed factors but were removed if fit and explanation of variance was not improved. The Karolinska sleepiness scale scores and SWEMWBS were covariates in the analysis. Jamovi (Version 2.4.11.0) was utilised for all statistical analyses and performed according to the methods in Chapter 3.

4.3. Results

Body composition and physical performance measures are illustrated in Table 7. Using Welch's t-test, statistical differences were observed for age ($p < 0.001$) and between age groups for 50-meter comfortable walk speed ($p = 0.010$).

Cardiometabolic and health risk indicators are illustrated in Table 8. Using Welch's t-test, statistical differences were observed between age groups, for systolic blood pressure ($p = 0.004$), fasted glucose ($p = 0.008$) and the SWEMWBS metric ($p = 0.038$). Although different at baseline between age groups, when used as a covariate, the SWEMWBS metric did not alter the significance of mood results.

Although DEXA was used to determine the potential effect of ALM ratios on primary outcomes, and to describe the sample population, inclusion did not affect any glucose or lipid panel results. It was therefore removed from the statistical models. All 17 participants completed all three trial conditions.

*Table 7 Gender, age, body composition and physical performance measures split by age group (Younger or older adults). BMI = Body Mass Index. * Stars indicate significant differences between age groups.*

	Age Group	N	Mean	SD
Gender	Older	8		
	Younger	9		
Age (years)*	Older	8	63	5
	Younger	9	30	7
Body Mass (kg)	Older	8	83.6	11.5
	Younger	9	76.6	18.0
Height (m)	Older	8	1.69	0.08
	Younger	9	1.69	0.10
Body Mass Index	Older	8	29.3	3.3
	Younger	9	27.1	6.9
Waist (cm)	Older	8	98.2	9.1
	Younger	9	86.9	16.6
Hip (cm)	Older	8	102.0	21.1
	Younger	9	105.2	12.3
Waist to hip ratio	Older	8	1.01	0.25
	Younger	9	0.82	0.12
Total Bone Mass (g)	Older	8	2629.9	495.2
	Younger	9	2541.6	271.7
Total Fat Mass (g)	Older	8	29925.0	7258.4
	Younger	9	27463.3	15432.0
Total Lean Mass (g)	Older	8	49446.8	10191.7
	Younger	9	45930.1	6878.4
Appendicular Lean Mass to Height Ratio	Older	8	7.8	1.4
	Younger	9	7.4	1.0
Appendicular Lean Mass to BMI Ratio	Older	8	0.8	0.2
	Younger	9	0.8	0.3
Maximum handgrip (kg)	Older	8	35.1	9.7
	Younger	9	35.0	8.7
50-meter comfortable walk (seconds)	Older	8	30.7	2.7
	Younger	9	36.5	5.0
50-meter brisk walk (seconds)	Older	8	23.6	1.6
	Younger	9	23.5	3.8

Table 8 Cardiometabolic and health risk indicators split by age group (Younger or older adults). Stars indicate significant differences between age groups. IPAQ 7 sedentary hours = International Physical Activity Questionnaire #7 (number of daily self-reported sedentary hours). SWEMWBS = Short Warwick-Edinburgh Mental Wellbeing Scale.

	Age Group	N	Mean	SD
Blood pressure systolic (mm Hg)*	Older	8	131.0	9.2
	Younger	9	113.8	11.3
Blood pressure diastolic (mm Hg)	Older	8	83.4	6.4
	Younger	9	76.2	11.0
Fasted glucose (mmol/L)*	Older	8	5.4	0.6
	Younger	9	4.6	0.3
IPAQ 7 sedentary (hours)	Older	8	11	3
	Younger	9	10	2
SWEMWBS Metric*	Older	8	26.7	3.8
	Younger	9	22.9	2.8

4.3.1. Glucose

4.3.1.1. 24-hour iGlucose

In Figure 16 the 15-minute iGlucose epoch values throughout the 24-hours are compared between the three exercise dose groups versus baseline. Fixed effects omnibus tests indicated that time of day had a significant effect on iGlucose ($F(95, 4357.1) = 27.50, p < 0.001$). Fixed effects parameter estimates indicated that baseline iGlucose values were significantly different (all $p < 0.05$) from the periods of 09:15 to 11:00, 12:15 to 13:45, 15:45 to 18:45 and 20:15 to 00:45 (excluding 23:30 to 00:00 which approached the significance threshold but did not achieve it i.e. $p > 0.05$ but < 0.10). SIT is largely obscured by 30WALK as can be identified from Figure 17.

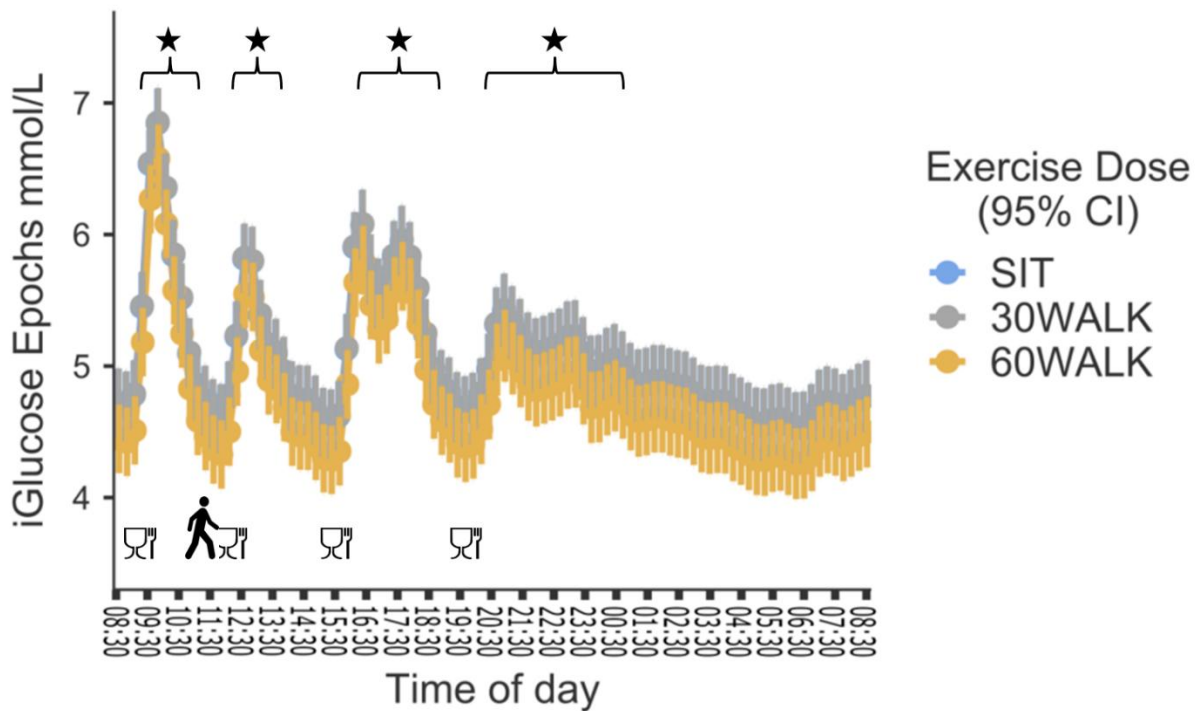


Figure 16 15-minute epoch interstitial flash glucose values (mmol/L) throughout the 24-hours (08:30-08:30) for the three exercise conditions: 0 minutes (SIT) in blue, 30 minutes walking (30WALK) in grey, and 60 minutes walking (60WALK) in gold. Breakfast began at 09:00. The exercise condition period began at 10:45 and ended at 11:45. Subsequently, meals were lunch at 12:00, afternoon snack at 15:30, dinner between 19:30 and 20:30. Meals are represented by cup and fork icons: Meal 1 (breakfast), Meal 2 (lunch), Meal 3, (snack), Meal 4 (dinner). The exercise condition period is represented by the walking person icon. Starred areas represent statistically significantly different values from baseline values at 08:30. Error bars represent 95% confidence intervals.

4.3.1.2. 24-hour interstitial flash glucose monitor total area under the curve

Fixed effects omnibus tests indicated that fasted baseline iGlucose values at the start of the 24-hour period were a significant predictor of iGlucose tAUC throughout the 24-hours ($F(1, 14.9) = 60.391, p < 0.001$). Additionally, exercise dose was also a significant predictor of iGlucose ($F(2, 21) = 4.660, p = 0.021$). However, age group was not a significant predictor of iGlucose ($F(1, 11) = 0.044, p = 0.837$). No significant interaction effect was observed between exercise dose and age groups ($F(2, 21.8) = 1.479, p = 0.250$). The marginal R^2 was 0.78, and the conditional R^2 was 0.87.

Fixed effects parameter estimates also indicated that the exercise dose comparison of 60WALK versus SIT was significantly different for iGlucose tAUC (Estimate = -28.02, SE = 9.14, 95% CI [-45.94, -10.1], $p = 0.005$). Furthermore, no significant differences were observed for the interaction effect of 60WALK versus SIT and age group (Estimate = 22.21, SE = 12.92, 95% CI [-3.11, 47.50], $p = 0.099$) although it approached the threshold of significance. Random-effect variance components indicated a participant-level random intercept standard deviation of 14.1 (variance = 198) and a residual standard deviation of 16.7 (variance = 279).

Post hoc comparisons indicated that 30WALK versus 60WALK were significantly different (Difference = 16.998, SE = 6.66, $p = 0.041$) with 30WALK having a higher tAUC than 60WALK. Furthermore, SIT versus 60WALK were significantly different (Difference = 16.920, SE = 6.36, $p = 0.041$) indicating a higher tAUC for SIT than 60WALK. Figure 17 represents these differences.

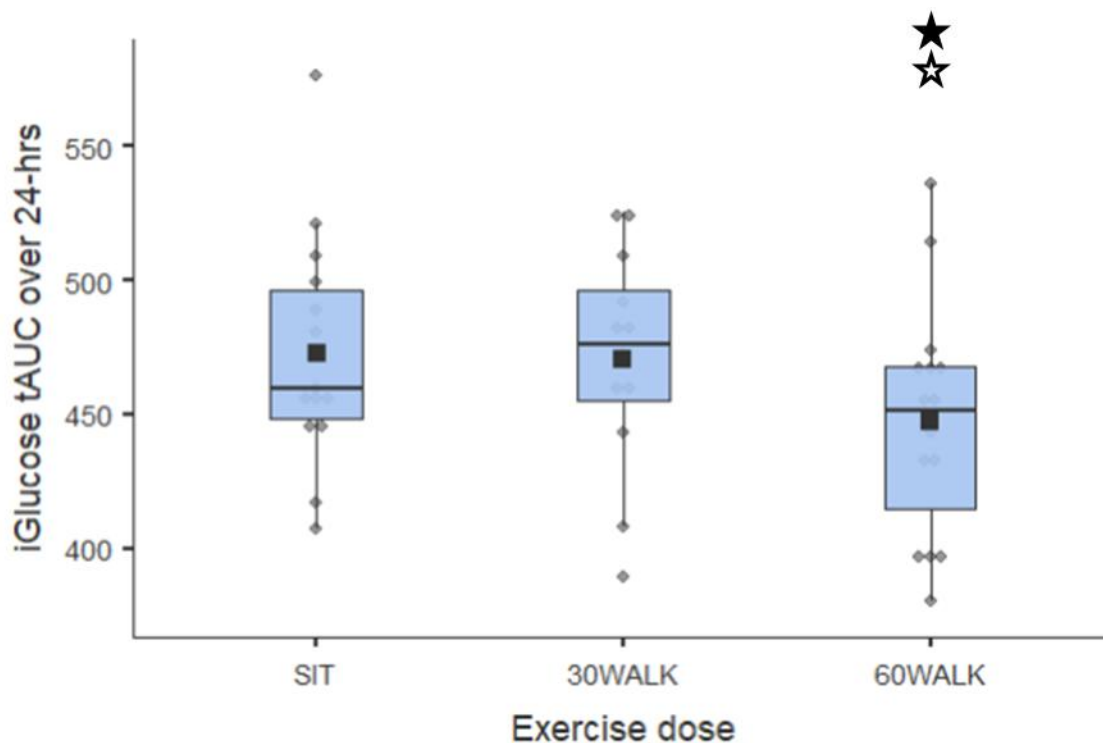


Figure 17 Box and whisker plot showing the relationship between exercise dose and interstitial glucose (iGlucose) total area under the curve (tAUC) throughout the 24-hour period for the three exercise conditions: 0 minutes (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. The black star represents significantly lower values than SIT. The empty star represents significantly lower values than 30WALK. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions.

4.3.1.3. Summary: Glucose

In Table 9, a summary is provided of the significant results for the primary glucose variables of interest which are exercise dose, and the potential interaction of exercise dose by age group. The main time of interest was the full 24-hour period. The *a priori* sub-analysis time periods are also presented to illustrate where other differences occurred. A detailed breakdown of the sub-period analysis can be found in 'Supplementary Material Chapter 4'.

*Table 9. Significant results for interstitial flash glucose monitor (iFGM) total area under the curve (tAUC) over the seven time periods analysed and significant capillary glucose (cGlucose) tAUC over the three time periods analysed. Three exercise conditions are represented: 0 minutes walking (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). Only primary variables of interest are included (exercise dose and exercise dose * age group) to improve table readability. Interaction effect comparisons are denoted by the * symbol.*

Time period	Main effects	Fixed effects parameter estimates	Post Hoc Tests
24-hours iFGM	Exercise dose	60WALK - SIT: higher tAUC for SIT	30WALK - 60WALK: higher tAUC for 30WALK - SIT - 60WALK: higher tAUC for SIT
Lab-Day iFGM	None	60WALK - SIT: higher tAUC for SIT 30WALK - SIT * Older – Younger: higher for older	None
Lab-Day cGlucose	None	None	None
Exercise iFGM	Exercise dose	None	SIT - 60WALK: higher tAUC for SIT
Exercise cGlucose	Exercise dose	60WALK - SIT: higher tAUC for 60WALK	30WALK - 60WALK: higher tAUC for 60WALK
Lunch iFGM	Exercise dose * age group	60WALK - SIT: higher tAUC for SIT 30WALK - SIT * Older – Younger: higher for older	None

		60WALK - SIT * Older – Younger: higher for older	
Lunch cGlucose	None	None	None
Snack iFGM	- Exercise dose - Exercise dose * age group	60WALK - SIT: higher tAUC for SIT 60WALK - SIT * Older – Younger: higher tAUC for older	30WALK - 60WALK: higher tAUC for 30WALK - SIT - 60WALK: higher tAUC for SIT 30WALK - 60WALK * Younger - Younger: higher tAUC for 30WALK 60WALK - 30WALK * Older - Younger: higher tAUC for 30WALK & older - SIT - 60WALK * Younger - Younger: higher tAUC for SIT
Dinner iFGM	None	60WALK - SIT: higher tAUC for SIT	None
Sleep iFGM	None	60WALK - SIT: higher tAUC for SIT	None

4.3.2. Lipid panel capillary bloods

4.3.2.1. Lab-day lipid panel

In Figure 18 A the 1-hour total cholesterol (TC) epoch values throughout the lab-day are compared between the three exercise dose groups versus baseline. Fixed effects omnibus tests indicated that TC values were not significantly different between time points in the lab-

day ($F(7, 368) = 1.395, p = 0.206$). Fixed effects parameter estimates also indicated that baseline value TC values were not significantly different from any other time point in the lab-day (all $p > 0.05$).

In Figure 18 B the 1-hour HDL cholesterol epoch values throughout the lab-day are compared between the three exercise dose groups versus baseline. Fixed effects omnibus tests indicated that time of day was significantly different between periods ($F(7, 368) = 2.225, p = 0.032$). However, fixed effects parameter estimates, and post hoc tests indicated that none of the differences were significantly different than baseline (all $p > 0.05$).

In Figure 18 C the 1-hour LDL cholesterol epoch values throughout the lab-day are compared between the three exercise dose groups versus baseline. Fixed effects omnibus tests indicated that LDL values were not significantly different between time points in the lab-day ($F(7, 368) = 1.671, p = 0.115$). Fixed effects parameter estimates also indicated that baseline value LDL values were not significantly different from any other time point in the lab-day (all $p > 0.05$).

In Figure 18 D the 1-hour triglyceride epoch values throughout the lab-day are compared between the three exercise dose groups versus baseline. Fixed effects omnibus tests indicated that time of day was significantly different between periods ($F(7, 368) = 11.576, p < 0.001$). Fixed effects parameter estimates indicated that the differences lay between baseline (08:45) and 12:45, 13:45, 14:45 and 15:30 (all $p < 0.05$) whereby triglycerides increased over time. Post hoc tests also indicated significant differences between baseline and 12:45, 13:45, 14:45 and 15:30 (all $p < 0.05$).

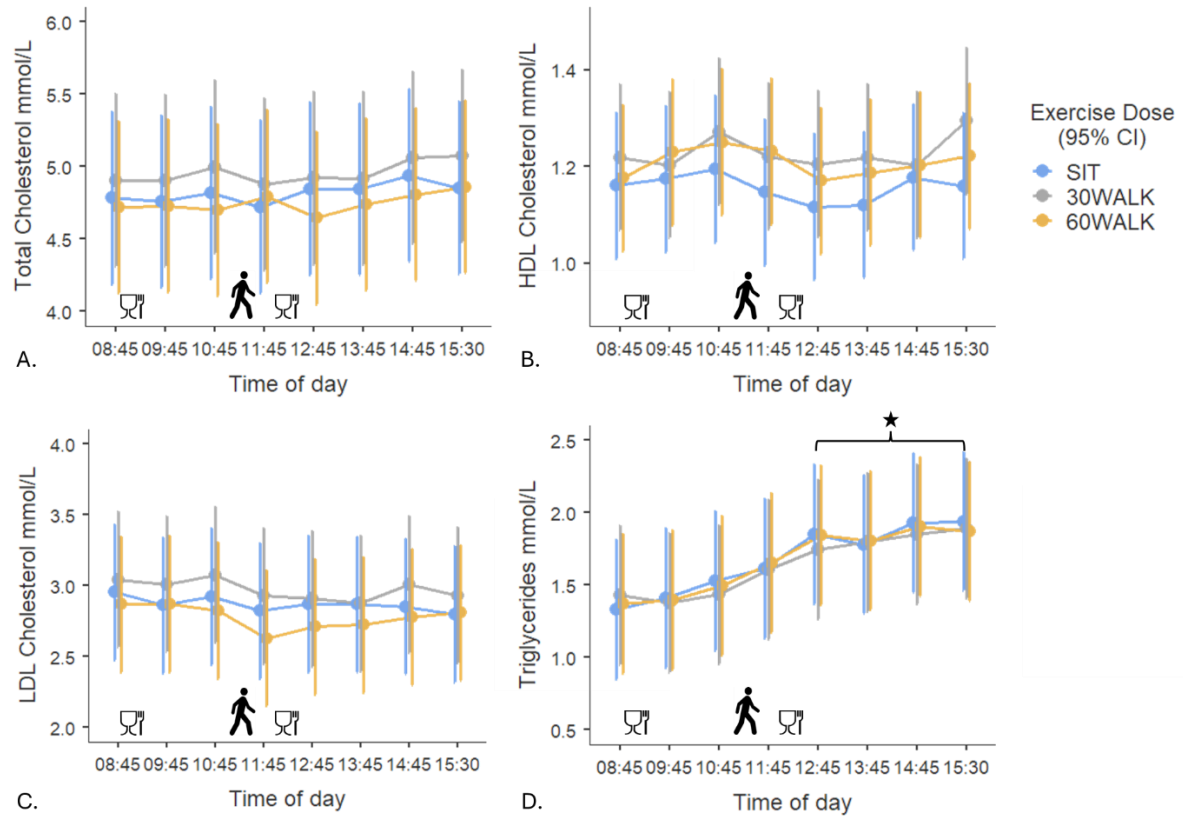


Figure 18 A-D Average epochs values (mmol/L) during the lab-day for total cholesterol (A), HDL cholesterol (B), LDL cholesterol (C) and triglycerides (D). The three exercise conditions are represented: 0 minutes (SIT) in blue, 30 minutes walking (30WALK) in grey, and 60 minutes walking (60WALK) in gold. The lab-day began at 08:30 and breakfast began at 09:00. The exercise condition period began at 10:45 and ended at 11:45. Subsequently, the lunch meal began at 12:00. Participants ended the lab-day at 15:30. Each time point (08:45-15:30) represents a capillary blood sample time. Stars represent statistically significantly different values from baseline (08:45). Meals are represented by cup and fork icons. The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals.

4.3.2.2. Lab-day capillary total cholesterol tAUC

Fixed effects omnibus tests indicated that fasted capillary total cholesterol baseline values at the start of the lab-day was a predictor of total cholesterol throughout the lab-day ($F(1, 17.5) = 521.766, p < 0.001$). In addition, BMI was a predictor of total cholesterol throughout the lab-day ($F(1, 11.8) = 7.353, p = 0.019$). Significance was not observed for exercise dose ($F(2, 27.5) = 0.219, p = 0.805$), nor age group ($F(1, 10.9) = 1.691, p = 0.220$) nor the interaction between

exercise dose and age group ($F(2, 27.3) = 0.845, p = 0.441$). The marginal R^2 was 0.96, and the conditional R^2 was 0.97. Other than baseline total cholesterol and BMI, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 0.91 (variance = 0.83) and a residual standard deviation of 1.29 (variance = 1.67). No post hoc tests were carried out.

4.3.2.3. Lab-day capillary HDL cholesterol tAUC

Fixed effects omnibus tests indicated that fasted HDL baseline values at the start of the lab-day were a predictor of HDL throughout the lab-day ($F(1, 41.50) = 101.611, p < 0.001$). The interaction of exercise dose by age group also showed significance ($F(2, 24.17) = 3.654, p = 0.041$). No significant difference was observed for exercise dose ($F(2, 23.51) = 2.477, p = 0.106$) or for age group ($F(1, 9.37) = 0.083, p = 0.780$). The marginal R^2 was 0.72, and the conditional R^2 was 0.94.

Parameter estimates indicated that the exercise dose comparison of 60WALK versus SIT was significantly different for HDL (Estimate = 0.309, SE = 0.114, 95% CI [0.03, 0.59], $p = 0.043$). Parameter estimates also indicated the interaction of exercise dose by age group for 30WALK versus SIT was significant (Estimate = 0.732, SE = 0.29, 95% CI [0.16, 1.30], $p = 0.019$) with older adults having higher values at 30WALK versus younger adults in SIT. This suggests that the 30WALK versus SIT may be moderated by age. Random-effect variance components indicated a participant-level random intercept standard deviation of 0.79 (variance = 0.62) and a residual standard deviation of 0.42 (variance = 0.18). However, post hoc tests did not show significance for the interaction of exercise dose by age group (all $p > 0.05$). Post hoc tests for exercise dose were not performed.

4.3.2.4. Lab-day capillary LDL cholesterol tAUC

Fixed effects omnibus tests indicated that fasted LDL baseline values at the start of the lab-day were a predictor of LDL throughout the lab-day ($F(1, 21.04) = 346.495, p < 0.001$). Age group approached the threshold but did not reach statistical significance ($F(1, 9.46) = 4.364, p = 0.065$). Similarly, the interaction of exercise dose by age group approached the threshold but did not reach statistical significance ($F(2, 24.41) = 3.045, p = 0.066$). Significant differences

were not observed between exercise doses ($F(2, 23.88) = 0.226, p = 0.799$). The marginal R^2 was 0.92, and the conditional R^2 was 0.95.

Fixed effects parameter estimates indicated statistically significant differences between the interaction of age group and 60WALK versus SIT (Estimate = 2.015, SE = 0.92, 95% CI [0.20, 3.82], $p = 0.039$). This suggests that the effects of 60 minutes of walking versus sitting may be moderated by age, with older adults seeing a higher level of LDL compared to younger adults. Results show 60WALK versus SIT approached the threshold but did not reach statistical significance (Estimate = -1.139, SE = 0.62, 95% CI [-2.36, 0.08], $p = 0.081$). Random-effect variance components indicated a participant-level random intercept standard deviation of 1.12 (variance = 1.25) and a residual standard deviation of 1.32 (variance = 1.74). No post hoc tests were performed.

4.3.2.5. Lab-day capillary triglycerides tAUC

Fixed effects omnibus tests indicated that fasted triglyceride baseline values at the start of the lab-day were a predictor of triglyceride throughout the lab-day ($F(1, 36.4) = 257.686, p < 0.001$). Subjects' BMI was also a predictor of triglyceride ($F(1, 12.8) = 6.611, p = 0.023$). Additionally, significant interaction effects were observed between exercise dose and age group ($F(2, 27.6) = 3.680, p = 0.038$). No significant differences were observed for exercise dose alone ($F(2, 27.2) = 2.004, p = 0.154$) or for age group ($F(1, 12.1) = 0.008, p = 0.928$). The marginal R^2 was 0.82, and the conditional R^2 was 0.96.

Parameter estimates indicated 30WALK was significantly different than SIT (Estimate = -1.620, SE = 0.55, 95% CI [-2.71, -0.53], $p = 0.007$). Furthermore, parameter estimates identified statistically significant differences between the interaction of age group and 30WALK versus SIT (Estimate = 1.666, SE = 0.806, 95% CI [0.09, 3.25], $p = 0.048$). This suggests that the effects of 30WALK versus SIT may be moderated by age, with older adults seeing a higher level of triglyceride compared to younger adults. Random-effect variance components indicated a participant-level random intercept standard deviation of 2.12 (variance = 4.49) and a residual standard deviation of 1.16 (variance = 1.35). Post hoc tests indicated revealed no statistically significant differences between parameters (all $p > 0.05$).

4.3.2.6. Summary: Non-glucose capillary bloods results

In Table 10, a summary is provided of the significant results for the primary capillary variables of interest which were exercise dose, and the potential interaction of exercise dose by age group. The main time of interest was the full lab period. The *a priori* sub-analysis time periods are also presented to illustrate where other differences occurred.

*Table 10 Significant results for capillary bloods total area under the curve (tAUC) over the three time periods analysed. The three exercise conditions are represented: 0 minutes walking (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). Only primary variables of interest are included (exercise dose and exercise dose * age group) to improve table readability. TC = total cholesterol. HDL = HDL cholesterol. LDL = LDL cholesterol. TRIG = triglycerides. Interaction effect comparisons are denoted by a * symbol.*

Time period	Main effects	Fixed effects parameter estimates	Post Hoc Tests
Lab-day TC	None	None	None
Lab-day HDL	Exercise dose * age group	60WALK – SIT: higher tAUC for 60WALK 30WALK – SIT * Older - Younger: higher tAUC for older	None
Lab-day LDL	None	60WALK – SIT * Older – Younger: higher tAUC for older	None
Lab-day TRIG	Exercise dose * age group	30WALK - SIT: higher tAUC for SIT 30WALK – SIT * Older - Younger: higher tAUC for older	None
Exercise TC	None	None	None

Exercise HDL	• None	• None	• None
Exercise LDL	• None	• None	• None
Exercise TRIG	• None	• None	• None
Post-exercise TC	• None	• None	• None
Post-exercise HDL	• Exercise dose	• None	• 30WALK – SIT: higher tAUC for 30WALK
Post-exercise LDL	• None	• None	• None
Post-exercise TRIG	• None	• None	• None

4.3.3. Blood pressure

4.3.3.1. Systolic and diastolic blood pressure during the lab-day

In Figure 19 A the 1-hour SBP epoch values throughout the lab-day are compared between the three exercise dose groups versus baseline. Fixed effects omnibus tests indicated that time of day was significantly different between periods ($F(7, 368) = 6.32, p < 0.001$). Gender was also significant ($F(1, 15) = 8.35, p = 0.011$). Post hoc tests indicated significant differences lay between baseline (08:45) and 11:45 ($p = 0.13$).

In Figure 19 B the 1-hour DBP epoch values throughout the lab-day are compared between the three exercise dose groups versus baseline. Fixed effects omnibus tests indicated that time of day was significantly different between periods ($F(7, 368) = 12.816, p < 0.001$). Post

hoc tests indicated significant differences lay between baseline (08:45) and 09:45, 10:45, 11:45, 12:45, 13:45 and 14:45 ($p < 0.05$).

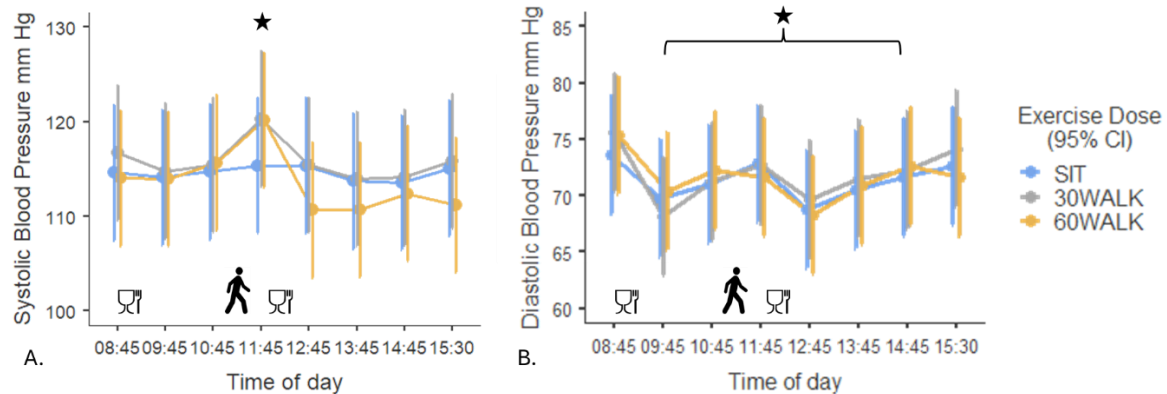


Figure 19 A-B Average epoch values (mm Hg) during the lab-day for systolic blood pressure (A) and diastolic blood pressure (B). The three exercise conditions are represented: 0 minutes (SIT) in blue, 30 minutes walking (30WALK) in grey, and 60 minutes walking (60WALK) in gold. The lab-day began at 08:30 and breakfast began at 09:00. The exercise condition period began at 10:45 and ended at 11:45. Subsequently, the lunch meal began at 12:00. Participants ended the lab-day at 15:30. Each time point (08:45-15:30) represents a blood pressure sample time. Stars represent statistically significantly different values from baseline (08:45). Meals are represented by cup and fork icons. The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals.

4.3.3.2. Lab-day systolic blood pressure tAUC

Fixed effects omnibus tests indicated that baseline SBP values at the start of the lab-day was a predictor of SBP throughout the lab-day ($F(1, 39.23) = 15.463, p < 0.001$). Gender was also a predictor of SBP ($F(1, 9.32) = 7.685, p = 0.021$). However, significant differences were not observed for exercise dose ($F(2, 20.94) = 0.584, p = 0.567$) or for age group ($F(1, 8.56) = 2.053, p = 0.187$). There was no significant interaction effect for exercise dose by age group ($F(2, 20.72) = 1.763, p = 0.196$). The marginal R^2 was 0.64, and the conditional R^2 was 0.93. Other than baseline SBP and gender, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). Random-effect variance components indicated

a participant-level random intercept standard deviation of 46.5 (variance = 2159) and a residual standard deviation of 22.2 (variance = 495). Post hoc tests indicated that gender (females versus males) was a predictor of lab-day SBP tAUC (Difference = -69.5, SE = 25.2, $p = 0.014$) with males having higher values compared to females.

4.3.3.3. *Lab-day diastolic blood pressure tAUC*

Fixed effects omnibus tests indicated that baseline DBP values at the start of the lab-day was a predictor of DBP throughout the lab-day ($F(1, 13.77) = 143.685, p < 0.001$). Results also indicated BMI was a predictor of DBP ($F(1, 10.05) = 6.906, p = 0.025$). In addition, age group was also a predictor of DBP ($F(1, 9.82) = 5.177, p = 0.047$). However, exercise dose was not a significant predictor ($F(2, 26.03) = 0.905, p = 0.417$), nor was the interaction between exercise dose and age group ($F(2, 25.64) = 0.689, p = 0.511$). The marginal R^2 was 0.89, and the conditional R^2 was 0.92.

Other than baseline DBP and BMI, fixed effects parameter estimates indicated age group was also a predictor of DBP (Estimate = 25.938, SE = 11.25, 95% CI [3.90, 47.98], $p = 0.028$). However, no differences were observed between the remaining parameters (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 10.7 (variance = 114) and a residual standard deviation of 19.3 (variance = 374). Post hoc tests illustrated that age group was a significant predictor of DBP (Difference = -18.4, SE = 8.13, $p = 0.040$) with the older group having higher values than younger group.

4.3.3.4. *Summary: Systolic and diastolic blood pressure results*

In Table 11 a summary is provided of the significant results for the primary blood pressure variables of interest which are exercise dose, and the potential interaction of exercise dose by age group. The main time of interest was the full lab period. The a priori sub-analysis time periods are also presented to illustrate where other differences occurred.

*Table 11 Significant results for blood pressure total area under the curve (tAUC). The three exercise conditions are represented: 0 minutes walking (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). Only primary variables of interest are included (exercise dose and exercise dose * age group) to improve table readability. SBP = systolic blood pressure. DBP = diastolic blood pressure. Interaction effect comparisons are denoted by a * symbol.*

Time period	Main effects	Fixed effects parameter estimates	Post Hoc Tests
Lab-day SBP	• None	• None	• None
Lab-day DBP	• None	• None	• None
Exercise SBP	• None	• None	• None
Exercise DBP	• None	• None	• None
Post-exercise SBP	• Exercise Dose	• None	• 30WALK – 60WALK: higher tAUC for 30WALK
Post-exercise DBP	• None	• 60WALK – SIT * Older – Younger: higher tAUC for older	• None

4.3.4. I-PANAS-SF results

4.3.4.1. I-PANAS-SF mood results

In Figure 20 A the average positive affect scores from the I-PANAS-SF between baseline (08:45) and after exercise condition (11:45) are compared between the three exercise dose groups. Fixed effects omnibus tests indicated that time of day was not significantly different between periods ($F(1, 80) = 2.768, p = 0.100$). Post hoc tests were not performed.

In Figure 20 B the average negative affect scores from the I-PANAS-SF between baseline (08:45) and after exercise condition (11:45) are compared between the three exercise dose

groups. Fixed effects omnibus tests indicated that time of day was not significantly different between periods ($F(1, 80) = 1.702, p = 0.196$). Post hoc tests were not performed.

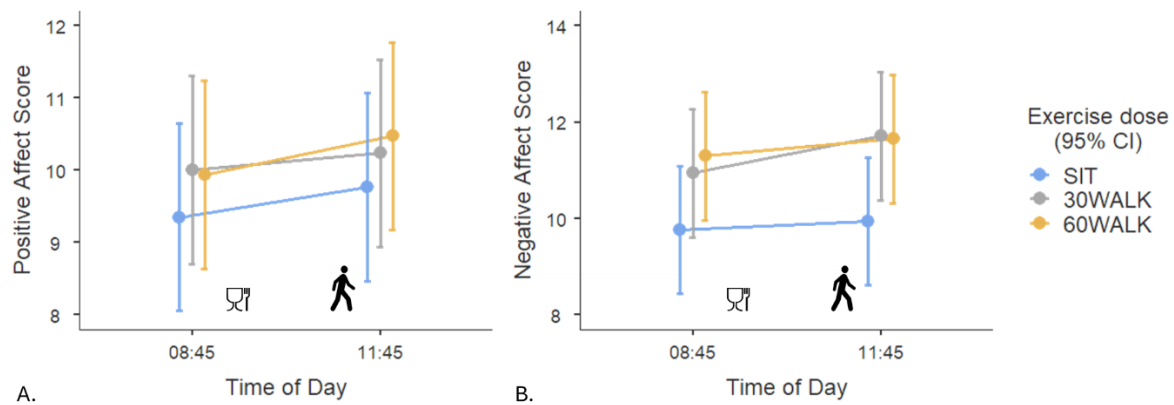


Figure 20 A-B Average positive affect scores (A) and negative affect scores (B) from the I-PANAS-SF between baseline (08:45) and after the exercise condition (11:45). The three exercise conditions are represented: 0 minutes (SIT) in blue, 30 minutes walking (30WALK) in grey, and 60 minutes walking (60WALK) in gold. The lab-day began at 08:30 and breakfast began at 09:00. The exercise condition period began at 10:45 and ended at 11:45. Meals are represented by cup and fork icons. The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals.

4.3.4.2. Change scores for I-PANAS SF positive affect scale before and after exercise.

Fixed effects omnibus tests indicated that the interaction of exercise dose and age group approached the threshold of significance but did not achieve it ($F(2, 28.6) = 3.271, p = 0.053$). Furthermore, the SWEMWBS was not a predictor of positive affect ($F(1, 14) = 0.003, p = 0.957$) nor was sleepiness score ($F(1, 14) = 0.151, p = 0.699$). In addition, no significant main effects were observed for exercise dose ($F(2, 28.2) = 0.106, p = 0.900$) or age group ($F(1, 13.4) = 2.629, p = 0.128$). The marginal R^2 was 0.16, and the conditional R^2 was 0.36.

Parameter estimates showed that the interaction between 60WALK versus SIT and age group was significant (Estimate = -2.594, SE = 1.02, 95% CI [-4.59, -0.60], $p = 0.017$), indicating some evidence that the effect of exercise dose on positive affect was moderated by age. Additionally, the effect of 60WALK versus SIT approached the threshold of significance but did not achieve significance (Estimate = 1.318, SE = 0.70, 95% CI [-0.05, 2.69], $p = 0.070$). Random-

effect variance components indicated a participant-level random intercept standard deviation of 0.81 (variance = 0.66) and a residual standard deviation of 1.48 (variance = 2.19). No post hoc tests were performed.

4.3.4.3. Change scores for I-PANAS SF negative affect scale before and after exercise.

Fixed effects omnibus tests indicated that age group approached the threshold of significance but did not achieve it ($F(1, 14.8) = 4.470, p = 0.052$). No significant main effects were observed for exercise dose ($F(2, 29.6) = 0.570, p = 0.572$), sleepiness score ($F(1, 38.7) = 0.391, p = 0.535$), or SWEMWBS ($F(1, 15.5) = 0.506, p = 0.488$). No significant interaction was observed between exercise dose and age ($F(2, 30) = 0.800, p = 0.459$). The marginal R^2 was 0.14, and the conditional R^2 was 0.31. Fixed effects parameter estimates did not reveal any significant differences in the parameters (all $p > 0.05$). Random-effect variance components indicated a participant-level random intercept standard deviation of 0.87 (variance = 0.76) and a residual standard deviation of 1.77 (variance = 3.12). No post hoc tests were performed.

4.4. Discussion

The primary aim of this study was to investigate the acute effects of breaking up a workday sitting with 30 and 60 minutes of LPA walking on iGlucose levels in apparently healthy, sedentary and inactive adults. The primary findings were twofold. Firstly, over 24-hours, breaking up a sedentary day with 60WALK decreased tAUC iGlucose compared to a control. Within the 24-hour period, specific differences in iGlucose occurred between 60WALK and SIT during both the exercise period and the snack period. Secondly, over 24-hours, 60WALK decreased tAUC iGlucose more than 30WALK. Within the 24-hour period, specific differences occurred between 60WALK and 30WALK during the snack period but not exercise period. These results have revealed a clear dose response relationship to LPA walking, with longer walking causing the greatest decrease in iGlucose tAUC. This reinforces the role of non-strenuous physical activity in glycaemic regulation and supports that the total duration of continuous physical activity plays a role in metabolic responses.

The secondary aim of this study was to investigate if age influenced the acute effects of breaking sitting with 60 and 30 minutes of LPA walking on iGlucose levels. The findings revealed that the influence of exercise dose was moderated by age group. During the snack period, younger adults exhibited significantly lower tAUC iGlucose levels following 60WALK compared to both 30WALK and SIT conditions, whereas this effect was not observed in older adults. Furthermore, tAUC iGlucose was lower for 60WALK compared to 30WALK in younger adults compared to older adults. These results indicate that while 60 minutes of LPA walking was effective in lowering iGlucose for sedentary and inactive younger adults, this response was not present in sedentary and inactive older adults during snack. This suggests longer duration LPA walking is required in a young sedentary inactive group to elicit decreases in iGlucose. Whereas in older adults, other physical activity variables may need to be altered to elicit an iGlucose lowering effect.

Both primary and secondary study findings indicate that LPA walking is sufficient to acutely lower iGlucose levels in adults, with longer periods of walking eliciting a greater glucose lowering effect. However, the responses are age dependent with older adult iGlucose levels not lowering in the manner of younger adults. As far as this author is aware, this is the first trial to observe a dose response when comparing the effects of breaking up a simulated workday with differing lengths of continuous LPA walking in sedentary and inactive adults. It

is also the first to compare older and younger adult groups within the same trial and report differing age-related responses.

