

**An exploration into the effects of prolonged sitting on
cardiovascular health and the influence of modifiable
lifestyle factors**

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Abstract

Time spent in sedentary behaviours, particularly prolonged sitting, has been identified as an independent risk factor for cardiovascular disease (CVD). However, the physiological mechanisms by which prolonged sitting contributes to CVD burden and what can be done to offset that burden remains unclear. Whilst a working model currently exists, further work is needed. In order to progress the working model, it is first necessary to consolidate the existing literature to identify the likely effect of prolonged sitting on the cardiovascular system. Identifying the impact of prolonged sitting is complicated by the fact that this behaviour is likely to cluster with other modifiable lifestyle behaviours, the most prominent of which are fat consumption, habitual physical activity, and cardiorespiratory fitness. Understanding into how these modifiable lifestyle factors may impact the cardiovascular system in conjunction with prolonged sitting is vital for the development of a robust biologically plausible model. As such, this thesis has two overarching aims: 1) to consolidate the existing evidence related to prolonged sitting, with and without interruption, and certain markers of cardiovascular health and function using systematic review and meta-analytic practices, and 2) to conduct experimental studies to investigate the interactions of prominent modifiable lifestyle factors on cardiovascular responses to bouts of prolonged sitting. This thesis demonstrated that: 1) acute bouts of prolonged uninterrupted sitting negatively impact cardiovascular function, but regularly interrupting bouts of prolonged sitting, particularly with aerobic activities, may offset these negative effects, and, 2) whilst cardiorespiratory fitness and habitual physical activity do not impact the cardiovascular responses to an acute sitting bout, the combined deleterious effects of prolonged sitting and consumption of a high-fat meal can be offset using regular interruptions. Collectively, this thesis identified a robust mechanism by which prolonged sitting contributes to cardiovascular burden, and further, this thesis offers insight into strategies to mitigate cardiovascular dysfunction. Finally, this thesis identified several methodological practices within the research area which should be improved.

Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Gloucestershire and is original except where indicated by specific reference in the text.

No part of this thesis has been submitted as part of any other academic award. The thesis has not been presented to any other education institution in the United Kingdom or overseas.

Any views expressed in the thesis are those of the author and in no way represent those of the University.

Signed:

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Publications and Presentations

Thesis Publications

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2. **Paterson C**, Fryer S, Zieff G, Stone K, Credeur DP, Barone Gibbs B, Padilla J, Parker JK, Stoner L. The Effects of Acute Exposure to Prolonged Sitting, With and Without Interruption, on Vascular Function Among Adults: A Meta-analysis. *Sports Med* 2020;50:1929–42. doi:10.1007/s40279-020-01325-5

Non-Thesis Publications

1. Fryer S, **Paterson C**, Stoner L, Brown MA, Faulkner J, Turner LA, Aguirre-Betolaza AM, Zieff G, Stone K. Leg Fidgeting Improves Executive Function following Prolonged Sitting with a Typical Western Meal: A Randomized, Controlled Cross-Over Trial. *Int J Environ Res Public Health* 2022;19:1357. doi:10.3390/ijerph19031357
2. Stone K, Fryer S, Faulkner J, Meyer ML, Heffernan K, Kucharska-Newton A, Zieff G, **Paterson C**, Masushita K, Hughes TM, Tanaka H, Stoner L. Associations of lower-limb atherosclerosis and arteriosclerosis with cardiovascular risk factors and disease in older adults: the atherosclerosis risk in communities (ARIC) study. *Atherosclerosis* 2022;340:53–60
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- between-day reliability. *Hypertens Res* 2021;:1–3. doi:10.1038/s41440-021-00712-3
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2. Adams NT, Poles J, **Paterson C**, Stoner L. The Time-Course of Blood Pressure Change in Adults During Prolonged Sitting: A Three-Level Meta-Analysis. American College of Sports Medicine. San Diego, CA. May 31, 2022.
3. Adams NT, Poles J, **Paterson C**, Stoner L. (2022). The Time-Course of Blood Pressure Change in Adults During Prolonged Sitting: A Three-Level Meta-Analysis. American College of Sports Medicine Southeast Regional Conference. Greenville, SC. Feb 2022.
4. Elliott D, Whitley G, Zieff G, Stone K, **Paterson C**, Fryer S, Diana J, Blackwell J, Stoner L. Reliability of Heart-Temple Pulse-Wave Velocity Assessments Derived From a Simple Photoplethysmography Device. American College of Sports Medicine Southeast Regional Conference. Greenville, SC. Feb, 2022.
5. Perks J, Zaccardi F, **Paterson C**, Houghton JS, Nickinson AT, Pepper CJ, Yates T, Sayers R. Effect of high-pain versus low-pain structured exercise on walking ability in people with intermittent claudication: A systematic review and network meta-analysis. American Heart Association Scientific Sessions. Boston, MA. Nov, 2021.
6. Adams NT, Poles J, Zieff G, Stone K, **Paterson C**, Fryer S, Meyer ML, Stoner L. Agreement of non-invasive blood pressure- and standard oscillometry-derived pulse wave velocities. ARTERY. Paris, France. Oct 21, 2021.
7. Zieff G, Stone K, **Paterson C**, Fryer S, Diana J, Blackwell J, Meyer ML, Stoner L. Photoplethysmography Pulse Wave Velocity: Reliability and Agreement with a Referent Device. SEACSM Regional Chapter Meeting. (Virtual format), 2021.
8. **Paterson C**, Giles D, Bird E, Stone K, Perkins IC, Potter JA, Willems MET, Fryer S. (2020). The effect of New Zealand blackcurrant extract on forearm performance

- and oxidative capacity in elite rock climbers. European Congress of Sports Science (ECSS). Seville, Spain. July 1-3, 2020 – Event cancelled due to Covid-19 pandemic.
9. Fanney L, Kelch E, Burnet K, Zieff G, Credeur DP, Stone K, **Paterson C**, Fryer S, Stoner L. (2020). Validation of heart-femoral pulse wave velocity as a measure of central arterial stiffness. American College of Sports Medicine (ACSM). San Francisco, CA, USA. May 26 – May 30, 2020 – Event cancelled due to Covid-19 pandemic.
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1

Introduction

Cardiovascular diseases (CVD)s are a range of disorders that affect the cardiovascular system, principally, the heart and the vascular system [1]. Currently, CVD represents the leading cause of death globally and is a leading cause of disability across all age groups and thus represents a substantial public health challenge [2]. Indeed, previous estimates suggest that CVD cost the UK economy nearly £30 billion annually between health care costs, productivity losses, and care-related costs [3]. Given the enormous public health burden that CVD creates, research has focused on identifying potential risk factors for the development of CVD which are typically separated into modifiable and non-modifiable categories. A prominent risk factor which has been thoroughly investigated is physical activity. Indeed, physical activity research dates back to the 1950s [4] and, at the time of writing, PubMed lists over 116,000 articles related to physical activity. Decades of research have identified that increasing physical activity is a cost-effective mean of improving health [5–7] and is associated with a decreased risk of not only CVD [8,9], but also hypertension [10], diabetes [11], and certain types of cancer [12]. Indeed, physical activity has been referred to as the “best buy” in public health [13]. The culmination of this research was the implementation of physical activity guidelines in 1996 [14] which have since been frequently updated and refined, leading to the current iteration of the World Health Organization (WHO) guidelines released in 2020 [15]. However, despite the volume of evidence regarding the health benefits of physical activity, and calls from the WHO to increase physical activity, levels of participation remain relatively low worldwide [16]. Thus, given the continued burden of CVD worldwide and the apparently limited effectiveness of attempts to increase physical activity as a means of offsetting that burden, research has begun to focus on other risk factors that may help to mitigate CVD risk.

One risk factor which is closely aligned with physical activity but remains in its relative infancy is sedentary behaviour. Sedentary behaviours are defined as any waking behaviour in a seated, reclined, or lying posture with a very low energy expenditure (≤ 1.5 metabolic equivalents) [17]. Large scale meta-analyses have identified that sedentary behaviour, rather than being synonymous with physical inactivity, represents a biologically distinct, independent risk factor for CVD and all-cause mortality [18–20]. Indeed, a landmark study by Ekelund et al., [18] identified that individuals who accrued the greatest amount of daily sedentary time (> 8 h·day⁻¹) would need to achieve 60 – 75 minutes of moderate intensity physical activity per day (more than double current WHO guidelines [15]) to offset the risk posed by sedentary time. These findings are particularly problematic as sedentary behaviours comprised a large portion of people’s waking hours worldwide, with ~ 60 - 80 % of waking hours spent sedentary [21–32]. Owing to the importance of sedentary behaviour as a CVD risk factor, the most recent WHO guidelines now feature recommendations to reduce time spent in sedentary behaviours and replace that time

with light intensity physical activity [15]. However, in contrast to the well-defined physical activity guidelines, the sedentary behaviour guidelines are vague and non-specific. Physical activity guidelines conform to the FITT principle (Frequency, Intensity, Time, and Type) allowing for effective dissemination and translation. Further, varying physical activity guidelines that conform to the FITT principle are available for different age demographics and populations. For example, physical activity recommendations for adults (18 – 64 yrs) are recommended to undertake 150 – 300 mins of moderate intensity, or 75 – 150 mins vigorous intensity physical activity per week, contrasted with pregnant women who are recommended to achieve at least 150 mins of moderate intensity physical activity, and children (5 – 17 yrs) are recommended to achieve 60 minutes of moderate-vigorous intensity physical activity per day [15]. By contrast, all the aforementioned demographics and populations receive the same advice regarding sedentary behaviour, i.e., reduce sedentary time and replace those activities with light intensity physical activity [15]. The lack of specificity for sedentary behaviour guidelines is likely due to the relatively limited evidence available compared to physical activity. Indeed, physical activity research has developed a biologically plausible model whereby physical activity levels influence health outcomes and research has shown a dose-response between physical activity levels and health outcomes [33,34]. To improve the evidence related to sedentary behaviour and to improve the specificity of future guidelines and recommendations, the WHO issued a call to researchers to develop a biologically plausible model whereby acute bouts of sedentary behaviour may contribute to CVD risk, which can subsequently be tested to investigate whether a dose-response exists [35,36].

Prior to the call by the WHO, and building on epidemiological evidence suggesting an association between increased sedentary time and CVD, many researchers had begun investigating the physiological effects of acute bouts of sedentary behaviour. These experimental studies have largely utilised bouts of prolonged uninterrupted sitting (uninterrupted sitting period \geq 60 mins) as an experimental model of sedentary behaviour. Sitting represents a ubiquitous behaviour in modernised societies and accounts for a large portion of sedentary time [21–23,37]. Experimental prolonged sitting studies have utilised several markers of cardiovascular health and function, including, but not limited to, vascular function assessed using flow-mediated dilation, peripheral blood pressure, and segmental measures of arterial stiffness using pulse wave velocity. Contemporary research in this area suggests that prolonged uninterrupted bouts of prolonged sitting may have detrimental effects on markers of cardiometabolic and cardiovascular health and function, including impaired endothelial function [38–51], impaired glucose and lipid metabolism [52,53], impaired cognition and cerebral blood flow [54,55], increased blood pressure [47,48,56–58], and increased arterial stiffness [59,60]. Whilst the long-term impact of these acute changes

is yet unknown, it is conceivable that repeated exposure to this negative phenotype may contribute to repeated and prolonged periods of increased cardiovascular burden which in turn may increase the risk of developing CVD in the future. Owing to the relative infancy of this research area, there have been divergent findings and research practices with no synthesis of the existing literature being performed. However, a working model of how sitting may contribute to cardiovascular burden and ultimately CVD risk now exists [61]. This model, discussed in greater detail within the next chapter, at its simplest, suggests that increased venous pooling in the lower limbs during a bout of sitting, as a consequence of reduced muscle activity, results in reduced venous return and as such reduced stroke volume. This reduction in stroke volume is thought to result in reduced shear stress within the aorta which then presents as increased aortic stiffness as a subclinical marker of increased cardiovascular dysfunction. In order to test this model, it is necessary to consolidate the current evidence of sitting-induced cardiovascular dysfunction and discern what the likely effect of prolonged sitting is on certain markers of cardiovascular health and function. By reviewing the existing evidence, it will also be possible to identify and evaluate current methodological practices that may be collectively hampering the research area. By identifying divergent practices, it may be possible to streamline future experimental studies towards fully realising a biologically plausible model and answer the call of the WHO.

A challenge particular to the sedentary behaviour research area is the fact that sedentary behaviours comprise a large portion of our waking hours in many countries. In contrast to bouts of physical activity which are typically planned and discrete, sedentary behaviour is likely to cluster with other lifestyle behaviours that may influence cardiovascular responses to prolonged sitting [61]. Thus, understanding the interaction of lifestyle behaviours and prolonged sitting may aid in our understanding of the impact of sedentary behaviour on the cardiovascular system and how we may offset any deleterious effects. For example, work by our research group has demonstrated that the combination of prolonged sitting with consumption of a typical high-fat Westernised meal results in greater increases in carotid-femoral pulse wave velocity compared to sitting combined with a low-fat Westernised control meal [62]. Whilst previous research has shown that interrupting sitting with stair-climbing sprints may offset the combined effect of prolonged sitting and a high-fat Westernised meal [63], this interruption is likely to be impractical for most settings and inaccessible for many. It may be possible to utilise the mechanisms contained within a biologically plausible model to develop effective sitting interruption strategies. Thus there is a need to develop a biologically plausible model to not only identify causes for disease but also to facilitate the development of accessible and efficacious sitting interruption strategies.

A further lifestyle factor that is necessary to explore is that of habitual physical activity and cardiorespiratory fitness. For example, increased habitual physical activity is known to attenuate sympathetic nervous system outflow and improve cardiovascular regulation [64,65]. Taken together, these factors may result in individuals of differing physical activity levels having different responses to an acute bout of prolonged sitting. Thus, understanding the interaction between certain lifestyle factors and cardiovascular responses to sitting may identify confounders that need to be considered for future large randomised controlled trials which will ultimately test any biologically plausible model and whether a dose response between sedentary behaviour and CVD exists.

Whilst it is beyond the scope of this thesis to fully develop a working model of how acute bouts of sedentary behaviour may contribute to CVD, and investigate every potential lifestyle factor that may influence such responses, it is possible to synthesise the existing literature to explore the plausibility of the current working model. Further, it is also possible to investigate how certain pertinent modifiable lifestyle factors may influence cardiovascular responses to prolonged sitting and evaluate what such findings mean within the context of the existing working model. To achieve these aims, several objectives have been identified:

- 1a)** to determine the effect of uninterrupted sitting on vascular function, with subgroup analysis to identify whether responses differ by artery
- 1b)** to determine the effect of regularly interrupting sitting on vascular function, with subgroup analysis to determine whether responses differed by interruption strategy
- 2a)** to determine the effect of uninterrupted sitting on peripheral blood pressure, with subgroup analysis to determine whether age of participants influence the observed result
- 2b)** to determine the effect of regularly interrupting sitting on peripheral blood pressure, with subgroup analysis to determine whether responses differ by interruption strategy
- 3)** consolidate the existing evidence regarding pulse wave velocity and prolonged sitting, with and without interruption
- 4)** to determine whether a simple desk-based leg fidgeting strategy is sufficient to confer a protective effect against the combined detrimental effects of a high-fat Westernised meal and prolonged sitting on cardiovascular health
- 5)** to investigate the association between cardiorespiratory fitness and habitual physical activity versus the changes in markers of central and peripheral blood pressure and pulse wave velocity

2

Literature Review

2.1 Prevalence of Cardiovascular Disease

Cardiovascular diseases (CVD)s are a group of disorders that affect the heart and vascular system [1]. In 2019, CVDs resulted in 18.6 million deaths worldwide, making CVDs the leading cause of death globally [2]. According to the Global Burden of Disease statistics, CVDs have represented the greatest cause of death globally since 1990 [2]. In the United Kingdom, CVD represents the greatest cause of death among both men and women and an estimated 6.5 million people are living with diagnosed CVD [66]. It should be noted that whilst the number of deaths attributed to CVD has decreased slightly in the previous decade, the number of disability-adjusted life years has increased and thus CVD represents a significant public health concern [66]. Indeed, it was estimated in 2006 that CVD cost the National Health Service nearly £16 billion representing a fifth of the Service's total expenditure, with this figure expected to rise [3]. Given the enormous cost and years of life lost as a result of CVD, it is important to be able to determine what risk factors can and cannot be modified in order to inform clear public health messages. Risk factors for CVD can be categorised into “modifiable” and “non-modifiable” risk factors which are briefly outlined below.