4.4.1. Glucose responses

Very few other lab studies have assessed the effects of breaking up a simulated workday with a continuous bout of LPA walking following a pre-trial period of controlled physical activity and dietary restrictions. One of those trials was carried out by Gao et al. (2024) who studied the effects of 30 minutes LPA walking on 18 males (age 21 ± 1.2 , BMI 28.8 ± 2.2). They reported lower glucose values for LPA when compared to sitting. With a well-controlled pre-trial period, strict lab conditions, and similar inclusion criteria (except age) their research is relatively comparable to ours. However, their walking speed (4 km/h) was slightly higher than ours (2.57 km/h), which may explain why they were able to see reduced glucose levels during the lab-day for 30 minutes walking. This contrasts with our results compared to SIT where we did not observe difference over the full lab period. Instead, we observed differences after 60WALK over the 24-hours, and specifically during exercise and during the 15:30 three-hour snack period. Additionally, their trial only used young adults, whereas ours utilised both younger and older adults. Our results observed interaction effects during the three-hour snack period between exercise dose and age, whereby younger adults reduced glucose levels and older adults did not. Therefore, the evidence by Gao et al. (2024) may support our research showing that responses may be age dependent, with younger adults being more able to lower glucose acutely after LPA exercise. The increase in insulin resistance with ageing is well established (Chia et al. 2018), and the possible lack of metabolic flexibility in our older group may help to explain the lack of an acute decrease in their glucose levels following LPA.

In a recent trial, Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. (2024) explored the effects of continuous walking on 17 young adults (Age 23.4 ± 3.3 years, 29.7 ± 3.8 kg/m², 47% females). They walked for the full eight-hour lab period, excluding allocated times for the toilet, and presumably for meals. Mean glucose for LPA walking was shown to be lower over the eight hours of the trial compared to sitting and specifically lower during the first two hours after breakfast. This contrasts with our study as our lab period alone was not shown to be significantly lower for LPA walking than SIT. However, their results do align in other ways. We observed lower glucose for 60WALK vs 30WALK and SIT over 24 hours (inclusive of the lab-day) and during the snack period (which was

immediately after the lab-day). Like Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Lackner et al. (2024), we also observed lower glucose levels in the exercise period between breakfast and lunch. Of note, their results were in young male and female adults, and our trial indicated that the response of LPA interventions may be modulated by age. Therefore, their trial reinforces that younger adults respond to continuous LPA bouts of considerable length.

Our research has shown that a same-day dose response relationship exists for a continuous bout of LPA breaking a sitting day, with longer walking causing the greatest decrease in iGlucose tAUC. Additionally, our research provides novel evidence that the effects of continuous bouts of LPA on glucose may be modulated by age. This has possible implications for exercise recommendations depending on age.

4.4.2. Lipid panel responses

A further secondary aim of this study was to assess responses of continuous LPA walking on capillary HDL cholesterol, LDL cholesterol, TC and triglycerides. We anticipated that both durations of LPA walking would not elicit a change in HDL cholesterol, LDL cholesterol or TC. As anticipated, we did not observe any significant main effects of exercise dose on LDL cholesterol or TC. HDL cholesterol was not different than SIT for 60WALK only, as expected. However, HDL cholesterol was higher than SIT for 30WALK. These results are novel as they 1) suggest that an increase in HDL cholesterol following continuous LPA may be dose dependent, and 2) provide limited evidence that triglyceride and HDL cholesterol responses may be modulated by age. Additionally, in contrast to our hypothesis, triglycerides were not different between exercise conditions.

When only the post-exercise period is considered, our trial showed evidence for an increase in HDL cholesterol tAUC for 30WALK compared to SIT. It appears counterintuitive that 30WALK had higher HDL than SIT, but 60WALK did not, as longer durations were expected to have greater effect on lipid metabolism. However, it may be that the response of HDL was dose dependent to exercise. A shorter bout (30 minutes) may have led to an increase in HDL, whereas a longer bout (60 minutes) may not have. However, the underlying mechanisms for such a response are not clear. Of note, when adding baseline HDL as a covariate, exercise condition became non-significant for main effects and post hoc analysis. Therefore, although

a dose dependent lipid response might occur acutely after LPA exercise, this should be explored further in future trials. This could be achieved by including larger sample sizes, adding more dose-response arms such as 15 and > 60 minutes of LPA, and additionally measuring evening and next day and responses.

In the trial by Katsanos et al. (2004), they assessed the response of ~ 240 minutes of LPA walking on 13 healthy active males (Age ~24, BMI ~24.3 kg/m²). They saw no difference between groups for HDL cholesterol. This contrasts with our results for 30WALK, but the LPA bouts between trial were considerably different in length. In a more recent study by Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Niedrist et al. (2024), researchers assessed breaking sitting with a continuous bout of LPA walking, lasting around eight hours, to a sitting control. They took a baseline blood sample at the beginning of the lab-day (at 08:00), one hour after the 12:00 lunch had started (at 13:00), and at the end of the lab-day (at 16:00). Their results contrasted to ours, as when compared to baseline, the postprandial lunch values were *lower* for HDL cholesterol. This effect also lasted until their final measure at the end of the lab-day. This recent evidence provides information suggesting that a dose response effect may indeed occur for HDL. Nevertheless, further studies are required to confirm or refute this hypothesis.

Aside from HDL cholesterol, a limited number of other trials have assessed triglyceride responses to continuous LPA bouts during the lab-day. Katsanos et al. (2004), reported no differences between LPA and sitting for triglycerides during the lab-day, which aligns with our research. In contrast, Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Niedrist et al. (2024) found that triglycerides were lower after lunch during eight hours of continuous walking. A limited number of trials assessed next day responses to LPA for triglycerides. In the trial by Tsetsonis and Hardman (1996), researchers assessed the responses to 180 minutes of LPA walking in nine physically active adults, aged between 25 and 32, with BMI values between 22.5 and 27.7 kg/m² (56% males). In the following day, they found that triglyceride levels were significantly lower than the sitting condition before breakfast and postprandially. However, Katsanos et al. (2004) also measured responses to LPA and did not observe differences between trials for triglycerides in the fasted state the following morning. These trials highlight that the effects of LPA exercise have the potential to occur during the lab-day and last into the following day, but the response is not consistent. Our research is

important it adds important time-specific information regarding triglyceride responses after LPA conditions.

In summary, considering the continuous bout LPA trials assessing *same-day* HDL cholesterol responses (Hoffmann, Schierbauer, Zimmermann, Voit, Grothoff, Wachsmuth, Rössler, Niedrist et al. 2024; Katsanos et al. 2004), as well as the evidence from our own research, the response to LPA walking may be dose dependent, with shorter bouts acutely increasing HDL and longer bouts potentially having a reducing effect on HDL. Furthermore, in the trials assessing next day HDL cholesterol responses to continuous LPA bouts (Katsanos et al. 2004; Tsetsonis and Hardman 1996), the effects of LPA are inconsistent. Both same day and next day HDL responses require further research to enrich the evidence available. Additionally, triglyceride responses are not consistent following LPA and require further research during and after the main lab intervention day.

4.4.3. Blood pressure responses

We hypothesised that due to the low intensity of walking activity, there would be no differences in SBP between conditions. However, this study illustrated that post-exercise SBP had a lower tAUC for 60WALK than 30WALK. See ‘Supplementary Material Chapter 4’ for detail. Initially it may seem counterintuitive that 60WALK was not also lower than SIT. However, both 30WALK and 60WALK had an elevated SBP immediately after exercise at 11:45 (Figure 19A). Whereas without exercise input SIT remained in status quo. Subsequently, 30WALK returned to pre-exercise SBP levels at 12:45 and remained there, closely shadowing the SIT condition’s SBP values, until 15:45. In contrast, when 60WALK peaked at 11:45 it subsequently dipped significantly lower throughout 12:45 to 15:45. The deeper nadir in 60WALK SBP lowered the tAUC for 60WALK and resulted in the comparably higher tAUC values for 30WALK. The post-exercise 60WALK condition was not lower than SIT because sitting did not elevate SBP at 11:45 and so there was no exercise-induced peak to contribute to an increased tAUC.

Overall, the results show that longer duration LPA elicits a stronger SBP lowering response than 30WALK, and this response lasted for several hours: indicating a dose response to LPA walking. It has previously been shown that relatively short durations of LPA (15 or 30 minutes) can reduce SBP in adults with elevated BP (Guidry et al. 2006). Our sample population was

normotensive, and we only observed a decrease in SBP after the longer duration 60-minute LPA. Therefore, our results support the notion that the SBP lowering effect of a continuous LPA break may be modulated by blood pressure status. This could have implications when designing exercise programmes designed to acutely lower SBP, where normotensive sedentary inactive adults may require a longer bout.

4.4.4. Positive and negative affect responses

A further secondary analysis of this trial was to determine whether different LPA intensities affected mood compared to sitting. We anticipated that LPA activity would improve both positive and negative affect after 30 and 60 minutes of activity. However, both emotional constructs were similar between groups. Therefore, both doses of LPA were acceptable to younger and older adults, without causing a significant increase or decrease in positive or negative affect scores. This indicates both lengths are tolerable in the current sedentary and inactive adult population, with no marked emotional changes in these dimensions. This finding has implications for the maintenance of physical activity interventions utilising these types of low-intensity breaks.

The study by Tamura et al. (2022) examined whether engaging in LPA during a university class would influence vigour and fatigue - both aspects of mood - using the Profile of Mood States. Their results indicated that participants reported higher vigour and lower fatigue during the class when they exercised, compared to control activities. However, our trial focused on positive and negative affect, and showed no significant differences in mood between groups using the I-PANAS-SF. This may suggest that the specific outcome measure utilised to assess mood changes, and the aspect of mood targeted, may be crucial in observing acute changes in mood following LPA breaks.

Future research should consider incorporating measures of other emotional constructs, such as vigour and fatigue, potentially using tools like the Profile of Mood States, to assess whether these constructs are influenced by a continuous bout of LPA. These emotional constructs could be more closely aligned with changes in motivational states, which are important in educational and task-oriented settings, such as office environments or universities.

4.4.5. Strengths, limitations and future research

This study benefitted from a robust design and well evidence participant restrictions. These included a well-controlled design in the non-lab and within-lab period. Furthermore, solid meals were utilised which replicate real life food intake in contrast with previous trials utilising liquid meals, which may influence nutrient handling and metabolism. Additionally, this trial assessed the responses according to different adult age groups, which proved to be significant for iGlucose.

Nevertheless, several limitations provide opportunity for future research. Firstly, this trial was initially powered to detect changes in glucose in a three-armed RCT. However, sub-group analysis of older versus younger adults was performed as an exploratory analysis, and full statistical power may not have occurred in this comparison. Future trials may incorporate a larger sample size into their design to further explore age-related differences. Longer term studies are needed to investigate the effects of LPA on these variables.

Finally, although this trial focussed on two important cognitive measures, positive and negative affect, including additional measures such as vigour or fatigue would incorporate a more comprehensive understanding of the cognitive effects of a continuous bout of LPA. These measures may be acutely influenced by LPA and examination could help to better assess the nuanced emotional responses to different doses of LPA and would further enrich the conclusions. This could provide valuable insights for refining intervention strategies to reduce sedentary time. In addition, measures of affect were performed immediately after exercise and latent responses may have become apparent later in the lab-day. Future trials should consider further repeating these measures at the end of the lab-day, or in the evening, to determine potential delayed effects.

4.5. Conclusions

In summary, this thesis indicated that 60 minutes of LPA walking is sufficient to lower iGlucose in sedentary and inactive adults compared to sitting, with longer duration LPA (60WALK) lowering iGlucose to a greater extent than shorter LPA (30WALK). The results of this trial also have age-related implications for exercise prescription for sedentary inactive adults. Namely, 30 and 60 minutes of continuous LPA walking is not sufficient in duration to acutely elicit a decrease in iGlucose for apparently healthy older adults several hours after exercise. This has

implications for exercise prescription according to age, indicating a one size fits all message may not be appropriate. Older adults may need to consider other ways to acutely alter iGlucose. This could include walking for a longer duration, or walking at a higher intensity. If the exercise programme aim is to acutely lower iGlucose, older adults may need to pursue MPA breaks to elicit changes in glucose levels (Kashiwabara et al. 2018; Miyashita et al. 2016) which have been shown to alter glucose levels in their sample populations. However, in healthy adults, a comparison of the long-term effects of intermittent versus continuous LPA breaks on iGlucose and other biomarkers is not yet established.

Additionally, compared to sitting, this trial provided evidence for an increase in HDL cholesterol after 30 minutes of LPA only, implying a possible dose-response relationship, but further research is required. We also determined that 60WALK has a greater SBP lowering effect than 30WALK, illustrating a dose response that may have implications for future programmes designed to lower SBP. The trial also indicated that 30 or 60 minutes of LPA walking does not alter positive or negative affect immediately after exercise compared to prolonged sitting, indicating that both lengths are tolerable in a sedentary and inactive adult population. Overall, to enrich conclusions, future research should further explore various lengths of continuous LPA and consider measuring biomarkers and cognitive measures throughout the 24 to 48 hours after baseline.

5. Chapter Five: General Discussion

5.1. Overview, aims and synthesis of findings

There is evidence globally at the population level that over the past century individuals are undertaking increasingly sedentary and inactive behaviours. Research over the past few decades has increasingly identified the combined negative health impacts of high sedentary time and low physical activity. Subsequently, researchers identified through observational studies that breaking up sitting time with physical activity can improve markers of health. As a result, researchers and healthcare professionals alike have increasingly explored ways to interrupt sitting time with physical activity to reduce sedentary time, ameliorate some of its effects, whilst gaining the additional benefits attained from physical activity.

Many studies now exist exploring ways to break up sitting time with physical activity in populations across the lifespan. However, to date, the optimal frequency, intensity, time and type (FITT) of physical activity to break up sitting time in children and adults is not yet clear. Nevertheless, school time and the adult workplace have been identified as prime intervention locations as time spent there is often particularly sedentary and inactive. However, there is a lack of high-quality acute research (Yin et al. 2024) and long-term research in adults (Hadgraft et al. 2021), meaning that relatively few high-quality studies are available to make conclusions regarding the efficacy of specific physical activities used to break up sitting. Furthermore, the evidence available for the efficacy of a single continuous bout of light-intensity physical activity (LPA) or moderate-intensity physical activity (MPA) to break up a sitting day in healthy sedentary inactive adults is even less studied. Similarly, other evidence suggests that school-based interventions can improve students' cardiorespiratory fitness, but the effects may not be consistent between certain groups (Hartwig et al. 2021). Furthermore, evidence also suggests that active breaks can reduce sedentary behaviours and increase physical activity in school children (Larose et al. 2024). School-based physical activity interventions may also reduce obesity in overweight children (Ronca et al. 2024). However, the optimal FITT in children has not been identified and more research is required to enhance knowledge.

As such, the overall aim of this thesis was to expand the knowledge and toolkit available for researchers, healthcare practitioners and policymakers alike, providing them with additional information to make informed decisions when implementing physical activity interventions to break up sedentary time across the lifespan. Building on the literature from **Chapter 1**, the following aims were established:

- Determine whether a physical activity intervention in children, aimed at breaking sedentary time, is sufficient to improve markers of health (**Chapter 2**).
- Investigate whether a single bout of LPA versus an energy matched bout of MPA, used to break up sedentary time, can alter key markers of health in adults including glucose, insulin, lipids and blood pressure (**Chapter 3**).
- To determine whether a dose response exists (shorter versus longer durations) when adults and older adults undertake a single continuous bout of LPA to break a sedentary day, and whether the responses differ according to age: specifically examining the effects on glucose, lipids, blood pressure and aspects of mood (**Chapter 4**).

These aims were explored in detail in their respective chapters, and their primary results are summarised here:

- A physical activity intervention in children - the Daily Mile intervention – reduced sedentary time, increased moderate-to-vigorous physical activity, increased physical fitness and improved body composition measured using skinfolds (**Chapter 2**).
- In sedentary inactive adults, interstitial glucose was higher during the MPA condition than during the sedentary condition, whereas an energy-matched LPA bout was not different compared to the sedentary condition (**Chapter 3**).
- Sixty minutes of LPA walking is sufficient to lower interstitial glucose in sedentary and inactive adults compared to sitting, with longer duration LPA lowering glucose to a greater extent than shorter duration LPA. Additionally, older adults did not respond to an LPA intervention in the same way as their younger counterparts (**Chapter 4**).

5.2. The effects of the Daily Mile on markers of health in children

Prior to the completion and publication of the original Daily Mile research (**Chapter 2**), only anecdotal evidence existed for the benefits of the Daily Mile. Upon publication of the research the knowledge gap was partially filled by showing that the Daily Mile intervention is effective at increasing levels of MVPA, reducing sedentary time, increasing physical fitness and improving body composition. Subsequently, several trials have been completed which have further explored the knowledge gap. This section analyses the available evidence and explores the novel and worthy contribution of **Chapters 2** to the evidence base.

Recent evidence, published after **Chapter 2's** article publication, has largely indicated that the Daily Mile leads to improvements in cardiorespiratory fitness, although the effects on adiposity appear more variable and may be dependent on measurement approach utilised. For example, the study by Brustio et al. (2019) examined the effects of three months of the Daily Mile on students versus a control group in Italy. In contrast to our measure, they used 6-minute run test to assess change in fitness. Nevertheless, after correction for age and gender, they found increased fitness in the Daily Mile group (estimated difference = 25.15m, percent change = 3.1%) compared with the control group (estimated difference = 4.44 m, percent change = 0.5%). Similar to our results, they also did not see a difference between groups for BMI. They did not utilise skinfolds as an outcome measure, which would have enhanced their conclusions regarding changes in adiposity between groups. Additionally, their intervention was relatively short (3-months) which may have limited the opportunity for changes to occur. Furthermore, the intervention was performed on three days per week, which may limit the effects of the Daily mile on primary outcomes in the period examined. Nevertheless, they observed an improvement in fitness over the study period which aligns with our research from **Chapter 2**.

The later research by Brustio et al. (2020), also carried out in Italy, showed a significant improvement for the Daily Mile groups compared to the control. Furthermore, they divided the intervention group into those who partook in the Daily Mile on average two days per week and those whose average was three days. The more regular Daily Mile group showed superior increase in fitness compared to the control than the less regular group. We did not measure adherence to the intervention in our trial (**Chapter 2**) and so it is not possible to ascertain whether this could have influenced our results. Future trials should attempt to measure this to improve comparability between studies. Nevertheless, their positive fitness results overall align with our results from **Chapter 2**.

Research by Breheny et al. (2020) examined multiple Daily Mile intervention schools and controls over a 12-month period in England. The main outcome of interest was BMI z-score which was analysed over the full research period. Additionally, fitness was measured at four and 12 months using the British Athletics Linear Track Test (British Athletics 2018) and no differences were observed. Like our research (**Chapter 2**), Breheny et al. (2020) saw no difference in BMI z scores between groups. However, although BMI z scores were not different

between groups after 12 months overall, there was evidence of a modest intervention effect on BMI z score for girls that was consistent with their findings for body fat percentage. This suggests further evidence that gender may moderate the health benefits of physical activity. Conversely, boys are generally less sedentary during school than girls (Breheny et al. 2020), so their capacity for change may be less. Missing data for BMI was low, however, missing data for fitness change was high (>56% missing) leading to difficulty in fully interpreting their fitness results. Furthermore, the method of fitness test they utilised (British Athletics Linear Track Test) differed from our research (20 m shuttle test) and may be in part responsible for the lack of differences observed to our results in **Chapter 2**. It is also possible that anthropometric assessment differences between our trials are a result of differences in body fat calculation (skinfolds versus bioimpedance monitor). Therefore, although differences were present between our trial and theirs, results were likely influenced by disparity between the outcome measures utilised.

The research by de Jonge et al. (2020) also assessed the effects of the Daily Mile on pupil's fitness and observed a greater improvement in the intervention group than the control. Their study measured pupil's responses from a range of schools across the Netherlands, improving generalisability. However, it was regrettable that they did not measure BMI or skinfolds which would have enhanced their conclusions. Nevertheless, their research shows some evidence that the Daily mile can be successful out with the Scottish school environment and supports our data from **Chapter 2**.

In a large citizen science study carried out by our inter-university Daily Mile research group, Booth et al. (2022) examined the longer-term impact of the Daily Mile on fitness in 6908 school pupils in the UK. They showed that longer-term participation (>3 months) in the Daily Mile resulted in higher fitness levels than the shorter participation group (<2 months) and the control group (no participation), whilst accounting for age, sex and socioeconomic status (SES). Fitness was measured according to multistage 20-m shuttle run test carried out by each school. As the research was carried out as a snapshot in the school year, pre- and post-fitness levels were not measured, and shuttle distance change scores are not available. However, this data aligns with our own research (**Chapter 2**) showing that shuttle distance (and associated fitness) is greater in the Daily Mile School than controls. Importantly, in the 2022 study, the fitness benefits of longer-term Daily Mile participation remained significant after adjusting for

SES, indicating that fitness was not explained by SES, supporting our previous research (**Chapter 2**). In a mixed methods non-RCT study by Marchant et al. (2020), researchers identified that SES led to equal increases in shuttle distances between baseline and follow-up. This further supports our research that the Daily Mile does not appear to be influenced by SES.

Dring et al. (2022) carried out research in England and found that after just five weeks of participating in the Daily Mile that the intervention group ran 140 m further than the control group (Intervention: 880 m, [820–940 m]; Control: 740 m, [680–800 m], $d = 0.39$, $p = 0.002$). The difference in shuttle distance achieved between intervention and control groups was much greater than found in the original Daily Mile study observed in **Chapter 2** where a difference of 39.1 m was seen after eight months. For Dring et al. (2022) this considerable difference might in part be explained as the intervention group was encouraged to attend every session (five days per week), with an average fidelity of $96 \pm 13\%$ being achieved (24 ± 4 days of participation). Whereas in our original Daily Mile study (**Chapter 2**), the adherence to the Daily mile was not recorded, although anecdotally it was consistently applied. It is also possible that, as intervention and control students were recruited from the same schools, that intervention students may have treated the Daily mile as a ‘training programme’, This could have resulted in behaviour modification whereby intervention students ran with more intensity and consistency than ordinarily seen in a school where everyone participates in the Daily Mile. Additionally, they also utilised ANCOVA, with baseline shuttle distance as covariate and no mention of the utilisation of impact of BMI, total skinfolds, WHR, gender or SIMD was stated. Their age was also slightly older than the cohort reported in **Chapter 2** (age 10.4 ± 0.8). Considering they completed a five-week intervention versus our eight-month intervention, the reasons above, may in part, explain the apparent differences between **Chapter 2** and theirs.

Unique research by Hatch et al. (2021) explored the difference achieved between less fit and more fit children when carrying out the Daily Mile. The highest fit children ran further than less fit children measured by the 20 m shuttle test. They also saw that on average ~ 1.5 miles distance was achieved and that the intensity was moderate-to-vigorous. The sample was older on average (10.4 ± 0.7 years) than our sample (8.4 ± 2.0 years), so direct comparisons cannot be made. However, their research demonstrates that given the opportunity, on average, older

primary aged children may choose to carry out moderate-to-vigorous intensity activity instead of lighter intensity activity. This provides an interesting opportunity for future research exploring the effects of the Daily Mile not performed in this thesis.

Overall, our research showed that a school-based intervention such as the Daily Mile is sufficient in breaking up sedentary time, increasing MVPA, preventing a rise in skinfolds and increasing fitness in children (**Chapter 2**): all positive health markers. Taken together, the recent evidence on the Daily mile appears to largely support the findings that cardiorespiratory fitness is improved following the intervention. However, detecting changes in body composition is less consistently supported, and may require higher resolution measures than BMI to detect changes, such as skinfolds. Additionally, due to limited objective assessment in recent trials, changes in MVPA and sedentary time cannot yet be corroborated.

5.3. The effects of LPA and MPA on markers of health in adults

To improve cardiometabolic health markers and reap positive health outcomes, the optimal FITT of physical activity break(s) for interrupting sedentary behaviours in adults is yet unknown. However, the results of the experimental **Chapters 3** and **4** have significantly expanded this knowledge and have added to the available evidence. Here, we describe the current evidence, how **Chapters 3** and **4** fit within the wider literature, and how our research provides novel and valuable evidence to the evidence base. In alignment with our research in **Chapter 3**, several studies have observed higher levels of glucose after MPA activity.

Of note, Kashiwabara et al. (2018) observed an increase in glucose after 30 minutes of MPA in older adults with hypertriglyceridemia. This suggests that in less healthy and/or older populations relatively short bouts may be sufficient to elicit a rise in glucose. Although they observed an increase during the laboratory period (whereas our effect was most prominent later during the post-lab snack window), together these findings emphasise the potential for population and timing-dependent responses. This supports the need for studies directly comparing apparently healthy versus metabolically compromised older adults, using measurement windows that extend into the evening. Wheeler et al. (2020) also examined older adults, albeit apparently healthy ones, and observed an increase in glucose during the lab period after 30 minutes of MPA, with the clearest elevations in glucose occurring around four hours after exercise and lunch. This aligns with our results, where glucose increases were

not immediate but became apparent later in the day. Notably, this is the same period that our LPA intervention also began to diverge from the control group. Further support comes from the trial by Holmstrup et al. (2014), who examined the responses to 60 minutes of post-breakfast MPA and similarly observed sustained elevations in glucose following morning MPA in adults at risk of diabetes. Taken together, these findings suggest that post-meal measurement windows must be sufficiently expansive to encompass any latent effects, as the glycaemic response to replacing sedentary time with a bout of physical activity may be delayed.

A potential physiological explanation for these delayed glucose responses may relate to the time-dependent dynamics of insulin following a bout of MPA. Wheeler et al. (2020) reported lower insulin levels following morning MPA exercise, whereas differences were not shown after lunch. The authors proposed that the post-breakfast exercise may have led to rapid and substantial declines in insulin, which may have facilitated hepatic glucose production during the post-lunch period, potentially via increased glucagon output. In **Chapter 3**, we did not characterise the insulin dynamics of the full post-lunch period, yet our iFGM data saw an increased glucose concentration, which was most apparent four hours after the bout of MPA ended. Taken together, these findings suggest that there may be a temporary lag between replacing sedentary time with physical activity and the glycaemic response, which highlights the importance of an extended measurement window after an intervention to capture delayed metabolic effects. Although the physiological mechanisms could not be directly corroborated in the present study, this observation highlights an important consideration for the design of future research.

Other research also aligns with our findings in the next-day pre-prandial period (**Chapter 3**), consistently showing no differences in fasting glucose between MPA and control groups following 30-, 60-, 90- and 180-minute bouts of MPA (Homer et al. 2017; Kim et al. 2014; Miyashita 2008; Miyashita et al. 2013; Miyashita et al. 2008; Tsetsonis and Hardman 1996). Additionally, these studies reported no differences in glucose during the postprandial period after breakfast. Furthermore, the only trial measuring next day responses to LPA similarly observed no differences between groups (Tsetsonis and Hardman 1996). Collectively, this implies that the window of opportunity to observe exercise-induced differences in glycaemic response may have passed by the following morning and is unlikely to be evident after

breakfast. This reinforces that the optimal window to detect glycaemic changes following a bout of exercise is likely to occur before the following morning breakfast and should therefore be prioritised in future trials.

However, the literature also presents data that does not align with our **Chapter 3** results. Trials whose MPA durations were between 30 and ~90 minutes saw no differences between intervention and control conditions (Benatti et al. 2017; Farah and Gill 2013; Katsanos et al. 2004; Maylor et al. 2018; Newsom et al. 2013; Peddie et al. 2013). However, these findings may be attributable to methodological and participant-related differences. For example, the duration of the bouts was often relatively short (e.g. 30 minutes MPA), some studies employed mixed modes of physical activity, and others differed substantially in outcome measurement timing, physical activity profiles and meal composition.

In contrast to our **Chapter 3** findings and much of the existing literature, some research has reported lower glucose following MPA. Bhammar et al. (2017) observed reductions in mean glucose after a 30-minute bout of MPA, although no differences were observed for glucose AUC. As discussed in **Chapter 3**, potential discrepancies in immediately pre-exercise baseline glucose between groups may partly explain these contrasting findings. Taken together, these divergent findings indicate that glycaemic responses to replacing sedentary time with a bout of physical activity are highly sensitive to activity duration, trial design, participant characteristics, timing of outcome measurements, and metabolic health status.

Overall, our findings indicate that only a 70-minute bout of MPA significantly increased iGlucose compared to a control in healthy inactive adults in our research (**Chapter 3**). Given that the increased glucose levels after MPA in our trial appears to have been driven by the evening period responses, this highlights the importance of regular glucose monitoring, via iFGM, as we were able to observe differences in glucose following MPA. Whereas many studies did not measure evening responses so could not observe potential differences between groups within the measurement window.

Regarding specific RCTs in adults replacing sedentary time with LPA, much less literature is available, and findings are not consistent. In the four-hour LPA arm of the RCT by Katsanos et al. (2004), no reductions in area under the curve glucose were observed during lab-time or the next morning. This broadly aligns with our results from **Chapter 3**, where our LPA trial arm

lasted for 150 minutes. However, the absence of evening measurements in Katsanos et al. (2004) limits conclusions regarding possible delayed responses.

In contrast, **Chapter 4** indicated that over 24-hours, 60 minutes of LPA walking is sufficient to lower iGlucose in sedentary and inactive adults compared to sitting, with this moderate-duration LPA bout eliciting a greater reduction than shorter-duration 30-minute LPA. This may suggest that there is a dose-response for observing changes in glucose, whereby short-to-moderate duration may cause a decrease in glucose, but longer duration may observe no clear changes in glucose. This theory is partly supported by Gao et al. (2024) who studied the response to 30 minutes of LPA, where they observed lower glucose levels in the LPA group during the lab-day. This provides further evidence to our overall results from **Chapters 3-4**, indicating a possible dose-response for LPA in adults. Further dose-response trials are required to confirm this hypothesis.

In contrast to this simple dose-response hypothesis presented above, Hoffman et al. (2024) studied the effects of approximately eight hours of continuous LPA compared with sitting in young adults and observed lower mean glucose levels. These findings suggest that glycaemic responses to LPA may not be governed solely by duration but instead may be influenced by the interaction between activity duration *and* small differences in intensity. It is possible that due to the very slow nature of their LPA arm (1 mph), substrate utilisation may have differed compared to **Chapter 4** which employed faster walking (1.6mph), resulting in the differing glucose responses observed. However, Hoffman et al. (2024) did not explore the post-lab period and so the possible presence of any delayed counterregulatory response in the evening cannot be ruled out in their study.

Nevertheless, we also observed in **Chapter 4** that 30 and 60 minutes of continuous LPA walking is not sufficient in duration to acutely elicit a decrease in iGlucose for apparently healthy older adults several hours after exercise. This has implications for prescription of physical activity breaks according to age, indicating recommendations may have to be personalised according to age. These findings are novel as no previous research has examined LPA glucose dose-responses to breaking up sitting in sedentary inactive older versus younger adults.

After a review of the literature, it appears that only two previous RCTs, by Katsanos et al. (2004) and Hoffmann et al. (2024), broke up a workday with a continuous bout of LPA and measured lipid responses in apparently healthy adults. Of these, only Hoffmann et al. (2024) observed lower lipid levels for LPA compared to sitting during the lab-day. This suggests lipid metabolism was significantly affected after LPA, which was not observed in our research (**Chapter 3** and **Chapter 4**). However, as previously stated, the physical activity was very long and very slow in the study by Hoffmann et al. (2024) which may have influenced substrate utilisation during their trial. Therefore, taking the results from the available literature, as well as our results from **Chapter 3** and **Chapter 4**, changes in lipids may be associated with a combination of LPA duration and intensity, with lower intensity and longer duration potentially lowering lipid values. Much more research is required to examine this, as so few studies are available. Regarding MPA and lipid responses, approximately half of the available RCTs observe no differences in triglyceride responses between MPA and controls (most measured triglycerides), which aligns with our research in **Chapter 3**. However, the other half of the RCTs observe lower triglyceride values following MPA trials. Therefore, more trials are required to determine why such differences are observed. Of note, it does not appear that any RCTs have measured lipid responses between the lab-day and the following morning, representing an opportunity for future research.

Very few RCTs have assessed the effects of a single continuous LPA or MPA break on blood pressure in healthy adults. To date, only two studies have previously examined blood pressure responses (Bhammar et al. 2017; Miyashita et al. 2008). In **Chapter 3**, we observed no differences between energy matched bouts of MPA and LPA, likely reflecting limited measurement timing. Building on this, **Chapter 4** incorporated more frequent blood pressure assessments. Consequently, the results revealed that longer duration (60 minutes) LPA elicited a more pronounced SBP lowering response than 30 minutes of LPA and this response lasted for several hours. These observations highlight the importance of repeated blood pressure measurement, which enabled the researcher to capture the transient and dose-dependent blood pressure responses to physical activity.

Very little research has also assessed the effects of continuous physical activity breaks on mood. However, the trial by Bergouignan et al. (2016) showed mood was improved for a sustained period after the intermittent bouts (30 mins total) of MPA, but not in a continuous

bout (30 mins). Our study (**Chapter 4**) also observed no differences for mood (measured by positive or negative affect) between either a continuous bout of 30 or 60 minutes of LPA. This might suggest that to observe acute changes in mood, bouts may need to be MPA intensity as well as intermittent. Nevertheless, our research and the available evidence suggests that a continuous bout is not detrimental to mood and is therefore likely acceptable for participants. More research is required to confirm this hypothesis.

Overall, research from **Chapters 3** and **4** shows that a single continuous bout of LPA or MPA, used to break up sitting, can influence markers of health in sedentary and inactive adults, while responses in older adults may be attenuated.

5.4. Strengths

The methodologies employed in the experimental chapters benefit from numerous strengths that have contributed to the overall quality of the studies and their data, some of which have already been discussed in **Chapter 2** including the higher resolution of skinfolds compared to BMI. However, following the publication of the **Chapter 2** study, additional strengths of our approach can be highlighted. For example, some schools tested fitness responses of the Daily Mile indoors. In our research, the same playground surfaces were used within each school during fitness testing, which simulated the common surface that students ran on during their normal Daily Mile (tarmac). This increased the consistency of conditions between testing measures and physical activity intervention, which would not occur if testing was performed inside using alternative fitness tests. A further major strength of the study was that we did not attempt to influence the regularity, intensity or adherence to the 15-minute Daily Mile intervention, which occurred in other subsequent research. This is a strength as it suggests that the overall effects observed from the Daily Mile in **Chapter 2** were not further driven by researcher-led biases and were rather due to the intrinsic response to the Daily Mile intervention.

In addition to **Chapter 2**, both **Chapter 3** and **Chapter 4** benefitted from several strengths. These included tight participant inclusion and exclusion characteristics, participation restrictions and the use of IFGMs, which have been discussed previously. However, a further strength of **Chapter 4** was the inclusion of sleepiness as a covariate to analyse mood outcomes. As sleepiness is known to directly affect mood states, its inclusion in analysis of the

I-PANAS-SF improved confidence that positive and negative affect were not confounded by variations in sleep-related fatigue (Lo et al. 2016). Sleepiness score did not affect the results of this trial, potentially indicating individuals adhered closely to pre-trial instructions and ensured a full night's sleep: further highlighting the rigour of the research design.

5.5. Limitations

Careful planning and consideration of the prior evidence base were used to reduce the number of limitations in experimental **Chapters 2-4**. However, some limitations were present in the research. In **Chapter 2**, we did not record whether students began new exercise or sports classes in their spare time after beginning (or not beginning) the Daily Mile. The influence of initiating such activities may have further increased the levels of fitness in those 'extra-active' children and not in others whose activity remained constant out with the Daily Mile intervention. Similarly, we did not record if children ceased partaking in exercise in the same time frame. Our research also did not record – between rounds of testing - whether children increased their levels of non-organised physical activity (not just sports) in non-school time. This could have occurred due to an increased general fitness caused by their regular participation in the Daily Mile. If this did occur, the 15-minute Daily Mile would have been indirectly, rather than directly responsible for these additional physical activities. Furthermore, we did not ask whether parents were more inclined to encourage their children to participate in further physical activity because of the increased publicity of the Daily Mile initiative. In either instance, if these additional activities did occur - possibly as a result of taking part in the Daily Mile - then these can be seen as positive lifestyle changes.

Due to the nature of the Daily Mile study in **Chapter 2**, participants could not be blinded to their intervention group. Therefore, participants may have been influenced to the Hawthorne effect (McCambridge et al. 2014). That is, simply being researched may have increased children's engagement or motivation beyond the 'normal' influence of partaking in the Daily Mile. Therefore, it is possible – due to taking part in the Daily Mile - that some students may have felt an increased competitiveness with the comparison school, knowing that their results would be directly compared at the end of the study. This competitiveness may have pushed them to 'do well' in follow up tests, to 'prove' the anecdotal worth of taking part in the Daily Mile. Therefore, they may have pushed their general fitness harder between testing rounds. This 'push' may have occurred during any organised or non-organised physical activities and

not just in the Daily Mile. Even in those that did not push fitness harder between rounds of testing, competitiveness may have led to an increase in the motivation of some Daily Mile students to push harder during the second shuttle run test. The presence of highly motivated classmates may have caused a strong peer influence, encouraging those less motivated to do better in testing. Likewise, peer influence of highly motivated Daily Mile school students could have increased the intensity of general Daily Mile participation in other less-motivated students. Nevertheless, these theories cannot be proved or disproved with the available data in **Chapter 2**.

Finally, while both **Chapter 3** and **Chapter 4** did not recruit the number of participants initially intended to ensure full sample power, statistically significant differences were still observed. This suggests that the observed effects were strong enough to be detected despite the smaller sample sizes available. Nevertheless, the relatively small sample sizes and lack of statistical power could also result in type 1 error (false positive outcomes). Therefore, larger scale studies are required to corroborate the findings.

5.6. Summary, implications and future directions

In summary, the findings of this thesis demonstrate that replacing sedentary time with physical activity can influence markers of health across the lifespan, while the thesis also identified important age-related differences in responses. Changes in health markers were clearer in children and adults, whereas comparable responses in older adults were not consistently observed, highlighting important gaps in the literature and directions for future research. The thesis further demonstrates that these interventions can be either less structured, such as during the Daily Mile (**Chapter 2**), or highly structured, such as under lab conditions in **Chapters 3-4**, and markers of health can still be influenced.

Building on the findings from **Chapters 2-4**, and considering the broader evidence base, several areas for future research are warranted. For the Daily Mile, and other studies in children, future research should focus on high resolution assessment of body composition. This may include skinfolds and advanced anthropometrists or, where feasible, imaging-based techniques (such as DEXA) may be utilised for a sub-set of participants if whole-cohort imaging is not practical. Furthermore, assessing the responses to the Daily Mile in other countries

outside Europe is warranted to ascertain whether similar physiological and behavioural effects are observed in different cultural and educational contexts.

Considering **Chapters 3** and **4**, there are several areas of research that could stem from our findings when viewed alongside the findings of other available literature. For example, several RCTs breaking up sitting time with physical activity in adults assessed the next-day postprandial period, which we did not, and many observed differences in cardiometabolic markers of health. Without measuring this period, **Chapters 3** and **4** could not conclude whether cardiometabolic patterns would have persisted or emerged the next day postprandially. Therefore, future research should consider including the next day responses to breaking up sedentary time with LPA or MPA, especially if participants have IFGMs whereby continued monitoring would be relatively easy. Furthermore, **Chapter 4** measured positive and negative affect and observed no differences between groups. However, future trials should consider utilising several measures of mood to determine whether different aspects of mood are affected. Lastly, considering the results of **Chapters 3** and **4** together, although glucose monitoring was robust and lipid panels were utilised, some questions remain regarding the specific substrates utilised following the different lengths and intensities of LPA and MPA breaks. Therefore, future research should consider incorporating measures of fat oxidation that would enrich the evidence and provide more comprehensive conclusions on the metabolic effect of LPA and MPA interventions. Furthermore, trials should assess the next day pre- and postprandial responses to MPA and LPA breaks when comparing older to younger adults. This would help to inform future recommendations regarding age specific guidance on breaking up sitting time.

Finally, the outcomes of the experimental chapters presented in this thesis are relevant to continuous physical activity periods that replace sedentary time. However, other research has shown in older adult non-exercisers, that any daily amount of incidental physical activity (which encompasses activities of daily living outside the leisure time domain), whether moderate or vigorous in intensity, is associated with lower cardiovascular risk in a dose-response manner (Stamatakis et al. 2025). Whereas LPA had weak associations with cardiovascular risk outcomes (Stamatakis et al. 2025). Therefore, it is also necessary to more fully explore and understand the effects of frequent intermittent exercise interventions, or varying duration and intensities, that may also produce positive health outcomes.