2.1.2 Non-Modifiable Risk Factors of Cardiovascular Disease

Non-modifiable risk factors are those factors known to influence the risk of developing CVD but cannot be modified. Such factors include; age, race, and family history of CVD. Age is the strongest predictor of CVD owing to morphological changes in arterial structure as well as extended exposure to other risk factors causing further burden, e.g., older individuals may experience high blood pressure, or high blood cholesterol (two risk factors of CVD) for a longer duration leading to the onset of CVD [67–71]. Race has generally been accepted as a non-modifiable risk factor, however, it is yet to be seen whether the associations between race and CVD are the result of genetic differences or social inequality [72]. Family history of CVD is a strong predictor of CVD with previous work suggesting that a paternal or sibling history of CVD increases the risk of developing CVD between 50 and 100 % [73–76]. There is also evidence to suggest that these associations may be moderated by sex [77]. Indeed, whilst sex has consistently been reported as a non-modifiable risk factor for CVD [78], emerging evidence from the Framingham Heart Study suggests that CVD and its time course may simply present differently between sexes [79]. This notion is supported by data from the Global Burden

of Disease study which suggests a similar level of prevalence between sexes in the UK and globally [66].

2.1.3 Modifiable Risk Factors of Cardiovascular Disease

Recent findings from the Prospective Urban Rural Epidemiology (PURE) study suggest that in excess of 70 % of CVD cases and deaths in a multinational cohort of 155,722 participants across 21 countries can be attributed to modifiable risk factors [80]. Modifiable risk factors are typically clustered as being metabolic, behavioural, psychosocial, and environmental.

Concerning metabolic risk factors, these may include, but not limited to, obesity, hypertension, abnormal lipid profiles, and type II diabetes mellitus (T2DM). Obesity, typically defined as a body mass index (BMI) of $\geq 30 \text{ kg}\cdot\text{m}^2$ [81] is associated with increased risk of developing CVD [82] as well as being associated with increased incidence of T2DM and abnormal blood lipids [83,84]. This constellation of metabolic risk factors is often referred to as metabolic syndrome and is a strong predictor of future CVD incidence [83]. Hypertension, that is persistently elevated blood pressure (systolic $>140 \text{ mmHg}$, diastolic $> 90 \text{ mmHg}$ [85]), is another component of metabolic syndrome but is also highly prevalent and often undiagnosed in large portions of the population with younger adults (18 - 31 years) being 33 % less likely to receive a correct diagnosis of hypertension compared to older adults (> 60 years) [83,86]. This is particularly concerning as hypertension is a typically asymptomatic condition which strongly predicts incidence of future CVD [2].

Psychosocial risk factors of CVD comprise a broad range of elements including, but not limited to, depression, anxiety, chronic and acute mental stress, and education level. Depression and anxiety, amongst other similar conditions, have been shown to exacerbate CVD risk in populations with and without pre-existing CVD [87–89]. Chronic stress is perhaps one of the most widely studied CVD risk factors with persistent evidence suggesting a link between CVD and elevated levels of chronic stress despite the heterogeneity in study design, measurement of stress, and sample demographics [90–94]. Additionally, extreme acute stress has demonstrated a strong link with rapid onset of CVD [95–97]. Number of years in education, sometimes used as a surrogate measure of socioeconomic status, have been shown to be associated with an increased risk of CVD

in countries of all income levels [80,98–100]. It should be noted that most evidence showing the association between modifiable risk factors and CVD originate from higher-income regions, particularly from North America, Western Europe, and China. This may be particularly problematic when considering some psychosocial risk factors but especially environmental risk factors that include consideration of household and ambient air pollution [101,102]. The income level of countries is likely to have a major influence on certain risk factors such as education level and air pollution and thus should be considered in a broader context [80].

2.1.4 Behavioural Risk Factors

Behavioural risk factors include habits such as tobacco use, nutrition, sodium intake, physical activity, and sedentary behaviour. Tobacco use is a well-established independent risk factor for CVD with evidence suggesting that it may have a multiplicative effect on other risk factors such as elevated plasma lipids and T2DM [103]. Gratefully, smoking prevalence within the United Kingdom appears to be declining in line with major public health campaigns and policies with the total number of smoking adults (> 18 years) reducing by ~30 % between 2011 and 2019 [104,105], however, the effect of regular tobacco use on CVD progression and all-cause mortality is still a cause for concern. Further, the increased use of electronic vaping cigarettes in the absence of traditional tobacco use may still pose a risk to cardiovascular health [106].

A number of nutritional factors are also associated with risk of CVD. Research suggests that increased consumption of saturated fat is associated with an increased risk of CVD, however it is important to consider saturated fat intake in the broader sense of total dietary intake [107]. Previous reviews have shown that isocaloric substitution of saturated fat with refined starches and added sugars conferred no change in CVD risk [108]. However, it should also be noted that evidence concerning carbohydrate intake and CVD risk is less clear, with foods of varying glycaemic index appearing to have no effect on CVD risk [109]. Additionally, increased intake of fruit and vegetables appears to be associated with a reduced risk of CVD [110,111] whilst increased sodium intake is well correlated with both increased CVD risk and hypertension [112,113].

2.2 Physical Activity and Sedentary Behaviour

Physical activity is defined as any bodily movement produced by skeletal muscles that requires energy expenditure and can be classified by intensity (e.g., light, moderate, and vigorous). Current national and international physical activity guidelines recommend at least 150-300 minutes of moderate intensity or 75-150 minutes of vigorous intensity physical activity per week for maximum benefit in adults aged 18 - 64 years [15,114-116]. There is substantial evidence that increased physical activity is associated with reduced risk of CVD, as well as obesity, T2DM, and all-cause mortality [15]. As an extension to this research area, recent work has begun to explore the effects of failing to reach physical activity guidelines, i.e., physical inactivity, and sedentary behaviour.

Evidence suggests that the importance of physical activity for health has been known for thousands of years, with texts ranging from 2600 BC in Eastern cultures, and 400 BC in Western cultures. As knowledge has grown over the centuries, we have begun to understand the mechanisms involved and why regular physical activity and structured exercise should represent important parts of people's lives. With the growing interest in the benefits of physical activity, so came the investigation into the effects that physical inactivity posed to health.

The earliest rigorous scientific exploration of the association between physical inactivity and negative health outcomes was carried out by Morris et al., [4]. This pioneering work was the first to demonstrate that individuals in less physically active jobs (bus drivers) were more at risk of developing coronary heart disease than their more occupationally-active colleagues (bus conductors). This research is believed to be the genesis of physical activity epidemiological research paving the way for future investigations [117]. Further work continued to demonstrate a clear link between occupational physical inactivity and negative health outcomes [118,119]. In time, investigations turned towards exploring leisure-time physical activities and the potential effects on long-term health. Work by Morris et al. [120] and Paffenbarger et al., [121] demonstrated that increased total physical activity, encompassing occupation and leisure-time physical activity, was inversely related to negative health outcomes such as coronary heart disease and myocardial infarction. These early investigations also suggested that higher intensity physical activity may confer more of a protective effect than lower intensity activities.

Throughout the latter half of the 20th century, evidence continued to build suggesting that physical activity may offer protective effects against obesity [122], some types of cancer [123], and CVD [124]. In time, the weight of this evidence became the cornerstone for both national and international physical activity guidelines [15,114,115]. Until the turn of the 20th century, physical activity and sedentariness were viewed as opposite ends of the same spectrum. To that end, physical inactivity and sedentariness were used and viewed interchangeably within the research area [125].

In 2000, Owen et al., [126] were the first to propose that sedentary behaviours may be a separate entity to physical activity. Owen et al., [126] proposed that sedentary behaviours were those behaviours where energy expenditure was not significantly greater than resting levels and where the individual was either sitting or lying down. The notion of sedentary behaviours and physical in/activity being separate was not immediately embraced and researchers continued to use sedentariness as an analogous term for physical inactivity [125]. It was not until twelve years after Owen et al., [126] proposed their definition that an accepted definition detailing energy expenditure (<1.5 metabolic equivalents [METs]) and posture (sitting or lying) from the Sedentary Behaviour Research Network (2012) was proposed and more widely adopted. This definition was subsequently updated to include a reclining posture [17]. By extension, the term “physically inactive” came to refer to an individual who failed to meet physical activity guidelines. With these definitions it was clear that an individual could be physically active (i.e., meets physical activity guidelines) but also highly sedentary (i.e., spending large periods of time in sedentary behaviours).