6. References

- Aadland, E. and Høstmark, A.T. (2008) Very light Physical Activity after a Meal Blunts the Rise in Blood Glucose and Insulin. *The Open Nutrition Journal*, (2), pp. 94–99.
- Adams, N.T., Paterson, C., Poles, J., Higgins, S. and Stoner, L. (2024) The Effect of Sitting Duration on Peripheral Blood Pressure Responses to Prolonged Sitting, With and Without Interruption: A Systematic Review and Meta-Analysis. *Sports Medicine (Auckland)*, 54 (1), pp. 169–183.
- Ainsworth, B.E., Haskell, W.L., Herrmann, S.D., Meckes, N., Bassett, D.R., Jr, Tudor-Locke, C., Greer, J.L., Vezina, J., Whitt-Glover, M.C. and Leon, A.S. (2011) 2011 Compendium of Physical Activities: a second update of codes and MET values. *Medicine and Science in Sports and Exercise*, 43 (8), pp. 1575–1581.
- Allen, M.S., Walter, E.E. and Swann, C. (2019) Sedentary behaviour and risk of anxiety: A systematic review and meta-analysis. *Journal of Affective Disorders*, 242, pp. 5–13.
- American Diabetes Association. (2001) Postprandial blood glucose. *American Diabetes Association. Diabetes Care*, 24 (4), pp. 775–778.
- Amor-Barbosa, M., Ortega-Martínez, A., Carrasco-Uribarren, A. and Bagur-Calafat, M.C. (2022) Active School-Based Interventions to Interrupt Prolonged Sitting Improve Daily Physical Activity: A Systematic Review and Meta-Analysis. *International Journal of Environmental Research and Public Health*, 19 (22), pp. 15409.
- Antunes, B.M., Rossi, F.E., Oyama, L.M., Rosa-Neto, J.C. and Lira, F.S. (2020) Exercise intensity and physical fitness modulate lipoproteins profile during acute aerobic exercise session. *Scientific Reports*, 10 (1), pp. 4160.
- Atkin, A.J., Sharp, S.J., Harrison, F., Brage, S. and Van Sluijs, E.M. (2016) Seasonal Variation in Children's Physical Activity and Sedentary Time. 48 (3), pp. 449–456.
- Bailey, D.P. (2021) Sedentary behaviour in the workplace: prevalence, health implications and interventions. *British Medical Bulletin*, 137 (1), pp. 42–50.
- Bailey, D.P., Boddy, L.M., Savory, L.A., Denton, S.J. and Kerr, C.J. (2012) Associations between cardiorespiratory fitness, physical activity and clustered cardiometabolic risk in children and adolescents: the HAPPY study. *European Journal of Pediatrics*, 171 (9), pp. 1317–1323.
- Bailey, D.P., Hewson, D.J., Champion, R.B. and Sayegh, S.M. (2019) Sitting Time and Risk of Cardiovascular Disease and Diabetes: A Systematic Review and Meta-Analysis. *American Journal of Preventive Medicine*, 57 (3), pp. 408–416.
- Barr-Anderson, D.J., AuYoung, M., Whitt-Glover, M.C., Glenn, B.A. and Yancey, A.K. (2011) Integration of short bouts of physical activity into organizational routine a systematic review of the literature. *American Journal of Preventive Medicine*, 40 (1), pp. 76–93.
- Basterfield, L., Adamson, A.J., Parkinson, K.N., Maute, U., Li, P.X. and Reilly, J.J. (2008) Surveillance of physical activity in the UK is flawed: validation of the Health Survey for England Physical Activity Questionnaire. *Archives of Disease in Childhood*, 93 (12), pp. 1054–1058.

- Benatti, F.B. and Ried-Larsen, M. (2015) The Effects of Breaking up Prolonged Sitting Time: A Review of Experimental Studies. *Medicine and Science in Sports and Exercise*, 47 (10), pp. 2053–2061.
- Benatti, F.B., Larsen, S.A., Kofoed, K., Nielsen, S.T., Harder-Lauridsen, N.M., Lyngbæk, M.P., Eriksen, D., Karstoft, K., Krogh-Madsen, R., Pedersen, B.K. and Ried-Larsen, M. (2017) Intermittent Standing but not a Moderate Exercise Bout Reduces Postprandial Glycemia. *Medicine and Science in Sports and Exercise*, 49 (11), pp. 2305–2314.
- Bennette, C. and Vickers, A. (2012) Against quantiles: categorization of continuous variables in epidemiologic research, and its discontents. *BMC Medical Research Methodology*, 12 (1), pp. 21–21.
- Bergouignan, A., Legget, K.T., De Jong, N., Kealey, E., Nikolovski, J., Groppel, J.L., Jordan, C., O'Day, R., Hill, J.O. and Bessesen, D.H. (2016) Effect of frequent interruptions of prolonged sitting on self-perceived levels of energy, mood, food cravings and cognitive function. *The International Journal of Behavioral Nutrition and Physical Activity*, 13 (1), pp. 113.
- Bhammar, D.M., Sawyer, B.J., Tucker, W.J. and Gaesser, G.A. (2017) Breaks in Sitting Time. *Medicine & Science in Sports & Exercise*, 49 (10), pp. 2119–2130.
- Biddle, S.J.H., Atkin, A.J., Cavill, N. and Foster, C. (2011a) Correlates of physical activity in youth: a review of quantitative systematic reviews. *International Review of Sport and Exercise Psychology*, 4 (1), pp. 25–49.
- Biddle, S.J., Gorely, T. and Stensel, D.J. (2004) Health-enhancing physical activity and sedentary behaviour in children and adolescents. *Journal of Sports Sciences*, 22 (8), pp. 679–701.
- Biddle, S.J., O'Connell, S. and Braithwaite, R.E. (2011b) Sedentary behaviour interventions in young people: a meta-analysis. *British Journal of Sports Medicine*, 45 (11), pp. 937–942.
- Bird, S.R. and Hawley, J.A. (2017) Update on the effects of physical activity on insulin sensitivity in humans. *BMJ Open Sport & Exercise Medicine*, 2 (1), pp. e–e000143.
- Bohr, A.D., Brown, D.D., Laurson, K.R., Smith, P.J.K. and Bass, R.W. (2013) Relationship Between Socioeconomic Status and Physical Fitness in Junior High School Students. *The Journal of School Health*, 83 (8), pp. 542–547.
- Booth, J.N., Brooks, N.E., Moran, C.N., Gallagher, I.J., Ryde, G.C. and Gorely, T. (2016) Is running a mile a day associated with children's behaviour and well-being? Exploring the impact of the Daily Mile and implications for policy. Available: <https://www.research.ed.ac.uk/en/publications/is-running-a-mile-a-day-associated-with-childrens-behaviour-and-w> [Accessed: 17 Apr 2018].
- Booth, J.N., Chesham, R.A., Brooks, N.E., Gorely, T. and Moran, C.N. (2022) The Impact of the Daily Mile™ on School Pupils' Fitness, Cognition, and Wellbeing: Findings From Longer Term Participation. *Frontiers in Psychology*, 13, pp. 812616.
- Breheny, K., Passmore, S., Adab, P., Martin, J., Hemming, K., Lancashire, E.R. and Frew, E. (2020) Effectiveness and cost-effectiveness of The Daily Mile on childhood weight outcomes and wellbeing: a cluster randomised controlled trial. *International Journal of Obesity*, 44 (4), pp. 812–822.

British Athletics (2018) British Athletics Linear Track Test. Available: <https://www.sportshall.org/wp-content/uploads/2013/06/Primary-Endurance-Poster.pdf> [Accessed: 16 April 2025].

Brooks, N.E., Booth, J.N., Chesham, R., Sweeney, E., Mitchell, I., Ryde, G., Gorely, T. and Moran, C.N. (2016) A Feasibility Study Investigating Body Composition and Physical Activity Levels in Children Taking Part In the Daily Mile. *The FASEB Journal*, 30 (S1), pp. lb669–lb669.

Brown, L., Campbell-Jack, D., Gray, L., Hovald, P., Kirkpatrick, G., Knudsen, L., Leyland, A.H., Montagu, I. and Rose, J. (2016) Scottish Health Survey 2015 - volume 1: main report. Available: <https://www.gov.scot/publications/scottish-health-survey-2015-volume-1-main-report/pages/0/> [Accessed: 17 April 2018].

Brustio, P.R., Mulasso, A., Lupo, C., Massasso, A., Rainoldi, A. and Boccia, G. (2020) The Daily Mile Is Able to Improve Cardiorespiratory Fitness When Practiced Three Times a Week. *International Journal of Environmental Research and Public Health*, 17 (6), pp. 2095.

Brustio, P.R., Mulasso, A., Marasso, D., Ruffa, C., Ballatore, A., Moisè, P., Lupo, C., Rainoldi, A. and Boccia, G. (2019) The Daily Mile: 15 Minutes Running Improves the Physical Fitness of Italian Primary School Children. *International Journal of Environmental Research and Public Health*, 16 (20), pp. 3921.

Butt, J., Weinberg, R.S., Breckon, J.D. and Claytor, R.P. (2011) Adolescent Physical Activity Participation and Motivational Determinants Across Gender, Age, and Race. *Journal of Physical Activity & Health*, 8 (8), pp. 1074–1083.

Cano, A., Ventura, L., Martinez, G., Cugusi, L., Caria, M., Deriu, F. and Manca, A. (2022) Analysis of sex-based differences in energy substrate utilization during moderate-intensity aerobic exercise. *European Journal of Applied Physiology*, 122 (1), pp. 29–70.

Carey, M., Markham, C., Gaffney, P., Boran, C. and Maher, V. (2006) Validation of a point of care lipid analyser using a hospital based reference laboratory. *Irish Journal of Medical Science*, 175 (4), pp. 30–35.

Casanova, F., O'Loughlin, J., Karageorgiou, V., Beaumont, R.N., Bowden, J., Wood, A.R. and Tyrrell, J. (2023) Effects of physical activity and sedentary time on depression, anxiety and well-being: a bidirectional Mendelian randomisation study. *BMC Medicine*, 21 (1), pp. 501.

Charisi, V., Zafeiroudi, A., Trigonis, I., Tsartsapakis, I. and Kouthouris, C. (2025) The Impact of Green Spaces on Workplace Creativity: A Systematic Review of Nature-Based Activities and Employee Well-Being. *Sustainability*, 17 (2), pp. 390.

Chastin, S.F.M., Egerton, T., Leask, C. and Stamatakis, E. (2015) Meta-analysis of the relationship between breaks in sedentary behavior and cardiometabolic health. *Obesity*, 23 (9), pp. 1800–1810.

Chesham, R.A., Booth, J.N., Sweeney, E.L., Ryde, G.C., Gorely, T., Brooks, N.E. and Moran, C.N. (2018) The Daily Mile makes primary school children more active, less sedentary and improves their fitness and body composition: a quasi-experimental pilot study. *BMC Medicine*, 16 (1), pp. 64.

Chia, C.W., Egan, J.M. and Ferrucci, L. (2018) Age-Related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk. *Circulation Research*, 123 (7), pp. 886–904.

Clemes, S.A., O'Connell, S.E. and Edwardson, C.L. (2014) Office Workers' Objectively Measured Sedentary Behavior and Physical Activity During and Outside Working Hours. *Journal of Occupational & Environmental Medicine*, 56 (3), pp. 298–303.

Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences*. 2 ed. United Kingdom: Routledge. Available: <http://digital.casalini.it/9781134742707> .

Conolly, A. (2015) Health Survey for England 2015 Children's body mass index, overweight and obesity. Available: <https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/health-survey-for-england-2015> [Accessed: 17 April 2018].

Cooper, A.R., Goodman, A., Page, A.S., Sherar, L.B., Esliger, D.W., van Sluijs, E.M., Andersen, L.B., Anderssen, S., Cardon, G., Davey, R., Froberg, K., Hallal, P., Janz, K.F., Kordas, K., Kreimler, S., Pate, R.R., Puder, J.J., Reilly, J.J., Salmon, J., Sardinha, L.B., Timperio, A. and Ekelund, U. (2015) Objectively measured physical activity and sedentary time in youth: the International children's accelerometry database (ICAD). *The International Journal of Behavioral Nutrition and Physical Activity*, 12 (1), pp. 113.

Crespo, N.C., Mullane, S.L., Zeigler, Z.S., Buman, M.P. and Gaesser, G.A. (2016) Effects of Standing and Light-Intensity Walking and Cycling on 24-h Glucose. *Medicine & Science in Sports & Exercise*, 48 (12), pp. 2503–2511.

de Jonge, M., Slot-Heijns, J.J., Prins, R.G. and Singh, A.S. (2020) The Effect of The Daily Mile on Primary School Children's Aerobic Fitness Levels After 12 Weeks: A Controlled Trial. *International Journal of Environmental Research and Public Health*, 17 (7), pp. 2198.

De Miguel-Etayo, P., Gracia-Marco, L., Ortega, F.B., Intemann, T., Foraita, R., Lissner, L., Oja, L., Barba, G., Michels, N., Tornaritis, M., Molnár, D., Pitsiladis, Y., Ahrens, W. and Moreno, L.A. (2014) Physical fitness reference standards in European children: the IDEFICS study. *International Journal of Obesity*, 38 (Suppl 2), pp. S57–S66.

Department of Health and Social Care (2011) *Department of Health: Start active, stay active: a report on physical activity from the four home countries' Chief Medical Officers*. 2011. UK: Available: <https://www.gov.uk/government/publications/start-active-stay-active-a-report-on-physical-activity-from-the-four-home-countries-chief-medical-officers> [Accessed: 17 Apr 2018].

Dias, D.F., Loch, M.R. and Ronque, E.R.V. (2015) Perceived barriers to leisure-time physical activity and associated factors in adolescents. *Ciência & Saude Coletiva*, 20 (11), pp. 3339–3350.

Dimkpa, U. and Ugwu, A.C. (2008) Age-Related Differences in Systolic Blood Pressure Recovery after a Maximal-Effort Exercise Test in Non-Athletic Adults. *International Journal of Exercise Science*, 1 (4), pp. 142–152.

Disclosure Scotland Disclosure Scotland Website. Scotland: Available: <https://www.mygov.scot/about-disclosure-scotland> [Accessed: 17 Apr 2018].

Disclosure Scotland (2016) Disclosure Scotland Website. Available: <https://www.disclosure.gov.scot/about> [Accessed: 17 April 2018].

Dobbins, M., Neil-Sztramko, S.E. and Caldwell, H. (2021) School-based physical activity programs for promoting physical activity and fitness in children and adolescents aged 6 to 18. *Cochrane Database of Systematic Reviews*, 2021 (9), pp. CD007651.

Dong, Y., Pan, Y., Zhang, X., He, Q., Chen, S., Du, L. and Yin, S. (2024) Impact of Prolonged Sitting Interruption on Blood Glucose, Insulin and Triacylglycerol in Adults: A Systematic Review and Meta-Analysis. *Applied Sciences*, 14 (8), pp. 3201.

Drenowatz, C., Ferrari, G., Greier, K. and Hinterkörner, F. (2021) Relative Age Effect in Physical Fitness during the Elementary School Years. *Pediatric Reports*, 13 (2), pp. 322–333.

Dring, K.J., Hatch, L.M., Williams, R.A., Morris, J.G., Sunderland, C., Nevill, M.E. and Cooper, S.B. (2022) Effect of 5-weeks participation in The Daily Mile on cognitive function, physical fitness, and body composition in children. *Scientific Reports*, 12 (1), pp. 14309.

Dunstan, D.W., Kingwell, B.A., Larsen, R., Healy, G.N., Cerin, E., Hamilton, M.T., Shaw, J.E., Bertovic, D.A., Zimmet, P.Z., Salmon, J. and Owen, N. (2012) Breaking Up Prolonged Sitting Reduces Postprandial Glucose and Insulin Responses. *Diabetes Care*, 35 (5), pp. 976–983.

Ekelund, U., Prof, Steene-Johannessen, J., PhD, Brown, W.J., Prof, Fagerland, M.W., PhD, Owen, N., Prof, Powell, K.E., MD, Bauman, A., Prof and Lee, I., Prof. (2016) Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. *The Lancet (British Edition)*, 388 (10051), pp. 1302–1310.

Ekelund, U., Brown, W.J., Steene-Johannessen, J., Fagerland, M.W., Owen, N., Powell, K.E., Bauman, A.E. and Lee, I. (2019) Do the associations of sedentary behaviour with cardiovascular disease mortality and cancer mortality differ by physical activity level? A systematic review and harmonised meta-analysis of data from 850 060 participants. *British Journal of Sports Medicine*, 53 (14), pp. 886–894.

Ekelund, U., Luan, J., Sherar, L.B., Esliger, D.W., Griew, P., Cooper, A. and International Children's Accelerometry Database (ICAD) Collaborators, for the. (2012) Moderate to Vigorous Physical Activity and Sedentary Time and Cardiometabolic Risk Factors in Children and Adolescents. *American Journal of Health Promotion*, 307 (7), pp. 704–712.

Ekelund, U., Tarp, J., Steene-Johannessen, J., Hansen, B.H., Jefferis, B., Fagerland, M.W., Whincup, P., Diaz, K.M., Hooker, S.P., Chernofsky, A., Larson, M.G., Spartano, N., Vasan, R.S., Dohrn, I., Hagströmer, M., Edwardson, C., Yates, T., Shiroma, E., Anderssen, S.A. and Lee, I. (2019) Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: systematic review and harmonised meta-analysis. *BMJ (Online)*, 366, pp. l4570.

Ekelund, U., Tomkinson, G. and Armstrong, N. (2011) What proportion of youth are physically active? Measurement issues, levels and recent time trends. *British Journal of Sports Medicine*, 45 (11), pp. 859–865.

Elizabeth Carpio-Rivera, José Moncada-Jiménez, Walter Salazar-Rojas and Andrea Solera-Herrera. (2016) Acute Effects of Exercise on Blood Pressure: A Meta-Analytic Investigation. *Arquivos Brasileiros De Cardiologia*, 106 (5), pp. 422–433.

Emini, B., Saiti, A., Gontarev, S., Baftiu, S. and Cemena, Y. (2022) Age and gender differences in a 10 × 4 m shuttle run test for primary school children aged 6 to 10. *Research in Physical Education, Sport and Health*, 11 (1), pp. 3–9.

Enríquez-del-Castillo, L.A., Ornelas-López, A., De León, L.G., Cervantes-Hernández, N., Quintana-Mendias, E. and Flores, L.A. (2022) Strength and VO₂max Changes by Exercise Training According to Maturation State in Children. *Children (Basel)*, 9 (7), pp. 938.

Esliger, D. and Hall, J. (2008) Health Survey for England - 2008. Available: <https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/health-survey-for-england-2008-physical-activity-and-fitness> [Accessed: 17 Apr 2018].

Evenson, K.R., Catellier, D.J., Gill, K., Ondrak, K.S. and McMurray, R.G. (2008) Calibration of two objective measures of physical activity for children. *Journal of Sports Sciences*, 26 (14), pp. 1557–1565.

Farah, N.M.F. and Gill, J.M.R. (2013) Effects of exercise before or after meal ingestion on fat balance and postprandial metabolism in overweight men. *British Journal of Nutrition*, 109 (12), pp. 2297–2307.

Fernández, I., Canet, O. and Giné-Garriga, M. (2017) Assessment of physical activity levels, fitness and perceived barriers to physical activity practice in adolescents: cross-sectional study. *European Journal of Pediatrics*, 176 (1), pp. 57–65.

Flouris, A.D., Metsios, G.S. and Koutedakis, Y. (2005) Enhancing the efficacy of the 20 m multistage shuttle run test. *British Journal of Sports Medicine*, 39 (3), pp. 166–170.

Foulds, H.J.A., Bredin, S.S.D., Charlesworth, S.A., Ivey, A.C. and Warburton, D.E.R. (2014) Exercise volume and intensity: a dose–response relationship with health benefits. *European Journal of Applied Physiology*, 114 (8), pp. 1563.

Frankenfield, D.C. (2013) Bias and accuracy of resting metabolic rate equations in non-obese and obese adults. *Clinical Nutrition (Edinburgh, Scotland)*, 32 (6), pp. 976–982.

Freckmann, G., Hagenlocher, S., Baumstark, A., Jendrike, N., Gillen, R.C., Rössner, K. and Haug, C. (2007) Continuous Glucose Profiles in Healthy Subjects under Everyday Life Conditions and after Different Meals. *Journal of Diabetes Science and Technology*, 1 (5), pp. 695–703.

Freedman, D.S., Mei, Z., Srinivasan, S.R., Berenson, G.S. and Dietz, W.H. (2007) Cardiovascular Risk Factors and Excess Adiposity Among Overweight Children and Adolescents: The Bogalusa Heart Study. *The Journal of Pediatrics*, 150 (1), pp. 12–17.e2.

Galinsky, T., Swanson, N., Sauter, S., Dunkin, R., Hurrell, J. and Schleifer, L. (2007) Supplementary breaks and stretching exercises for data entry operators: A follow-up field study. *American Journal of Industrial Medicine*, 50 (7), pp. 519–527.

Gao, Y., Li, Q., Finni, T. and Pesola, A.J. (2024) Enhanced muscle activity during interrupted sitting improves glycemic control in overweight and obese men. *Scandinavian Journal of Medicine & Science in Sports*, 34 (4), pp. e14628.

Garber, C.E., Blissmer, B., Deschenes, M.R., Franklin, B.A., Lamonte, M.J., Lee, I.M., Nieman, D.C. and Swain, D.P. (2011) American College of Sports Medicine position stand: Quantity and

quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults. *Medicine & Science in Sports & Exercise*, 43 (7), pp. 1334–1359.

Giattino, C., Ortiz-Ospina, E. and Roser, M. (2020) Working Hours. Available: <https://ourworldindata.org/working-hours> [Accessed: 2020].

Gillen, J.B., Estafanos, S. and Govette, A. (2021) Exercise-nutrient interactions for improved postprandial glycemic control and insulin sensitivity. *Applied Physiology, Nutrition, and Metabolism*, 46 (8), pp. 856–865.

Giné-Garriga, M., Sansano-Nadal, O., Tully, M.A., Caserotti, P., Coll-Planas, L., Rothenbacher, D., Dallmeier, D., Denking, M., Wilson, J.J., Martin-Borràs, C., Skjødt, M., Ferri, K., Farche, A.C., McIntosh, E., Blackburn, N.E., Salvà, A. and Roqué-i-Figuls, M. (2020) Accelerometer-Measured Sedentary and Physical Activity Time and Their Correlates in European Older Adults: The SITLESS Study. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 75 (9), pp. 1754–1762.

Gouldrup, H. and Ma, T. (2021) Why are physical activity breaks more effective than a single session of isoenergetic exercise in reducing postprandial glucose? A systemic review and meta-analysis. *Journal of Sports Sciences*, 39 (2), pp. 212–218.

Griffiths, L.J., Cortina-Borja, M., Sera, F., Poulou, T., Geraci, M., Rich, C., Cole, T.J., Law, C., Joshi, H., Ness, A.R., Jebb, S.A. and Dezaux, C. (2013) How active are our children? Findings from the Millennium Cohort Study. *BMJ Open*, 3 (8), pp. e–e002893.

Guidry, M.A., Blanchard, B.E., Thompson, P.D., Maresh, C.M., Seip, R.L., Taylor, A.L. and Pescatello, L.S. (2006) The influence of short and long duration on the blood pressure response to an acute bout of dynamic exercise. *American Heart Journal*, 151 (6), pp. 1322.e5–1322.e12.

Hadgraft, N.T., Winkler, E., Climie, R.E., Grace, M.S., Romero, L., Owen, N., Dunstan, D., Healy, G. and Dempsey, P.C. (2021) Effects of sedentary behaviour interventions on biomarkers of cardiometabolic risk in adults: systematic review with meta-analyses. *British Journal of Sports Medicine*, 55 (3), pp. 144–154.

Hands, B., Parker, H., Larkin, D., Cantell, M. and Rose, E. (2016) Male and Female Differences in Health Benefits Derived from Physical Activity: Implications for Exercise Prescription. *Journal of Womens Health, Issues and Care*, 5 (4), pp. 1–5.

Hänggi, J.M., Phillips, L.R.S. and Rowlands, A.V. (2013) Validation of the GT3X ActiGraph in children and comparison with the GT1M ActiGraph. *Journal of Science and Medicine in Sport*, 16 (1), pp. 40–44.

Harber, M.P., Kaminsky, L.A., Arena, R., Blair, S.N., Franklin, B.A., Myers, J. and Ross, R. (2017) Impact of cardiorespiratory fitness on all-cause and disease-specific mortality: Advances since 2009. *Progress in Cardiovascular Diseases*, 60 (1), pp. 11–20.

Hardman, A. and Aldred, H. (1995) Walking during the postprandial period decreases alimentary lipaemia. *Journal of Cardiovascular Risk*, (2), pp. 71–78.

Harris, K.C., Kuramoto, L.K., Schulzer, M. and Retallack, J.E. (2009) Effect of school-based physical activity interventions on body mass index in children: a meta-analysis. *Canadian Medical Association Journal*, 180 (7), pp. 719–726.

Hartwig, T.B., Sanders, T., Vasconcellos, D., Noetel, M., Parker, P.D., Lubans, D.R., Andrade, S., Ávila-García, M., Bartholomew, J., Belton, S., Brooks, N.E., Bugge, A., Cavero-Redondo, I., Christiansen, L.B., Cohen, K., Coppinger, T., Dyrstad, S., Errisuriz, V., Fairclough, S., Gorely, T., Javier Huertas-Delgado, F., Issartel, J., Kriemler, S., Kvalø, S.E., Marques-Vidal, P., Martinez-Vizcaino, V., Møller, N.C., Moran, C., Morris, J., Nevill, M., Ochoa-Avilés, A., O'Leary, M., Peralta, L., Pfeiffer, K.A., Puder, J., Redondo-Tébar, A., Robbins, L.B., Sanchez-Lopez, M., Tarp, J., Taylor, S., Tercedor, P., Toftager, M., Villa-González, E., Wedderkopp, N., Weston, K.L., Yin, Z., Zhixiong, Z., Lonsdale, C. and del Pozo Cruz, B. (2021) School-based interventions modestly increase physical activity and cardiorespiratory fitness but are least effective for youth who need them most: an individual participant pooled analysis of 20 controlled trials. *British Journal of Sports Medicine*, 55 (13), pp. 721–729.

Harvey, J.A., Chastin, S.F.M. and Skelton, D.A. (2015) How Sedentary Are Older People? A Systematic Review of the Amount of Sedentary Behavior. *Journal of Aging and Physical Activity*, 23 (3), pp. 471–487.

Hashimoto, S., Hayashi, S., Yoshida, A. and Naito, M. (2013) Acute Effects of Postprandial Aerobic Exercise on Glucose and Lipoprotein Metabolism in Healthy Young Women. *Journal of Atherosclerosis and Thrombosis*, 20 (2), pp. 204–213.

Hashimoto, S., Ootani, K., Hayashi, S. and Naito, M. (2011) Acute Effects of Shortly Pre- Versus Postprandial Aerobic Exercise on Postprandial Lipoprotein Metabolism in Healthy but Sedentary Young Women. *Journal of Atherosclerosis and Thrombosis*, 18 (10), pp. 891–900.

Haskell, W.L., Montoye, H.J. and Orenstein, D. (1985) Physical Activity and Exercise to Achieve Health-Related Physical Fitness Components. *Public Health Reports* (1974), 100 (2), pp. 202–212.

Hatch, L.M., Williams, R.A., Dring, K.J., Sunderland, C., Nevill, M.E. and Cooper, S.B. (2021) Activity patterns of primary school children during participation in The Daily Mile. *Scientific Reports*, 11 (1), pp. 7462.

Health Survey for England (2023) Health Survey for England, 2021 part 2. Available: <https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/2021-part-2/physical-activity> [Accessed: March 2025].

Healy, G.N., Dunstan, D.W., Salmon, J., Cerin, E., Shaw, J.E., Zimmet, P.Z. and Owen, N. (2007) Objectively Measured Light-Intensity Physical Activity Is Independently Associated With 2-h Plasma Glucose. *Diabetes Care*, 30 (6), pp. 1384–1389.

Healy, G.N., Dunstan, D.W., Salmon, J., Cerin, E., Shaw, J.E., Zimmet, P.Z. and Owen, N. (2008) Breaks in Sedentary Time: Beneficial associations with metabolic risk. *Diabetes Care*, 31 (4), pp. 661–666.

Hesketh, K.R., Brage, S., Inskip, H.M., Crozier, S.R., Godfrey, K.M., Harvey, N.C., Cooper, C. and Van Sluijs, E.M.F. (2022) Activity Behaviors in British 6-Year-Olds: Cross-Sectional Associations and Longitudinal Change During the School Transition. *Journal of Physical Activity & Health*, 19 (8), pp. 558–565.

Hoffmann, S.W., Schierbauer, J., Zimmermann, P., Voit, T., Grothoff, A., Wachsmuth, N., Rössler, A., Lackner, H.K. and Moser, O. (2024a) Effects of light-intensity physical activity on cardiometabolic parameters in young adults with overweight and obesity: The SED-ACT

randomized controlled crossover trial. *Diabetes, Obesity & Metabolism*, 26 (9), pp. 3849–3859.

Hoffmann, S.W., Schierbauer, J., Zimmermann, P., Voit, T., Grothoff, A., Wachsmuth, N.B., Rössler, A., Niedrist, T., Lackner, H.K. and Moser, O. (2024b) Effects of Interrupting Prolonged Sitting with Light-Intensity Physical Activity on Inflammatory and Cardiometabolic Risk Markers in Young Adults with Overweight and Obesity: Secondary Outcome Analyses of the SED-ACT Randomized Controlled Crossover Trial. *Biomolecules (Basel, Switzerland)*, 14 (8), pp. 1029.

Holm, S. (1979) A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics*, 6 (2), pp. 65–70.

Holmstrup, M., Fairchild, T., Keslacy, S., Weinstock, R. and Kanaley, J. (2014) Multiple short bouts of exercise over 12-h period reduce glucose excursions more than an energy-matched single bout of exercise. *Metabolism*, 63 (4), pp. 510–519.

Holtz, K.A., Stephens, B.R., Sharoff, C.G., Chipkin, S.R. and Braun, B. (2008) The effect of carbohydrate availability following exercise on whole-body insulin action. *Applied Physiology, Nutrition, and Metabolism*, 33 (5), pp. 946–956.

Homer, A.R., Fenemor, S.P., Perry, T.L., Rehner, N.J., Cameron, C.M., Skeaff, C.M. and Peddie, M.C. (2017) Regular activity breaks combined with physical activity improve postprandial plasma triglyceride, nonesterified fatty acid, and insulin responses in healthy, normal weight adults: A randomized crossover trial. *Journal of Clinical Lipidology*, 11 (5), pp. 1268–1279.e1.

Høstmark, A.T., Ekeland, G.S., Beckstrøm, A.C. and Meen, H.D. (2006) Postprandial light physical activity blunts the blood glucose increase. *Preventive Medicine*, 42 (5), pp. 369–371.

Inchley, J., Currie, D., Jewell, J., Breda, J. and Barnekow, V. (2007) Adolescent Obesity and Related Behaviours: Trends and Inequalities in the WHO European Region, 2002-2014. Observations from the Health Behaviour in School-Aged Children (HBSC) WHO Collaborative Cross-National Study. World Health Organization Regional Office for Europe. Available: <http://eprints.gla.ac.uk/220299>.

Inchley, J., Currie, D., Young, T., Samdal, O., Torsheim, T., Augustson, L., Mathison, F., Aleman-Diaz, A., Molcho, M., Weber, M. and Barnekow, V. (2016) Growing Up Unequal: Gender and Socioeconomic Differences in Young People's Health and Well-Being. Health Behaviour in School-Aged Children (HBSC) Study: International Report from the 2013/2014 Survey. World Health Organization. Available: <http://eprints.gla.ac.uk/220304>.

Jadallah, R. and Hammad, S.S. (2024) Comparison of the effect of medium-chain fatty acids and long-chain fatty acids on postprandial appetite and lipemia: a randomised crossover trial. *BMJ Nutrition, Prevention & Health*, 7 (2), pp. 385–393.

Jetté, M., Sidney, K. and Blümchen, G. (1990) Metabolic equivalents (METs) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clinical Cardiology (Mahwah, N.J.)*, 13 (8), pp. 555–565.

Jiménez-Pavón, D., Ortega, F.B., Ruiz, J.R., Chillón, P., Castillo, R., Artero, E.G., Martínez-Gómez, D., Vicente-Rodríguez, G., Rey-López, J.P., Gracia, L.A., Noriega, M.J., Moreno, L.A. and Gonzalez-Gross, M. (2010) Influence of socioeconomic factors on fitness and fatness in

Spanish adolescents: The AVENA study. *International Journal of Pediatric Obesity*, 5 (6), pp. 467–473.

Jingjie, W., Yang, L., Jing, Y., Ran, L., Yiqing, X. and Zhou, N. (2022) Sedentary time and its association with risk of cardiovascular diseases in adults: an updated systematic review and meta-analysis of observational studies. *BMC Public Health*, 22 (1), pp. 286–9.

Kashiwabara, K., Kidokoro, T., Yanaoka, T., Burns, S.F., Stensel, D.J. and Miyashita, M. (2018) Different Patterns of Walking and Postprandial Triglycerides in Older Women. *Medicine & Science in Sports & Exercise*, 50 (1), pp. 79–87.

Katsanos, C.S., Grandjean, P.W. and Moffatt, R.J. (2004) Effects of low and moderate exercise intensity on postprandial lipemia and postheparin plasma lipoprotein lipase activity in physically active men. *Journal of Applied Physiology*, 96 (1), pp. 181–188.

Kido, M., Asakawa, A., Koyama, K.K., Takaoka, T., Tajima, A., Takaoka, S., Yoshizaki, Y., Okutsu, K., Takamine, K.T., Sameshima, Y. and Inui, A. (2016) Acute effects of traditional Japanese alcohol beverages on blood glucose and polysomnography levels in healthy subjects. *PeerJ*, 4, pp. e1853–e1853.

Kim, I., Park, S., Trombold, J.R. and Coyle, E.F. (2014) Effects of Moderate- and Intermittent Low-Intensity Exercise on Postprandial Lipemia. *Medicine and Science in Sports and Exercise*, 46 (10), pp. 1882–1890.

King, A.C., Parkinson, K.N., Adamson, A.J., Murray, L., Besson, H., Reilly, J.J. and Basterfield, L. (2011) Correlates of objectively measured physical activity and sedentary behaviour in English children. *European Journal of Public Health*, 21 (4), pp. 424–431.

Kohl, H.W., Craig, C.L., Lambert, E.V., Inoue, S., Alkandari, J.R., Leetongin, G. and Kahlmeier, S. (2012) The pandemic of physical inactivity: global action for public health. *The Lancet (British Edition)*, 380 (9838), pp. 294–305.

Kokkinos, P., Myers, J., Faselis, C., Panagiotakos, D.B., Doumas, M., Pittaras, A., Manolis, A., Kokkinos, J.P., Karasik, P., Greenberg, M., Papademetriou, V. and Fletcher, R. (2010) Exercise capacity and mortality in older men: a 20-year follow-up study. *Circulation*, 122 (8), pp. 790–797.

Konstabel, K., Veidebaum, T., Verbestel, V., Moreno, L.A., Bammann, K., Tornaritis, M., Eiben, G., Molnár, D., Siani, A., Sprengeler, O., Wirsik, N., Ahrens, W. and Pitsiladis, Y. (2014) Objectively measured physical activity in European children: the IDEFICS study. *International Journal of Obesity*, 38 (Suppl 2), pp. S135–S143.

Ku, P., Steptoe, A., Liao, Y., Hsueh, M. and Chen, L. (2018a) A cut-off of daily sedentary time and all-cause mortality in adults: a meta-regression analysis involving more than 1 million participants. *BMC Medicine*, 16 (1), pp. 74–74.

Ku, P., Steptoe, A., Liao, Y., Hsueh, M. and Chen, L. (2018b) A cut-off of daily sedentary time and all-cause mortality in adults: a meta-regression analysis involving more than 1 million participants. *BMC Medicine*, 16 (1), pp. 74.

Larose, D., Massie, C., St-Aubin, A., Boulay-Pelletier, V., Boulanger, E., Lavoie, M.D., Yessis, J., Tremblay, A. and Drapeau, V. (2024) Effects of flexible learning spaces, active breaks, and active lessons on sedentary behaviors, physical activity, learning, and musculoskeletal health

in school-aged children: a scoping review. *Journal of Activity, Sedentary and Sleep Behaviors*, 3 (1), pp. 30.

Lee, D.H., Rezende, L.F.M., Joh, H., Keum, N., Ferrari, G., Rey-Lopez, J.P., Rimm, E.B., Tabung, F.K. and Giovannucci, E.L. (2022) Long-Term Leisure-Time Physical Activity Intensity and All-Cause and Cause-Specific Mortality: A Prospective Cohort of US Adults. *Circulation*, 146 (7), pp. 523–534.

Lee, I., Shiroma, E.J., Lobelo, F., Puska, P., Blair, S.N. and Katzmarzyk, P.T. (2012) Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *The Lancet*, 380 (9838), pp. 219–229.

Lee, K., Brownstein, J.S., Mills, R.G. and Kohane, I.S. (2010) Does Collocation Inform the Impact of Collaboration? *PLoS ONE*, 5 (12), pp. e14279–e14279.

Leese, C., Abraham, K. and Smith, B.H. (2023) Narrative review – Barriers and facilitators to promotion of physical activity in primary care. *Lifestyle Medicine*, 4 (3), pp. e21.

Léger, L.A., Mercier, D., Gadoury, C. and Lambert, J. (1988) The multistage 20 metre shuttle run test for aerobic fitness. *Journal of Sports Sciences*, 6 (2), pp. 93–101.

Lichtenstein, E., Nebiker, L. and Faude, O. (2023) Improvements in fitness and motor performance from the first to fifth grade in Basel. *Current Issues in Sport Science*, 8 (2), pp. 81.

Lo, J.C., Ong, J.L., Leong, R.L.F., Gooley, J.J. and Chee, M.W.L. (2016) Cognitive Performance, Sleepiness, and Mood in Partially Sleep Deprived Adolescents: The Need for Sleep Study. *Sleep*, 39 (3), pp. 687–698.

Loh, R., Stamatakis, E., Folkerts, D., Allgrove, J.E. and Moir, H.J. (2020) Effects of Interrupting Prolonged Sitting with Physical Activity Breaks on Blood Glucose, Insulin and Triacylglycerol Measures: A Systematic Review and Meta-analysis. *Sports Medicine (Auckland)*, 50 (2), pp. 295–330.

Lunde, M.S.H., Hjellset, V.T. and Høstmark, A.T. (2012) Slow Post Meal Walking Reduces the Blood Glucose Response: An Exploratory Study in Female Pakistani Immigrants. *Journal of Immigrant and Minority Health*, 14 (5), pp. 816–822.

MacEwen, B.T., MacDonald, D.J. and Burr, J.F. (2015) A systematic review of standing and treadmill desks in the workplace. *Preventive Medicine*, 70, pp. 50–58.

Macías, N., Monterrubio-Flores, E., Salmerón, J., Meneses-León, J., Flores, Y.N., Jáuregui, A., Salvo, D., Villa, U., Olvera, A.G. and Gallegos-Carrillo, K. (2024) Exploring the effect of sedentary behavior on increased adiposity in middle-aged adults. *BMC Public Health*, 24 (1), pp. 2228–11.

Mamen, A., Lindberg, M. and Fredriksen, P.M. (2022) Increase in peak oxygen uptake and Andersen test performance in children from age six to ten: The Health Oriented Pedagogical Project (HOPP). *Frontiers in Physiology*, 13, pp. 976505.

Marchant, E., Todd, C., Stratton, G. and Brophy, S. (2020) The Daily Mile: Whole-school recommendations for implementation and sustainability. A mixed-methods study. *PloS One*, 15 (2), pp. e0228149.

Marion-Latard, F., Crampes, F., Zakaroff-Girard, A., De Glisezinski, I., Harant, I., Stich, V., Thalamas, C., Rivi re, D., Lafontan, M. and Berlan, M. (2003) Post-Exercise Increase of Lipid Oxidation after a Moderate Exercise Bout in Untrained Healthy Obese Men. *Hormone and Metabolic Research*, 35 (2), pp. 97–103.

Matthews, C.E., Chen, K.Y., Freedson, P.S., Buchowski, M.S., Beech, B.M., Pate, R.R. and Troiano, R.P. (2008) Amount of Time Spent in Sedentary Behaviors in the United States, 2003–2004. *American Journal of Epidemiology*, 167 (7), pp. 875–881.

Mauvais-Jarvis, F. (2018) Gender differences in glucose homeostasis and diabetes. *Physiology & Behavior*, 187, pp. 20–23.

Maylor, B.D., Zakrzewski-Fruer, J.K., Orton, C.J. and Bailey, D.P. (2018) Beneficial postprandial lipaemic effects of interrupting sedentary time with high-intensity physical activity versus a continuous moderate-intensity physical activity bout: A randomised crossover trial. *Journal of Science and Medicine in Sport*, 21 (12), pp. 1250–1255.

McCambridge, J., Witton, J. and Elbourne, D.R. (2014) Systematic review of the Hawthorne effect: New concepts are needed to study research participation effects. *Journal of Clinical Epidemiology*, 67 (3), pp. 267–277.

Mei, H., Xiong, Y., Xie, S., Guo, S., Li, Y., Guo, B. and Zhang, J. (2016) The impact of long-term school-based physical activity interventions on body mass index of primary school children – a meta-analysis of randomized controlled trials. *BMC Public Health*, 16 (209), pp. 205.

Mendis, S. (2014) Global status report on noncommunicable diseases 2014. Geneva: Available: <https://www.who.int/publications/i/item/9789241564854> [Accessed: 17 Apr 2018].