Further evidence suggests that sedentary behaviour and physical activity have independent impacts on health. Growing evidence indicates that increased time spent in sedentary behaviours is associated with increased CVD incidence and all-cause mortality, as well as being associated with incidence of cardiometabolic diseases, such as T2DM [18–20,34,128–133]. Sedentary behaviour, in particular sitting, has garnered significant attention due in no small part to its prevalence in modern life within economically-developed countries. Observational data from multiple economically developed countries report that a significant portion of adults’ waking hours are spent in sedentary behaviours, predominantly sitting [21–32]. Sitting is a particularly problematic sedentary behaviour as it is omnipresent in many people’s lives and forms an

unconscious part of many daily activities such as working, driving, and watching television [37]. Lab-based studies investigating the effects of prolonged sitting, that is a bout of sitting equal to or greater than 30 mins in duration, have examined many different factors relating to health, from musculoskeletal dysfunction and pain to feelings of wakefulness and focus, as well as cardiovascular dysfunction.

Given the historical context, prevalence and continued increase in CVD-related pathologies, it is pertinent to understand the structure and function of a healthy cardiovascular system and how sedentary behaviour may negatively affect the system and thus contribute to CVD risk.

2.3 Cardiovascular System

The cardiovascular system is comprised of the heart, blood, and blood vessels (arteries and veins) and serves to connect and supply all bodily tissues with the necessary nutrients. The heart serves as a pump to deliver blood carrying nutrients to tissues via the arterial system and is then returned to the heart via the venous system.

2.3.2 Artery Structure and Function

The vascular system is comprised of an arterial and venous side, however, the fundamental structure of vessels on either side, with the exception of capillaries and venules, is similar, comprising of three layers; the tunica intima, tunica media, and tunica externa (or adventitia) (**Figure 2.1**). The innermost layer, the tunica intima, is comprised of the endothelium, which will be discussed in greater detail later in [this Chapter](#), and a connective tissue basement membrane. The middle tunica media layer consists of smooth muscle, collagen, and elastin. Finally, the outermost layer, the tunica externa, is comprised principally of connective tissue. The relative thickness of each layer depends on the blood vessel type.

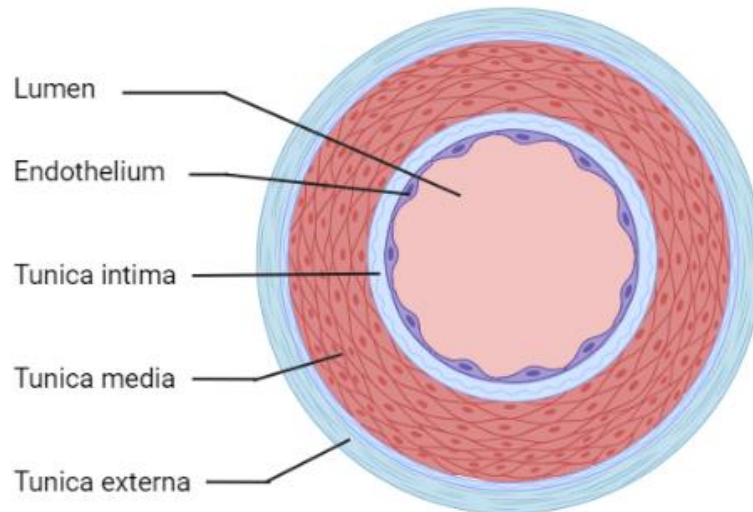


Figure 2.1. Artery structure

The arterial system is comprised of large elastic arteries, and medium to small muscular arteries. The thickness and structure of the tunica intima differs very little between vessel types. By contrast, the relative thickness and structure of the tunica media and externa change to reflect the differing roles of various arteries. In large elastic arteries, such as the aorta, the tunica media is comprised of a greater proportion of elastic tissue and a lower proportion of vascular smooth muscle cells (VSMC) and collagen. By contrast, medium and small muscular arteries feature greater proportions of VSMC and collagen and reduced elastic tissues. The variation in structure is owed to the differing roles of and stresses experienced by each artery type.

Owing to the greater proportion of VSMC and collagen in the tunica media, medium and small arteries are stiffer and serve as distributing arteries [134], able to partially regulate blood supply to different regions of the body [135]. By contrast, large elastic arteries represent the capacitive side of the arterial system [136]. During systole, approximately 50 % of the stroke volume is momentarily stored in the aorta and other elastic arteries, causing them to distend [137]. In a healthy arterial system, approximately 10 % of the energy produced by the heart during contraction is diverted to the distension of these vessels [138]. The energy is then used to recoil the elastic arteries during diastole. This pressure dampening action, known as the Windkessel effect, ensures a smooth flow of blood around the body [139].

For the Windkessel effect to be maximally efficient, the energy needed to distend and recoil the elastic arteries needs to be as low as possible [137]. This efficiency is largely dependent on the viscoelastic properties of the vessel walls as well as their geometry, principally their length and diameter [137,138]. The viscoelastic properties of arteries can be described in terms of compliance, stiffness, and distensibility. Compliance is expressed as $C = \Delta V / \Delta P$, where ΔV describes a change in volume for a given change in pressure (ΔP) and is a marker of an artery's ability to store volume and buffer changes in pressure throughout the cardiac cycle [137,140]. Stiffness is the reciprocal value of compliance ($\text{Stiffness} = \Delta P / \Delta V$) and represents the slope of the pressure-volume relationship [137,138]. Distensibility, expressed as $\Delta V / V / \Delta P$, where V is the initial volume, describes the relative change in volume for a change in pressure and represents the mechanical load placed on the vessel wall [137,140]. Owing to the vessel wall structure the pressure-volume relationship in elastic arteries is non-linear. At lower distending pressures, the strain is borne by elastic tissues, whereas at higher pressures, strain moves to the stronger but less elastic collagen fibres making the artery stiffer [136,137].

In a normal arterial system, the central arteries are more distensible compared to peripheral arteries, creating a physiologically advantageous stiffness gradient [136]. To better contextualise this phenomenon, it is useful to consider pulse wave velocity (PWV), a measure of arterial stiffness. Pulse wave velocity relates to the transmission of energy down the arterial tree and represents the time taken for a pulse wave to travel, or propagate, from a proximal to a distal point within the vascular system [137]. Pulse wave velocity (typically expressed as $\text{m}\cdot\text{s}^{-1}$) travels down the arterial tree significantly faster than blood flows throughout the tree (a measure usually expressed in $\text{cm}\cdot\text{s}^{-1}$). During systole, the left ventricle ejects blood into the ascending aorta pushing forward the blood already present and increasing pressure within the proximal portion of vessel. The increase in pressure in the aorta creates a pressure gradient whereby the proximal pressure (closer to the heart) is greater than downstream distal portions. This pressure gradient (or pulse wave) rapidly travels down the arterial tree shunting blood forwards and making space for the subsequent stroke volume [136–139].

As a pulse wave travels forward, through the elastic to the peripheral muscular arteries, it is partially reflected by changes in arterial geometry and elasticity, creating a

backwards traveling wave [141]. A measured pressure wave therefore is the summation of both the forward traveling (incident) wave (Pf) and reflected wave (Pb) (**Figure 2.2**) [136]. The timing of the reflected wave can influence several important factors, including coronary perfusion. In a healthy vascular system with the aforementioned stiffness gradient, PWV is significantly lower in the elastic arteries, and greater in the peripheral muscular arteries [136,142]. This stiffness gradient ensures that the reflected wave arrives back at the aorta during diastole causing an increase in diastolic pressure which aids coronary perfusion without increasing left ventricular afterload [136] (**Figure 2.3**). By contrast, if central arterial stiffness increases, either transiently or chronically, the timing of the reflected wave can be augmented and arrives sooner, during systole. In this scenario, owing to the summation of the pressure waves, aortic systolic pressure increases whilst diastolic pressure decreases [136]. The increase in aortic systolic pressure increases left ventricular afterload, increasing myocardial oxygen demand, whilst the decrease in diastolic pressure inhibits coronary perfusion during diastole [136].

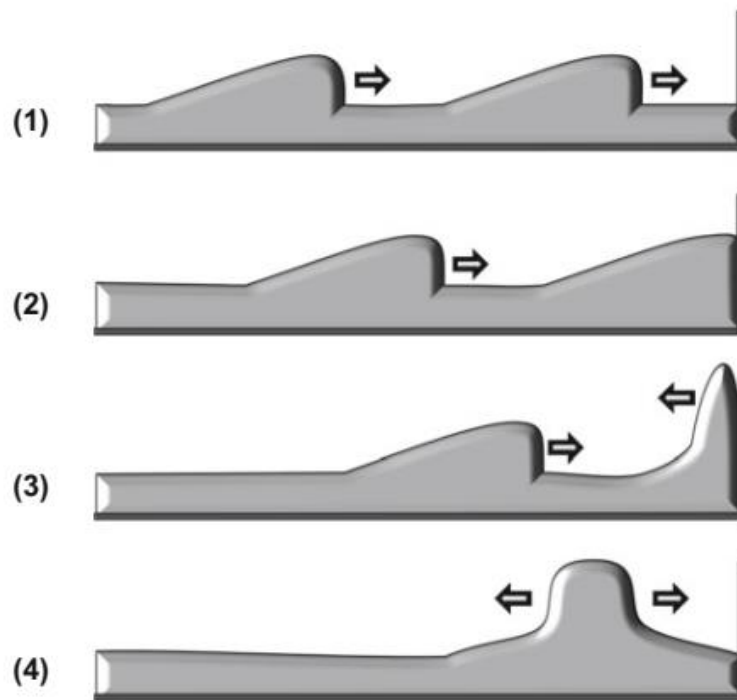


Figure 2.2 Illustrative diagram of the superimposition of forward and backward travelling waves. (1) two forward travelling waves approach a reflection point before (2) the first wave arrives at the reflection point. (3) the initial forward travelling wave becomes a reflected wave and changes directions before (4) augmenting the second forward travelling wave to create a larger overall wave. Within the context of the arterial system, reflection points consist of changes in artery geometry such as bifurcations or transitions from elastic to muscular arteries. Adapted from Salvi, 2017 [143].

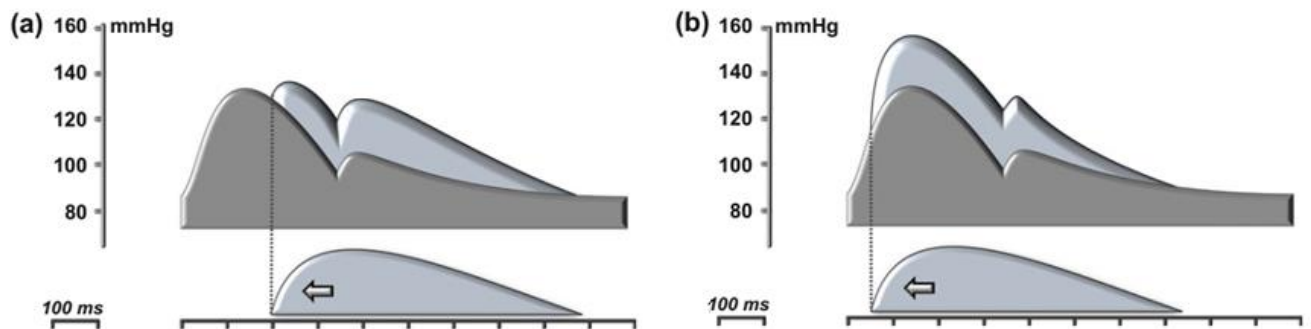


Figure 2.3 Illustrative diagram of superimposition of backward travelling waves (light grey) on forward waves (dark grey). (a) Assuming a heart rate of 60 bpm in a healthy elastic aorta ($PWV = 5 \text{ m}\cdot\text{s}^{-1}$), the backward travelling wave arrives 200 ms after left ventricular contraction, resulting in the superimposition of pressure waves occurring during late systole and lasting almost the entire diastolic phase thus facilitating coronary perfusion. Further, the superimposition of forward and backwards waves has little effect on the observed blood pressure. (b) Assuming a heart rate of 60 bpm in a stiffened aorta ($PWV = 20 \text{ m}\cdot\text{s}^{-1}$), the backward travelling wave arrives 50 ms after left ventricular contraction, resulting the superimposition of pressure waves occurring early in systole. This early arrival reduces diastolic pressure, hindering coronary perfusion whilst augmenting the observed pressure to a higher overall value. Adapted from Salvi, 2017 [143].

Further, the increase in stiffness can result in increased pulsatile pressure reaching the microcirculation, particularly in high-flow organs such as the brain and kidneys, causing damage [144,145]. In high flow organs such as the brain and kidneys, blood flow is controlled by vascular tone and in the presence of increased perfusion pressure, the local arteries and arterioles constrict to maintain a constant flow [146]. In the presence of chronic elevated pulse pressure, these smaller vessels can undergo morphological changes fostering increased stiffness in order to offset the high pressure [147]. This change limits flow in order to lessen risk of damage from pulsatile pressure but increases the risk of ischemic episodes whereby the target organ receives an insufficient blood supply [146,148]. Also noteworthy that the increased pulsatile strain can result in [endothelial activation](#) [149,150], potentially leading to further issues.

Similarly, chronic increases in blood pressure and stiffness may result in morphological changes in elastic arteries. It is important to note that arterial stiffness and blood pressure are interdependent, and it can be difficult to separate the two components. In the presence of increased blood pressure, potentially resulting from an increase in elastic

artery stiffness, circumferential strain shifts from the elastic fibres to less distensible collagen fibres [151]. The shift to less distensible collagen fibres can cause transient increases in stiffness, however, chronic elevation of blood pressure, i.e., hypertension, can cause morphological changes to artery structure, chronically increasing stiffness [151]. It should be noted that morphological changes to artery structure whereby the central elastic arteries become stiffer occurs naturally with increasing age [142,152,153]. However, hypertension and therefore increased arterial stiffness in earlier life may exacerbate this process. Further, hypertension, is associated with both an increase in oxidative stress as well as an impairment of endothelium function [154,155]. These factors may combine to increase the risk of atherosclerotic plaques developing within arteries [156].

2.3.3 Endothelium

The endothelium is a monocellular layer of epithelial cells that lines the entire vascular system. The endothelium, and its healthy function, is integral to the maintenance of the vascular system and homeostasis. The endothelium fulfils the role of a receptor-effector structure, responding to different physical and chemical stimuli to release a large variety of different molecules which may serve as either agonists and antagonists, moderating vascular tone, thrombotic process, and maintaining homeostasis [157]. Endothelial cell function is influenced by two distinct haemodynamic forces; circumferential stress and shear stress [158,159] (**Figure 2.4**). Circumferential stress (**Figure 2.4A**) acts perpendicular to the vessel wall, applying stress to all layers of the vessel. By contrast, shear stress (**Figure 2.4B**) is the tangential force created by blood flowing past the vessel wall and thus only influences the innermost layer, the endothelium. Shear stress is thought to be the primary stimulus for endothelial cell function [160]. If shear stress is reduced or disturbed, endothelial dysfunction can occur which can present as increased arterial stiffness [161,162].