Mikkelsen, M., Wilsgaard, T., Grimsgaard, S., Hopstock, L.A. and Hansson, P. (2023) Associations between postprandial triglyceride concentrations and sex, age, and body mass index: cross-sectional analyses from the Troms  study 2015–2016. *Frontiers in Nutrition (Lausanne)*, 10, pp. 1158383.

Mittendorfer, B. and Klein, S. (2003) Physiological factors that regulate the use of endogenous fat and carbohydrate fuels during endurance exercise. *Nutrition Research Reviews*, 16 (1), pp. 97–108.

Miyashita, M. (2008) Effects of continuous versus accumulated activity patterns on postprandial triacylglycerol concentrations in obese men. *International Journal of Obesity*, 32 (8), pp. 1271–1278.

Miyashita, M., Park, J.-., Takahashi, M., Suzuki, K., Stensel, D. and Nakamura, Y. (2013) Postprandial Lipaemia: Effects of Sitting, Standing and Walking in Healthy Normolipidaemic Humans. *International Journal of Sports Medicine*, 34 (1), pp. 21–27.

Miyashita, M., Burns, S. and Stensel, D. (2008) Accumulating short bouts of brisk walking reduces postprandial

plasma triacylglycerol concentrations and resting blood pressure in

healthy young men. *Am J Clin Nutr*, 88 (5), pp. 1225–1231.

Miyashita, M., Edamoto, K., Kidokoro, T., Yanaoka, T., Kashiwabara, K., Takahashi, M. and Burns, S. (2016) Interrupting Sitting Time with Regular Walks Attenuates Postprandial Triglycerides. *International Journal of Sports Medicine*, 37 (2), pp. 97–103.

Must, A. and Tybor, D.J. (2005) Physical activity and sedentary behavior: a review of longitudinal studies of weight and adiposity in youth. *International Journal of Obesity*, 29 (S2), pp. 84–96.

Newsom, S.A., Everett, A.C., Hinko, A. and Horowitz, J.F. (2013) A Single Session of Low-Intensity Exercise Is Sufficient to Enhance Insulin Sensitivity Into the Next Day in Obese Adults. *Diabetes Care*, 36 (9), pp. 2516–2522.

Nguyen, Q.M., Xu, J., Chen, W., Srinivasan, S.R. and Berenson, G.S. (2012) Correlates of Age Onset of Type 2 Diabetes Among Relatively Young Black and White Adults in a Community: The Bogalusa Heart Study. *Diabetes Care*, 35 (6), pp. 1341–1346.

Norris, E., Shelton, N., Dunsmuir, S., Duke-Williams, O. and Stamatakis, E. (2015) Physically active lessons as physical activity and educational interventions: A systematic review of methods and results. *Preventive Medicine*, 72, pp. 116–125.

Norton, K., Carter, L., Olds, T. and Marfell-Jones, M. (2011) Standards for anthropometry assessment. In: *Kinanthropometry and Exercise Physiology*. 4 ed. World: International Society for the Advancement of Kinanthropometry (ISAK), pp. 1–71.

Nygaard, H., Tomten, S.E. and Høstmark, A.T. (2009) Slow postmeal walking reduces postprandial glycemia in middle-aged women. *Applied Physiology, Nutrition, and Metabolism*, 34 (6), pp. 1087–1092.

Obesity Action Scotland (2024) Childhood Obesity Briefing October 2024. Online: Available: <https://www.obesityactionsotland.org/media/qc4bpoal/obesity-action-scotland-childhood-obesity-briefing-october-2024.pdf> [Accessed: February 2025].

Pan, H. and Cole, T.J. (2011) LMS growth, a Microsoft Excel add-in to access growth references based on the LMS method. Available: v 2.74 .

Parr, J., Winwood, K., Hodson-Tole, E., Deconinck, F., Hill, J., Teunissen, J.W. and Cumming, S. (2020) The Main and Interactive Effects of Biological Maturity and Relative Age on Physical Performance in Elite Youth Soccer Players. *Journal of Sports Medicine*, 2020, pp. 1957636.

Peddie, M.C., Bone, J.L., Rehrer, N.J., Skeaff, C.M., Gray, A.R. and Perry, T.L. (2013) Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. *The American Journal of Clinical Nutrition*, 98 (2), pp. 358–366.

Penpraze, V., Reilly, J.J., MacLean, C.M., Montgomery, C., Kelly, L.A., Paton, J.Y., Aitchison, T. and Grant, S. (2006) Monitoring of Physical Activity in Young Children: How Much Is Enough? *Pediatric Exercise Science*, 18 (4), pp. 483–491.

Peralta, L.R., Mhrshahi, S., Bellew, B., Reece, L.J. and Hardy, L.L. (2019) Influence of School-Level Socioeconomic Status on Children's Physical Activity, Fitness, and Fundamental Movement Skill Levels. *The Journal of School Health*, 89 (6), pp. 460–467.

Poitras, V.J., Gray, C.E., Borghese, M.M., Carson, V., Chaput, J., Janssen, I., Katzmarzyk, P.T., Pate, R.R., Connor Gorber, S., Kho, M.E., Sampson, M. and Tremblay, M.S. (2016) Systematic

review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Applied Physiology, Nutrition, and Metabolism*, 41 (6 (Suppl. 3)), pp. 197–239.

Public Health England (2016) Guidance Health matters: getting every adult active every day. Available: <https://www.gov.uk/government/organisations/public-health-england> [Accessed: 1 March 2022].

Qaisar, R., Hussain, M.A., Franzese, F., Karim, A., Ahmad, F., Awad, A. and Alkahtani, S.A. (2025) Accelerometry 24-hour movement behaviours and health markers in the European old population: iso-temporal substitution regression. *Archives of Public Health = Archives Belges De Santé Publique*, 83 (1), pp. 41–12.

Quan, M., Xun, P., Wu, H., Wang, J., Cheng, W., Cao, M., Zhou, T., Huang, T., Gao, Z. and Chen, P. (2021) Effects of interrupting prolonged sitting on postprandial glycemia and insulin responses: A network meta-analysis. *Journal of Sport and Health Science*, 10 (4), pp. 419–429.

Reilly, J.J., Penpraze, V., Hislop, J., Davies, G., Grant, S. and Paton, J.Y. (2008) Objective measurement of physical activity and sedentary behaviour: review with new data. *Archives of Disease in Childhood*, 93 (7), pp. 614–619.

Reis, C.E.G., Dórea, J.G. and da Costa, T.H.M. (2019) Effects of coffee consumption on glucose metabolism: A systematic review of clinical trials. *Journal of Traditional and Complementary Medicine*, 9 (3), pp. 184–191.

Reyes-Amigo, T., Salinas-Gallardo, G., Mendoza, E., Ovalle-Fernández, C., Ibarra-Mora, J., Gómez-Álvarez, N., Carrasco-Beltrán, H., Páez-Herrera, J., Hurtado-Almonacid, J., Yañez-Sepúlveda, R., Zapata-Lamana, R., Sepúlveda-Figueroa, F., Olivares-Arancibia, J. and Mota, J. (2025) Effectiveness of school-based active breaks on classroom behavior, executive functions and physical fitness in children and adolescent: a systematic review. *Frontiers in Public Health*, 13, pp. 1469998.

Robusto, K.M. and Trost, S.G. (2012) Comparison of three generations of ActiGraph™ activity monitors in children and adolescents. *Journal of Sports Sciences*, 30 (13), pp. 1429–1435.

Rodriguez-Sanchez, N. and Galloway, S.D.R. (2015) Errors in Dual Energy X-Ray Absorptiometry Estimation of Body Composition Induced by Hypohydration. *International Journal of Sport Nutrition and Exercise Metabolism*, 25 (1), pp. 60–68.

Ronca, F., Burgess, P.W., Savage, P., Senaratne, N., Watson, E. and Loosemore, M. (2024) Decreasing Sedentary Time during Lessons Reduces Obesity in Primary School Children: The Active Movement Study. *Obesity Facts*, 17 (3), pp. 243–254.

Rosselli, M., Ermini, E., Tosi, B., Boddì, M., Stefani, L., Toncelli, L. and Modesti, P.A. (2020) Gender differences in barriers to physical activity among adolescents. *Nutrition, Metabolism, and Cardiovascular Diseases*, 30 (9), pp. 1582–1589.

Rouyard, T., Yoda, E., Akksilp, K., Dieterich, A.V., Kc, S., Dabak, S.V. and Müller, A.M. (2025) Effects of workplace interventions on sedentary behaviour and physical activity: an umbrella review with meta-analyses and narrative synthesis. *The Lancet. Public Health*, 10 (4), pp. e295–e308.

Ruxton, G.D. (2006) The unequal variance t-test is an underused alternative to Student's t-test and the Mann–Whitney U test. *Behavioral Ecology*, 17 (4), pp. 688–690.

Sanchez, B.N., Volek, J.S., Kraemer, W.J., Saenz, C. and Maresh, C.M. (2024) Sex Differences in Energy Metabolism: A Female-Oriented Discussion. *Sports Medicine (Auckland)*, 54 (8), pp. 2033–2057.

Sarria, A., Garcia-Llop, L.A., Moreno, L.A., Fleta, J., Morellon, M.P. and Bueno, M. (1998) Skinfold thickness measurements are better predictors of body fat percentage than body mass index in male Spanish children and adolescents. *European Journal of Clinical Nutrition*, 52 (8), pp. 573–576.

Saunders, T.J., Atkinson, H.F., Burr, J., MacEwen, B., Skeaff, C.M. and Peddie, M.C. (2018) The Acute Metabolic and Vascular Impact of Interrupting Prolonged Sitting: A Systematic Review and Meta-Analysis. *Sports Medicine*, 48 (10), pp. 2347–2366.

Schmelzeisen-Redeker, G., Schoemaker, M., Kirchsteiger, H., Freckmann, G., Heinemann, L. and del Re, L. (2015) Time Delay of CGM Sensors. *Journal of Diabetes Science and Technology*, 9 (5), pp. 1006–1015.

Scottish Government (2015) Daily Mile roll out. Scotland: Available: <https://news.gov.scot/news/daily-mile-roll-out> [Accessed: 17 Apr 2018].

Scottish Government (2016) Postcode to Scottish Index of Multiple Deprivation (SIMD) rank. Scottish Government. Available: <https://webarchive.nrscotland.gov.uk/20190708060827/https://www2.gov.scot/Topics/Statistics/SIMD> [Accessed: 17 Apr 2018].

Scottish Government (2017) The Scottish Attainment Challenge. Available: <https://www.gov.scot/policies/schools/pupil-attainment/> [Accessed: 17 Apr 2018].

Scottish Government (2021) Early learning and childcare: statutory guidance - July 2021. Available: <https://www.gov.scot/publications/early-learning-childcare-statutory-guidance-july-2021/pages/9/> [Accessed: March 2022].

Shah, R.V., Murthy, V.L., Colangelo, L.A., Reis, J., Venkatesh, B.A., Sharma, R., Abbasi, S.A., Goff, D.C., Carr, J.J., Rana, J.S., Terry, J.G., Bouchard, C., Sarzynski, M.A., Eisman, A., Neilan, T., Das, S., Jerosch-Herold, M., Lewis, C.E., Carnethon, M., Lewis, G.D. and Lima, J.A.C. (2016) Association of Fitness in Young Adulthood With Survival and Cardiovascular Risk: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *JAMA Internal Medicine*, 176 (1), pp. 87–95.

Singh, A.S., Mulder, C., Twisk, J.W.R., Van Mechelen, W. and Chinapaw, M.J.M. (2008) Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obesity Reviews*, 9 (5), pp. 474–488.

Singh, B., Olds, T., Curtis, R., Dumuid, D., Virgara, R., Watson, A., Szeto, K., O'Connor, E., Ferguson, T., Eglitis, E., Miatke, A., Simpson, C.E. and Maher, C. (2023a) Effectiveness of physical activity interventions for improving depression, anxiety and distress: an overview of systematic reviews. *British Journal of Sports Medicine*, 57 (18), pp. 1203–1209.

Singh, J., Nguyen, T., Kerndt, C., Dhamoon, A.M. and . (2023b) Physiology, Blood Pressure Age Related Changes. Treasure Island (FL): StatPearls Publishing. Available: <https://www.ncbi.nlm.nih.gov/books/NBK537297/> [Accessed: March 2025].

Smart, N.A., Downes, D., van der Touw, T., Hada, S., Dieberg, G., Pearson, M.J., Wolden, M., King, N. and Goodman, S.P.J. (2025) The Effect of Exercise Training on Blood Lipids: A Systematic Review and Meta-analysis. *Sports Medicine (Auckland)*, 55 (1), pp. 67–78.

Somerville, S.A. (2021) Education - Achieving Excellence and Equity: national improvement framework and improvement plan 2022. Scottish Government. Available: <https://webarchive.nrsotland.gov.uk/20220307034337/http://www.gov.scot/publications/achieving-excellence-equity-2022-national-improvement-framework-improvement-plan/> [Accessed: March 2024].

Stamatakis, E., Biswas, R.K., Koemel, N.A., Sabag, A., Pulsford, R., Atkin, A.J., Stathi, A., Cheng, S., Thøgersen-Ntoumani, C., Blodgett, J.M., Bauman, A., Celis-Morales, C., Hamer, M., Gill, J.M.R. and Ahmadi, M.N. (2025) Dose Response of Incidental Physical Activity Against Cardiovascular Events and Mortality. *Circulation (New York, N.Y.)*, 151 (15), pp. 1063–1075.

Stanish, H., Curtin, C., Must, A., Phillips, S., Maslin, M. and Bandini, L. (2015) Enjoyment, Barriers, and Beliefs About Physical Activity in Adolescents With and Without Autism Spectrum Disorder. *Adapted Physical Activity Quarterly*, 32 (4), pp. 302–317.

Stirling Council (2015) Stirling Council: The Daily Mile - Health and Wellbeing for free. Available: <https://www.stirling.gov.uk/> [Accessed: 17 April 2018].

Takaishi, T., Imaeda, K., Tanaka, T., Moritani, T. and Hayashi, T. (2012) A short bout of stair climbing–descending exercise attenuates postprandial hyperglycemia in middle-aged males with impaired glucose tolerance. *Applied Physiology, Nutrition, and Metabolism*, 37 (1), pp. 193–196.

Talma, H., Chinapaw, M.J.M., Bakker, B., HiraSing, R.A., Terwee, C.B. and Altenburg, T.M. (2013) Bioelectrical impedance analysis to estimate body composition in children and adolescents: a systematic review and evidence appraisal of validity, responsiveness, reliability and measurement error. *Obesity Reviews*, 14 (11), pp. 895–905.

Tamura, A., Murayama, K., Ishii, R., Sakaki, M. and Tanaka, A. (2022) The effect of low-intensity exercise on emotional and cognitive engagement in the classroom. *NPJ Science of Learning*, 7 (9), pp. 1–7.

Telama, R., Yang, X., Viikari, J., Välimäki, I., Wanne, O. and Raitakari, O. (2005) Physical activity from childhood to adulthood: a 21-year tracking study. *American Journal of Preventive Medicine*, 28 (3), pp. 267–273.

Telford, R.M., Telford, R.D., Olive, L.S., Cochrane, T. and Davey, R. (2016) Why Are Girls Less Physically Active than Boys? Findings from the LOOK Longitudinal Study. *Plos One*, 11 (3), pp. e0–e0150041.

The Daily Mile (2016) The Daily Mile. Available: <https://thedailymile.co.uk/> [Accessed: 17 April 2018].

Thosar, S.S., Bielko, S.L., Wiggins, C.C. and Wallace, J.P. (2014) Differences in brachial and femoral artery responses to prolonged sitting. *Cardiovascular Ultrasound*, 12 (50), pp. 12–50.

- Tiihonen, K., Rautonen, N., Alhoniemi, E., Ahotupa, M., Stowell, J. and Vasankari, T. (2015) Postprandial triglyceride response in normolipidemic, hyperlipidemic and obese subjects – the influence of polydextrose, a non-digestible carbohydrate. *Nutrition Journal*, 14 (1), pp. 23.
- Tomkinson, G.R., Lang, J.J., Blanchard, J., Léger, L.A. and Tremblay, M.S. (2019) The 20-m Shuttle Run: Assessment and Interpretation of Data in Relation to Youth Aerobic Fitness and Health. *Pediatric Exercise Science*, 31 (2), pp. 152–163.
- Tomkinson, G.R., Leger, L.A., Olds, T.S. and Cazorla, G. (2003) Secular trends in the performance of children and adolescents (1980-2000): An analysis of 55 studies of the 20m Shuttle Run test in 11 countries. *Sports Medicine (Auckland)*, 33 (4), pp. 285–300.
- Trautmann, S., Rehm, J. and Wittchen, H. (2016) The economic costs of mental disorders: Do our societies react appropriately to the burden of mental disorders? *EMBO Reports*, 17 (9), pp. 1245–1249.
- Trefts, E., Williams, A.S. and Wasserman, D.H. (2015) Exercise and the Regulation of Hepatic Metabolism. *Molecular and Cellular Regulation of Adaptation to Exercise*, 135, pp. 203–225.
- Tremblay, M.S., LeBlanc, A.G., Kho, M.E., Saunders, T.J., Larouche, R., Colley, R.C., Goldfield, G. and Connor Gorber, S. (2011) Systematic review of sedentary behaviour and health indicators in school-aged children and youth. *The International Journal of Behavioral Nutrition and Physical Activity*, 8 (1), pp. 98–492.
- Truesdale, B.C. and Jencks, C. (2016) The Health Effects of Income Inequality: Averages and Disparities. *Annual Review of Public Health*, 37 (1), pp. 413–430.
- Tsetsonis, N.V. and Hardman, A.E. (1996) Reduction In Postprandial Lipemia After Walking: Influence Of Exercise Intensity. *Medicine and Science in Sports*, 28, pp. 1235–1242.
- UK Chief Medical Officers (2019) UK Chief Medical Officers' physical activity guidelines. Available: <https://www.gov.uk/government/collections/physical-activity-guidelines> [Accessed: 1 March 2022].
- UK Parliament (2016a) Early day motion 1074: Five Year Anniversary of Daily Mile. Available: <http://www.parliament.uk/edm/2016-17/1074> [Accessed: 17 Apr 2018].
- UK Parliament (2016b) Obesity: Children: Written question - 46791. Available: <http://www.parliament.uk/business/publications/written-questions-answers-statements/written-question/Commons/2016-10-07/46791> [Accessed: 17 Apr 2018].
- van Ballegooijen, A.J., van der Ploeg, H.P. and Visser, M. (2019) Daily sedentary time and physical activity as assessed by accelerometry and their correlates in older adults. *European Review of Aging and Physical Activity*, 16 (3), pp. 1–12.
- Viir, R. and Verakšitš, A. (2012) Discussion of “Letter to the Editor: Standardized use of the terms sedentary and sedentary behaviours” — Sitting and reclining are different states. *Applied Physiology, Nutrition, and Metabolism*, 37 (6), pp. 1256–1257.
- Vos, M.B. and Welsh, J. (2010) Childhood Obesity: Update on Predisposing Factors and Prevention Strategies. *Current Gastroenterology Reports*, 12 (4), pp. 280–287.

Wang, Y. and Lim, H. (2015) The global childhood obesity epidemic and the association between socio-economic status and childhood obesity. *International Review of Psychiatry*, 24 (3), pp. 176–188.

Waongenngarm, P., Areerak, K. and Janwantanakul, P. (2017) The effects of breaks on low back pain, discomfort, and work productivity in office workers: A systematic review of randomized and non-randomized controlled trials. *Applied Ergonomics*, 68, pp. 230–239.

Weiss, R., Dziura, J., Burgert, T.S., Tamborlane, W.V., Taksali, S.E., Yeckel, C.W., Allen, K., Lopes, M., Savoye, M., Morrison, J., Sherwin, R.S. and Caprio, S. (2004) Obesity and the Metabolic Syndrome in Children and Adolescents. *New England Journal of Medicine*, 350 (23), pp. 2362–2374.

Wheeler, M.J., Green, D.J., Cerin, E., Ellis, K.A., Heinonen, I., Lewis, J., Naylor, L.H., Cohen, N., Larsen, R., Dempsey, P.C., Kingwell, B.A., Owen, N. and Dunstan, D.W. (2020) Combined effects of continuous exercise and intermittent active interruptions to prolonged sitting on postprandial glucose, insulin, and triglycerides in adults with obesity: a randomized crossover trial. *The International Journal of Behavioral Nutrition and Physical Activity*, 17 (1), pp. 152.

Whitton, C., Nicholson, S.K., Roberts, C., Prynne, C.J., Pot, G.K., Olson, A., Fitt, E., Cole, D., Teucher, B., Bates, B., Henderson, H., Pigott, S., Deverill, C., Swan, G. and Stephen, A.M. (2011) National Diet and Nutrition Survey: UK food consumption and nutrient intakes from the first year of the rolling programme and comparisons with previous surveys. *British Journal of Nutrition*, 106 (12), pp. 1899–1914.

Wilmot, E.G., Edwardson, C.L., Achana, F.A., Davies, M.J., Gorely, T., Gray, L.J., Khunti, K., Yates, T. and Biddle, S.J.H. (2012) Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. *Diabetologia*, 55 (11), pp. 2895–2905.

Wolever, T.M.S. and Jenkins, D.J.A. (1986) The use of the glycemic index in predicting the blood glucose response to mixed meals. *The American Journal of Clinical Nutrition*, 43 (1), pp. 167–172.

World Health Organisation (2017) World Health Organisation: Physical activity fact sheet. World Health Organisation. Available: <https://www.who.int/news-room/fact-sheets/detail/physical-activity> [Accessed: 17 April 2018].

World Health Organization (2020a) Basic documents. World Health Organization. Available: <https://iris.who.int/handle/10665/339554> [Accessed: March 2024].

World Health Organization (2020b) WHO guidelines on physical activity and sedentary behaviour. Available: <https://www.who.int/publications/i/item/9789240015128> [Accessed: Mar, 2021].

Wu, Y., Van Gerven, P.W.M., Groot, R.H.M., Eijnde, B.O., Winkens, B. and Savelberg, H.H.C.M. (2023) Effects of breaking up sitting with light-intensity physical activity on cognition and mood in university students. *Scandinavian Journal of Medicine & Science in Sports*, 33 (3), pp. 257–266.

Yang, Y., Chen, L. and Filippidis, F.T. (2025) Accelerometer-measured physical activity, frailty, and all-cause mortality and life expectancy among middle-aged and older adults: a UK Biobank longitudinal study. *BMC Medicine*, 23 (1), pp. 125–12.

Yin, M., Xu, K., Deng, J., Deng, S., Chen, Z., Zhang, B., Zhong, Y., Li, H., Zhang, X., Toledo, M.J.L., Diaz, K.M. and Li, Y. (2024) Optimal Frequency of Interrupting Prolonged Sitting for Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Crossover Trials. *Scandinavian Journal of Medicine & Science in Sports*, 34 (12), pp. e14769.

Young, D.R., Hivert, M., Alhassan, S., Camhi, S.M., Ferguson, J.F., Katzmarzyk, P.T., Lewis, C.E., Owen, N., Perry, C.K., Siddique, J. and Yong, C.M. (2016) Sedentary Behavior and Cardiovascular Morbidity and Mortality: A Science Advisory From the American Heart Association. *Circulation*, 134 (13), pp. e262–e279.

Youssef, R.M., Shafie, K.A., Mukhaini, M.A. and Balushi, H.A. (2013) Physical activity and perceived barriers among high-school students in Muscat, Oman/Activite physique des lycéens et obstacles perçus par ces derniers a Mascate (Oman). *Eastern Mediterranean Health Journal*, 19 (9), pp. 759–768.

Zelenović, M., Manić, M., Stamenković, A., Čaprić, I. and Božić, D. (2021) Barriers to physical activity in adolescents: A systematic review. *Turkish Journal of Kinesiology*, 7 (1), pp. 22–30.

Zhou, S., Raat, H., You, Y., Santos, S., van Grieken, A., Wang, H. and Yang-Huang, J. (2024) Change in neighborhood socioeconomic status and childhood weight status and body composition from birth to adolescence. *International Journal of Obesity*, 48 (5), pp. 646–653.

Zhu, B., Shi, C., Park, C.G., Zhao, X. and Reutrakul, S. (2019) Effects of sleep restriction on metabolism-related parameters in healthy adults: A comprehensive review and meta-analysis of randomized controlled trials. *Sleep Medicine Reviews*, 45, pp. 18–30.

Zhu, E., Li, W., Zhao, C. and Wang, J. (2024) Promoting the Sustainable Development of Enterprises: Unraveling the Dual Impact of Informal Communication on Employee Affect and Problem-Solving Capabilities. *Sustainability*, 16 (13), pp. 5710.

7. Appendices

Appendix 1: Participant Information Sheet



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Participant Information Sheet (Full version)

Investigators: Mr Ross Chesham, Dr Naomi Brooks, Dr Lee Hamilton, Dr Gemma Ryde and Dr Trish Gorely

1. Study title

'Examining the effect of breaking up prolonged sitting with light versus moderate physical activity on glucose handling and insulin in sedentary, inactive adults.'

i.e. Research to see the effect of breaking up long periods of sitting with exercise on the body's ability to handle the sugar in a meal. A period of light exercise, moderate exercise and no exercise will be compared. This is in people who often sit for long periods and do less than the 150 minutes of advised exercise per week.

2. Why am I being asked to take part in this research?

You have been asked to take part in this study as you sit for long time periods on a regular basis. We are looking for 18 adults to complete the study. You do not have to take part if you do not want to. Please read this information which will help you to decide. Please discuss with others or ask one of our group if anything is unclear (see contact details in section 15).

3. What is the purpose of the research?

People who sit for long periods of time (prolonged sitting), on a daily basis, have a greater chance of developing diseases. These can include cardiovascular disease (CVD) and type 2 diabetes.

CVD is a term used to describe impaired functioning of the heart and blood vessels. Broadly, CVD includes heart disease, stroke and other diseases involved with blood travelling around the body: such as the growth of fatty plaques in the blood vessels.

In type 2 diabetes, sugar builds up in your blood to unsafe levels. Over the long-term this can increase the chance of developing health problems. Problems can include damage to the blood vessels of the eye, foot ulcers, kidney disease, and CVD.

People who break up prolonged sitting have a lower chance of developing these health problems. Breaking up prolonged sitting can be done at work, a place where large amounts of people in the UK

6. What will I have to do if I take part in the research?

Taking part in the study involves 2 pre-research periods and 3 research periods (figure 1):

1) Pre-research period 1: First contact

You are at this stage. This involves having the study explained to you in detail. You can ask any questions at this time, but also at any other time during the study. We will ask a few quick questions and begin to see if you are able to do the study (see section 5). This should take around 30 minutes. You will then be granted as much time as you need to decide if you want to take part.

Pre-research period 1	Pre-research period 2	Research period 1	Research period 2	Research period 3
First contact 30 minutes	Familiarisation Up to 1 hour	Experimental days Lab time • Day 1: 15 minutes • Day 2: 0 minutes • Day 3: 9 hours • Day 4: 30 minutes	Experimental days Lab time • Day 1: 15 minutes • Day 2: 0 minutes • Day 3: 9 hours • Day 4: 30 minutes	Experimental days Lab time • Day 1: 15 minutes • Day 2: 0 minutes • Day 3: 9 hours • Day 4: 30 minutes

Figure 1. Brief outline of study time frames

2) Pre-research period 2: Familiarisation

First you must verbally confirm you fully understand what is needed of you. Then we will ask for you to sign a consent form showing you agree to do the study: aka written informed consent.

At this visit we will ask you to arrive in the morning after a 12 hour fast (no food or drinks, other than water, after 8pm the night before). You will then fill in a pre-participation health and activity questionnaire. After filling these in, we will assess your blood sugar level using a finger prick (figure 2.A.). We will also assess your blood pressure (figure 2.B.). You will then walk on a treadmill at 1.6mph and then 3.5mph, to get you used to the speed required in the study (figure 2.C.). Your height, weight, waist and hip girth will then be taken. You will need to wear loose fitting clothing and comfortable shoes, preferably sports clothing if possible (not essential). This should take less than 1 hour. We will also ask you about your exercise history and ask you to fill in a 3 day food diary.

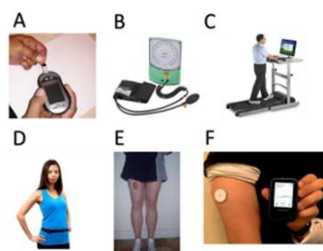


Figure 2. Equipment you will use or wear

sit for prolonged periods. Yet, the best type of exercise to break up prolonged sitting is unknown. It is also unknown for how long, how often, or how hard people should exercise.

In our research, we want to break up a day of prolonged sitting. We want to compare a long period of light intensity walking to a shorter period of moderate intensity walking. These 2 walks will be compared to a day where no walking is done. This will allow us to see which of these walks can improve the body's control of sugar levels best. This sugar, known as glucose, builds up in the blood after a meal. How long and how much glucose appears in the body after a meal tells us how well the body can cope with the sugar in that meal.

The intensity of the walking in this study may cause unique sugar responses in the body after the meal. We hope to find out if one walking intensity has a better response than the other does. In the future, this will help tell us what people should do in the workplace to offset the harmful effects of prolonged sitting on health.

4. Do I have to take part?

No. It is entirely up to you to decide. If you do not want to take part that's OK. If you do decide to take part, you will be asked to sign a consent form. If you do decide to take part you are free to withdraw at any time, without giving a reason. Just contact Ross Chesham to let him know you've withdrawn (r.a.chesham@stir.ac.uk, 01786 466296 or 07794774183).

5. Am I eligible to take part?

Adults that sit for a number of hours each day can take part in this study. However, they must also fulfil these conditions.

Volunteers must:

- ❖ Be 18-59 years of age.
- ❖ Sit for a total of 5 hours or more per day (we will assess this with you).
- ❖ Have normal blood pressure (we will assess this for you).
- ❖ Have normal blood sugar (we will assess this for you).
- ❖ Speak and understand written English.

Volunteers must not:

- ❖ Have risk-factors, illnesses or injuries stopping safe light or moderate exercise.
- ❖ Have known disorders such as type 2 diabetes that would affect the main study outcome (glucose / sugar levels).
- ❖ Smoke.
- ❖ Be pregnant.
- ❖ Be taking blood pressure drugs.
- ❖ Be taking drugs that lower fat and/or blood sugars.
- ❖ Lift weights 2 or more times per week.
- ❖ Do more than 150 minutes of moderate-to-vigorous exercise per week (we will assess this with you).

3) Research periods 1, 2 & 3

Each research period is carried out over 4 consecutive days from any time between 8am and 8pm on Day 1, to 8:30am on Day 4 (between 60.5 and 72.5 hours total). **Importantly, although the total length of each research period is between 60.5 and 72.5 hours, you do not need to be in the lab the whole time.** (See figure 1 above).

Within each research period you will be asked to carry out a single experimental protocol. These protocols are called EP-light, EP-moderate and EP-sit. EP-light involves 150 minutes of slow walking (1.6mph). EP-moderate involves 70 minutes of faster walking (3.5mph). EP-sit involves no walking, just sitting. These protocols will be done in a random order. You need to complete all 3 research periods. The research periods are spread 1 week apart, but this gap can be longer if needed.

On Day 1 you only need to be in the lab (or suitable private room, potentially your house) sometime between 8am and 8pm for 15 minutes so that we can administer you with equipment.

This equipment includes 2 activity monitors and a glucose monitor (Figure 2.D, E & F.). We will show you how to use and apply them. Once you leave on Day 1, you can do most of your normal activities at home. However, there is a list of activities we want you to do and others we want you to avoid before 8am on Day 3 (the main study day). Please see section 7 for the list.

We will also give you a meal to eat the following evening (Day 2) between 7pm and 8pm.

On Day 2 you are not required to be in the lab. You only need to wear the equipment applied on Day 1, limit some activities and eat the meal provided (see section 7).

Day 3 is the main research day and you need to be in the University of Stirling Cottrell exercise lab/workspace for 9 hours, from 8am to 5pm.

At 8am we will take a small blood sample (10ml) from your arm, take your blood pressure and feed you breakfast. You will then sit at a desk.

We will endeavour to minimise the impact of this research period on your normal work productivity by providing you with a workspace. You may choose not to work during this research period. That is OK too

Later in the morning you will either be asked to walk for slowly 150 minutes, walk slightly faster for 70 minutes, or not walk at all (EP-light, EP-moderate, EP-sit). The walks will take place on a treadmill in the Cottrell exercise lab, with access to a laptop/TV. We will then give you lunch at 1pm. About 45 minutes later we will take a second small blood sample and take your blood pressure. You will then sit and work/relax until 5pm when you will be given a snack and sent home. We'll give you another meal to eat between 7pm and 8pm. Again, you can do most of your normal activities at home. However, there is a list of activities we want you to do and others we want you to avoid before 8am on Day 4. Please see section 7 for the list.

During most of Day 3 you will simply be asked to stay seated at a desk. I (Ross Chesham) will also sit in the same office and work during this period. This will allow me to transport you to the lab at appropriate times and ensure your comfort and adherence to the research protocol. You also have the option to use your own office desk instead of ours. However, using the dedicated office workplace located next to the lab is simpler for us. It also wastes less time for you travelling between your office and the lab.

If you choose to work in a workplace with others, we can put up a partition screen for you whilst taking the blood pressure and blood samples.

On Day 4, you only need to be in the lab for 30 minutes at 8am.

At 8am we will take a final blood sample and take your blood pressure. You will then be free to go to work/home.

These blood samples, along with the monitor on your arm will allow us to study the effects of exercise on your body's sugar response. Without these measurements we will not be able to measure your body's response to exercise and a meal.

All equipment and measurements will be applied/taken by fully qualified and experienced researchers.

7. What else do I have to do/avoid/know?

Please see the attached 'Example week' sheet for things to do/avoid. It is very important that you follow the eating, drinking and exercise instructions on the 'Example week' sheet. If not, then this will make our results much less accurate.

If you choose to take part in this study, you will be involved for 4-6 weeks. The entire research project will last for 1 year (August 2017 – August 2018).

It is possible that some people will have blood sugar or blood pressure levels that are outside of normal ranges. If this happens, these will be highlighted and then you will be advised to contact your doctor.

8. What will happen to my blood samples?

Blood samples collected for the purposes of the research will be stored securely in the University of Stirling labs in the Faculty of Health Sciences and Sport. A unique study identification number will be used to label samples, rather than your name or contact details. They will be frozen at -70°C so that they can be analysed in batches. Stored samples will include separate parts of your blood (e.g. plasma, serum, and DNA) which will be:

- ❖ Used by the named researchers for the purpose of this study to measure health markers such as glucose, insulin and lipids. They will not be labelled with your name or contact details, but only with a unique study number.
- ❖ Defrosted and analysed in the University of Stirling labs.

Samples will be stored until all participants have finished, all analysis is complete and when research has been published in a peer-reviewed scientific journal. They will be disposed of in the appropriate clinical waste. We will NOT be analysing your DNA.

However, we may use your blood samples in the future to analyse other markers in your blood. We will only analyse samples if you have given us consent to do so and where ethical approval has been given. We will ask if you consent for this. Otherwise, the samples will be discarded in clinical waste.

13. Will my taking part in this study be kept confidential?

All information about you that is collected during the course of the research study shall be kept strictly confidential

- ❖ During the familiarisation session, your consent to take part will be recorded on a form that will collect details such as name, email address and phone number. These will be stored in a secure location within the University of Stirling.
- ❖ Any data and samples collected will be recorded using a unique study identification number, rather than your name. Electronic study data will be stored in restricted access, password protected study computers.
- ❖ Only the named researchers will have access to your data. Your GP will NOT be informed that you are taking part in this study. If a researcher has any concerns regarding observations made during the study you will be advised to contact your GP.
- ❖ We will not give your information, samples or test results to insurance companies or employers.

14. What will happen to the results of the research study?

Results of the study will be used in order to inform future workplace exercise practice. They will also hopefully lead to publication in a scientific journal. If you wish, you will receive a copy of your own specific results on completion of the study. You will not be identified in any data collected or report that is written.

15. Who has funded and reviewed/approved the study?

The study was funded by the University of Stirling. The University of Stirling NHS, Invasive or Clinical Research (NICR) Ethics Committee reviewed/approved the study. This committee is there to protect your safety, rights, wellbeing and dignity.

16. Contact for Further Information

Mr Ross Chesham (PhD Student). Telephone: 01786466296 or 07794774183. r.a.chesham@stir.ac.uk

Dr Naomi Brooks (Senior Lecturer). Telephone: 01786 466478. n.e.brooks@stir.ac.uk

Should you have concerns regarding the study or wish to make a formal complaint, you should contact Professor Jayne Donaldson, Dean of the Faculty of Health Sciences & Sport. 01786 466345. jayne.donaldson@stir.ac.uk

17. What should I do if I want to take part?

Contact either Mr Ross Chesham or Dr Naomi Brooks. We will then agree a date for you to come for a familiarisation session.

9. What are the possible disadvantages and risks of taking part?

There are only minimal risks involved in this research.

Finger prick blood glucose (sugar) screening test: A small risk of soreness and infection is possible. This will be minimised by using the correct technique, sterile equipment and a trained individual.

Blood pressure cuff: These may cause brief discomfort, but you will be warned before starting.

Exercise: There are no complications expected during this study apart from those normally seen following any light to moderate exercise. This could include light headedness, mild fatigue, thirst or muscle aches. Risk of serious complication such as heart attack, palpitations or nausea will be low. This will be low due to appropriate pre-research health questions, blood glucose checks and blood pressure checks. Close attention of the researcher during exercise will further reduce risk. I (Ross Chesham) am a qualified Physiotherapist and Gym Instructor. As a result, I have experience in spotting whether a person should be stopped during exercise. I am also a First Aider. In the unlikely event of a severe adverse reaction, we have systems in place to alert the emergency services.

Sedentary monitor: This is worn on your leg. The adherent pad may cause skin irritation in some sensitive individuals. You will be informed of this possibility. If irritation occurs you will be asked to remove the item. Low allergy tape will be used to reduce this risk.

Continuous glucose monitor and sensor: The sensor is applied to the skin on your arm. Small bruising and/or some small bleeding may be caused upon application of the sensor. However, this will be carried out by trained individuals. To prevent transmission of infection, each sensor will only be used once by you.

Blood samples: Blood draws will be performed by experienced people who are trained to draw blood. So, complications are likely to be minimal. You may feel a discomfort or a sharp scratch when the needle is inserted. This pain should be small and minimised by the trained individual. The needle will only be inserted for a matter of seconds to allow a blood sample to be obtained. Bruising and soreness is possible around the site. This will be minimised by applying pressure to the site upon needle removal.

10. What are the possible benefits of taking part?

If you wish, you will find out about your blood pressure, fasted glucose status, body mass index score and waist to hip ratio. You will also be helping advance science. Although you will not be reimbursed for your time, research like this helps to improve the strength of advice made by healthcare and fitness experts about how we do our daily physical activity.

11. What happens when the research study stops?

When you finish your final lab visit, your role in the study is complete. If you wish, you will be given your results on an individual basis, when they are ready.

12. What if I have complaints or concerns?

If you would like to complain, or have any concerns about how you have been approached or treated during any part of this study, you should do so by contacting the University of Stirling, Faculty of Health Sciences and Sport or the Chief Investigator (details provided in section 16.)

Thank you for taking the time to read this information sheet.

Appendix 2: IPAQ-SF

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

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

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

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

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

____ hours per day
____ minutes per day

☐ Don't know/Not sure

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

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

____ days per week

☐ No walking → **Skip to question 7**

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

____ hours per day
____ minutes per day

☐ Don't know/Not sure

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

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

____ hours per day
____ minutes per day

☐ Don't know/Not sure

Name _____ Date _____ Signature _____

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

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

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

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

____ days per week

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

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

____ hours per day
____ minutes per day

☐ Don't know/Not sure

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

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

____ days per week

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

Appendix 3: Participant Information Sheet



UNIVERSITY of STIRLING
Physiology, Exercise & Nutrition Research Group
Faculty of Health Sciences and Sport
University of Stirling
Stirling FK9 4LA
Tel: 01786 466478

Participant Information Sheet (Full version)

Investigators: Mr Ross Chesham, Dr Naomi Brooks, Dr Gemma Ryde & Dr Stuart Galloway

1. Study title

'Breaking up prolonged sitting: how much light physical activity is required to improve health?'

2. Why am I being asked to take part in this research?

You have been asked to take part in this study as you sit for long time periods on a regular basis. We are looking for 18 eligible adults to complete the study (see section 5). You do not have to take part if you do not want to. Please read this information which will help you to decide. Please discuss with others or ask one of our group if anything is unclear (see contact details in section 15).

3. What is the purpose of the research?

People who sit for long periods of time (prolonged sitting), on a daily basis, have a greater chance of developing diseases. These can include cardiovascular disease (CVD) and type 2 diabetes.

CVD is a term used to describe impaired functioning of the heart and blood vessels. Broadly, CVD includes heart disease, stroke and other diseases involved with blood travelling around the body: such as the growth of fatty plaques in the blood vessels.