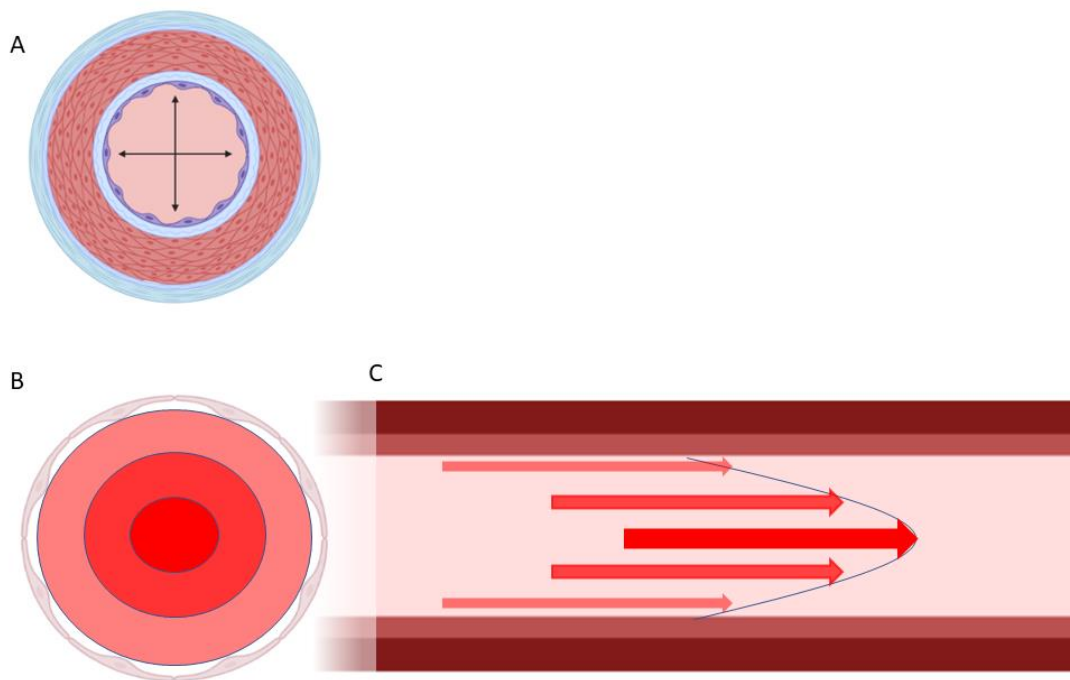


Figure 2.4 A) Circumferential stress within the artery whereby force is exerted outwards, applying stress to all layers of the artery. Circumferential stress is primarily related to variation within in pulse pressure within the vessel. B) Shear stress is created by the flow of blood cells against the endothelium. The darkening concentric circles represent the changing flow velocity within the vessel. As the red blood cells flow through the vessel, a natural velocity gradient occurs whereby flow is fastest in the centre of the lumen and gradually slows closer to the vessel wall. C) The flow velocity profiles are presented as a parabola with the same colour coding as B and represent the optimum laminar flow within the vessel facilitating proper endothelial function. Shear stress can become disturbed by arterial tortuosity or atherosclerotic deposits on the vessel wall, leading to endothelial dysfunction.

The endothelium helps to maintain both vaso-dilation and -constriction via the release of several molecules, most notably, nitric oxide (NO). Nitric oxide is expressed in response to increase in shear stress against the endothelium [163–165]. Increases in shear stress are sensed by mechanotransducers in the endothelium causing an influx of calcium ions (Ca^{2+}) into the endothelial cells [166]. This increase in Ca^{2+} stimulates the Ca^{2+} -dependent enzyme endothelial-NO synthase (eNOS) to react with the amino acid L-Arginine, ultimately synthesising NO [167]. Nitric oxide diffuses into the sub-endothelial space and enters the VSMC below [168]. Nitric oxide then binds to vascular smooth muscle cell guanylate cyclase, increasing cyclic guanosine monophosphate (cGMP) and activating cGMP-dependent protein kinases, leading to phosphorylation of potassium (K^+) channels

and consecutive hyperpolarisation and extrusion of Ca^{2+} ions, resulting in VSMC relaxation [169–171].

In addition to NO, the endothelium also influences vasodilation via the release of prostacyclin (PGI_2). Similarly to NO synthesis, the production of PGI_2 is initially stimulated by an increased intracellular Ca^{2+} [172]. Increased Ca^{2+} activates phospholipase A_2 which in turn liberates arachidonic acid from membrane-bound lipids [173]. Once liberated, arachidonic acid is metabolised by cyclo-oxygenase via two steps; an initial oxygenase step whereby prostaglandin (PG) G_2 is formed, and a subsequent peroxidase step which forms PGH_2 from PGG_2 . PGH_2 then serves as the substrate for prostacyclin synthetase, ultimately producing PGI_2 [173]. PGI_2 then binds to cell surface prostacyclin (IP) receptors, resulting in G-protein-mediated activation of adenylate cyclase, prompting formation of cyclic adenosine monophosphate (cAMP) and subsequent phosphorylation of protein kinase A and an ultimate reduction of Ca^{2+} in target cells [174].

It has been suggested that another pathway, or pathways, may exist which facilitate endothelium-dependent dilation, independent of the established NO and PGI_2 pathways [175]. Initially this unknown mechanism was termed endothelium-derived hyperpolarising factor (EDHF), however, subsequent research has identified that several other mechanisms contribute to endothelium-dependent vasodilation [176]. Largely these additional mechanisms are similarly activated by an influx of Ca^{2+} and may serve as back-up systems when the primary vasodilator, NO, is inhibited although there is still debate in this area [177,178]. It should be noted however, that the NO pathway still appears to be the greatest contributor to the maintenance of vascular tone in healthy individuals [179]. Also, of note, the effects of NO and PGI_2 are not exclusively found in the VSMC. Both molecules also diffuse from the endothelium into the blood affecting platelet aggregation, reducing the risk of thrombosis, and platelet aggregation [180,181].

Endothelial dysfunction is characterised by an imbalance in the production of vasodilatory and vasoconstricting factors. Importantly, endothelial dysfunction typically results from a downregulation of NO production or availability. Acutely, the downregulation of NO production can occur as a result of reduced shear stress caused by reduced or disturbed blood flow [162]. Additionally, reductions in NO availability may be the result of increased NO degradation by reactive oxygen species (ROS) (or free

radicals). Within the normal functioning endothelium, the ROS superoxide anion (O_2^-) is produced by the partial reduction of oxygen in the mitochondrial electron transport chain and serves as a signalling molecule. When the endothelium is in a state of redox balance, O_2^- is converted to hydrogen peroxide (H_2O_2), a vasodilator, by superoxide dismutases, a potent ROS scavenger enzyme [178]. When in a state of redox balance, endothelial cells are able to clear ROS at an equal or greater rate than their production [182]. However, in the presence of diminished NO availability, this balance can be altered, leading to oxidative stress [183]. In a state of oxidative stress, excess ROS are produced at a rate greater than their clearance and the scavenging ability of enzymes such as superoxide dismutases is not sufficient [184]. In this state, NO binds to the highly reactive O_2^- to form peroxynitrate ($ONOO^-$), another potent ROS. Increased $ONOO^-$ in turn leads to $ONOO^-$ -mediated oxygenation of tetrahydrobiopterin (BH_4) (a critical cofactor for eNOS), leading to eNOS uncoupling [185–187]. eNOS uncoupling results in eNOS producing O_2^- rather than NO [185,186,188]. In the presence of increased O_2^- , any further NO is quickly scavenged forming more $ONOO^-$ and therefore fails to diffuse to VSMC. The lack of NO diffusing to VSMC in turn leads to changes in vascular tone, causing the artery to become stiffer and less reactive to vasodilatory stimuli [154].

In the absence of sufficient NO, the balance of vasodilatory and vasoconstricting factors is disturbed. In a state of dysfunction, the endothelium shifts towards an inflammatory, pro-thrombotic, and pro-atherogenic phenotype, sometimes referred to as endothelial activation [189,190]. Indeed, chronic endothelial dysfunction and activation is thought to be an early predictor of future CVD [191–195]. Endothelial activation can be subdivided into two distinct components: Type I and type II activation [196]. Type I activation is typically rapid in onset and transient, not resulting in any sustained morphological or functional changes [197]. Conversely, the effects of type II activation are more sustained, mediated by the cytokines tumour-necrosis factor and interleukin (IL)-1 derived from activated leukocytes at the activated endothelial cell [197]. Type II activation and the corresponding inflammatory response can then ultimately lead to atherosclerosis [198,199].

2.3.4 Atherosclerosis

Atherosclerosis is an inflammatory disease characterised by the formation of plaques within arteries and represents one of the leading causes of vascular disease in the world

[200]. Atherosclerosis can occlude arteries by the overdevelopment or rupture of plaques [200] (**Figure 2.5**). The development of atherosclerosis stems from endothelial activation, specifically type II activation [197]. As previously mentioned, endothelial activation is characterised by the shifting of the endothelium from an anti-adhesive, anti-thrombotic phenotype to an inflammatory pro-adhesive, pro-thrombotic phenotype [190,199]. In this state, endothelial-leukocyte adhesion molecules, principally vascular cell adhesion molecule-1 (VCAM-1) and E-selectin are expressed by the endothelium in tandem with the secretion of chemokines such as interleukin-8 [197]. This cascade fosters the arrival of leukocytes to the affected area which then bind to the activated endothelium. Once bound to the endothelium, the leukocytes “crawl” towards endothelial junctions, before migrating to the underlying tunica intima [190,201].

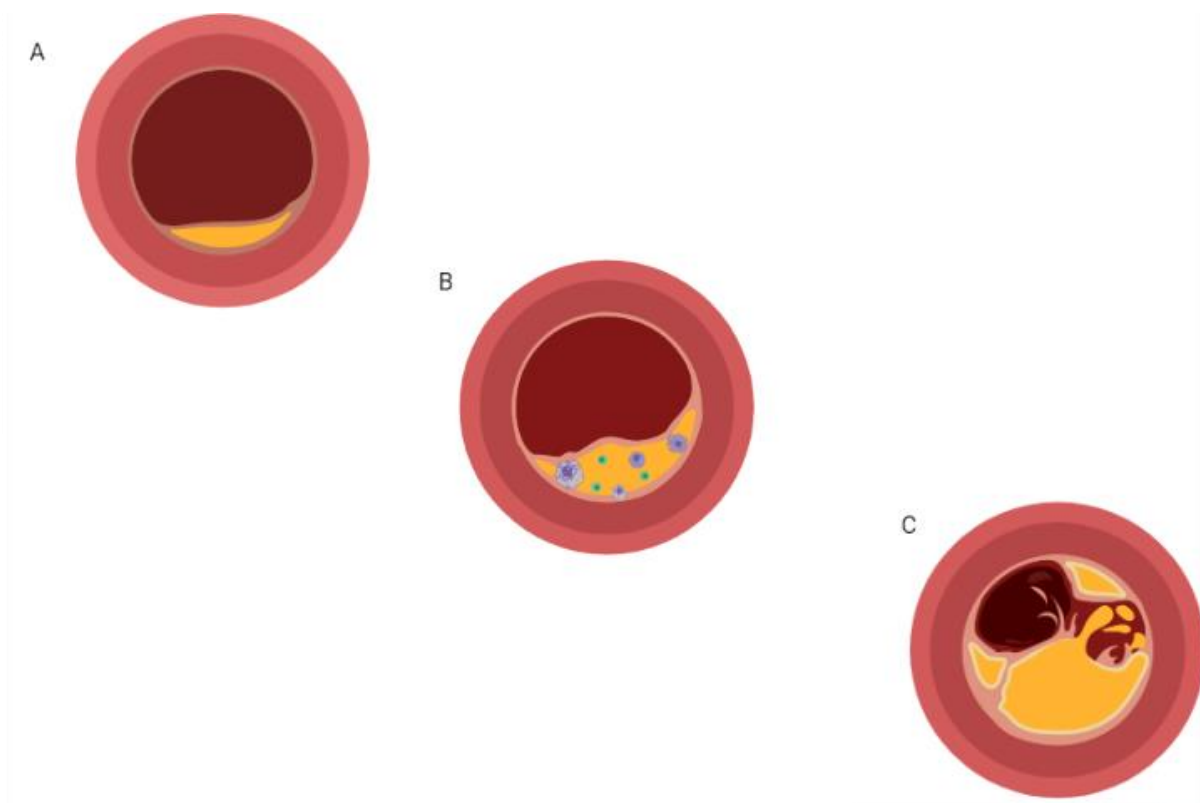


Figure 2.5 Atherosclerosis timeline

Once leukocytes collect in the intima, they accumulate lipoproteins and mature into macrophages, eventually becoming foam cells [202]. The accumulation of foam cells and T lymphocytes eventually form a fatty streak (**Figure 2.5A**), the precursor of atherosclerosis [199,202]. It should be noted that fatty streaks can be common and may not pose long-lasting threat to the vascular system. Further, fatty streaks may also be

reversible, however, in the presence of chronic inflammation or high levels of circulating low-density lipoproteins, fatty streaks can continue to develop into atheroma [203,204]. Continued inflammation can then cause further aggregation of macrophages and T lymphocytes in the vessel wall via the release of macrophage colony-stimulating factor and IL-2 [201,203]. The process of inflammation also causes circulating platelets to bind to the activated endothelium or macrophages. As the platelets are activated they release their granules containing cytokines and growth factors that facilitate the migration and proliferation of smooth muscle cells [202], forming a fibrous cap on the atherosclerotic lesion (**Figure 2.5B**).

In advanced atherosclerotic lesions, a cycle of macrophage and T lymphocyte migration and eventual apoptosis (programmed cell death), may lead to the formation of a necrotic core of dead cells in the centre of the lesion [203]. Potentially owing to the unique environment created within the core, dead cells are not cleared away leading to growth of the necrotic core [203,205]. Certain cytokines, notably interferon- γ , tumor-necrotic factor- α , and IL-1 β [206], released by necrotic core can then inhibit the normal function of smooth muscle cells [207], resulting in apoptosis of the smooth muscle cells [208], weakening the lesion and in particular the fibrous cap. Weakening of the fibrous cap can result in rupture and subsequent thrombosis, resulting in unstable coronary syndromes or myocardial infarction (**Figure 2.5C**) [209].

Whilst the long-term evidence linking prolonged sedentary behaviour and CVD is limited to cross-sectional studies, it is conceivable that repeated acute bouts of sedentary behaviour, namely sitting, may consistently expose the vascular system to a recurring and sustained negative phenotype. These repeated exposures may, throughout life, accumulate resulting in the aforementioned links between sedentary behaviour and CVD.

2.4 Prolonged Sitting-Induced Cardiovascular Dysfunction

In order to understand how repeated bouts of prolonged sitting may detrimentally affect the cardiovascular system in the longer term, it is important to understand how acute bouts of prolonged uninterrupted sitting affect aspects of the cardiovascular system. Of equal importance is the need to understand the mechanisms by which these detrimental outcomes present themselves and what mitigation strategies may be beneficial. A schematic of the potential mechanisms and pathways are presented in **Figure 2.6**.