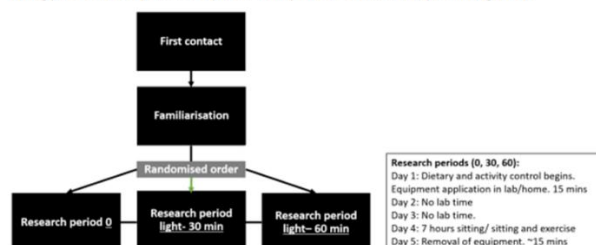
In type 2 diabetes, sugar builds up in your blood to unsafe levels. Over the long-term this can increase the chance of developing health problems. Problems can include damage to the blood vessels of the eye, foot ulcers, kidney disease, and CVD.

People who break up prolonged sitting have a lower chance of developing these health problems. Breaking up prolonged sitting can be done at work, a place where large amounts of people in the UK sit for prolonged periods. Yet, the best type of exercise to break up prolonged sitting is unknown. It is also unknown for how long, how often, or how hard people should exercise.

In our research, we want to break up a day of prolonged sitting. We want to compare 2 different durations of light intensity walking. These 2 walks will be compared to a day where no walking is done. This will allow us to see which of these walk durations can improve the body's control of sugar levels best. This sugar, known as glucose, builds up in the blood after a meal. How long and how much glucose appears in the body after a meal tells us how well the body can cope with the sugar in that meal.

6. What will I have to do if I take part in the research?

Taking part in the study involves 2 pre-research periods and 3 research periods (figure 1):



Experimental design overview

1) Pre-research period 1: First contact

You are at this stage. This involves having the study explained to you in detail. You can ask any questions at this time, but also at any other time during the study. We will ask a few quick questions and begin to see if you are able to do the study (see section 5). This should take around 30-60 minutes. You will then be granted as much time as you need to decide if you want to take part.

2) Pre-research period 2: Familiarisation

First you must verbally confirm you fully understand what is needed of you. Then we will ask for you to sign a consent form. This shows you agree to do the study: aka written informed consent.

At this visit we will ask you to arrive in the morning after a 12 hour fast. This means no food or drinks, other than water, after 8pm the night before. You will fill in a pre-participation health and activity questionnaire. We will assess your blood sugar level using a small finger prick (figure 2.A). We will also assess your blood pressure (figure 2.B). We will provide you with a letter to your doctor if either of these 2 measures are abnormal. You will then walk on a treadmill at 1.6mph to get you used to the study walking speed (figure 2.C). We will also test your walking function using a validated walking test. Your height, weight, waist and hip girth as well as handgrip strength will also be taken. You will need to wear loose fitting clothing and comfortable shoes (shorts are preferable but not essential). We will also ask you about your exercise history and ask you to fill in a food diary. Lastly, you will undergo a DEXA scan. This is a scan that tells us the density of your bones, how much muscle you have and how much fat you have. A low bone density increases the risk for osteoporosis (a medical condition in which bones become weak and fragile). Should this be the case, we will provide you with a letter for your doctor. We will also ask you to complete a short wellbeing questionnaire. Familiarisation should take 1-2 hours.

The duration of the walks in this study may cause unique sugar responses in the body after the meal. We hope to find out if one walking duration has a better response than the other does. In the future, this will help tell us what people should do in the workplace to offset the harmful effects of prolonged sitting on their health.

4. Do I have to take part?

No. It is entirely up to you to decide. If you do not want to take part that's OK. If you do decide to take part, you will be asked to sign a consent form. If you do decide to take part you are free to withdraw at any time, without giving a reason. Just contact Ross Chesham to let him know you've withdrawn (r.a.chesham@stir.ac.uk, 01786 466296 or 07794774183).

5. Am I eligible to take part?

Adults that sit for a number of hours each day can take part in this study. However, they must also fulfil these conditions.

Volunteers must:

- ❖ Be 18-40 or over 60 years of age.
- ❖ Sit for a total of 5 hours or more per day (we will assess this with you).
- ❖ Speak and understand written English.
- ❖ Have a body mass index of less than 40 (we will assess this with you).

Volunteers must not:

- ❖ Have diagnosed disorders such as diabetes or type 2 diabetes that would affect the main study outcome (glucose / sugar levels).
- ❖ Have diagnosed hypertension (high blood pressure).
- ❖ Do more than 150 minutes of moderate-to-vigorous exercise per week (we will assess this with you).
- ❖ Lift weights 2 or more times per week.
- ❖ Have significant memory impairment that will effect study outcomes
- ❖ Have risk-factors, illnesses or injuries stopping safe light walking exercise.
- ❖ Smoke.
- ❖ Be pregnant. For DEXA scans, females must take a pregnancy test for safety (DEXA scans are explained in section 6)

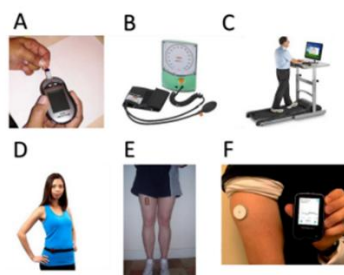


Figure 2. Equipment you will use or wear

3) Research periods 1, 2 & 3

Each research period lasts 5 days each. There are 3 research periods. You are required to complete all 3 research periods (15 days total). *****Please note: although the research periods last 5 days each, you are NOT required to be in the lab all this time. ONLY for 15 minutes on Day 1, 7 hours on Day 4 and 5 minutes on Day 5.*****

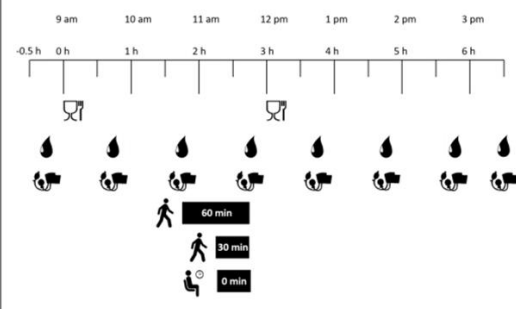
Day 1: We require to see you for 15 minutes. This can be in your home or at the lab. We will administer you with monitors (Figure 2.D, E & F). This equipment includes 2 activity monitors and a glucose monitor. We will show you how to use and apply them. Avoid exercise and alcohol. You can eat as normal on this day.

Day 2: You are not required to be in the lab. Avoid exercise and alcohol. You will continue wearing the monitors. You can eat as normal on this day.

Day 3: You are not required to be in the lab. Avoid exercise and alcohol. You will eat your breakfast, lunch and any snacks as normal. This should be recorded in the food and activity diary provided. However, you must only eat the meal we provide you with between 7:30 and 8:30pm. You will then avoid eating or drinking (except water) after 8:30pm until 8:30am the next day. Please also avoid caffeine (coffee, tea etc) from 8:30pm. You will continue wearing the monitors. We ask you to ensure you get a full night sleep.

Day 4 MAIN LAB DAY: You must NOT eat breakfast. Avoid exercise and alcohol. Avoid caffeine. You will attend the University of Stirling Cottrell lab. Please arrive by car if possible to minimise walking. If you feel unable to drive due to fasting, please ask a friend/family member to drive you or use public transport. If this is not possible, then we can delay the beginning of the trial. From 8:30am you will undergo the main lab trial outlined in the box below:

The lab component of the trial starts at 8:30am and ends at 3:30pm. You will arrive at 8:30am or earlier. You will then rest for 15 minutes. We will then take your resting blood pressure and a small blood sample from the tip of your finger. We will then feed you breakfast. Before lunch we will ask you to do 1 of 3 exercise conditions. 1) walk for 60 minutes 2) walk for 30 minutes 3) not walk at all. You can watch TV/read/work during the walks as the walking treadmill setup permits laptop/TV/book use. We will then provide you with lunch. When not doing the exercise conditions, you will sit in our dedicated research space. At this time, you can watch DVDs, use a laptop/PC, do normal work, or any activity that involves just sitting. You will be transported via wheelchair to the toilet when necessary to minimise excessive walking. During this 7-hour period we will take a total of 8 blood samples from the tip of your finger. A total of 8 blood pressures will also be taken. Your sleep, mood and cognition will also be assessed using 3 short tests to assess the effect of the exercise on them.



Day 4 continued: When you finish at 3:30pm, you will be given a snack to eat at the lab. Continue to avoid caffeine. Do not eat again until between 7:30 and 8:30pm. We will provide you with this evening meal. Record that you have eaten it. You will avoid eating or drinking (except water) after 8:30pm until 8:30am the next day. Continue to avoid caffeine. You will continue wearing the monitors. We will ask you to ensure you get a full night sleep.

The food provided on Day 3 evening and Day 4 is made of 17% protein, 36% fat and 47% carbohydrate. The calories (energy) of each meal will be evenly spread across the day and based on your height, weight, gender and activity level. All food allergies are taken into account. Meals can be tailored to your preferences within the boundaries of the study protocol. The same food will be eaten during each trial to minimise the effect on results. These will be provided in the nutrition lab.

DEXA: Firstly, your body composition will be assessed using a dual energy X-ray absorptiometry (DEXA) scan which will expose you to a small amount of radiation. The total amount of radiation you will be exposed to is 1 µGy which equates to a little less than 1/4 of the daily background radiation that you are exposed to in the UK.

Finger prick blood sampling: A small risk of soreness and infection is possible. This will be minimised by using the correct technique, sterile equipment and a trained individual.

Blood pressure cuff: These may cause brief discomfort (tightness), but you will be warned before starting.

Exercise: There are no complications expected during this study apart from those normally seen following any light exercise. This could include light headedness, mild fatigue, thirst or muscle aches. Risk of serious complication such as heart attack, palpitations or nausea will be low. This will be low due to appropriate pre-research health questions, blood glucose checks and blood pressure checks. Close attention of the researcher during exercise will further reduce risk. I (Ross Chesham) am a qualified Physiotherapist and Gym Instructor. As a result, I have experience in spotting whether a person should be stopped during exercise. In the unlikely event of a severe adverse reaction, we have systems in place to alert the emergency services.

Sedentary monitors: These are worn on your leg and hip. The adherent pad may cause skin irritation in some sensitive individuals. You will be informed of this possibility. If irritation occurs you will be asked to remove the item. Low allergy tape will be used to reduce this risk.

Continuous glucose monitor and sensor: The sensor is applied to the skin on your arm. Small bruising and/or some small bleeding may be caused upon application of the sensor. However, this will be carried out by trained individuals. To prevent transmission of infection, each sensor will only be used once by you.

10. What are the possible benefits of taking part?

You will receive detailed information on your bone density, muscle mass and fat mass through a non-diagnostic DEXA scan: This can indicate your bone mineral density (strength). This can cost around £150 privately. You will also learn your blood pressure, blood glucose status, body mass index and waist to hip ratio: important health indicators. You will also be helping advance science. You will be reimbursed £100 for your time.

11. What happens when the research study stops?

When you finish your final lab visit, your role in the study is complete. If you wish, you will be given your results on an individual basis, when they are ready.

12. What if I have complaints or concerns?

If you would like to complain, or have any concerns about how you have been approached or treated during any part of this study, you should do so by contacting the University of Stirling, Faculty of Health Sciences and Sport or the Chief Investigator (details provided in section 16.)

Day 5: We require to see you for just 15 minutes. We can remove the monitors at this time: please do not do this yourself. We will assess your sleep quality with a questionnaire. This 15 minutes can be at any time after 8:30am. HOWEVER, please do not eat before 8:30am. Please do not drink caffeine or alcohol before 8:30am. Please do not do any exercise before 8:30am. You can still drink water before 8:30am. We can discuss whether we meet at home or at the lab for this 15 minutes.

General points: The order of exercise (60 minutes 30 minutes or 0 minutes) will be done in a random order. i.e. 60 minute walk could be done on research period 1, 2 or 3. The research periods are usually spread 1 week apart, but this gap can be longer if needed.

The finger blood samples, along with the monitor on your arm will allow us to study the effects of exercise on your body's sugar response. Without the food and activity restrictions as well as the blood measurements, we will not be able to measure your body's response to exercise and a meal reliably.

If necessary, a researcher will drive you from the local bus stops/train station in the morning and/or at the end of the experimental day.

All equipment and measurements will be applied/taken by fully qualified and experienced researchers.

7. What else do I have to do/avoid/know?

Please see section 6 and the attached 'Research period example and instructions' sheet for things to do/avoid. It is very important that you follow the instructions. If not, then this will make our results much less accurate.

It is possible that some people will have blood sugar, blood pressure and bone density levels that are outside of normal ranges. If this happens, these will be highlighted to you, and your GP will be provided with a letter.

If you choose to take part in this study, you will be involved for 4-6 weeks.

8. What will happen to my blood samples?

Blood samples collected for the purposes of the research will be analysed immediately and disposed of in clinical waste as per standard protocol. We will NOT be analysing your DNA. Results will be stored securely in the University of Stirling labs in the Faculty of Health Sciences and Sport. A unique study identification number will be used to label results, rather than your name or contact details. The samples will be:

- Used by the named researchers for the purpose of this study to measure health markers such as glucose and lipids. Results will not be labelled with your name or contact details, but only with a unique study number.

Results will be stored for 10 years after all participants have finished, all analysis is complete and when research has been published in a peer-reviewed scientific journal. This is standard procedure.

9. What are the possible disadvantages and risks of taking part?

There are only minimal risks involved in this research.

13. Will my taking part in this study be kept confidential?

All information about you that is collected during the course of the research study shall be kept strictly confidential

- During the familiarisation session, your consent to take part will be recorded on a form that will collect details such as name, email address and phone number. These will be stored in a secure location within the University of Stirling.
- Any data collected will be recorded using a unique study identification number, rather than your name. Electronic study data will be stored in restricted access, password protected study computers.
- Only the named researchers will have access to your data. Your GP will NOT be informed that you are taking part in this study. However, if a researcher has any concerns regarding observations made during the study, we will provide you with a letter to show your GP.
- We will not give your information, samples or test results to insurance companies or employers.
- If you wish to withdraw from the study, we will dispose of all of your data in a secure fashion and recruit another participant to maintain our required study numbers.

14. What will happen to the results of the research study?

Results of the study will be used in order to inform future workplace exercise practice. They will also likely lead to publication in a scientific journal. If you wish, you will receive a copy of your own specific results on completion of the study. You will not be identified in any data collected or report that is written.

15. Who has funded and reviewed/approved the study?

The East of Scotland Research Ethics Service REC 2, which has responsibility for scrutinising all proposals for medical research on humans, has examined the proposal and has raised no objections from the point of view of research ethics. It is a requirement that our records (not including participant personal data) in this research be made available for scrutiny by monitors from the University of Stirling Research Office and NHS Forth Valley, whose role is to check that research is properly conducted and the interests of those taking part are adequately protected.

The study was funded by the University of Stirling.

16. Contact for Further Information

Dr Naomi Brooks (Senior lecturer). Telephone: 01786 466478. n.e.brooks@stir.ac.uk
Dr Ross Chesham (PhD Candidate). Telephone: 07794774183. r.a.chesham@stir.ac.uk
Should you have concerns regarding the study or wish to make a formal complaint, you should contact Professor Jayne Donaldson, Dean of the Faculty of Health Sciences & Sport. 01786 466345. jayne.donaldson@stir.ac.uk

17. What should I do if I want to take part?

Contact either Mr Ross Chesham or Dr Naomi Brooks. We will then agree a date for you to come for a familiarisation session.

Thank you for taking the time to read this information sheet.

Appendix 4: SWEMWBS



The Short Warwick-Edinburgh Mental Well-being Scale (SWEMWBS)

Below are some statements about feelings and thoughts.

Please tick the box that best describes your experience of each over the last 2 weeks

STATEMENTS	None of the time	Rarely	Some of the time	Often	All of the time
I've been feeling optimistic about the future	1	2	3	4	5
I've been feeling useful	1	2	3	4	5
I've been feeling relaxed	1	2	3	4	5
I've been dealing with problems well	1	2	3	4	5
I've been thinking clearly	1	2	3	4	5
I've been feeling close to other people	1	2	3	4	5
I've been able to make up my own mind about things	1	2	3	4	5

Sleep Diary

Guess the approximate times. Do not worry if your figures are not exactly correct. We are interested in your opinion of how you slept.

	Day 1 Date ____ / ____ / ____							
Did you take any naps yesterday? If so, how long were they?								
What time did you go to bed last night?								
How long did it take you to fall asleep?								
How many times did you wake up during the night?								
How many minutes were you awake during the night?								
What time did you wake up this morning?								
What time did you get out of bed this morning?								
How many hours of sleep did you get last night?								
How well did you sleep? (1-10=best)								
Rate your sleepiness (below):								

- 1 = Extremely alert
- 2 = Very alert
- 3 = Alert
- 4 = Rather alert
- 5 = Neither alert nor sleepy

- 6 = Some signs of sleepiness
- 7 = Sleepy, but no effort to keep awake
- 8 = Sleepy, some effort to keep awake
- 9 = Very sleepy, great effort to keep awake
- 10 = Extremely sleepy, falling asleep

Appendix 6: I-PANAS-SF

PANAS Questionnaire

Name

ID

Date

Time

This scale consists of a number of words that describe different feelings and emotions. Read each item and then list the number from the scale below next to each word. Indicate to what extent you feel this way right now, that is, at the present moment.

1	2	3	4	5
Very slightly or not at all	A little	Moderately	Quite a bit	Extremely

	1. Active
	2. Determined
	3. Attentive
	4. Inspired
	5. Alert

	6. Afraid
	7. Nervous
	8. Upset
	9. Hostile
	10. Ashamed

RESEARCH ARTICLE

Open Access



The Daily Mile makes primary school children more active, less sedentary and improves their fitness and body composition: a quasi-experimental pilot study

Ross A. Chesham¹, Josephine N. Booth², Emma L. Sweeney¹, Gemma C. Ryde¹, Trish Gorely^{1,3}, Naomi E. Brooks¹ and Colin N. Moran^{1*}

Abstract

Background: The Daily Mile is a physical activity programme made popular by a school in Stirling, Scotland. It is promoted by the Scottish Government and is growing in popularity nationally and internationally. The aim is that each day, during class time, pupils run or walk outside for 15 min (~1 mile) at a self-selected pace. It is anecdotally reported to have a number of physiological benefits including increased physical activity, reduced sedentary behaviour, increased fitness and improved body composition. This study aimed to investigate these reports.

Methods: We conducted a quasi-experimental repeated measures pilot study in two primary schools in the Stirling Council area: one school with, and one without, intention to introduce the Daily Mile. Pupils at the control school followed their usual curriculum. Of the 504 children attending the schools, 391 children in primary classes 1–7 (age 4–12 years) at the baseline assessment took part. The follow-up assessment was in the same academic year. Outcomes were accelerometer-assessed average daily moderate to vigorous intensity physical activity (MVPA) and average daily sedentary behaviour, 20-m shuttle run fitness test performance and adiposity assessed by the sum of skinfolds at four sites. Valid data at both time points were collected for 118, 118, 357 and 327 children, respectively, for each outcome.

Results: After correction for age and gender, significant improvements were observed in the intervention school relative to the control school for MVPA, sedentary time, fitness and body composition. For MVPA, a relative increase of 9.1 min per day (95% confidence interval or 95%CI 5.1–13.2 min, standardised mean difference SMD = 0.407, $p = 0.027$) was observed. For sedentary time, there was a relative decrease of 18.2 min per day (10.7–25.7 min, SMD = 0.437, $p = 0.017$). For the shuttle run, there was a relative increase of 39.1 m (21.9–56.3, SMD = 0.236, $p = 0.037$). For the skinfolds, there was a relative decrease of 1.4 mm (0.8–2.0 mm, SMD = 0.246, $p = 0.036$). Similar results were obtained when a correction for socioeconomic groupings was included.

Conclusions: The findings show that in primary school children, the Daily Mile intervention is effective at increasing levels of MVPA, reducing sedentary time, increasing physical fitness and improving body composition. These findings have relevance for teachers, policymakers, public health practitioners, and health researchers.

Keywords: Daily Mile, Children, Physical activity, Primary school, Fitness, Body composition

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Background

The Daily Mile is a physical activity intervention developed at St Ninian's Primary School (Stirling, Scotland) in 2012 [1]. The initial aim was to improve the fitness of children; although, there have since been many additional benefits anecdotally reported by children, teachers and parents [1]. These include improved physical activity, sedentary time, physical fitness, body composition, sleep, diet, concentration, well-being and obesity levels. However, no objectively measured scientific evidence has yet been gathered on the validity of these reports.

The successful implementation of the Daily Mile at many schools, its continued maintenance and its increasing popularity seem to be a result of the simplicity of the activity, the autonomy given to classroom teachers over when they do it during the school day and the pupil-determined pace. Since its development, the Daily Mile has been rolled out across the country by the Scottish government [2]. It is estimated by Education Scotland that, from local authorities who responded to their query, ~ 50% of primary schools in Scotland are already doing the Daily Mile with a further 18% planning to do the Daily Mile soon (personal communication). It has also become popular in the rest of the UK with interest from the UK government [3, 4]. Additionally, it has been introduced in the Netherlands, Belgium and parts of the USA with interest from many other countries [5], despite the lack of rigorous evidence on the efficacy of taking part.

Globally, physical activity levels are low [6]. Furthermore, low physical activity levels in childhood are predictive of low physical activity levels in adulthood [7]. The World Health Organisation (WHO) considers policies and interventions to increase physical activity levels to be important in all age groups and that any potential harm of increasing physical activity is outweighed by the associated benefits [8, 9]. In a recent accelerometer study on children (2–11 years old) from eight European countries, the proportion of children achieving 60+ minutes per day (recommended for 5–18 year olds [10]) of moderate to vigorous physical activity (MVPA) ranged from 9.5% to 34.1% in boys and from 2.0% to 14.7% in girls [11]. MVPA levels generally decline with age in both genders as sedentary time increases [12]. MVPA levels are also believed to be influenced by socioeconomic status; however, systematic reviews are unclear on the consistency of this relationship and more evidence needs to be gathered [13]. School-based physical activity interventions like the Daily Mile are appealing because they include whole classes, therefore they reach many children regardless of socioeconomic status, physical activity level or fitness level. They also break up sedentary time as they occur during lessons and have the potential to reach much larger proportions of the population than opt-in groups like sports clubs. Therefore, understanding the impact of the Daily Mile on

physical activity and sedentary behaviour levels is of key importance.

Overweight and obesity rates are of pandemic proportions and considered to be a key target for the WHO [8]. In Scotland, 30% of children (29% of boys and 32% of girls) aged 7–11 years were overweight or obese in 2015 [14], a figure similar to that in England [15]. At the same time, there is evidence of a decline in the performance of children in the 20-m shuttle run (an indicator of physical endurance fitness) [16]. Low fitness and low levels of physical activity in adults and children are associated with a number of risk factors for non-communicable diseases and adverse health outcomes including obesity, cardiovascular disease, diabetes, some cancers, low mood and poor cognitive function [17–21]. Additionally, both overweight and low fitness are also known to be related to lower socioeconomic status [22, 23]. Studies into the impact of the Daily Mile on fitness and body composition are important for understanding its potential to improve public health, including health inequalities, and for developing future public health policies.

The Daily Mile at least has the potential to impact on key areas of global public health. Whilst published evidence shows a positive health relationship between physical activity, particularly MVPA, and these outcomes [24], it is unknown whether 15 min of exercise, particularly with no expectation of intensity, will provide such benefits. Given the associated loss of academic classroom time (up to 75 min per week), it is paramount that evidence on the impact of the Daily Mile on each anecdotally reported benefit is gathered to ensure that government policies are appropriate. Furthermore, any associated benefits in physiological health are likely to be small [25]; thus, it is essential to use gold standard measurement techniques. Therefore, the aims of this study are to assess the anecdotally reported physiological benefits of participation in the Daily Mile. Specifically, using a repeated measures design and gold standard measurement techniques, we will assess whether the introduction of the Daily Mile into a primary school setting leads to increased MVPA, reduced sedentary time, improved fitness and improved body composition.

Methods

Study design and ethics

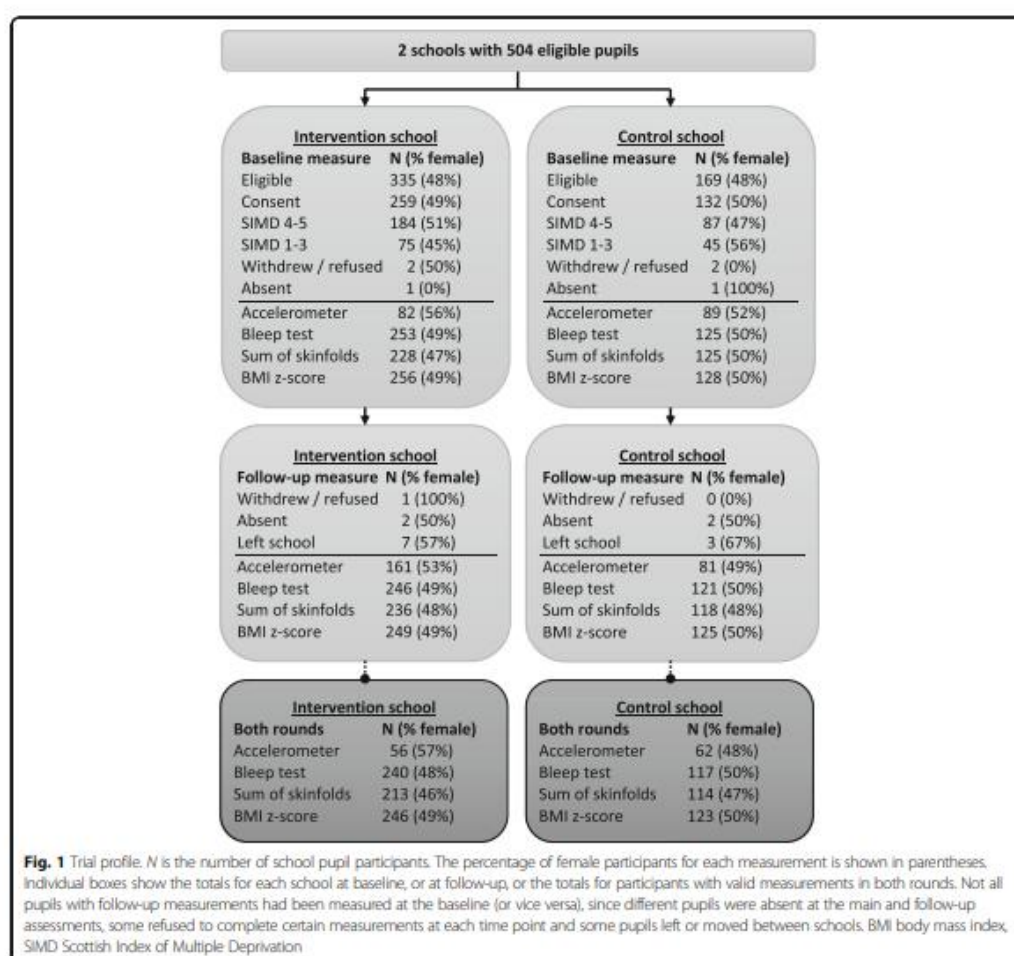
"Using the Daily Mile to turn the WHEEL" (Well-being, Health, Exercise, Enjoyment and Learning) is a school-based quasi-experimental study designed to assess the anecdotally reported benefits of taking part in the Daily Mile. Ethical approval was obtained from the University of Stirling, School of Sport Research Ethics Committee (reference number 760). Approval was also obtained from the Director of Children, Young People and Education at Stirling Council.

Eligibility and recruitment

State primary schools in the Stirling Council areas were eligible for inclusion. Recruiting from only one council area reduced any potential variance in the delivery of education that may have impacted on outcome measurements. Two local primary schools were identified and approached: one that was not doing the Daily Mile but was intending to introduce it (the intervention school) and one that was not doing the Daily Mile and did not intend to introduce it (the control school). Both schools had expressed a desire to introduce the Daily Mile although the control school felt that it would not be possible due to the layout of its playground. Both schools had a range of levels of deprivation, although the majority of pupils were from higher socioeconomic quintiles (Fig. 1). Participants

were children in all years (age 4–12 years) at the time of recruitment.

Once the schools had agreed to participate in the study, parents and guardians of the children were sent a letter and information sheet about the study with an opt-in consent form. For children in primary classes 4–7 (age 7–12 years), an additional child consent form was included. Information sessions were held in both schools to allow parents to ask questions and see the study equipment. They were also given the opportunity to contact the research team by email or phone to discuss the study. Information about being able to withdraw from the study at any stage was given in the information sheet, consent form and verbally. All of the children were also asked to confirm verbally that they were happy to take part on each day of testing.



Intervention

The Daily Mile is a school-based physical activity intervention made popular by a primary school in the Stirling Council area, Scotland [1]. It involves children going outside, at a time of the classroom teacher's choosing, for ~15 min of exercise at a pace self-selected by each individual child. This is done during normal classroom time and is in addition to time spent in physical education or scheduled breaks. Typically, it involves laps of a football pitch or playground area. Children often talk as they go and perform a mixture of walking and running. Those who run the whole time will complete ~1 mile in 15 min. Children wear their normal school clothes; most wear their normal school shoes and jackets are only worn in cold or wet weather. It is completed on most days regardless of weather conditions. A leaflet produced by the originator school was given to the school implementing the Daily Mile. No additional instructions for initiation of the Daily Mile were given by the research team.

Participant involvement

The outcome measures selected for the study were chosen based on anecdotal reports of the influence of taking part in the Daily Mile. This information had been gathered from the children, their parents and their teachers by the originator school. The research question, study design and specific outcome measures were developed in part during meetings with the head teacher at the originator school, who was familiar with this information. After publication, the results from this study will be disseminated to the schools involved as copies of the manuscript and infographics, and during question and answer sessions in the schools.

Outcome measures

The primary physiology-related outcome measurements for the WHEEL project were accelerometer-assessed MVPA and sedentary time, fitness assessed using a 20-m shuttle run, and body composition assessed using skinfolds. Additional, cognitive- and well-being-related outcome measurements will be reported elsewhere.

Participant assessments

Baseline assessments (before the intervention) were carried out in October 2015 for the intervention school and March 2016 for the control school. Outcome assessments were completed in May 2016 for the intervention school and June 2016 for the control school. Identical protocols and procedures were used at both assessments. They were undertaken by trained fieldworkers. The fieldworkers worked in groups such that at least one member of each group was disclosure-checked under the Protecting Vulnerable Groups scheme [26]. Children completed tasks in a random order and were given stickers for completing

each measurement type. Testing sessions lasted between 1 and 2 h and were carried out over 2 weeks, depending on class size and school timetable.

ActiGraph accelerometers were used to assess physical activity and sedentary time. Five models of accelerometer were used: ActiGraph wGT3X-BT, wGT3X+, GT3X+, GT3X and GT1M. The GT1M and GT3X monitors are comparable when classifying total time spent in specific intensity categories [27]. There is also strong agreement between the GT1M, GT3X and GT3X+ accelerometers, making it acceptable to use them in the same study [28]. The primary difference between the wGT3X-BT and GT3X+ accelerometers is the ability of the wGT3X-BT to communicate wirelessly and all measurements should function identically (personal communication from ActiGraph). Accelerometers were worn around the waist on the left side with the same orientation to standardise the position. Children were asked to wear the belt for eight consecutive days during waking hours (except when bathing or swimming). A poster and instruction sheet with visual and written prompts were provided to each child to remind them of how and when to wear the accelerometer. Upon collection by the research team, the data were downloaded with the ActiLife 6 software (ActiGraph LLC, USA). A valid measurement required at least 10 h wear for 3 days [29]. A 60-s epoch was used and non-wear time was defined as strings of consecutive zeros lasting 60 min or more [30]. The accelerometer output is in counts per minute (cpm). Evenson cut points [31] were used to define time spent being sedentary (≤ 100 cpm) and time spent in MVPA (≥ 2296 cpm). The accelerometer data were corrected for wear time in addition to gender and age in days on the day of testing in the main analysis.

The maximal multistage 20-m shuttle run test [32] was assessed using the Multistage Fitness Test CD (Sports Coach UK, UK) using the standard procedure. Cones marked shuttle boundaries and lanes. Between four (younger) and eight (older) children completed the test at the same time depending on their age group. Instructions and a demonstration of the test were provided prior to each group participating and additional verbal instructions were provided during the test as necessary. The test began at $8.5 \text{ km}\cdot\text{h}^{-1}$ and after each minute increased by $0.5 \text{ km}\cdot\text{h}^{-1}$. When a child was unable to reach the 20-m line prior to the bleep twice in a row, they were asked to stop and their level and shuttle score was recorded. Shuttle run tests were performed outside on tarmac. Age-corrected $\dot{V}\text{O}_2$ max scores were created according to the method of [32] for comparison with other studies (Additional file 1: Tables S1–S3). However, this corrects for age in years and therefore, it was not used for the main analysis. Instead, shuttle distance corrected for gender and age in days on day of testing was used to give an improved resolution to the correction in the main analysis.

All skinfold measurements were completed with the child behind a privacy screen with at least two disclosure-checked fieldworkers present. Fieldworkers taking skinfold measurements were trained by an International Society for the Advancement of Kinanthropometry (ISAK) Level 3 instructor prior to involvement in the study and all measurements were taken according to standard ISAK procedures [33]. Triceps, biceps, iliac crest and subscapular skinfolds were measured using Harpenden skinfold calipers (Baty International, UK). All measurements were taken from the right-hand side with the child standing. Where appropriate, they stood on an anthropometric box. Skinfold measurements were summed prior to analysis to give a sum of skinfolds (in millimetres). Skinfold data were corrected for gender and age in days on day of testing in the main analysis.

Height was measured, to the nearest 1 mm, without shoes using the Leicester Height Measure (Seca, UK) according to standard ISAK procedures [33]. Body weight was measured without shoes in light clothing to the nearest 0.1 kg using electronic Sensa 804 scales (Seca, UK). Height (m) and weight (kg) were used to calculate the body mass index ($BMI = \text{weight}/\text{height}^2$). BMI *z* scores relative for age were calculated using UK 1990 reference data in the LMS Growth add-in for Microsoft Excel [34]. Healthy weight was defined as BMI *z* score < 1.04, overweight as BMI *z* score of 1.04–1.63 and obesity as BMI *z* score ≥ 1.64 .

All anthropometric measurements were taken twice and the average taken for the analyses. Where there was a substantial difference between the two measurements, a third measurement was taken and the median value was used for the analyses.

Additional Information

Schools provided the date of birth and postcodes for all consented pupils. The date of birth allowed the analyses to be corrected for age on day of testing. The postcode allowed the assignment of the Scottish Index of Multiple Deprivation (SIMD) [35]. SIMD combines data from seven different domains of deprivation into a single score: income, employment, health, education, access to services, crime and housing. However, it should be noted that this gives a postcode-specific deprivation score that may not reflect that of an individual household.

Statistical analyses

Descriptive statistics, Pearson χ^2 and odds ratios were calculated in Excel 2013. Baseline group frequency comparisons were performed using a Pearson χ^2 test. Baseline group mean comparisons were performed using Student's *t* test (uncorrected) or general linear model ANOVA (corrected for age at time of testing, gender and age*gender). The main analyses were performed in SPSS Statistics (version 21.0.0.1). General linear model regression

analyses with repeated measures were used to investigate the effect of doing the Daily Mile. Analyses of all outcome measures included an adjustment for the common confounders: age at time of testing, gender and age*gender. This controls for the effects of age and gender and any different effects of age in the two genders as well as any differences in length of time in the study. Analyses for MVPA and sedentary time were additionally corrected for accelerometer wear time as a covariate. Analyses were conducted first without and then with a correction for SIMD. Standardised mean differences (SMDs), or effect sizes, are calculated as a change measured in the intervention school relative to the control school as a proportion of the pooled standard deviation of the change. An SMD of 0.2–0.5 is considered to be small, an SMD of 0.5–0.8 is considered to be medium and an SMD of 0.8 or above is considered to be large [36]. No correction for multiple testing was made since all four primary outcome measures were anecdotally reported to be influenced by the Daily Mile.

Results

Figure 1 shows the study profile. Overall 77.6% of eligible pupils consented to the study (77.3% in the intervention school and 78.1% in the control school). A total of 371 pupils (247 and 124 in the intervention and control schools respectively) provided at least one measurement in both rounds of testing. All consented children, irrespective of whether they had all measurements or not, are included in Fig. 1. For each outcome measurement, the information in Tables 1 and 2 is based only on children who had measurements at both time points.

Consent for a complete set of measurements was given in the majority of cases; although, on the day of testing, some pupils did not wish to give verbal consent for skinfold measurements, had worn inappropriate clothing for some skinfold measurements or were unable to complete individual tests due to pre-existing minor injuries or ailments. In total, at both baseline and follow-up, between 84% and 94% of originally consented participants had data on fitness and body composition outcomes and the proportions were similar at both baseline and follow-up, in the intervention and control schools and in males and females (Fig. 1).

Three pupils refused to wear accelerometers. The proportion of pupils with valid accelerometer data was lower than other measurements although the proportion of males and females was similar. Only 32% of participants in the intervention school and 67% in the control school had valid accelerometer data at baseline whilst at follow-up 65% and 64% respectively had valid accelerometer data (Fig. 1). This was partially because of the requirement that wearers had at least 10 hours of valid wear time on at least 3 days [31]. However, due to a desire to start doing the Daily Mile as soon as possible in the intervention school

Table 1 Baseline characteristics of participants by study group and gender

	Total	Males	Females	Intervention vs control school (uncorrected)	Intervention vs control school (corrected)
Age (years)	8.4 ± 2.0 (379)	8.3 ± 2.0 (192)	8.4 ± 1.9 (187)	$t = 3.18$	
Intervention school	8.1 ± 2.0 (252)	8.2 ± 2.0 (129)	8.0 ± 1.9 (123)	$SMD = 0.346$	
Control school	8.8 ± 1.8 (127)	8.6 ± 1.8 (63)	9.0 ± 1.8 (64)	$p = 0.002$	
Daily MVPA (min)	55 ± 22 (118)	60 ± 20 (56)	50 ± 22 (62)	$t = 0.766$	$F = 1.982$
Intervention school	53 ± 22 (56)	53 ± 19 (24)	53 ± 24 (32)	$SMD = 0.141$	$SMD = 0.258$
Control school	56 ± 22 (62)	66 ± 19 (32)	47 ± 20 (30)	$p = 0.445$	$p = 0.162$
Daily sedentary time (min)	345 ± 74 (118)	338 ± 80 (56)	352 ± 67 (62)	$t = 1.142$	$F = 7.543$
Intervention school	337 ± 77 (56)	327 ± 84 (24)	344 ± 71 (32)	$SMD = 0.210$	$SMD = 0.493$
Control school	352 ± 71 (62)	346 ± 78 (32)	360 ± 63 (30)	$p = 0.256$	$p = 0.007$
Total shuttle distance (m)	670 ± 351 (357)	748 ± 399 (183)	589 ± 271 (174)	$t = 1.944$	$F = 0.294$
Intervention school	645 ± 351 (240)	719 ± 397 (124)	566 ± 276 (116)	$SMD = 0.219$	$SMD = 0.061$
Control school	722 ± 347 (117)	807 ± 400 (59)	635 ± 258 (58)	$p = 0.053$	$p = 0.588$
Sum of skinfolds (mm)	35.1 ± 14.7 (327)	32.6 ± 14.9 (175)	38.1 ± 13.9 (152)	$t = 2.214$	$F = 2.117$
Intervention school	33.8 ± 12.7 (213)	31.7 ± 13.5 (115)	36.4 ± 11.2 (98)	$SMD = 0.257$	$SMD = 0.169$
Control school	37.6 ± 17.6 (114)	34.4 ± 17.3 (60)	41.1 ± 17.4 (54)	$p = 0.028$	$p = 0.147$
% meeting physical activity guidelines	36.4% (118)	46.4% (56)	27.4% (62)	$\chi^2 = 4.29, p = 0.038$	
Intervention school	26.8% (56)	25.0% (24)	28.1% (32)	$OR = 2.251 (1.037-4.884),$	
Control school	45.2% (62)	62.5% (32)	26.7% (30)	$p = 0.040$	
% overweight or obese	16.8% (369)	18.6% (188)	14.9% (181)	$\chi^2 = 0.01, p = 0.922$	
Intervention school	16.7% (246)	19.0% (126)	14.2% (120)	$OR = 1.029 (0.578-1.833),$	
Control school	17.1% (123)	17.7% (62)	16.4% (61)	$p = 0.928$	

Values in columns 2–4 are means ± SD (n value) or percentage (n value). Comparisons are by t test or χ^2 and odds ratio. Corrected values are from ANOVA including correction for age, gender and age*gender. Shuttle distance is given to the nearest metre. Accelerometer minutes are given to the nearest minute. It was not possible to correct percentage values for age and gender. MVPA and sedentary time were also corrected for wear time. This table includes only participants with valid measurements both before and after the intervention
MVPA moderate to vigorous intensity physical activity, OR odds ratio, SD standard deviation, SMD standardised mean difference

and a limited number of accelerometers (117), it was possible to collect true baseline data (i.e. prior to beginning the Daily Mile) from only a portion of the intervention school participants. These participants were selected at random based on the availability of pupils for the other physiological tests.

The baseline characteristics of the participants are given in Table 1. Age was significantly higher in the control school at baseline due to the difference in the time of baseline measurements meaning that the pupils were slightly further through the academic year at the control school (Table 1). The percentage meeting the physical activity guidelines of 60+ min MVPA per day and the sum of skinfolds were both higher in the control school. However, after correction for age, gender and age*gender, only sedentary time differed significantly between the schools at baseline, suggesting that the correct confounders were accounted for in the analysis. Additionally, the intra-school differences between genders and year groups were similar (Additional file 1: Table S1).

SIMD scores were similar across schools ($\chi^2 = 1.299$, $p = 0.254$). For quintiles 4–5 (least deprived), the scores

were 71% versus 65%, respectively, for the intervention school and control school. For quintiles 1–3 (most deprived), the scores were 29% versus 35%. These reflect the lower deprivation than the average across Scotland [35], since ~20% would be expected in each quintile. However, this excess of children from less deprived areas reduces the likelihood of observing an impact of the Daily Mile, rather than creating any potential artefacts, as the children are more likely to be fitter and less likely to be overweight or obese [22, 23] at baseline. Nonetheless, children from areas with lower socioeconomic scores (quintiles 1–3) had similar minutes of MVPA and sedentary time compared to those with higher socioeconomic scores (quintiles 4–5). They were also equally likely to meet the physical activity guidelines. However, they had lower shuttle distance, and a higher sum of skinfolds and rates of overweight and obesity (Table 2). A full breakdown of baseline characteristics by socioeconomic group, school and gender is given in Additional file 1: Table S2.

In the main analysis, after adjustment for the common confounders of age, gender and age*gender, significant improvements were observed in the intervention school

Table 2 Baseline characteristics of participants by study group and SIMD

	Total	SIMD 4–5	SIMD 1–3	SIMD 1–3 vs SIMD 4–5
Age (years)	8.4 ± 2.0 (379)	8.4 ± 1.9 (264)	8.4 ± 2.0 (115)	$t = 0.033$
Intervention school	8.1 ± 2.0 (252)	8.1 ± 2.0 (182)	8.2 ± 2.1 (70)	SMD = 0.004
Control school	8.8 ± 1.8 (127)	8.9 ± 1.8 (82)	8.6 ± 1.8 (45)	$p = 0.974$
Daily MVPA (min)	55 ± 22 (118)	56 ± 21 (92)	51 ± 25 (26)	$t = 1.024$
Intervention school	53 ± 22 (56)	52 ± 19 (42)	57 ± 29 (14)	SMD = 0.227
Control school	56 ± 22 (62)	60 ± 22 (50)	44 ± 17 (12)	$p = 0.308$
Daily sedentary time (min)	345 ± 74 (118)	342 ± 70 (92)	356 ± 86 (26)	$t = 0.879$
Intervention school	337 ± 77 (56)	337 ± 70 (42)	337 ± 97 (14)	SMD = 0.195
Control school	352 ± 71 (62)	346 ± 70 (50)	378 ± 70 (12)	$p = 0.381$
Total shuttle distance (m)	670 ± 351 (357)	702 ± 368 (252)	593 ± 294 (105)	$t = 2.695$
Intervention school	645 ± 351 (240)	658 ± 365 (173)	613 ± 314 (67)	SMD = 0.313
Control school	722 ± 347 (117)	800 ± 359 (79)	559 ± 255 (38)	$p = 0.007$
Sum of skinfolds (mm)	35.1 ± 14.7 (327)	32.9 ± 13.0 (229)	40.4 ± 16.9 (98)	$t = 4.350$
Intervention school	33.8 ± 12.7 (213)	32.4 ± 11.9 (154)	37.5 ± 14.0 (59)	SMD = 0.525
Control school	37.6 ± 17.6 (114)	33.8 ± 15.0 (75)	44.8 ± 20.0 (39)	$p < 0.001$
% meeting physical activity guidelines	36.4% (118)	39.1% (92)	26.9% (26)	$\chi^2 = 1.304, p = 0.253$
Intervention school	26.8% (56)	26.2% (42)	28.6% (14)	OR = 0.573 (0.219–1.500), $p = 0.260$
Control school	45.2% (62)	50.0% (50)	25.0% (12)	
% overweight or obese	16.8% (369)	12.4% (259)	27.3% (110)	$\chi^2 = 12.291, p < 0.001$
Intervention school	16.7% (246)	12.4% (178)	27.9% (68)	OR = 2.660 (1.520–4.655), $p < 0.001$
Control school	17.1% (123)	12.3% (81)	26.2% (42)	

Values in columns 2–4 are means ± SD (n value) or percentage (n value). Comparisons are by t test or χ^2 and odds ratio. Shuttle distance is given to the nearest metre. Accelerometer minutes are given to the nearest minute. This table includes only participants with valid measurements both before and after the intervention

MVPA moderate to vigorous intensity physical activity, OR odds ratio, SD standard deviation, SIMD Scottish Index of Multiple Deprivation, SMD standardised mean difference

relative to the control school for MVPA (+9.1 min), sedentary time (−18.2 min), fitness (+39.1 m) and body composition (−1.4 mm; Table 3). These relationships persisted after including a correction for SIMD (Table 3). Baseline values and change in BMI z score and age-corrected $\dot{V}O_2$ max scores are given in Additional file 1: Table S1–S3 only for comparison with other studies.

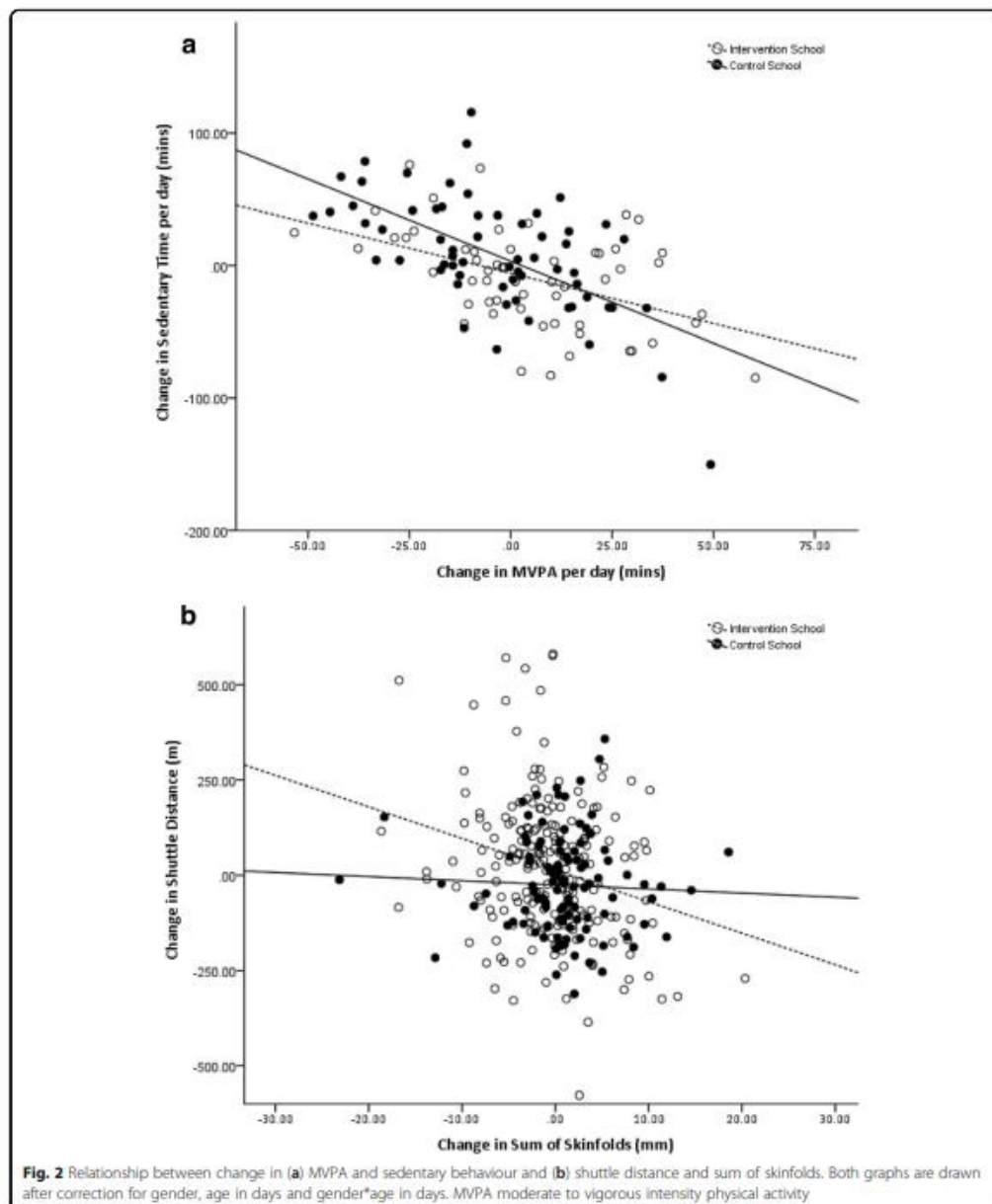
A change in MVPA predicted a change in sedentary behaviour ($r = -0.559, n = 118, p < 0.001$; Fig. 2a) but did not predict changes in other primary outcome variables. A change in shuttle distance predicted a change in sum of skinfolds ($r = -0.203, n = 317, p < 0.001$; Fig. 2b) but did not predict changes in other primary outcome variables. These correlations were not significantly altered by the

Table 3 Effect of introducing the Daily Mile on outcomes assessed immediately after the end of the intervention period

Outcome	Difference in change between schools after correction for age, gender and age*gender			Difference in change between schools after correction for age, gender, age*gender and SIMD		
	Mean (95% CI)	SMD	p value	Mean (95% CI)	SMD	p value
Mean MVPA per day (min)	9.1 (5.1 to 13.2)	0.407	0.027	9.5 (5.4 to 13.5)	0.422	0.021
Mean sedentary time per day (min)	−18.2 (−10.7 to −25.7)	0.437	0.017	−18.1 (−10.6 to −25.6)	0.435	0.018
Total shuttle distance (m)	39.1 (21.9 to 56.3)	0.236	0.037	37.2 (20.1 to 54.3)	0.225	0.046
Sum of skinfolds (mm)	−1.4 (−2.0 to −0.8)	0.246	0.034	−1.4 (−2.0 to −0.8)	0.258	0.026

SMD is calculated as the change in the intervention school relative to the control school as a proportion of the standard deviation of the change. Analyses were conducted using GLM-ANOVA corrected for age, gender, age*gender ± SIMD with repeated measures for the outcome. Sedentary time and MVPA were also corrected for accelerometer wear time

95% CI 95% confidence interval, MVPA moderate to vigorous intensity physical activity, SIMD Scottish Index of Multiple Deprivation, SMD standardised mean difference



inclusion of SIMD ($r = -0.564$ and -0.212 , respectively) although a change in MVPA additionally predicted a change in shuttle distance ($r = 0.187$, $n = 115$, $p = 0.046$). However, the relationship between a change in sum of

skinfolds and a change in shuttle distance differed by school (interaction $p = 0.043$). The relationship was stronger for the intervention school than the control school ($r = -0.245$ and -0.046 , respectively). The relationship

between a change in MVPA and a change in sedentary behaviour was not different by school (interaction $p = 0.896$). However, it should be noted that the calculations for MVPA and sedentary time are linked by the finite number of minutes in a day and a change in one is likely to result in a change in the other.

Discussion

In this primary-school-based quasi-experimental pilot study, which investigated the effects of taking part in the Daily Mile, we found evidence of a positive effect on our four primary outcomes—accelerometer-assessed time spent in MVPA, accelerometer-assessed time spent in sedentary behaviour, physical fitness and body composition—after correcting for the common confounders of age and gender with or without socioeconomic grouping.

Comparisons with other studies

Whilst no other studies have investigated the effect of taking part in the Daily Mile, some have investigated the effect of increasing physical activity throughout the school day or introducing short physical activity breaks into the school day itself [37, 38]. On the whole, they have had mixed results, with some finding alterations in MVPA and others not. This is likely in part due to the different methods used to assess these behaviours, in part due to the different interventions involved and in part due to the different accelerometer cut-points that can be found in the literature. Additionally, some studies use self-reported physical activity measures, which although easier to administer on a large scale, can lead to differing estimates in comparison to accelerometry [39]. Undoubtedly, the age and demographic of the children also has an influence and an intervention that works in one setting may not work in another.

Similarly, some studies have found changes in body composition or fitness whilst others have not [40–42]. The observed effect of the Daily Mile on fitness in the current study may be a result of the type of intervention activity involved (i.e. running) being similar to the fitness test. However, few studies have taken detailed physiological measurements and often assess BMI only. Changes in BMI are observed with some physical activity interventions but mostly in high-BMI groups. The decrease in the sum of skinfolds observed in this study without a concomitant change in BMI z score is likely due to the higher resolution of skinfolds and its utility in assessing body fatness without the confounding effect of muscle mass.

Meaning of study findings

Scottish government figures suggest that 73% of children in Scotland (77% of boys and 69% of girls) meet the physical activity guidelines [14]. However, this figure is based on self-reported questionnaire results rather than

accelerometer assessment and is likely to contain bias [43, 44]. Estimates by accelerometer of the percentage of children meeting the physical activity guidelines vary across Europe, from as low as 2% to as high as 63% [11, 45]. The children in this study fall within this range and are likely typical of Scottish and European primary school children [46, 47]. Regardless of how many meet the minimum recommended guidelines (at least 60 min per day), higher levels of MVPA are generally considered to be better. This study shows that introducing the Daily Mile into a primary school setting does increase the MVPA of children by 9.1 min (SMD = 0.407). Although the Daily Mile is a 15-min physical activity intervention, an increase of ~9 min is consistent with the pattern of running interspersed with periods of walking and chatting that is observed in children taking part (personal observations). Although the SMD would be considered to be small according to Cohen [36], small effects on a prevalent behaviour, such as physical inactivity, may have a high impact at the level of population health [48]. In addition, a change of this magnitude is close to the 10 min increase in MVPA previously associated with meaningful reductions in cardiometabolic risk in children and adolescents [49].

Sedentary time is less well studied than physical activity. Nonetheless, the available evidence suggests that the children in this study are typical of European children [11, 45]. In some studies, sedentary time appears to be a predictor of chronic disease independent of physical activity levels [50]. Two aspects of sedentary behaviour appear to be key to this: total sedentary time and prolonged blocks of sedentary time. The Daily Mile is potentially able to address both these issues although the present analysis only investigates total sedentary time. Although children at the intervention school were less sedentary at the baseline (after correction for the common confounders), this would make it harder to observe a reduction in sedentary behaviour rather than easier. Despite this, this study shows an ~18 min reduction (SMD = 0.437) in average daily sedentary time with the introduction of the Daily Mile. Again, this is consistent with a target of 15 min of physical activity since the children will at least be up from their chairs for a slightly longer period. However, if done correctly, the Daily Mile also breaks up the sedentary time, as it should happen in the middle of lessons, so that the children are likely to be sitting before and after their Daily Mile. As for MVPA, the SMD would be considered to be small but may well have significant impacts on population health due to mass participation. Additionally, the data also show a strong correlation between increasing MVPA and reducing sedentary time. This suggests that children are not compensating for the increase in MVPA during the Daily Mile by sitting more at other times of the day: they are replacing sedentary time with MVPA. However, note that the calculations for MVPA and sedentary time are linked

by the finite number of minutes in a day and may be more appropriately analysed in future studies using a compositional data analysis.

The children in the IDEFICS study [51] have median values of age-corrected $\dot{V}O_2$ max scores between 46.7 and 48.1 ml·kg⁻¹·min⁻¹ for boys and between 45.4 and 47.4 ml·kg⁻¹·min⁻¹ for girls between the ages of 6 and 9 years. Relatively, the children in the current study could be considered to have high aerobic fitness (see Additional file 1: Tables S1 and S2 for age-corrected $\dot{V}O_2$ max scores). This high baseline fitness would make it less likely that a change in fitness could be observed after a small increase in physical activity. Nonetheless, an improvement in fitness, as measured by shuttle distance (39.1 m, SMD = 0.236), was observed with the introduction of the Daily Mile. $\dot{V}O_2$ max is linked with cardiovascular health and all-cause mortality [52]. Although the SMD would be considered to be small, it may have a significant impact on a population scale. The CARDIA study in young adults suggests that having a $\dot{V}O_2$ max of 3.5 ml·kg⁻¹·min⁻¹ (approximately 1 metabolic equivalent) higher gives a reduction in all-cause mortality of ~15% [53]. Whilst we only see a relative increase of ~0.35 ml·kg⁻¹·min⁻¹ (Additional file 1: Table S3) with the Daily Mile, this is still predictive of an ~1.5% reduction in all-cause mortality risk. Note that the conversion from shuttle distance to $\dot{V}O_2$ max includes age in years and has, therefore, relatively lower resolution. It has also been suggested that having a higher cardiorespiratory fitness at a younger age confers the greatest survival benefit [52]. Furthermore, those with lower starting values appear likely to benefit to a greater extent [54]. This suggests that there are potentially useful health benefits associated with taking part in the Daily Mile.

The children at both schools in this study had lower rates of overweight and obesity than are typical of Scottish children. The Scottish Health Survey reports overweight and obesity rates in 7–11 year olds as 30% (29% for boys and 32% for girls) [14]. Again, this makes it less likely that a change in adiposity could be observed after a small increase in physical activity. Still, a reduction in adiposity as measured by skinfold (1.4 mm, SMD = 0.246) was observed with the introduction of the Daily Mile. Again, although the SMD would be considered to be small, at the population level it may have significant impacts on levels of adiposity. It is also possible that the impact of the Daily Mile on body composition may be larger still in children with higher rates of overweight and obesity. This intervention may be a useful component within measures designed to help tackle the obesity pandemic [8]. The strong correlation between those who reduced their skinfolds the most and those who gained the most fitness may indicate a common cause. Given that the pace each child completes the Daily Mile at is self-determined, it is possible that the children who gained the most benefits took a particular

approach to the Daily Mile. An insight into this may come when we interview the children taking part in the Daily Mile about their experiences.

Evidence linking socioeconomic status to MVPA and sedentary behaviour is unclear [13]. This is in part due to the use of different methods of capturing these outcome measures but also due to different ways of assessing socioeconomic status in different countries. Nonetheless, these outcome measures do appear to associate with specific aspects of socioeconomic status in some studies. However, clear differences between higher and lower socioeconomic groupings could be seen in the current study for fitness and body composition: children from postcodes with higher deprivation had lower levels of fitness, higher sums of skinfolds and higher rates of overweight and obesity. This is consistent with the widely recognised health inequality gap [55]. However, no differences were seen between the socioeconomic groupings in response to the introduction of the Daily Mile, suggesting that it may be beneficial to all groups regardless of background. Note that this study was not intended to investigate this, and larger more powerful studies are needed to investigate this aspect of the Daily Mile. A summary of the study and its implications can be found in Box 1.

Strengths and limitations of study

This study is the first to investigate the widely publicised and adopted Daily Mile physical activity intervention. The intervention appears to be increasingly popular and has now been maintained in the originator school for more than five years. Thus, it is undoubtedly feasible to deliver and has been adopted locally in many areas. What was unknown was the efficacy for the anecdotally reported physiological benefits of taking part in the Daily Mile. Consent rates were high (>77% in both schools) as were the number of children successfully assessed at both time points for most outcome measures. We assessed MVPA and sedentary time using the gold standard accelerometer technique, we assessed fitness using the bleep test (which has been validated in this age group) and we assessed body composition using labour-intensive skinfold assessments rather than the more straightforward but lower resolution BMI.

We acknowledge that there was a difference in sedentary time between the schools at baseline and the socioeconomic groupings were not reflective of the whole of Scotland, which are limitations of our study. However, these differences would be predicted to make any effects of the Daily Mile harder to observe, not easier. Changes were observed despite these differences. It would have been preferable to assess both the intervention and control schools at the same time of year to avoid any seasonal impact on physical activity. However, we believe that October and March should be similar enough to allow comparison [56]. Additionally, it would have been better to have had both

Box 1: What this study adds

Why was this study done?

- Low physical activity, high sedentary behaviour, declining fitness levels and high levels of overweight and obesity are global problems that have been targeted by the World Health Organisation.
- The Daily Mile is an increasingly popular school-based physical activity intervention, backed by the Scottish government, which is anecdotally reported to lead to increased physical activity, reduced sedentary time, improved fitness and improved body composition. Pupils run or walk laps of the playground at a self-selected pace for 15 min during normal classroom time. It is increasingly popular throughout the UK, in parts of Europe and some schools in the USA.
- However, these reported benefits remain anecdotal and need to be quantitatively and objectively assessed to ensure that the loss of academic classroom time is providing the reported alternative benefits.

What did the researchers do and find?

- Two schools in the Stirling Council area, Scotland, were recruited: one with intention to start the Daily Mile, the other without.
- Researchers assessed the physical activity and sedentary behaviour of children using accelerometers, their fitness using the bleep test and their body composition using skinfolds. This was done in both schools before and after the intervention school introduced the Daily Mile.
- This quasi-experimental pilot study found that, after correcting for age, gender and socioeconomic grouping, taking part in the Daily Mile did lead to an improvement in physical activity, sedentary behaviour, fitness and body composition of children in the intervention school relative to the control school.

What do these findings mean?

- This suggests that the Daily Mile is a worthwhile intervention to introduce in schools and that it should be considered for inclusion in government policy.
- This study can underpin the policy already introduced by the Scottish government and the development of future policy in other parts of the UK and abroad.

schools involved in the study for the same length of time, although, correcting for age and gender should account for this difference. It is also possible that differences in the health and well-being policies within the schools contributed to differences in the results. However, the schools were selected to be from the same local authority and to be of similar socioeconomic make-up to minimise potential differences. As predicted, changes in outcome variables had effect sizes at the smaller end of the distribution (0.2–0.5). However, given the involvement of whole classes, small effects could have an important impact on population health. To gain further confidence in the results, this study should be replicated in a larger number of schools. Furthermore, no monitoring of adherence, or level of adherence, to the intervention was carried out, although the results suggest adherence was sufficient.

Unanswered questions and future research

Additional anecdotally reported benefits to the Daily Mile (cognition, behaviour and well-being) are currently being investigated [57, 58]. It is essential that the current studies are replicated in a larger number of schools and countries to ensure that the findings are both robust and repeatable in different educational contexts. Future studies should include diet and sleep quality, which we are not yet investigating, to explore the potential mechanisms of impact. More attention should be given to when the Daily Mile is being done during the school day and whether it is breaking up sedentary time. Additionally, future studies should investigate whether MVPA and sedentary behaviour are changing on weekdays and/or weekend days.

In 2015, the Scottish government launched the Scottish Attainment Challenge with the aim of achieving equity in educational outcomes for all Scottish children [59]. Whilst the current study found no difference in the response to the Daily Mile by socioeconomic grouping, both schools were heavily weighted towards less deprived catchment areas. Furthermore, the current study was not powered to detect such a complex interaction. The Daily Mile is a free, simple intervention that can be rolled out to schools regardless of socioeconomic status. It is necessary to conduct carefully designed studies to understand the impact of the Daily Mile in different socioeconomic settings and to understand whether it can have any impact on the attainment gap.

The sample of children participating in this study included a number with challenging behaviours including autism spectrum disorders. Nonetheless, they took part in the Daily Mile and our investigations. Understanding the impact of the Daily Mile on children with differing learning needs should also be a future priority.

This study shows the value of introducing the Daily Mile into schools. Whilst the Daily Mile has been introduced as

policy across Scotland, many schools do not have appropriate outdoor facilities to allow their children to take part. One of the challenges for policymakers and other stakeholders is to consider how to introduce the Daily Mile or alternative interventions that have been shown to increase MVPA and fitness into such schools or how to adapt those schools to allow the introduction of appropriate interventions.

Conclusions

In conclusion, introducing the Daily Mile to the primary school day appears to be an effective intervention for increasing MVPA and reducing sedentary time and it has measurable impacts on key aspects of metabolic health: body composition and physical fitness. This study provides the first assessment of the Daily Mile and it will allow the development of evidence-based policy around introducing the Daily Mile to more schools.

Additional file

Additional file 1: Table S1. Baseline characteristics of participants by year group and study group. **Table S2.** Baseline characteristics of participants by socioeconomic group, school and gender. **Table S3.** Effect of introducing the Daily Mile on additional outcomes assessed immediately after the end of the intervention period for comparison with other studies. (DOCX 65 kb)

Abbreviations

95%CI: 95% confidence interval; BMI: Body mass index; cpm: Counts per minute; ISAK: International Society for the Advancement of Kinanthropometry; MVPA: Moderate to vigorous intensity physical activity; PE: Physical education; SIMD: Scottish Index of Multiple Deprivation; SMD: Standardised mean difference; WHEEL: Well-being, Health, Exercise, Enjoyment and Learning; WHO: World Health Organisation

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Availability of data and materials

The datasets generated and analysed during the current study are not publicly available due to the risk of identifying individuals from their age in days at testing but are available from the corresponding author on reasonable request.

Authors' contributions

JNB, GCR, TG, NEB and CNM were involved in the design of the study and in seeking funding for it. JNB, RAC, ES, NEB and CNM were responsible for conducting the study. RAC and CNM wrote the first draft of the paper, and CNM coordinated contributions from other co-authors. CNM wrote the

analysis plan used for this paper, and RAC and CNM completed all analyses. JNB, RAC, NEB and CNM managed the data collection with input from other members of the study team. All authors made critical comments on drafts of the paper. CNM is the guarantor. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the University of Stirling, School of Sport Research Ethics Committee (reference number 760). Approval was also obtained from the Director of Children, Young People and Education at Stirling Council. This study was performed in accordance with the Declaration of Helsinki.

Competing interests

The authors declare that they have no competing interests.

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References

1. Stirling Council: The Daily Mile - Health and Wellbeing for free. 2015.
2. Daily Mile roll out [<https://news.gov.scot/news/daily-mile-roll-out>]. Accessed 17 Apr 2018.
3. Obesity: Children: Written question - 46791 [<http://www.parliament.uk/business/publications/written-questions-answers-statements/written-question/Commons/2016-10-07/46791>]. Accessed 17 Apr 2018.
4. Early day motion 1074: Five Year Anniversary of Daily Mile [<http://www.parliament.uk/edm/2016-17/1074>]. Accessed 17 Apr 2018.
5. The Daily Mile [<http://thedailymile.co.uk/>]. Accessed 17 Apr 2018.
6. Kohl HW, Craig CL, Lambert EV, Inoue S, Alkandari JR, Leetongin G, Kahlmeier S. The pandemic of physical inactivity: global action for public health. *Lancet*. 2012;380(9838):294–305.
7. Telama R, Yang X, Viikari J, Valimäki I, Wanne O, Raitakari O. Physical activity from childhood to adulthood: a 21-year tracking study. *Am J Prev Med*. 2005;28(3):267–73.
8. Mendis S. Global status report on noncommunicable diseases 2014. Geneva: WHO; 2014.
9. World Health Organisation: Physical activity fact sheet. 2017.
10. Department of Health: Start active, stay active: a report on physical activity from the four home countries' Chief Medical Officers. 2011.
11. Korstabel K, Veidebaum T, Verbestel V, Moreno LA, Bammann K, Tomaritis M, Eiben G, Molnar D, Siani A, Sprengeler O, et al. Objectively measured physical activity in European children: the IDEFICS study. *Int J Obes*. 2014; 38(Suppl 2):S135–43.
12. Esliger D, Hall J. Health Survey for England. In: Centre TNI, editor, vol. 1. London: Department of Epidemiology and Public Health, UCL Medical School; 2008.
13. Biddle SJH, Atkin AJ, Cavill N, Foster C. Correlates of physical activity in youth: a review of quantitative systematic reviews. *Int Rev Sport Exer P*. 2011;4(1):25–49.
14. Brown L, Campbell-Jack D, Gray L, Hovold P, Kirkpatrick G, Knudsen L, Leyland AH, Montagu I, Rose J. The Scottish Health Survey. In: Scotland ANSP, editor. 2016. <http://www.gov.scot/Publications/2016/09/2764/0>.
15. Conolly A. Health Survey for England 2015 Children's body mass index, overweight and obesity. In: Centre HaSCL, editor. London: Health and Social Care Information Centre; 2016.
16. Tomkinson GR, Leger LA, Olds TS, Cazorla G. Secular trends in the performance of children and adolescents (1980–2000): an analysis of 55 studies of the 20m shuttle run test in 11 countries. *Sports Med*. 2003;33(4):285–300.

17. Biddle SJ, Gorely T, Stensel DJ. Health-enhancing physical activity and sedentary behaviour in children and adolescents. *J Sports Sci.* 2004;22(8):679–701.
18. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, Lancet Physical Activity Series Working G. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet.* 2012;380(9838):219–29.
19. Must A, Tybor DJ. Physical activity and sedentary behavior: a review of longitudinal studies of weight and adiposity in youth. *Int J Obes.* 2005;29(Suppl 2):S84–96.
20. Vos MB, Welsh J. Childhood obesity: update on predisposing factors and prevention strategies. *Curr Gastroenterol Rep.* 2010;12(4):280–7.
21. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med.* 2004;350(23):2362–74.
22. Jimenez-Pavon D, Ortega FB, Ruiz JR, Chillon P, Castillo R, Artero EG, Martinez-Gomez D, Vicente-Rodriguez G, Rey-Lopez JP, Gracia LA, et al. Influence of socioeconomic factors on fitness and fatness in Spanish adolescents: the AVENA study. *Int J Pediatr Obes.* 2010;5(6):467–73.
23. Wang Y, Lim H. The global childhood obesity epidemic and the association between socio-economic status and childhood obesity. *Int Rev Psychiatry.* 2012;24(3):176–88.
24. Poitras VJ, Gray CE, Borghese MM, Carson V, Chaput JP, Janssen I, Katzmarzyk PT, Pate RR, Connor Gorber S, Kho ME, et al. Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Appl Physiol Nutr Metab.* 2016;41(6 Suppl 3):S197–239.
25. Foulds HJ, Bredin SS, Charlesworth SA, Ivey AC, Warburton DE. Exercise volume and intensity: a dose-response relationship with health benefits. *Eur J Appl Physiol.* 2014;114(8):1563–71.
26. Disclosure Scotland Website [<https://www.mygov.scot/about-disclosure-scotland/>]. Accessed 17 Apr 2018.
27. Hanggi JM, Phillips LR, Rowlands AV. Validation of the GT3X ActiGraph in children and comparison with the GT1M ActiGraph. *J Sci Med Sport.* 2013;16(1):40–4.
28. Robusto KM, Trost SG. Comparison of three generations of ActiGraph activity monitors in children and adolescents. *J Sports Sci.* 2012;30(13):1429–35.
29. Penpraze V, Reilly JJ, MacLean CM, Montgomery C, Kelly LA, Paton JY, Aitchison T, Grant S. Monitoring of physical activity in young children: How much is enough? *Pediatr Exerc Sci.* 2006;18(4):483–91.
30. Cooper AR, Goodman A, Page AS, Sherar LB, Esliger DW, van Sluijs EM, Andersen LB, Anderssen S, Cardon G, Davey R, et al. Objectively measured physical activity and sedentary time in youth: the International children's accelerometry database (ICAD). *Int J Behav Nutr Phys Act.* 2015;12:113.
31. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. *J Sports Sci.* 2008;26(14):1557–65.
32. Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci.* 1988;6(2):93–101.
33. Norton K, Carter L, Olds T, Marfell-Jones M: International standards for anthropometric assessment; 2011.
34. Pan H, Cole TJ: LMSgrowth, a Microsoft Excel add-in to access growth references based on the LMS method. Version 2.74. 2011.
35. Postcode to Scottish Index of Multiple Deprivation (SIMD) rank [<http://www.gov.scot/Topics/Statistics/SIMD>]. Accessed 17 Apr 2018.
36. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. Routledge Academic; New York, NY; 1988.
37. Barr-Anderson DJ, AuYoung M, Whitt-Glover MC, Glenn BA, Yancey AK. Integration of short bouts of physical activity into organizational routine: a systematic review of the literature. *Am J Prev Med.* 2011;40(1):76–93.
38. Norris E, Shelton N, Dunsmuir S, Duke-Williams O, Stamatakis E. Physically active lessons as physical activity and educational interventions: a systematic review of methods and results. *Prev Med.* 2015;72:116–25.
39. Reilly JJ, Penpraze V, Hislop J, Davies G, Grant S, Paton JY. Objective measurement of physical activity and sedentary behaviour: review with new data. *Arch Dis Child.* 2008;93(7):614–9.
40. Dobbins M, Husson H, DeCorby K, LaRocca RL. School-based physical activity programs for promoting physical activity and fitness in children and adolescents aged 6 to 18. *Cochrane Database Syst Rev.* 2013(2):CD007651. <https://doi.org/10.1002/14651858.CD007651.pub2>.
41. Harris KC, Kuramoto LK, Schulzer M, Retallack JE. Effect of school-based physical activity interventions on body mass index in children: a meta-analysis. *CMAJ.* 2009;180(7):719–26.
42. Mei H, Xiong Y, Xie S, Guo S, Li Y, Guo B, Zhang J. The impact of long-term school-based physical activity interventions on body mass index of primary school children - a meta-analysis of randomized controlled trials. *BMC Public Health.* 2016;16:205.
43. Ekelund U, Tomkinson G, Armstrong N. What proportion of youth are physically active? Measurement issues, levels and recent time trends. *Br J Sports Med.* 2011;45(11):859–65.
44. Basterfield L, Adamson AJ, Parkinson KN, Maute U, Li PX, Reilly JJ. Gateshead Millennium Study Core T: Surveillance of physical activity in the UK is flawed: validation of the Health Survey for England Physical Activity Questionnaire. *Arch Dis Child.* 2008;93(12):1054–8.
45. Griffiths LJ, Cortina-Borja M, Sera F, Poulou T, Geraci M, Rich C, Cole TJ, Law C, Joshi H, Ness AR, et al. How active are our children? Findings from the Millennium Cohort Study. *BMJ Open.* 2013;3(8):e002893.
46. World Health Organisation. Growing up unequal: gender and socioeconomic differences in young people's health and well-being. In: Inchley J, Currie D, Young T, Samdal O, Torsheim T, Augustsson L, Mathison F, Aleman-Diaz A, Molcho M, Weber M, et al, editors. *Health Behaviour in School-aged Children (HBSC) study: international report from the 2013/2014 survey*. Copenhagen: WHO Regional Office for Europe; 2016.
47. World Health Organisation. Adolescent obesity and related behaviours: trends and inequalities in the WHO European Region, 2002–2014. In: Inchley J, Currie D, Jewell J, Breda J, Barnekow V, editors. *Observations from the Health Behaviour in School-aged Children (HBSC) WHO collaborative cross-national study*. Copenhagen: WHO Regional Office for Europe; 2017.
48. Biddle SJ, O'Connell S, Braithwaite RE. Sedentary behaviour interventions in young people: a meta-analysis. *Br J Sports Med.* 2011;45(11):937–42.
49. Ekelund U, Luan J, Sherar LB, Esliger DW, Griew P, Cooper A. International Children's Accelerometry Database C. Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. *JAMA.* 2012;307(7):704–12.
50. Endorsed by The Obesity S, Young DR, Hivert MF, Alhassan S, Camhi SM, Ferguson JF, Katzmarzyk PT, Lewis CE, Owen N, Perry CK, et al. Sedentary Behavior and Cardiovascular Morbidity and Mortality: A Science Advisory From the American Heart Association. *Circulation.* 2016;134(13):e262–79.
51. De Miguel-Etayo P, Gracia-Marco L, Ortega FB, Intemann T, Foraita R, Lissner L, Oja L, Barba G, Michels N, Tomatis M, et al. Physical fitness reference standards in European children: the IDEFICS study. *Int J Obes.* 2014;38(Suppl 2):S57–66.
52. Harber MP, Kaminsky LA, Arena R, Blair SN, Franklin BA, Myers J, Ross R. Impact of cardiorespiratory fitness on all-cause and disease-specific mortality: advances since 2009. *Prog Cardiovasc Dis.* 2017;60(1):11–20. <https://doi.org/10.1016/j.pcad.2017.03.001>.
53. Shah RV, Murthy VL, Colangelo LA, Reis J, Venkatesh BA, Sharma R, Abbasi SA, Goff DC Jr, Carr JJ, Rana JS, et al. Association of Fitness in Young Adulthood With Survival and Cardiovascular Risk: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *JAMA Intern Med.* 2016;176(1):87–95.
54. Kokkinos P, Myers J, Faselis C, Panagiotakos DB, Doumas M, Pittaras A, Manolis A, Kokkinos JP, Karasik P, Greenberg M, et al. Exercise capacity and mortality in older men: a 20-year follow-up study. *Circulation.* 2010;122(8):790–7.
55. Truesdale BC, Jencks C. The Health Effects of Income Inequality: Averages and Disparities. *Annu Rev Public Health.* 2016;37:413–30.
56. Atkin AJ, Sharp SJ, Harrison F, Brage S, Van Sluijs EM. Seasonal Variation in Children's Physical Activity and Sedentary Time. *Med Sci Sports Exerc.* 2016;48(3):449–56.
57. Booth JN, Brooks NE, Moran CN, Gallagher U, Ryde GC, Gorely T. Is running a mile a day associated with children's behaviour and well-being? Exploring the impact of the Daily Mile and implications for policy. In: *British Psychological Society Annual Meeting*. Belfast: British Psychological Society; 2016.
58. Brooks NE, Booth JN, Chesham R, Sweeney E, Mitchell I, Ryde GC, Gorely T, Moran CN. A feasibility study investigating body composition and physical activity levels in children taking part in the daily mile. In: *Experimental Biology*. vol. 30. FASEB J; 2016. p. lb669. https://www.fasebj.org/doi/abs/10.1096/fasebj.30.1_supplement.lb669.
59. The Scottish Attainment Challenge [<http://www.gov.scot/Topics/Education/Schools/Raisingeducationalattainment>]. Accessed 17 Apr 2018.

CORRESPONDENCE

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Response to Daly-Smith et al.'s commentary on 'The Daily Mile makes primary school children more active, less sedentary and improves their fitness and body composition: a quasi-experimental pilot study'



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Abstract

We thank Daly-Smith et al. for taking the time to read the results of our pilot research study, describing it as an important and welcome contribution. Nonetheless, the authors argue six points against our conclusion. We contend that we addressed three of these points in our original discussion and disagree with their remaining points. Overall, their Commentary adds little to the topic of research into the Daily Mile™ that we had not already raised in our discussion. Additionally, they attribute statements to us that we did not make and ignore the raising of key issues in our original article. Given this, we stand by our original peer-reviewed conclusion that introducing the Daily Mile™ to the primary school day appears to be an effective intervention for increasing levels of moderate to vigorous physical activity, reducing sedentary time, increasing physical fitness and improving body composition, and that these findings have relevance for teachers, policy-makers, public health practitioners and health researchers.

Keywords: The Daily Mile™, Children, Primary school, Physical activity, MVPA, Sedentary, Fitness, Body composition

Background

We thank Daly-Smith et al. [1] for taking the time to read the results of our pilot research study [2], describing it as an important and welcome contribution. Low physical activity and high sedentary behaviour are important issues currently on the global health policy agenda [3] (e.g. Active Scotland Outcomes Framework [4]). The Daily Mile™ is a run-walk intervention with growing popularity and global reach, now being implemented in more than 8300 schools in over 50 countries [5]. Our study was not intended or designed to compare

the Daily Mile™ to classroom movement breaks or physically active learning programmes; instead, we sought to investigate the effectiveness of the Daily Mile™ in terms of the anecdotally reported physiology-related benefits [2]. Our quasi-experimental pilot study provides the first assessment of the Daily Mile™, setting the starting point for the development of an evidence base.

Response

Daly-Smith et al. [1] make six key points in their argument against our conclusion. We contend that we made three of these points in our original discussion and provide a rebuttal to their remaining points. We comment on each one in turn below.

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Correcting for gender and age on day of testing is appropriate when accounting for potentially unequal dose–response conditions

Daly-Smith et al. [1] describe our measures of moderate to vigorous intensity physical activity (MVPA), fitness and body composition as valid and reliable; these measures are all known to change with age and to differ by gender [6]. Physical activity recommendations vary for pre-school children, school children and adults [7], with fitness and body composition having a range of age- and gender-based norms and corrections (e.g. [8–10]). As acknowledged in our original article (Discussion, Strengths and limitation section –[2], p. 10–11), it would be preferable to have simultaneously included intervention and control schools for the same length of time. However, correcting for age on day of testing and gender effectively makes these variables independent of time. Thus, our correction for age and gender is both common and necessary when studying children and accounts for any differences in the dose.

Seasonal effects are expected to be minimal, whilst correcting for gender and age on day of testing reduces any unequal opportunity for benefits

Daly-Smith et al. [1] suggest that the between-group differences in baseline outcome measures may unduly influence the study findings and that time of year may influence the measurements. It was expected that slightly older children would have different values for some of our outcome measures at baseline; to account for this, we corrected for age and gender, as clearly stated in our article (Results section, [2], p. 6). The direction of the remaining difference in sedentary time made it less, rather than more, likely that we would find a change associated with the Daily Mile™. Although we were not able to control for daily weather patterns, we already acknowledged that, “It would have been preferable to assess both the intervention and control schools at the same time of year to avoid any seasonal impact on physical activity. However, we believe that October and March should be similar enough to allow comparison” ([2], p. 10). We discussed this during the review process with reference to Atkin et al. [11] (Discussion, Strengths and limitation section [2], p. 10, and see also author response in ‘Open Peer Review Reports’ link in our original article); thus, we believe that we have adequately addressed Daly-Smith et al.’s comment in our original article and during the peer-review process.

The variability in the individual response to the exercise intervention is exactly as would be expected based on a large body of literature

Daly-Smith et al. [1] attributed a statement implying that we claimed a universal benefit of the Daily Mile™ – we made no such statement. Variability in response to an exercise intervention (indeed, any intervention) is well

established. A good example of this can be seen in the HERITAGE study [12], where 481 people took part in a 20-week exercise intervention. The average increase in VO_2 max was $393 \text{ mL} \cdot \text{min}^{-1}$, with a range of responses from $> 1000 \text{ mL} \cdot \text{min}^{-1}$ to a decrease of approximately $-100 \text{ mL} \cdot \text{min}^{-1}$. Importantly, there were no distinct responder and non-responder groups, but instead a continuum of response size. Coupled with the facts that we did not control for pupils who took up or dropped out of sports classes and that the Daily Mile™ is a self-paced activity of only 15 min duration, then the size and spread of the changes observed were exactly as would be expected. This spread in response does not detract from the typical value of taking part in the Daily Mile™. Nevertheless, we do agree that understanding why some people have larger responses than others is highly important and should be further assessed in future studies.

Our accelerometer sample was randomly selected across the participants and should be representative of the entire sample

As we already discussed during peer review, the smaller number of children in the accelerometer analysis was due to the intervention school’s desire to start the Daily Mile™. We only included accelerometer data that we were certain was collected before the school began the intervention (Results section [2], p. 5–6). These pupils were not selected, they were simply the first to receive accelerometers because they were the first to be available for the other physiological tests, which made them relatively well spread throughout the school (see Table S1 in [2]). As stated in our article, all children involved in the study received accelerometers; however, those that received them after the introduction of the Daily Mile™ were excluded from the analysis. Whilst sensitivity analyses may be interesting, the smaller number of participants with valid accelerometer data was not due to attrition. Furthermore, we already stated that future studies should recruit larger samples across multiple schools (Discussion and Strengths and limitations sections [2], p. 11).

Using 60 s epochs in our accelerometer analysis allows us to directly compare our results to large international studies such as the international Children’s Accelerometry database [13]

Daly-Smith et al. [1] suggest that there are four issues with our approach to accelerometers, namely that (1) we analysed data in 60 s rather than 15 s epochs; (2) we did not analyse data in epochs of ≤ 15 s; (3) we did not report whether participants were given the same accelerometer model in pre- and post-intervention measurements; and (4) we included weekend days in our analysis of MVPA and sedentary time. On points 1 and 2, the review by

Migueles et al. [14] cited by Daly-Smith et al. [1] demonstrates that there are mixed reports on epoch length in the literature. We certainly agree that altering epoch length could change the conclusions. Thus, we chose to use the Evenson cut points [15], as recommended for school age children [16], with 60 s epochs and 60 min of non-wear time to allow direct comparison with data from the largest collection of accelerometer measurements in children – the International Children's Accelerometry Database [13]. To address point 3, we note that the International Children's Accelerometry Database includes studies with variable accelerometer models. It is vital that data from different studies can be compared. Further, as stated on page 4 of our original article (Methods, Participant assessments section [2], others have compared different accelerometer models and found their use acceptable in the same study. Finally, regarding point 4, Daly-Smith et al. [1] pose a good question about differences in MVPA between days on which the Daily Mile™ is completed versus days on which it is not (i.e. weekend days) and the potential for compensation; this is why we chose to look at overall MVPA and highlight (Discussion, Strengths and limitations section [2], p. 11) that further assessment of this issue is an important question for future research.

Assessing the effectiveness of the Daily Mile™ as interpreted by the schools is a strength of our study

Daly-Smith et al. [1] suggest that we should have confirmed the contribution of the Daily Mile™ to acute MVPA responses and outcomes using a segmented day analysis. However, our study sought to assess the veracity of some of the anecdotally reported benefits to doing the Daily Mile™, including reports of children being more willing to walk and run to places outside school. Additionally, there was potential for a compensatory decrease in MVPA at other times of the day which we wished to be able to discount. Although a segmented day analysis may provide more insight into our data and be a useful approach in future studies, our more straightforward analysis was entirely appropriate to assess the impact of the Daily Mile™ on overall MVPA. Additionally, Daly-Smith et al. [1] see our lack of a treatment fidelity measure as a weakness; however, we see it as a strength. As we state in our methods (Intervention section [2], p. 4), a leaflet produced by the originator school was given to the school implementing the Daily Mile™ without additional instructions. Therefore, the form of the Daily Mile™ that we assessed is likely to be the form closest to that being taken up and maintained by schools across the globe and thus more ecologically valid than a strict prescription. Nevertheless, we also see careful assessment of the exercise dose as an important area for future research.

Summary and conclusion

The Daily Mile™ has become a popular intervention worldwide, with many benefits being anecdotally reported. The aim of our research was, as clearly stated, to investigate only some of these anecdotally reported physiological benefits, whilst we highlighted that many more benefits remain to be assessed. We have performed additional research into successful implementation of the Daily Mile™ [17] and on its cognitive benefits [18]. We describe our work as a quasi-experimental pilot study with a clear description of our methods and results. We had already raised the majority of the criticisms of Daly-Smith et al. [1] in our article as weaknesses and have addressed their remaining issues herein. Our article states that current studies should be replicated in more schools and countries and that additional anecdotally reported benefits should be investigated. Consequently, we stand by our original conclusion that *"introducing the Daily Mile to the primary school day appears to be an effective intervention for increasing MVPA and reducing sedentary time and it has measurable impacts on key aspects of metabolic health: body composition and physical fitness"* ([2], p. 12). We believe that these findings have relevance for teachers, policy-makers, public health practitioners and health researchers.

Abbreviation

MVPA: moderate to vigorous intensity physical activity

Authors' contributions

All authors contributed to drafting and editing the response. All authors read and approved the final version.

Competing interests

CNM and JNB have been members of the Daily Mile Foundation Expert Steering Group since March 28, 2018. The authors declare no other competing interests.

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References

1. Daly-Smith A, Morris JL, Hobbs M, McKenna J. Commentary on a recent article on the effects of the 'daily mile' on physical activity, fitness and body composition: addressing key limitations. <https://doi.org/10.1186/s12916-019-1335-4>.
2. Chesham RA, Booth JN, Sweeney EL, Ryde GC, Gorely T, Brooks NE, Moran CN. The daily mile makes primary school children more active, less sedentary and improves their fitness and body composition: a quasi-experimental pilot study. *BMC Med*. 2018;16:64.

3. Kohl HW, Craig CL, Lambert EV, Inoue S, Alkandari JR, Leetongin G, Kahlmeier S. The pandemic of physical inactivity: global action for public health. *Lancet*. 2012;380(9838):294–305.
4. Active Scotland Outcomes Framework. <https://www2.gov.scot/About/Performance/scotPerforms/partnerstories/Outcomes-Framework>. Accessed 23 Apr 2019.
5. The Daily Mile. <http://thedailymile.co.uk/>. Accessed 23 Apr 2019.
6. WHO Regional Office for Europe. Growing up unequal: gender and socioeconomic differences in young People's and well-being. Health behaviour in school-aged children (HBSC) study: international report from the 2013/2014 survey. 2016. http://www.euro.who.int/__data/assets/pdf_file/0014/303440/HBSC-No.7-Growing-up-unequal-PART-1.pdf. Accessed 23 Apr 2019.
7. Department of Health and Social Care. Start Active, Stay Active: A Report on Physical Activity from the Four Home Countries' Chief Medical Officers. 2011. <https://www.gov.uk/government/publications/start-active-stay-active-a-report-on-physical-activity-from-the-four-home-countries-chief-medical-officers>. Accessed 23 Apr 2019.
8. Leger LA, Merdier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci*. 1988;6(2):93–101.
9. Tomkinson GR, Lang JJ, Tremblay MS, Dale M, LeBlanc AG, Belanger K, Ortega FB, Leger L. International normative 20 m shuttle run values from 1 142 026 children and youth representing 50 countries. *Br J Sports Med*. 2017;51(21):1545–54.
10. Weststrate JA, Deurenberg P. Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. *Am J Clin Nutr*. 1989;50(5):1104–15.
11. Atkin AJ, Sharp SJ, Harrison F, Brage S, Van Sluys EM. Seasonal variation in children's physical activity and sedentary time. *Med Sci Sports Exerc*. 2016; 48(3):449–56.
12. Bouchard C, An P, Rice T, Skinner JS, Wilmore JH, Gagnon J, Perusse L, Leon AS, Rao DC. Familial aggregation of VO₂(max) response to exercise training: results from the HERITAGE family study. *J Appl Physiol*. 1999;87(3):1003–8.
13. Sherar LB, Griew P, Esliger DW, Cooper AR, Ekelund U, Judge K, Riddoch C. International Children's Accelerometry database (ICAD): design and methods. *BMC Public Health*. 2011;11:485.
14. Migueles JH, Cadenas-Sanchez C, Ekelund U, Delisle Nystrom C, Mora-Gonzalez J, Lof M, Labayen I, Ruiz JR, Ortega FB. Accelerometer data collection and processing criteria to assess physical activity and other outcomes: a systematic review and practical considerations. *Sports Med*. 2017;47(9):1821–45.
15. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. *J Sports Sci*. 2008;26(14): 1557–65.
16. Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of accelerometer cut points for predicting activity intensity in youth. *Med Sci Sports Exerc*. 2011;43(7):1360–8.
17. Ryde GC, Booth JN, Brooks NE, Chesham RA, Moran CN, Gorely T. The daily mile: what factors are associated with its implementation success? *PLoS One*. 2018;13(10):e0204988.
18. Gorely T, Booth J, Brooks N, Chesham R, Moran C. Does exercise during the school day boost children's attention and memory? *J Phys Act Health*. 2018; 15(10):55.

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8. Supplementary Materials

8.1. Supplementary Material Chapter Two

Supplementary Table S1. Baseline characteristics of participants by year group and study group.

	Year Group	Intervention School						Control School					
		Total		Males		Females		Total		Males		Females	
Age (years)	P1	5.2 ± 0.3	(34)	5.2 ± 0.3	(15)	5.2 ± 0.3	(19)	5.7 ± 0.3	(11)	5.9 ± 0.1	(7)	5.4 ± 0.2	(4)
	P2	6.2 ± 0.3	(40)	6.2 ± 0.4	(25)	6.1 ± 0.3	(15)	6.7 ± 0.3	(17)	6.7 ± 0.3	(10)	6.6 ± 0.3	(7)
	P3	7.3 ± 0.3	(39)	7.3 ± 0.3	(17)	7.2 ± 0.3	(22)	7.6 ± 0.4	(20)	7.7 ± 0.4	(11)	7.5 ± 0.2	(9)
	P4	8.3 ± 0.2	(37)	8.3 ± 0.2	(20)	8.3 ± 0.2	(17)	8.7 ± 0.3	(18)	8.6 ± 0.3	(8)	8.7 ± 0.3	(10)
	P5	9.2 ± 0.3	(34)	9.2 ± 0.3	(16)	9.2 ± 0.3	(18)	9.6 ± 0.3	(27)	9.6 ± 0.3	(12)	9.6 ± 0.3	(15)
	P6	10.2 ± 0.4	(36)	10.3 ± 0.3	(17)	10.1 ± 0.4	(19)	10.7 ± 0.3	(23)	10.7 ± 0.3	(11)	10.7 ± 0.3	(12)
	P7	11.2 ± 0.3	(32)	11.3 ± 0.3	(19)	11.0 ± 0.3	(13)	11.7 ± 0.3	(11)	11.8 ± 0.4	(4)	11.7 ± 0.3	(7)
Average MVPA per day (mins)	P1	54 ± 13	(5)	43 ± 2	(2)	62 ± 11	(3)	NR	(1)	NR	(1)	NA	(0)
	P2	55 ± 22	(18)	49 ± 16	(11)	64 ± 28	(7)	70 ± 20	(8)	69 ± 23	(6)	74 ± 4	(2)
	P3	56 ± 33	(9)	72 ± 42	(2)	52 ± 32	(7)	62 ± 21	(10)	71 ± 15	(4)	57 ± 24	(6)
	P4	54 ± 8	(6)	63 ± 7	(2)	50 ± 3	(4)	56 ± 22	(12)	74 ± 14	(6)	38 ± 11	(6)
	P5	52 ± 25	(13)	62 ± 34	(3)	49 ± 23	(10)	54 ± 21	(12)	64 ± 18	(7)	40 ± 17	(5)
	P6	37 ± 13	(3)	44 ± 9	(2)	NR	(1)	45 ± 21	(14)	52 ± 22	(6)	41 ± 21	(8)
	P7	52 ± 9	(2)	52 ± 9	(2)	NA	(0)	62 ± 21	(5)	78 ± 5	(2)	52 ± 22	(3)
Sedentary time per day (mins)	P1	267 ± 39	(5)	293 ± 57	(2)	249 ± 16	(3)	NR	(1)	NR	(1)	NA	(0)
	P2	281 ± 38	(18)	275 ± 31	(11)	291 ± 49	(7)	280 ± 71	(8)	258 ± 68	(6)	346 ± 28	(2)
	P3	332 ± 72	(9)	271 ± 25	(2)	350 ± 72	(7)	304 ± 47	(10)	322 ± 58	(4)	292 ± 38	(6)
	P4	333 ± 20	(6)	323 ± 25	(2)	338 ± 18	(4)	343 ± 38	(12)	343 ± 34	(6)	343 ± 45	(6)
	P5	410 ± 62	(13)	440 ± 98	(3)	401 ± 52	(10)	402 ± 68	(12)	409 ± 72	(7)	392 ± 68	(5)
	P6	405 ± 32	(3)	397 ± 41	(2)	NR	(1)	386 ± 64	(14)	372 ± 64	(6)	397 ± 66	(8)
	P7	468 ± 29	(2)	468 ± 29	(2)	NA	(0)	393 ± 29	(5)	405 ± 50	(2)	385 ± 12	(3)
Total Shuttle Distance (m)	P1	269 ± 135	(32)	280 ± 161	(13)	261 ± 119	(19)	376 ± 106	(10)	393 ± 106	(6)	350 ± 116	(4)
	P2	404 ± 157	(36)	452 ± 164	(24)	310 ± 87	(12)	439 ± 196	(16)	432 ± 234	(10)	450 ± 127	(6)
	P3	572 ± 194	(38)	606 ± 193	(17)	545 ± 196	(21)	653 ± 280	(18)	784 ± 274	(10)	490 ± 200	(8)
	P4	639 ± 238	(35)	682 ± 246	(20)	583 ± 222	(15)	776 ± 234	(17)	820 ± 211	(7)	746 ± 255	(10)
	P5	765 ± 281	(33)	823 ± 265	(15)	718 ± 293	(18)	808 ± 344	(26)	942 ± 389	(12)	693 ± 261	(14)
	P6	894 ± 344	(36)	1074 ± 373	(17)	733 ± 220	(19)	863 ± 374	(23)	1080 ± 378	(11)	665 ± 245	(12)
	P7	1004 ± 403	(30)	1120 ± 474	(18)	830 ± 159	(12)	1123 ± 329	(7)	1393 ± 316	(3)	920 ± 148	(4)
Sum of skinfolds (mm)	P1	30.3 ± 7.8	(21)	27.2 ± 4.9	(10)	33.1 ± 9.1	(11)	33.6 ± 15.7	(7)	33.8 ± 17.2	(6)	NR	(1)
	P2	28.3 ± 8.4	(34)	24.9 ± 6.7	(22)	34.5 ± 7.8	(12)	34.5 ± 17.0	(16)	36.1 ± 20.6	(10)	31.8 ± 9.4	(6)
	P3	30.4 ± 7.0	(36)	29.0 ± 8.0	(16)	31.6 ± 6.1	(20)	31.4 ± 11.0	(17)	24.9 ± 6.2	(9)	38.7 ± 11.0	(8)
	P4	31.5 ± 12.3	(32)	27.2 ± 8.1	(17)	36.4 ± 14.6	(15)	33.2 ± 14.7	(17)	29.0 ± 9.8	(8)	37.0 ± 17.7	(9)

	P5	32.2 ± 10.8 (28)	28.3 ± 7.2 (14)	36.0 ± 12.6 (14)	41.5 ± 18.3 (25)	35.1 ± 14.5 (12)	47.4 ± 20.0 (13)
	P6	40.5 ± 14.0 (31)	42.1 ± 17.7 (17)	38.5 ± 7.5 (14)	46.9 ± 22.4 (22)	47.5 ± 24.0 (11)	46.4 ± 21.8 (11)
	P7	43.5 ± 17.3 (31)	41.2 ± 19.2 (19)	47.2 ± 13.6 (12)	33.0 ± 12.8 (10)	25.7 ± 2.7 (4)	37.9 ± 14.8 (6)
Percent meeting 60+ mins per day activity guidelines (%)	P1	40.0% (5)	00.0% (2)	66.7% (3)	NR (1)	NR (1)	NA (0)
	P2	27.8% (18)	27.3% (11)	28.6% (7)	75.0% (8)	66.7% (6)	100.0% (2)
	P3	44.4% (9)	50.0% (2)	42.9% (7)	50.0% (10)	75.0% (4)	33.3% (6)
	P4	16.7% (6)	50.0% (2)	00.0% (4)	41.7% (12)	83.3% (6)	00.0% (6)
	P5	23.1% (13)	33.3% (3)	20.0% (10)	41.7% (12)	57.1% (7)	20.0% (5)
	P6	00.0% (3)	00.0% (2)	NR (1)	28.6% (14)	33.3% (6)	25.0% (8)
	P7	00.0% (2)	00.0% (2)	NA (0)	60.0% (5)	100.0% (2)	33.3% (3)
Percent overweight or obese (%)	P1	21.4% (28)	23.1% (13)	20.0% (15)	21.7% (23)	22.2% (9)	21.4% (14)
	P2	31.0% (29)	26.7% (15)	35.7% (14)	25.0% (20)	10.0% (10)	40.0% (10)
	P3	16.1% (31)	16.7% (18)	15.4% (13)	17.4% (23)	23.1% (13)	10.0% (10)
	P4	37.5% (24)	23.1% (13)	54.5% (11)	35.3% (17)	18.2% (11)	66.7% (6)
	P5	32.1% (28)	23.1% (13)	40.0% (15)	25.0% (16)	12.5% (8)	37.5% (8)
	P6	39.5% (38)	50.0% (22)	25.0% (16)	34.8% (23)	53.8% (13)	10.0% (10)
	P7	47.4% (19)	61.5% (13)	16.7% (6)	56.3% (16)	66.7% (12)	25.0% (4)
$\dot{V}O_{2max}$ (ml·kg ⁻¹ ·min ⁻¹)	P1	25.1 ± 2.7 (32)	25.3 ± 3.2 (13)	25.0 ± 2.4 (19)	27.3 ± 2.0 (10)	27.6 ± 2.0 (6)	26.8 ± 2.3 (4)
	P2	27.8 ± 3.0 (36)	28.7 ± 3.1 (24)	26.0 ± 1.7 (12)	28.4 ± 3.7 (16)	28.2 ± 4.4 (10)	28.7 ± 2.4 (6)
	P3	30.9 ± 3.5 (38)	31.5 ± 3.4 (17)	30.4 ± 3.5 (21)	32.2 ± 4.9 (18)	34.5 ± 4.6 (10)	29.4 ± 3.7 (8)
	P4	32.0 ± 4.1 (35)	32.8 ± 4.2 (20)	31.0 ± 4.0 (15)	34.4 ± 3.9 (17)	35.2 ± 3.5 (7)	33.9 ± 4.2 (10)
	P5	34.2 ± 4.7 (33)	35.2 ± 4.5 (15)	33.3 ± 4.9 (18)	34.8 ± 5.7 (26)	37.0 ± 6.3 (12)	32.9 ± 4.6 (14)
	P6	36.3 ± 5.6 (36)	39.1 ± 5.9 (17)	33.7 ± 3.8 (19)	35.7 ± 6.1 (23)	39.2 ± 5.9 (11)	32.5 ± 4.3 (12)
	P7	38.0 ± 6.3 (30)	39.8 ± 7.4 (18)	35.4 ± 2.7 (12)	40.0 ± 5.0 (7)	44.1 ± 4.6 (3)	36.9 ± 2.3 (4)
BMI z-score	P1	0.298 ± 0.721 (33)	0.279 ± 0.692 (14)	0.312 ± 0.761 (19)	0.011 ± 1.142 (10)	0.311 ± 1.254 (6)	-0.437 ± 0.918 (4)
	P2	0.123 ± 0.828 (37)	-0.076 ± 0.715 (24)	0.491 ± 0.924 (13)	0.138 ± 1.299 (17)	0.236 ± 1.593 (10)	-0.003 ± 0.809 (7)
	P3	0.239 ± 0.701 (39)	0.343 ± 0.827 (17)	0.158 ± 0.593 (22)	0.006 ± 0.641 (20)	0.005 ± 0.572 (11)	0.008 ± 0.753 (9)
	P4	-0.045 ± 0.950 (37)	-0.059 ± 0.983 (20)	-0.029 ± 0.940 (17)	0.033 ± 0.960 (18)	-0.120 ± 1.026 (8)	0.155 ± 0.941 (10)
	P5	0.072 ± 0.956 (33)	0.029 ± 0.808 (15)	0.109 ± 1.086 (18)	0.139 ± 0.970 (26)	0.091 ± 0.783 (12)	0.180 ± 1.134 (14)
	P6	0.297 ± 0.889 (36)	0.649 ± 0.879 (17)	-0.019 ± 0.791 (19)	0.465 ± 1.278 (22)	0.808 ± 1.135 (11)	0.121 ± 1.372 (11)
	P7	0.298 ± 0.952 (31)	0.478 ± 1.032 (19)	0.013 ± 0.765 (12)	-0.557 ± 0.906 (10)	-0.572 ± 0.652 (4)	-0.547 ± 1.106 (6)

Values are means ± SD (n-value). MVPA is moderate to vigorous physical activity. Shuttle distance is given to nearest metre. Accelerometer minutes are given to nearest minute. NA = no individuals in that category. NR = not reported to prevent association of individual with their score. Note that this table only includes participants with valid measurements both before and after the intervention.

Supplementary Table S2. Baseline characteristics of participants by socioeconomic group, school and gender.

	Intervention School						Control School					
	Total		Males		Females		Total		Males		Females	
Age (years)	8.1 ± 2.0	(252)	8.2 ± 2.0	(129)	8.0 ± 1.9	(123)	8.8 ± 1.8	(127)	8.6 ± 1.8	(63)	9.0 ± 1.8	(64)
SIMD 4-5	8.1 ± 2.0	(182)	8.3 ± 2.0	(91)	7.9 ± 1.9	(91)	8.9 ± 1.8	(82)	8.9 ± 1.7	(43)	8.9 ± 1.9	(39)
SIMD 1-3	8.2 ± 2.1	(70)	8.1 ± 2.2	(38)	8.4 ± 1.9	(32)	8.6 ± 1.8	(45)	7.9 ± 1.8	(20)	9.1 ± 1.7	(25)
Daily MVPA (mins)	53 ± 22	(56)	53 ± 19	(24)	53 ± 24	(32)	56 ± 22	(62)	66 ± 19	(32)	47 ± 20	(30)
SIMD 4-5	52 ± 19	(42)	54 ± 19	(15)	51 ± 20	(27)	60 ± 22	(50)	70 ± 17	(28)	47 ± 21	(22)
SIMD 1-3	57 ± 29	(14)	52 ± 22	(9)	68 ± 41	(5)	44 ± 17	(12)	39 ± 12	(4)	46 ± 20	(8)
Daily Sedentary time (mins)	337 ± 77	(56)	327 ± 84	(24)	344 ± 71	(32)	352 ± 71	(62)	346 ± 78	(32)	360 ± 63	(30)
SIMD 4-5	337 ± 70	(42)	319 ± 71	(15)	347 ± 69	(27)	346 ± 70	(50)	338 ± 75	(28)	356 ± 64	(22)
SIMD 1-3	337 ± 97	(14)	341 ± 105	(9)	330 ± 90	(5)	378 ± 70	(12)	396 ± 86	(4)	369 ± 65	(8)
Total Shuttle Distance (m)	645 ± 351	(240)	719 ± 397	(124)	566 ± 276	(116)	722 ± 347	(117)	807 ± 400	(59)	635 ± 258	(58)
SIMD 4-5	658 ± 365	(173)	747 ± 407	(88)	565 ± 290	(85)	800 ± 359	(79)	923 ± 406	(41)	667 ± 242	(38)
SIMD 1-3	613 ± 314	(67)	651 ± 367	(36)	569 ± 237	(31)	559 ± 255	(38)	542 ± 227	(18)	574 ± 283	(20)
Sum of skinfolds (mm)	33.8 ± 12.7	(213)	31.7 ± 13.5	(115)	36.4 ± 11.2	(98)	37.6 ± 17.6	(114)	34.4 ± 17.3	(60)	41.1 ± 17.4	(54)
SIMD 4-5	32.4 ± 11.9	(154)	30.6 ± 13.4	(82)	34.5 ± 9.6	(72)	33.8 ± 15.0	(75)	28.6 ± 10.5	(42)	40.5 ± 17.3	(33)
SIMD 1-3	37.5 ± 14.0	(59)	34.3 ± 13.7	(33)	41.6 ± 13.5	(26)	44.8 ± 20.0	(39)	47.9 ± 22.3	(18)	42.1 ± 18.0	(21)
% meeting PA guidelines	26.8%	(56)	25.0%	(24)	28.1%	(32)	45.2%	(62)	62.5%	(32)	26.7%	(30)
SIMD 4-5	26.2%	(42)	26.7%	(15)	25.9%	(27)	50.0%	(50)	71.4%	(28)	22.7%	(22)
SIMD 1-3	28.6%	(14)	22.2%	(9)	40.0%	(5)	25.0%	(12)	00.0%	(4)	37.5%	(8)
% overweight or obese	16.7%	(246)	19.0%	(126)	14.2%	(120)	17.1%	(123)	17.7%	(62)	16.4%	(61)
SIMD 4-5	12.4%	(178)	14.6%	(89)	10.1%	(89)	12.3%	(81)	9.3%	(43)	15.8%	(38)
SIMD 1-3	27.9%	(68)	29.7%	(37)	25.8%	(31)	26.2%	(42)	36.8%	(19)	17.4%	(23)
$\dot{V}O_{2max}$ (ml·kg ⁻¹ ·min ⁻¹)	51.4 ± 3.2	(240)	52.2 ± 3.4	(124)	50.6 ± 2.8	(116)	51.3 ± 3.5	(117)	52.6 ± 3.3	(59)	50.1 ± 3.3	(58)
SIMD 4-5	51.6 ± 3.1	(173)	52.5 ± 3.2	(88)	50.8 ± 2.7	(85)	52.1 ± 3.4	(79)	53.3 ± 3.4	(41)	50.7 ± 3.0	(38)
SIMD 1-3	50.8 ± 3.4	(67)	51.5 ± 3.7	(36)	50.0 ± 2.7	(31)	49.8 ± 3.1	(38)	50.8 ± 2.4	(18)	48.8 ± 3.5	(20)
BMI z-score	0.180 ± 0.858	(246)	0.217 ± 0.880	(126)	0.142 ± 0.837	(120)	0.093 ± 1.052	(123)	0.178 ± 1.072	(62)	0.007 ± 1.033	(61)
SIMD 4-5	0.054 ± 0.857	(178)	0.091 ± 0.928	(89)	0.017 ± 0.784	(89)	-0.028 ± 0.929	(81)	-0.067 ± 0.826	(43)	0.015 ± 1.043	(38)
SIMD 1-3	0.511 ± 0.774	(68)	0.519 ± 0.674	(37)	0.502 ± 0.890	(31)	0.327 ± 1.235	(42)	0.730 ± 1.356	(19)	-0.006 ± 1.040	(23)

Values are means ± SD. MVPA is moderate to vigorous physical activity. After correction for gender and age at time of testing, no significant differences were observed between the schools. Shuttle distance is given to nearest metre. Accelerometer minutes are given to nearest minute. NA = no individuals in that category. Note that this table only includes participants with valid measurements both before and after the intervention.

Supplementary Table S3. Analyses to investigate the effect of introducing the Daily Mile on additional outcomes assessed immediately after the end of the intervention period for comparison with other studies.

Outcome	Difference in change between schools after correction for age, gender and age*gender			Difference in change between schools after correction for age, gender, age*gender and SIMD		
	Mean (95%CI)	SMD	p-value	Mean (95%CI)	SMD	p-value
$\dot{V}O_{2max}$ (ml·kg ⁻¹ ·min ⁻¹)	0.35 (0.10 to 0.59)	0.145	0.196	0.31 (0.07 to 0.55)	0.132	0.243
BMI z-score	-0.008 (-0.035 to 0.019)	0.030	0.785	-0.008 (-0.035 to 0.019)	0.030	0.784

SMD is standardised mean differences. SMD are calculated as change in intervention school relative to the control school as a proportion of the standard deviation of the change. 95%CI is 95% confidence interval. Analyses were conducted using GLM-ANOVA corrected for age, gender, age*gender ± SIMD with repeated measures for the outcome.

8.2. Supplementary Material Chapter Three

Lab-day interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicated that fasted baseline iGlucose values at the start of the 24-hour period were a significant predictor of iGlucose tAUC throughout the lab period ($F(1, 18.3) = 65.13$, $p < 0.001$). In contrast, the exercise conditions did not have a significant effect on iGlucose tAUC throughout the lab period ($F(2, 13.3) = 3.51$, $p = 0.060$) although the result approached the threshold of significance.

Fixed effects parameter estimates indicated that the MPA condition versus the SIT condition was significantly different for iGlucose tAUC (Estimate = 9.17, SE = 3.81, 95% CI [1.69, 16.60], $p = 0.032$). However, no significant differences were observed for the LPA condition versus the SIT condition (Estimate = 8.32, SE = 3.85, 95% CI [0.772, 15.9], $p = 0.050$), although the result approached the threshold of significance. Post hoc tests indicated no significant differences between exercise conditions (all $p > 0.05$), although the comparison of SIT versus MPA approached the threshold of significance (Difference = -9.169, SE = 3.81, $p = 0.095$). Figure 21 illustrates this relationship.

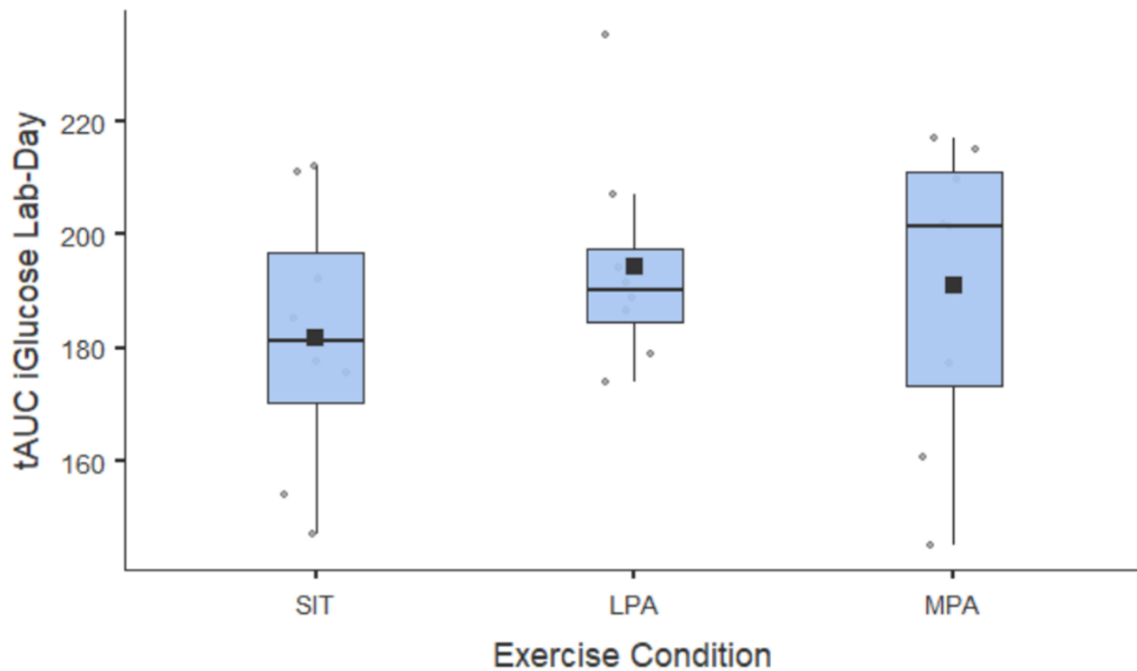


Figure 21 Box and whisker plot showing the relationship between exercise condition and interstitial glucose (iGlucose) total area under the curve (tAUC) throughout the lab-day period for the three exercise conditions: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions.

Exercise period interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicated that pre-exercise baseline iGlucose values were a significant predictor of iGlucose tAUC during the exercise period ($F(1, 19) = 7.902, p = 0.011$). In contrast, the exercise conditions did not have a significant effect on iGlucose tAUC throughout the exercise period ($F(2, 13.7) = 0.071, p = 0.932$). Fixed effects parameter estimates indicated no significant differences for exercise conditions (all $p > 0.05$). No post hoc tests were performed.

Lunch interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicated that there were no significant differences between exercise conditions ($F(2, 14) = 1.62, p = 0.234$). Additionally, fixed effects parameter estimates

indicated no significant differences for exercise conditions (all $p > 0.05$). No post hoc tests were performed.

Snack interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicated that exercise condition was a significant predictor of iGlucose tAUC for the snack period ($F(2, 14) = 5.84, p = 0.014$). Additionally, fixed effects parameter estimates indicated that MPA was significantly different than SIT (Estimate = 5.36, SE = 1.60, 95% CI [2.23, 8.49], $p = 0.005$), with MPA values being higher. Furthermore, results indicated that LPA was also significantly different than SIT (Estimate = 3.59, SE = 1.60, 95% CI [0.46, 6.72], $p = 0.041$), with LPA values being higher. However, subsequent post hoc tests indicated that only the comparison of SIT versus MPA was significantly different (Difference = -5.36, SE = 1.60, $p = 0.014$), with MPA values being significantly higher. Figure 22 illustrates this relationship. The comparison of SIT versus LPA was not significantly different (Difference = -3.59, SE = 1.60, $p = 0.083$), although the comparison approached the threshold of statistical significance.

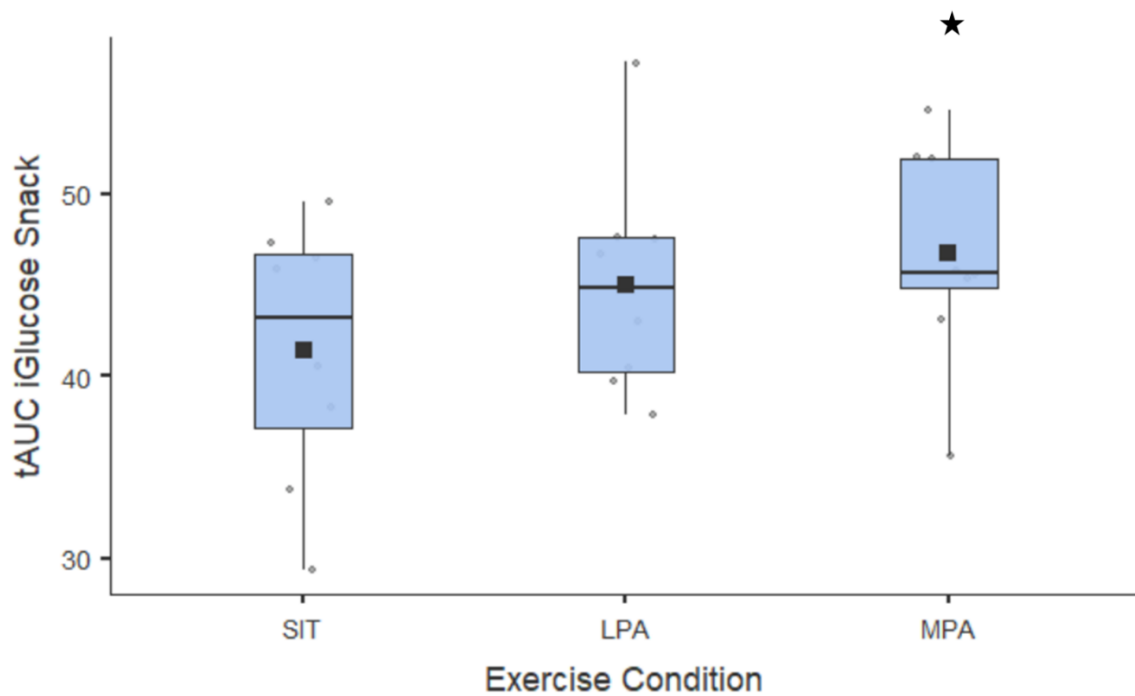


Figure 22 Box and whisker plot showing the relationship between exercise condition and interstitial glucose (iGlucose) total area under the curve (tAUC) throughout the snack period for the three exercise conditions: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions. * black star represents significantly lower values than SIT.

Dinner interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicated that there were no significant differences between exercise conditions for iGlucose tAUC over the dinner period ($F(2, 13.5) = 3.18, p = 0.074$), although the result approached the threshold of significance. However, fixed effects parameter estimates indicated that MPA was significantly different than SIT (Estimate = 6.32, SE = 2.62, 95% CI [1.81 11.46], $p = 0.031$). Post hoc tests indicated no significant differences between exercise conditions (all $p > 0.05$), although the comparison of MPA versus SIT approached the threshold of statistical significance (Difference = -6.32, SE = 2.63, $p = 0.095$). Figure 23 represents this relationship.

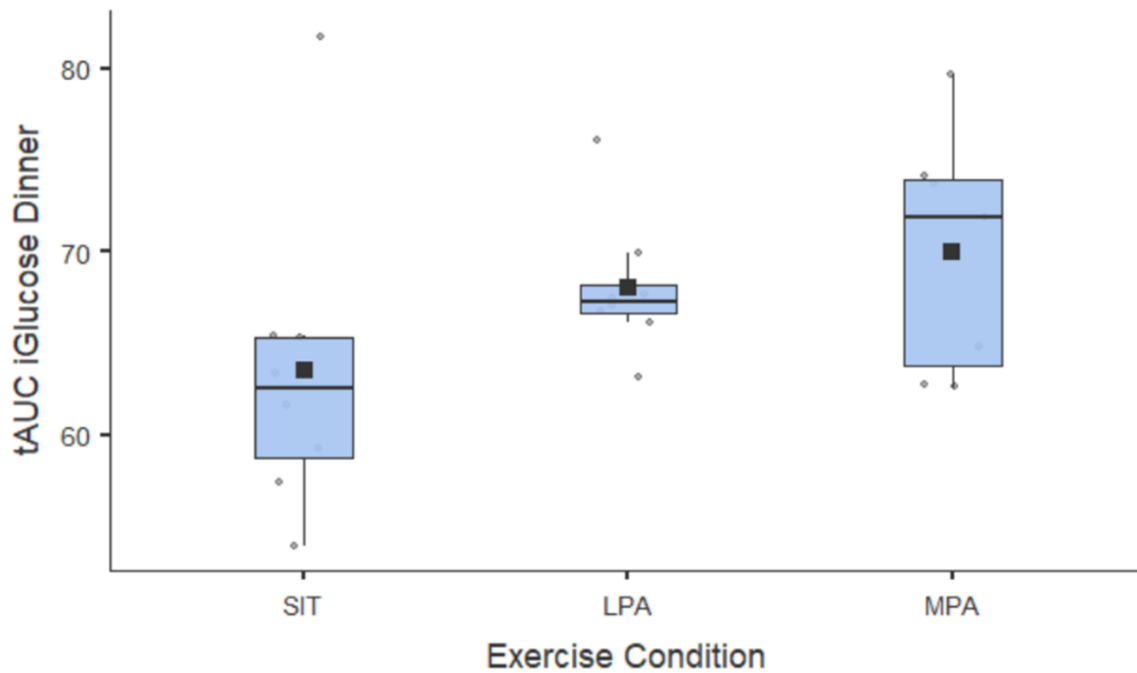


Figure 23 Box and whisker plot showing the relationship between exercise condition and interstitial glucose (iGlucose) total area under the curve (tAUC) throughout the dinner period for the three exercise conditions: no walking (SIT), light-intensity walking (LPA), and moderate-intensity walking (MPA). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions.

Sleep interstitial flash glucose monitor tAUC

Fixed effects omnibus tests revealed that exercise condition was not a significant predictor of iGlucose tAUC during sleep ($F(2, 14) = 0.403, p = 0.676$). Additionally, fixed effects parameter estimates indicated no significant differences for exercise conditions (all $p > 0.05$). No post hoc tests were performed.

8.3. Supplementary Material Chapter Four

Lab-day iGlucose and cGlucose

Lab-day iGlucose epochs can be observed within Figure 3 of Chapter 4, where significantly different iGlucose values from baseline have already been indicated in the periods between 09:15 to 11:00 and 12:15 to 13:45. In Figure 24 hourly average cGlucose tAUC values throughout the lab-day are compared between the three exercise dose groups. Fixed effects omnibus tests indicated that time of day had a significant effect on cGlucose ($F(7, 368) = 95.153, p < 0.001$). Fixed effects parameter estimates, and post hoc tests indicated that cGlucose values were significantly different from 08:45 baseline cGlucose values at 09:45, 10:45 and 12:45 (all $p < 0.05$).

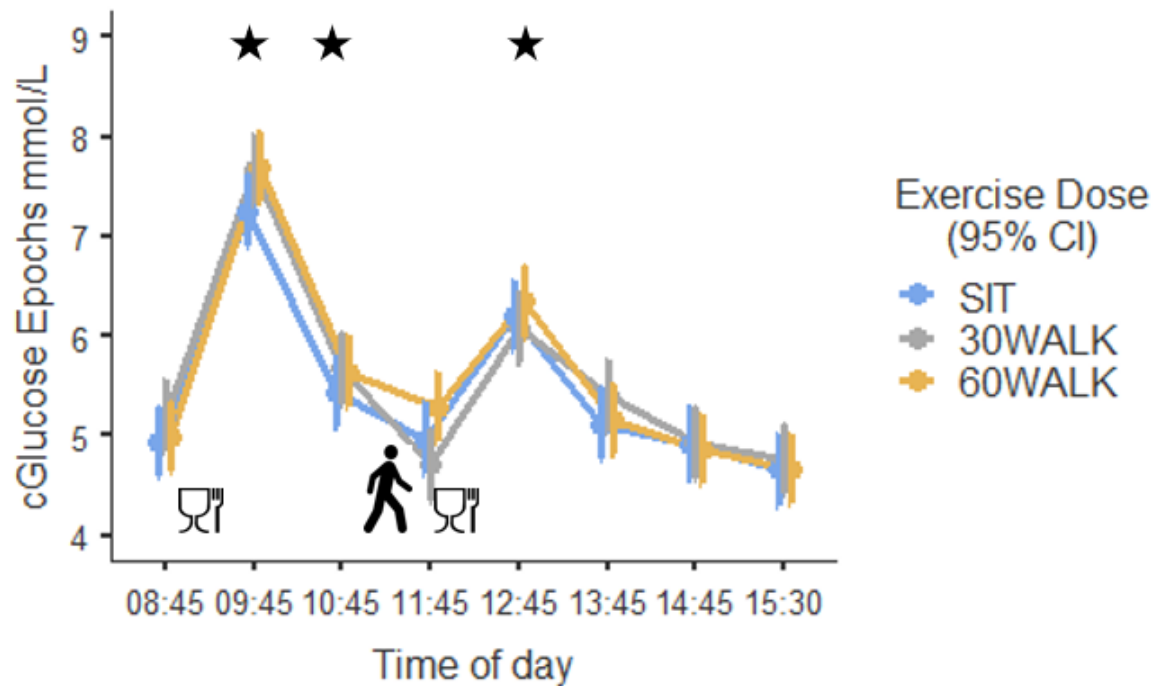


Figure 24 Average capillary glucose (cGlucose) epochs values (mmol/L) during the lab-day. The three exercise conditions are represented: 0 minutes (SIT) in blue, 30 minutes walking (30WALK) in grey, and 60 minutes walking (60WALK) in gold. The lab-day began at 08:30 and breakfast began at 09:00. The exercise condition period began at 10:45 and ended at 11:45. Subsequently, the lunch meal began at 12:00. Participants ended the lab-day at 15:30. Each time point (08:45-15:30) represents a capillary blood sample time. Meals are represented by cup and fork icons. The exercise condition period is represented by the walking person icon. Error bars represent 95% confidence intervals. * Stars represent statistically significantly different cGlucose values from baseline (08:45).

Lab-day interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicate that fasted baseline iGlucose values at the start of the lab-day were a significant predictor of iGlucose tAUC throughout the lab-day ($F(1, 28.2) = 85.638$, $p < 0.001$). No significant interaction effect was observed between exercise dose and age group ($F(2, 26.8) = 2.624$, $p = 0.091$), although it did approach the threshold of significance. Additionally, exercise dose did not reach significance ($F(2, 26.1) = 1.612$, $p = 0.219$) nor did age group ($F(1, 11.9) = 0.078$, $p = 0.789$).

Fixed effects parameter estimates indicated there was a significant difference between 60WALK versus SIT (Estimate = -5.83, SE = 2.44, 95% CI [-10.62, -1.04], $p = 0.024$). In addition, there was a significant interaction effect between 30WALK versus SIT and age group (Estimate = 7.73, SE = 3.47, 95% CI [0.93, 14.54], $p = 0.035$), with the older group having higher iGlucose for 30WALK compared to the younger group. However, the difference between 30WALK and SIT alone was not significant (Estimate = -4.49, SE = 2.34, 95% CI [-9.08, 0.09], $p = 0.066$), although it did approach the threshold of significance. No post hoc tests were performed.

Lab-day capillary glucose tAUC

Fixed effects omnibus tests indicated that fasted cGlucose baseline values at the start of the lab-day was a predictor of cGlucose throughout the lab-day ($F(1, 43.1) = 16.35$, $p < 0.001$). Exercise dose approached but did not reach the threshold of significance ($F(1, 27.7) = 3.12$, $p = 0.060$). Significance was not observed for age group ($F(1, 14.3) = 0.001$, $p = 0.974$) or the interaction of exercise dose by age group ($F(2, 26.3) = 0.25$, $p = 0.782$). Parameter estimates did not show any significance for the main outcomes of interest ($p > 0.05$). Post hoc tests were not carried out.

Exercise period interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicate that pre-exercise baseline iGlucose values were a significant predictor of iGlucose tAUC during the exercise period ($F(1, 41.5) = 46.033$, $p < 0.001$). Additionally, exercise dose was a significant predictor of tAUC ($F(2, 30.3) = 4.170$, $p = 0.025$). However, age group was not a significant predictor ($F(1, 14.8) = 0.513$, $p = 0.485$). The interaction effect between exercise dose and age group was also not significant ($F(2, 30.5) = 0.101$, $p = 0.904$).

Fixed effects parameter estimates indicated no significant differences for 30WALK versus SIT (Estimate = -1.108, SE = 0.625, 95% CI [-2.33, 0.12], $p = 0.086$) or for 60WALK versus SIT (Estimate = -1.168, SE = 0.64, 95% CI [-2.43, 0.09], $p = 0.079$) although both comparisons approached the threshold of statistical significance.

Post hoc tests indicated that SIT versus 60WALK were significantly different (Difference = 1.242, SE = 0.485, $p = 0.033$) with SIT having a higher tAUC than 60WALK. Figure 25 represents

these results. There was no significant difference between SIT versus 30WALK (Difference = 0.980, SE = 0.454, $p = 0.078$) although it approached the threshold of statistical significance.

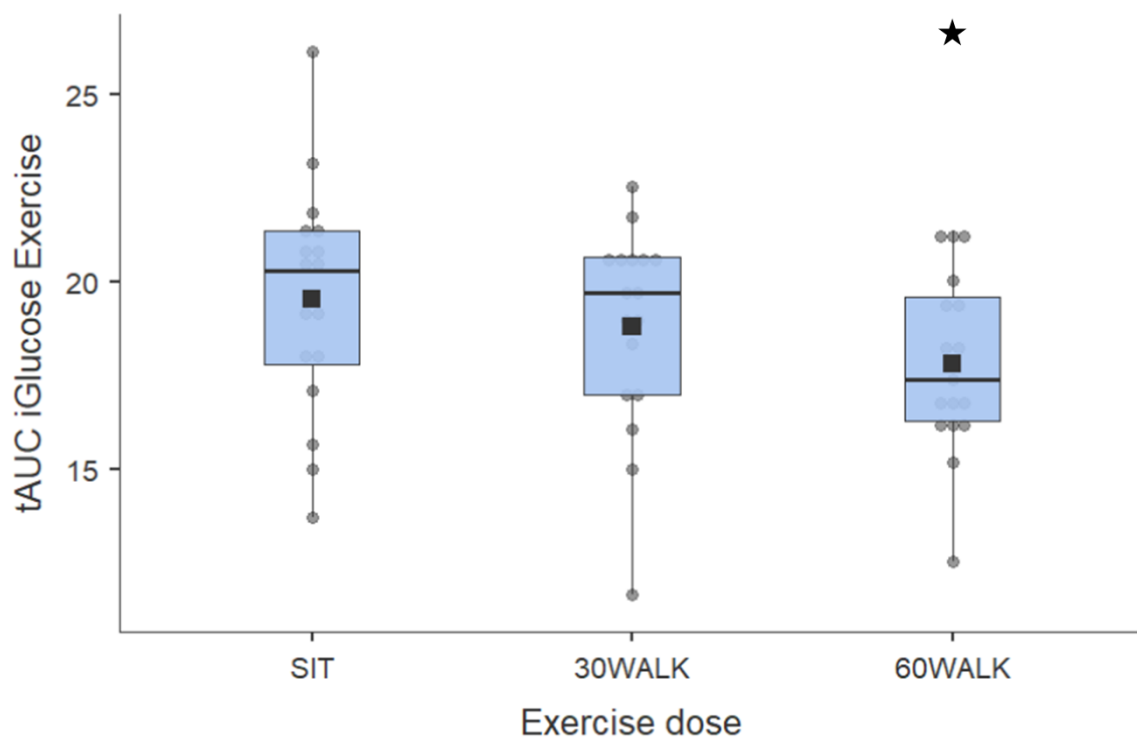


Figure 25 Box and whisker plot showing the relationship between exercise dose and interstitial glucose (iGlucose) total area under the curve (tAUC) throughout exercise period for the three exercise conditions: 0 minutes (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions. * black star represents significantly lower values for 60WALK than SIT.

Exercise period capillary glucose tAUC

Fixed effects omnibus tests indicated that cGlucose baseline values immediately before exercise was a predictor of cGlucose throughout the exercise period ($F(1, 29.2) = 182.32, p < 0.001$). Exercise dose was also a predictor of cGlucose ($F(2, 29.1) = 8.89, p < 0.001$). Significance was not observed for age group ($F(1, 13.5) = 2.11, p = 0.169$), or for the interaction of exercise dose by age group ($F(2, 28.8) = 1.09, p = 0.350$).

Fixed effects parameter estimates significant differences for 60WALK versus SIT (Estimate = 0.237, SE = 0.09, 95% CI [0.05, 0.42], $p = 0.018$), suggesting the 60-minute walking condition had higher cGlucose values compared to the sitting condition.

Post hoc analysis for exercise dose showed significantly higher cGlucose values for 60WALK versus 30WALK (Difference = -0.290, SE = 0.07, $p < 0.001$). No differences were observed between SIT versus 30WALK (Difference = 0.154, SE = 0.07, $p = 0.071$) or for SIT versus 60WALK (Difference = -0.137, SE = 0.07, $p = 0.071$), although both approached the threshold of significance. This highlights that, while parameter estimates show an overall difference between 60WALK and SIT, post hoc tests show a significant pairwise difference between 30WALK and 60WALK. Figure 26 shows the relationship between exercise dose and cGlucose tAUC throughout the exercise period.

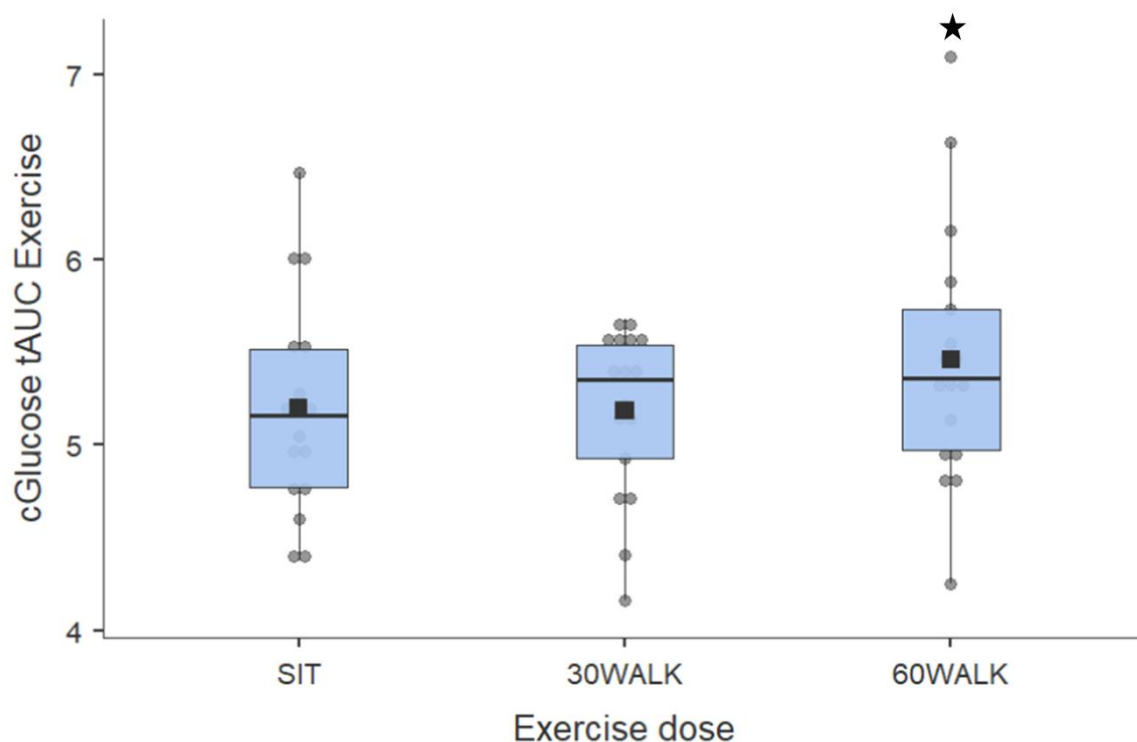


Figure 26 Box and whisker plot showing the relationship between exercise dose and capillary glucose (iGlucose) total area under the curve (tAUC) throughout exercise period for the three exercise conditions: 0 minutes (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions. * black star represents significantly lower values for 60WALK than 30WALK.

Lunch interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicate there was an interaction effect for exercise dose by age group ($F(2, 30) = 5.72, p = 0.008$). However, there was no main effect of age group alone ($F(1, 15) = 3.44, p = 0.084$) although this did approach the threshold of significance. No significant main effect was observed for exercise dose alone ($F(2, 30) = 1.29, p = 0.290$).

Fixed effects parameter estimates indicated that exercise dose for 60WALK versus SIT was significantly different (Estimate = -4.806, SE = 1.60, 95% CI [-7.94, -1.67], $p = 0.005$).

Additionally, significance was observed for the interaction effect of 30WALK versus SIT by age group (Estimate = 6.721, SE = 2.33, 95% CI [2.15, 11.29], $p = 0.007$) and for 60WALK versus SIT by age group (Estimate = 6.949, SE = 2.33, 95% CI [2.38, 11.52], $p = 0.006$). Both interaction effects indicate that the older group had a higher tAUC than the younger group for the walk conditions compared to sitting. In contrast, 30WALK versus SIT alone was not significantly different (Estimate = -2.883, SE = 1.60, 95% CI [-6.02, 0.25], $p = 0.082$) although this did approach the threshold of significance.

Post hoc comparisons indicated two interaction effects approached but did not achieve significance. These were 1) 60WALK versus 30WALK by age group (Difference = -8.633, SE = 2.80, $p = 0.072$) where the younger group had a lower tAUC for 60WALK compared to 30WALK in the older group and 2) SIT versus 60WALK for age group (Difference = 4.806, SE = 1.61, $p = 0.080$) where the younger group had a higher tAUC for SIT compared to 60WALK in the younger group. No post hoc tests for exercise dose alone were performed.

Lunch capillary glucose tAUC

To investigate the potential effects of exercise dose and age on cGlucose levels in the lunch period of the lab-day, a mixed-effects model was performed. Fixed effects omnibus tests did not indicate significant differences for exercise dose ($F(2, 30) = 0.604$, $p = 0.553$), or age group ($F(1, 15) = 1.944$, $p = 0.184$). The interaction between exercise dose and age group was also not significant ($F(2, 30) = 0.429$, $p = 0.655$). Parameter estimates showed no significant differences between any parameters. No post hoc tests were performed.

Snack interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicated that exercise dose was a significant predictor of iGlucose tAUC for the snack period ($F(2, 29.3) = 5.09$, $p = 0.013$). Additionally, the interaction effect of exercise dose by age group was significant ($F(2, 29.3) = 6.65$, $p = 0.004$). However, the main effect of age group alone was not statistically significant ($F(1, 15.1) = 2.55$, $p = 0.131$).

Fixed effects parameter estimates indicated that 60WALK versus SIT was strongly significantly different (Estimate = -9.27, SE = 2.07, 95% CI [-13.32, 5.22], $p < 0.001$). In addition, the interaction effect of 60WALK versus SIT and age group was significantly different (Estimate = 10.95, SE = 3.05, 95% CI [5.05, 16.85], $p = 0.001$). This indicates that the older group had a

higher tAUC for 60WALK when compared to the younger group. However, the effect of 30WALK versus SIT and age group was not significantly different (Estimate = 6.10, SE = 3.07, 95% CI [0.08, 12.12], $p = 0.056$) although it did approach the threshold of significance.

Post hoc comparisons for exercise dose showed that the 30WALK versus 60WALK was significantly different (Difference = 4.516, SE = 1.54, $p = 0.019$). Additionally, SIT versus 60WALK was also significantly different (Difference = 3.795, SE = 1.51, $p = 0.035$). Figure 27 illustrates this relationship.

Post hoc comparisons for the interaction effect of exercise dose by age group showed several differences. Firstly, there was a significant interaction effect between SIT versus 60WALK within the younger group (Difference = 6.944, SE = 2.15, $p = 0.040$), as well as between 30WALK versus 60WALK within the younger group (Difference = 9.272, SE = 3.35, $p = 0.002$). These results suggest that sitting resulted in a higher tAUC than 60 minutes of walking, and 30 minutes of walking had a higher tAUC than 60 minutes of walking. This indicates a dose response within the younger group, with more walking was associated with lower iGlucose tAUC values. In addition, a significant interaction effect was observed for 60WALK versus 30WALK between younger and older groups (Difference = -11.932, SE = 3.35, $p = 0.020$). This indicates that younger adults had a lower iGlucose tAUC for 60WALK than older adults compared to 30WALK. One other interaction effect, comparing 60WALK between younger and older adults, approached the threshold of significance but did not reach it (Difference = -9.844, SE = 3.35, $p = 0.081$). These relationships are illustrated by Figure 28.

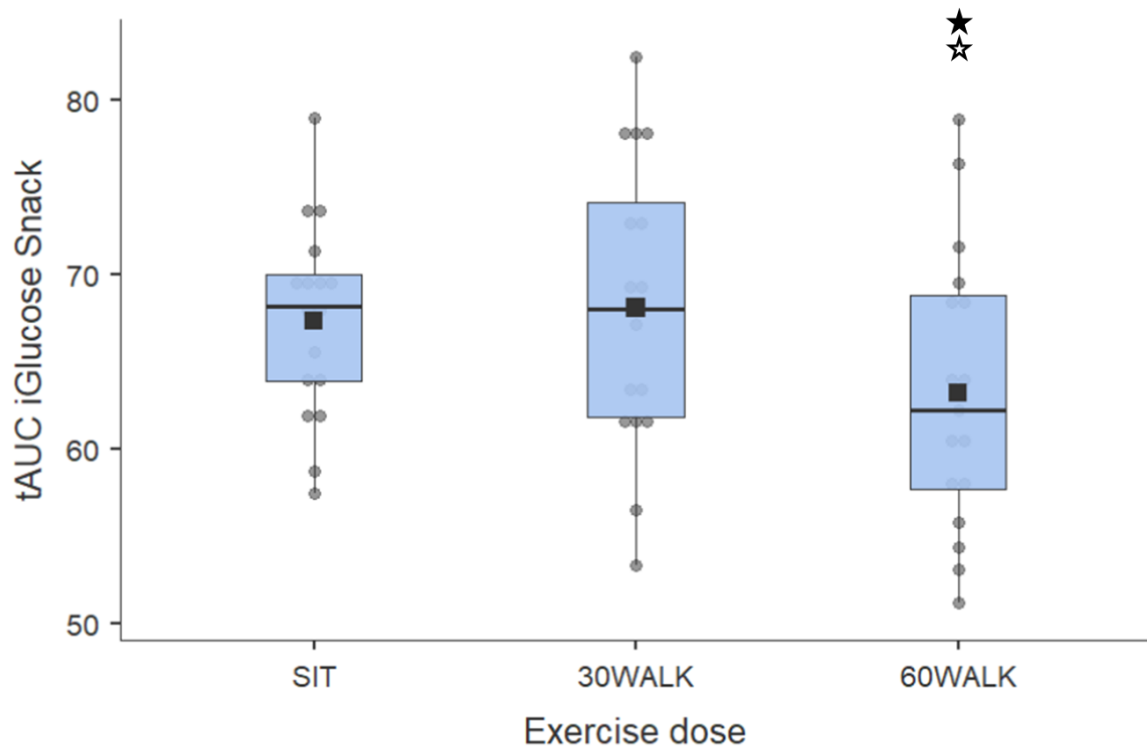


Figure 27 Box and whisker plot showing the relationship between exercise dose and interstitial glucose (iGlucose) total area under the curve (tAUC) after snack for the three exercise conditions: 0 minutes (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions. * black star represents significantly lower values for 60WALK than SIT. The empty * star represents significantly lower values for 60WALK than 30WALK.

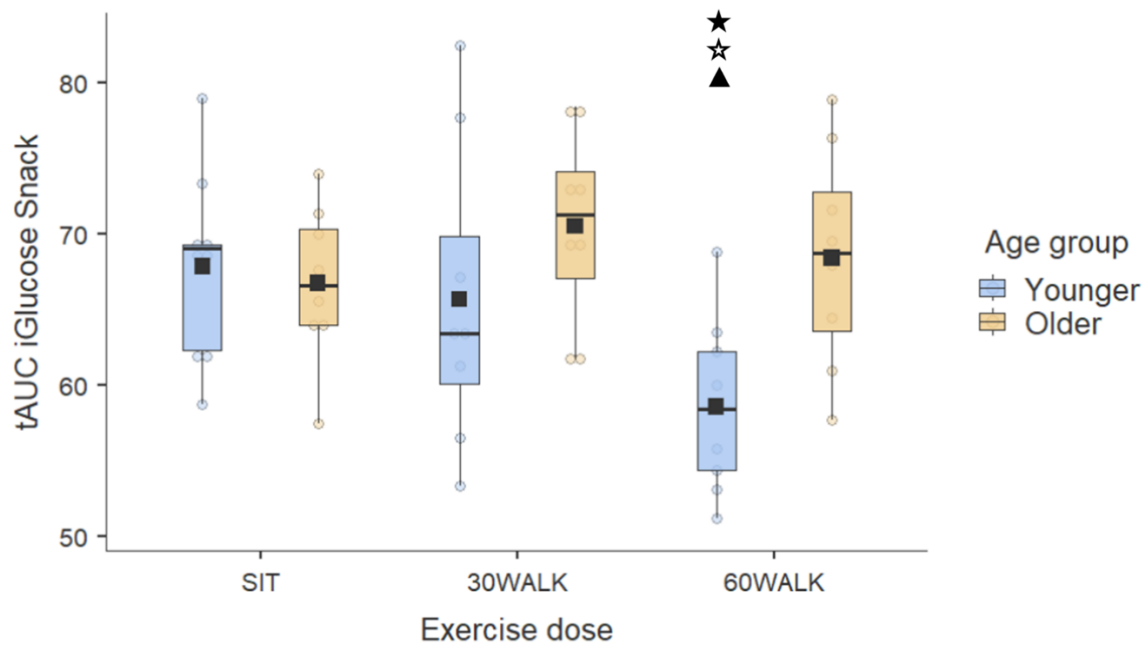


Figure 28 Box and whisker plot showing the relationship between exercise dose and age group for interstitial glucose (iGlucose) total area under the curve (tAUC) during snack for the three exercise conditions: 0 minutes (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). The black triangle represents significantly lower 60WALK values for younger adults compared to older adults at 30WALK. The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's iGlucose tAUC during the respective exercise conditions colour coded by age. * black star represents significantly lower 60WALK values than SIT within the younger group. The empty * star represents significantly lower 60WALK values than 30WALK within the younger group. Δ Triangle represents significantly lower 60WALK versus 30WALK between younger and older groups.

Dinner interstitial flash glucose monitor tAUC

Fixed effects omnibus tests indicated that body mass index (BMI) is a significant predictor of iGlucose tAUC ($F(1, 12.8) = 5.431, p = 0.037$). Additionally, age group was also a significant predictor ($F(1, 11.8) = 14.279, p = 0.003$). However, results indicate that exercise dose was not a significant predictor of iGlucose ($F(2, 22.9) = 2.766, p = 0.084$), although this approached

the threshold of significance. The interaction effect of exercise dose by age group was not significant ($F(2, 22.9) = 0.387, p = 0.684$).

Fixed effects parameter estimates indicated that BMI is a significant predictor of iGlucose tAUC (Estimate = -0.440, SE = 0.19, 95% CI [-0.81, -0.07], $p = 0.037$). Additionally, significant differences were observed for 60WALK versus SIT (Estimate = -4.524, SE = 2.12, 95% CI [-8.68, -0.36], $p = 0.044$). Furthermore, significant differences were observed for age group (Estimate = 6.620, SE = 2.71, 95% CI [1.31, 11.93], $p = 0.021$).

Sleep interstitial flash glucose monitor tAUC

Fixed effects omnibus tests revealed that age group was not a significant predictor of iGlucose tAUC for sleep ($F(2, 24.1) = 3.77, p = 0.075$), although it did approach the threshold of significance. The main effect of exercise dose was not significant ($F(2, 24.1) = 1.99, p = 0.169$). Additionally, the interaction effect between exercise dose and age group was not significant ($F(2, 24.1) = 1.73, p = 0.198$).

Fixed effects parameter estimates indicated that the effect of 60WALK versus SIT was significantly different (Estimate = -14.63, SE = 5.85, 95% CI [-26.10, -3.17], $p = 0.020$). No significant difference was observed for the interaction effect between 60WALK versus SIT and age group (Estimate = 15.42, SE = 8.56, 95% CI [-1.36, 32.21], $p = 0.084$) although this approached the threshold of significance.

Exercise period capillary total cholesterol tAUC

Fixed effects omnibus tests indicated that total cholesterol baseline values immediately before exercise were a predictor of total cholesterol throughout the exercise period ($F(1, 17.7) = 698.896, p < 0.001$). Age group approached but did not reach the threshold for significance ($F(1, 14.3) = 4.139, p = 0.061$). Significance was not observed for exercise dose ($F(2, 30.3) = 0.925, p = 0.407$) or for the interaction of exercise dose and age group ($F(2, 29.8) = 0.700, p = 0.505$). Other than baseline total cholesterol, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). No post hoc tests were performed.

Exercise period capillary HDL cholesterol tAUC

Fixed effects omnibus tests indicated that HDL cholesterol baseline values immediately before exercise were a predictor of HDL cholesterol throughout the exercise period ($F(1, 44) =$

895.298, $p < 0.001$). No significant differences were observed for exercise dose ($F(2, 44) = 0.468$, $p = 0.629$), nor age group ($F(1, 44) = 0.701$, $p = 0.407$), nor the interaction of exercise dose and age group ($F(2, 44) = 0.658$, $p = 0.523$). Other than baseline HDL cholesterol, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). No post hoc tests were performed.

Exercise period capillary LDL cholesterol tAUC

Fixed effects omnibus tests indicated that LDL baseline values immediately before exercise were a predictor of LDL throughout the lab-day ($F(1, 44) = 2206.613$, $p < 0.001$). Significant differences were not observed for age group exercise dose ($F(2, 44) = 0.582$, $p = 0.563$), or for age group ($F(1, 44) = 2.737$, $p = 0.105$) nor the interaction between exercise dose and age group ($F(2, 44) = 0.025$, $p = 0.975$). Other than baseline LDL cholesterol, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). No post hoc tests were performed.

Exercise-period capillary triglycerides tAUC

Fixed effects omnibus tests indicated that triglyceride baseline values immediately before exercise were a predictor of triglyceride throughout the exercise period ($F(1, 28) = 3947.161$, $p < 0.001$). Significance was not observed for exercise dose ($F(2, 28.9) = 2.022$, $p = 0.151$) or for age group ($F(1, 13.6) = 0.831$, $p = 0.378$). No significant interaction effect was observed between exercise dose and age group ($F(2, 29) = 0.307$, $P = 0.738$). Other than baseline triglyceride values, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). No post hoc tests were performed.

Post-exercise period capillary total cholesterol tAUC

Fixed effects omnibus tests indicated that BMI was a predictor of total cholesterol throughout the post-exercise period ($F(1, 14) = 5.911$, $p = 0.029$). Significant differences were not observed for exercise dose ($F(2, 32) = 1.949$, $p = 0.159$) or for age group ($F(1, 14) = 0.369$, $p = 0.553$). Other than baseline total cholesterol, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). No post hoc tests were carried out.

Post-exercise period capillary HDL cholesterol tAUC

Fixed effects omnibus tests indicated that exercise dose was a predictor of HDL cholesterol throughout the post-exercise period ($F(2, 30) = 3.666, p = 0.038$). Significant differences were not observed between age groups (Estimate (1, 15) = 0.034, $p = 0.856$) or for the interaction of exercise dose and age group (Estimate (2, 30) = 0.870, $p = 0.429$). Figure 29 shows the relationship between exercise dose and HDL tAUC during the post-exercise period. Other than baseline HDL cholesterol, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). Post hoc analysis for exercise dose showed significantly higher HDL values for 30WALK versus SIT (Difference = -0.323, SE = 0.12, $p = 0.040$).

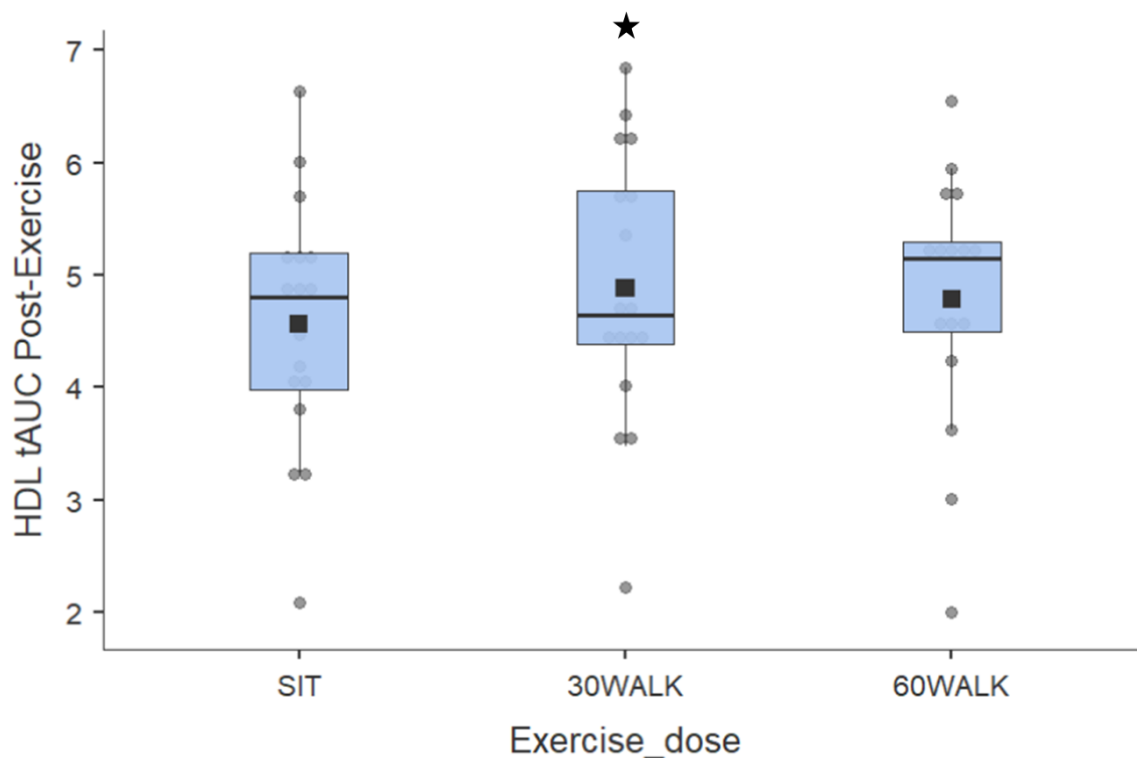


Figure 29 Box and whisker plot showing the relationship between exercise dose and HDL cholesterol tAUC total area under the curve (tAUC) throughout the post-exercise period for the three exercise conditions: 0 minutes (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's HDL cholesterol tAUC during the exercise conditions. * black star represents significantly higher values for 30WALK than SIT.

Post exercise period capillary LDL cholesterol tAUC

Fixed effects omnibus tests indicated no significant differences for exercise dose ($F(2, 30) = 1.511, p = 0.237$), or for age group ($F(1, 15) = 1.431, p = 0.250$). Additionally, no significant interaction effects were observed between exercise dose and age group ($F(2, 30) = 0.100, p = 0.905$). Fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). No post hoc tests were performed.

Post-exercise period capillary triglycerides tAUC

Fixed effects omnibus tests indicated that BMI was a predictor of triglycerides throughout the post exercise ($F(1, 14) = 5.520, p = 0.034$). No significant differences were observed for exercise dose ($F(2, 30) = 0.064, p = 0.938$), nor age group ($F(1, 14) = 0.012, p = 0.914$). Additionally, no significant difference was observed for the interaction effect of exercise dose and age group ($F(2, 30) = 0.218, p = 0.805$). Other than BMI, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). No post hoc tests were performed.

Exercise period systolic blood pressure tAUC

Fixed effects omnibus tests indicated that pre-exercise SBP values were a predictor of SBP during the exercise period ($F(1, 44) = 895.396, p < 0.001$). However, no significant differences were observed for exercise dose ($F(2, 44) = 2.358, p = 0.106$) or for age group ($F(1, 44) = 0.169, p = 0.683$). Similarly, there were no significant interaction effects for exercise dose compared to age group ($F(2, 44) = 0.406, p = 0.669$). Other than pre-exercise SBP, fixed effects parameter estimates showed no significant differences between parameters (all $p > 0.05$). The effect of 30WALK versus SIT approached the threshold of significance but did not achieve significance (Estimate 2.763, SE = 1.44, 95% CI [-0.07, 5.59], $p = 0.062$). Post hoc tests were not performed.

Exercise period diastolic blood pressure tAUC

Fixed effects omnibus tests indicated that pre-exercise DBP values were a predictor of DBP during the exercise period ($F(1, 16.5) = 602.987, p < 0.001$). However, exercise dose was not a predictor of DBP ($F(2, 26.4) = 1.459, p = 0.251$), nor was age group ($F(1, 11.7) = 2.024, p = 0.181$). The interaction effect of exercise dose and age group was also not significant ($F(2,$

26.8) = 0.984, $p = 0.387$). Other than baseline DBP, fixed effects parameter estimates indicated no differences between the remaining parameters (all $p > 0.05$). No post hoc tests were performed.

Post-exercise period systolic blood pressure tAUC

Fixed effects omnibus tests indicated that exercise dose was a significant predictor of SBP in the post-exercise period ($F(2, 30) = 3.50$, $p = 0.043$). Additionally, gender was also a predictor of SBP ($F(1, 14) = 10.03$, $p = 0.007$). However, age group approached the threshold of significance ($F(1, 14) = 3.76$, $p = 0.073$) but did not achieve it. There were no significant interaction effects between exercise dose and age group ($F(2, 30) = 1.76$, $p = 0.189$).

Fixed effects parameter estimates indicated gender was a significant predictor of SBP during the post-exercise period (Estimate = 60.54, SE = 19.12, 95% CI [23.07, 98.01], $p = 0.007$). None of the other parameters were significantly different (all $p > 0.05$). However, the effect of age group approached the threshold for significance but did not reach it (Estimate = 39.88, SE 19.93, 95% CI [0.82, 78.94], $p = 0.062$). Post hoc tests indicated that 30WALK versus 60WALK was significantly different with 60WALK having a lower tAUC (Difference = 12.61, SE = 4.88, $p = 0.045$). Figure 30 illustrates the relationship between SBP and exercise dose.

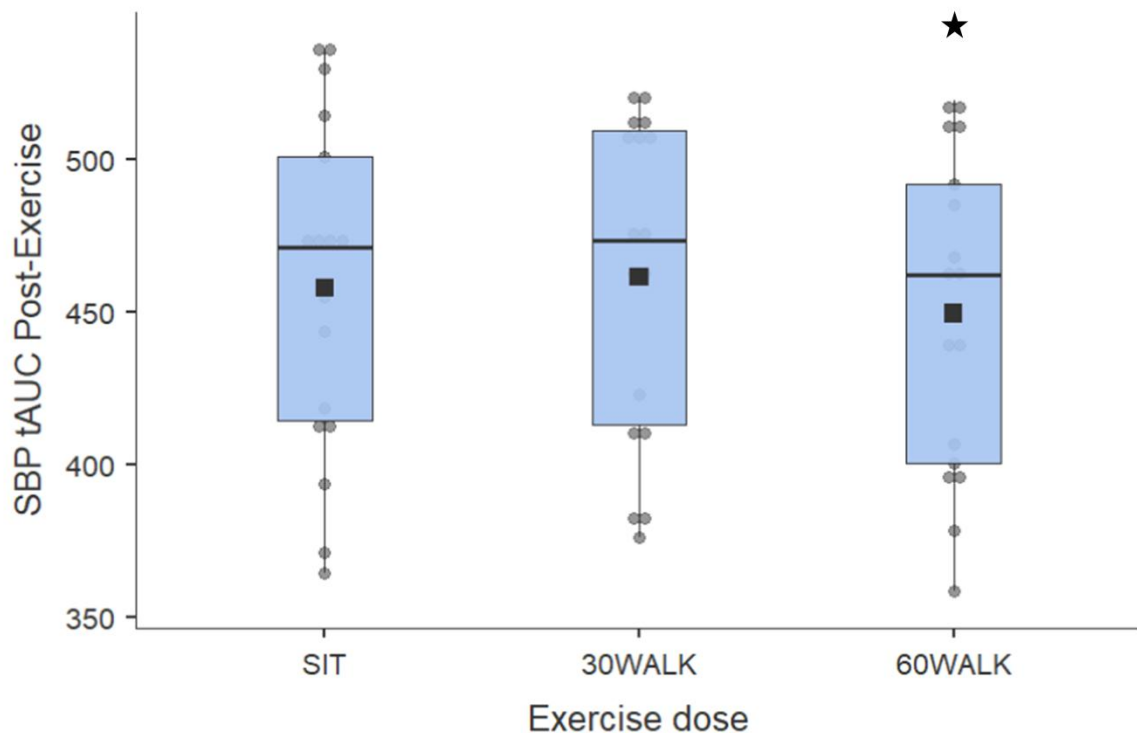


Figure 30 Box and whisker plot showing the relationship between exercise dose and systolic blood pressure (SBP, mm Hg) tAUC total area under the curve (tAUC) during the post-exercise period for the three exercise conditions: 0 minutes (SIT), 30 minutes walking (30WALK) and 60 minutes walking (60WALK). The black horizontal line inside each blue box represents the median, inner filled black boxes represent the mean, the outer boxes represent the interquartile range, and the whiskers extend to the minimum and maximum values not considered outliers. Individual dots represent each subject's SBP tAUC during the post-exercise period. * black star represents significantly lower values for 60WALK than 30WALK.

Post exercise period diastolic blood pressure tAUC

Fixed effects omnibus tests indicated that BMI was a significant predictor of DBP in the post-exercise period ($F(1, 14) = 6.326, p = 0.025$). The effect of age group approached the threshold but did not reach significance ($F(1, 14) = 4.526, p = 0.052$). The interaction effect of exercise dose and age group also approached the threshold but did not reach significance ($F(2, 30) = 3.113, p = 0.059$). However, exercise dose alone was not a predictor of DBP ($F(2, 30) = 0.857, p = 0.434$).

Other than BMI, fixed effects parameter estimates indicated age group was also a significant predictor of DBP (Estimate = 38.88, SE = 15.03, 95% CI [9.42, 68.35], $p = 0.020$). The interaction effect of 60WALK versus SIT and age group was also a significant predictor of DBP (estimate = -15.19, SE = 6.10, 95% CI [-27.14, -3.25], $p = 0.018$). No post hoc tests were performed.