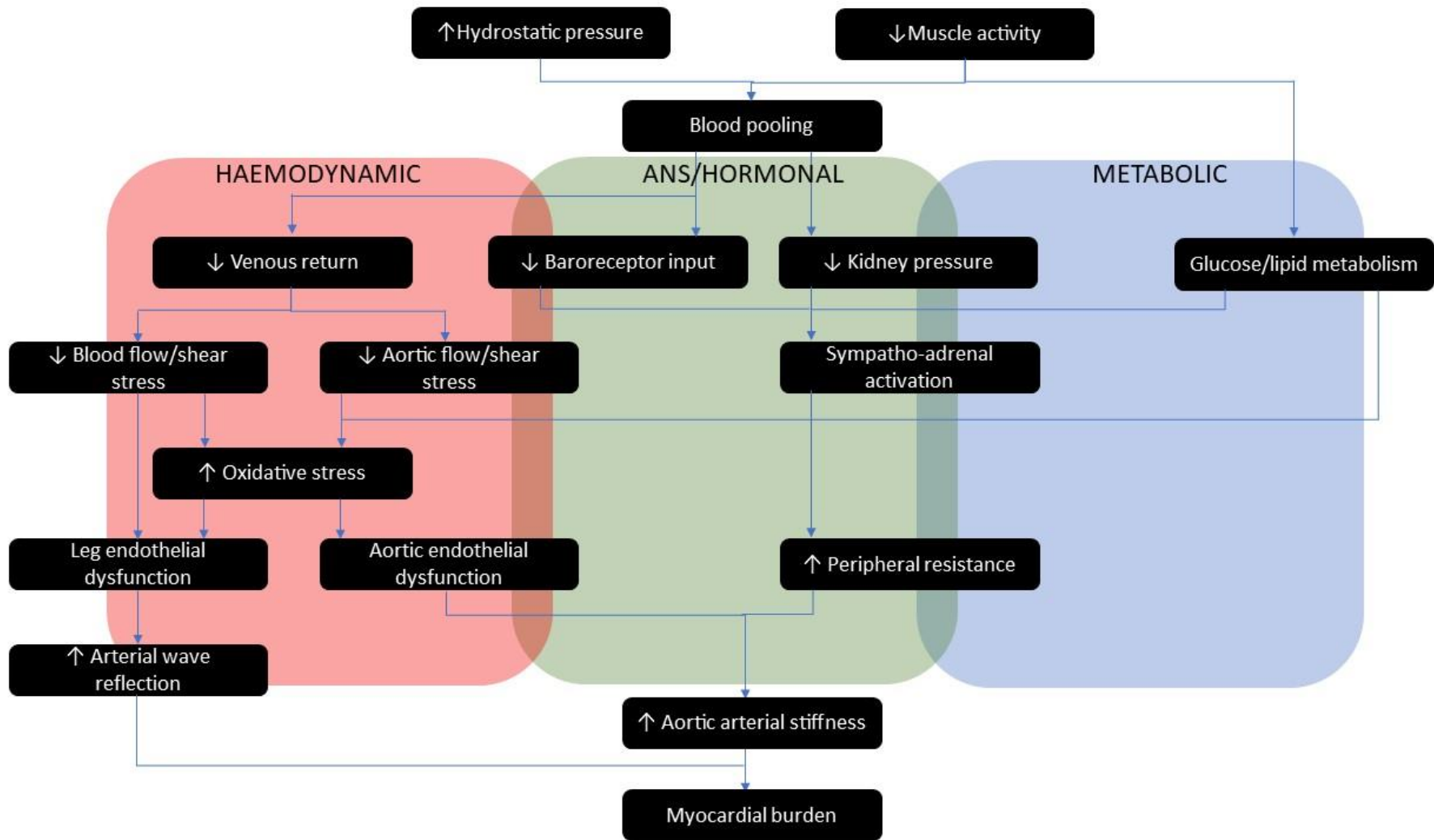


Figure 2.6 Working model of how prolonged sitting impacts aortic arterial stiffness and increases myocardial burden. Adapted from Stoner et al., [61]

2.4.2 Prolonged Sitting and Blood Pooling

The primary stimulus for sitting-induced cardiovascular dysfunction appears to be a reduction in lower limb muscle activity coupled with increased hydrostatic pressure within the lower limbs [210]. These factors are posited to result in venous pooling, or more accurately, congestion, whereby venous return is reduced and via the Starling mechanism, stroke volume and blood flow is reduced. Several studies have attempted to quantify the degree of blood pooling within the lower limb in order to elucidate this mechanism. Non-invasive measures have ranged from simplistic calf circumference measures [42,46,48–50,57,58,211,212], to the use of near-infrared spectroscopy to quantify the total haemoglobin within the gastrocnemius and/or soleus muscles [59,60]. Whilst these studies have identified apparent increases in blood pooling, only a limited number have attempted to relate the degree of pooling to changes in cardiac output. Of the studies that have attempted to do so, only one has found a significant correlation between the degree of pooling and change in stroke volume [211].

The study by Horiuchi and Stoner [211] identified that an acute bout of prolonged sitting resulted in a significant increase in lower limb pooling, as evidenced by increased calf circumference, deoxyhaemoglobin, and total haemoglobin within the gastrocnemius [211]. Total haemoglobin was significantly inversely correlated with decreases in stroke volume ($r = -0.58$, 95 % Confidence Interval [CI]: -0.68 to -0.45) and positively correlated with heart rate ($r = 0.47$, 95 % CI: 0.33 to 0.59). These results provide support for the notion that in the presence of increased blood pooling within the lower limb, stroke volume may be reduced. Additionally, this study, utilising a crossover design examined the effect of compression socks as a means of reducing venous pooling and showed that by attenuating venous pooling (total haemoglobin increased 4.3 % vs 10.5 % in control condition), stroke volume did not reduce to the same degree (-7.6 % vs 14.8 %). However, the results of this analysis, whilst promising, should be interpreted with caution. Firstly, this study sampled young healthy adults (age: 22 ± 4 yrs, body mass index: 22.1 ± 2 kg·m²) using the Modelflow method which estimates cardiac output from arterial pulse waves detected in the finger [213]. The young sample coupled with an estimation technique which has shown varied reliability and validity in different groups [213–215] may introduce doubt regarding the conclusions and generalisability to broader groups within

the population. However, these results are still promising and need further replication in larger, more diverse samples.

2.4.3 Blood Pooling and Lower Limb Arterial Dysfunction

Venous pooling, the associated drop in venous return, and increased hydrostatic pressure have been posited as a mechanism by which both lower limb and aortic endothelial dysfunction may occur during prolonged sitting [61,210]. Focusing firstly on the lower limb; the gold standard, non-invasive technique for assessing endothelial function is flow-mediated dilation (FMD). FMD is an ultrasound technique developed by Celermajer et al., [216] used to evaluate endothelium-dependent vasodilation in response to a hyperaemic stimulus. This technique involves placing an ultrasound probe over a peripheral artery (most commonly the brachial artery) and creating an artificial hyperaemic stimulus by occluding the artery downstream for a given period (**Figure 2.7**). Upon release of the occlusion, the increase in blood flow results in a corresponding increase in shear stress which, in a healthy artery, results in an increase in dilation. As vasodilation is mediated by both endothelium-dependent and -independent mechanisms, to accurately assess endothelial function, participants should then be given an NO donor, typically glyceryl trinitrate, to assess the maximal vasodilatory capacity of the artery being assessed after which the maximal vasodilation between the two conditions can be compared [217,218].

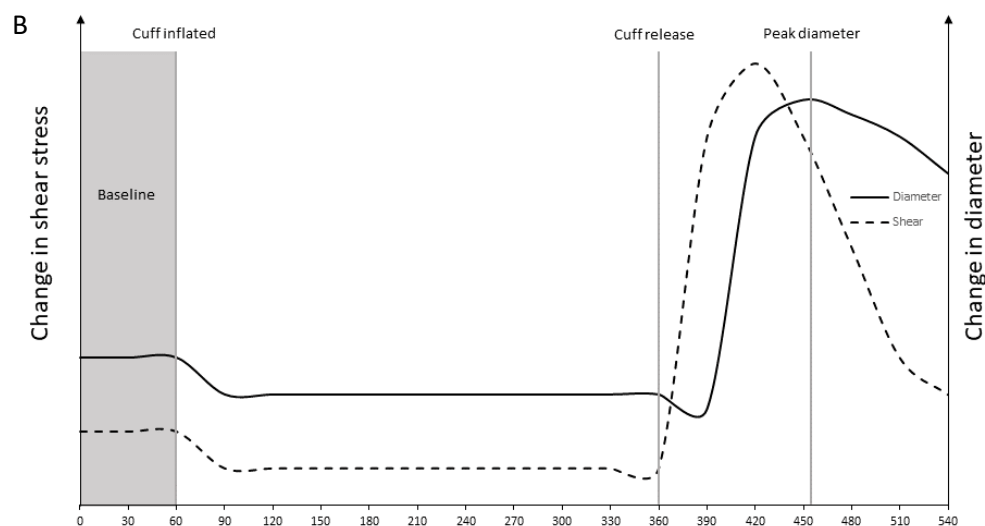


Figure 2.7 A) Schematic of brachial artery FMD setup. A high-resolution B-mode linear ultrasound probe is placed over the brachial artery (typical diameter 3 – 5 mm) providing longitudinal images of the artery and, importantly, diameter measures. Modern ultrasound units also facilitate concurrent assessment of flow velocity facilitated by pulsed wave Doppler ultrasound as a means of calculating shear rate, the primary stimulus for the FMD response. When assessing the brachial artery, an occlusion cuff is typically placed distal to the elbow on the forearm. Occlusion cuff placement can alter the magnitude [219], duration [219,220], and clinical value [221] of the observed dilation response and thus is an important consideration across studies. B) Schematic trace of shear rate and diameter traces during the FMD protocol adapted from Thijssen et al. [218]. Baseline diameter and shear are collected for ~ 60 secs before the cuff is inflated to supra-systolic pressure, fully occluding the downstream artery. After an occlusion period of 5 mins, cuff pressure is rapidly released prompting a near immediate hyperaemic response and subsequent increase in shear rate. In the case of brachial FMD assessments, diameter and shear rate data are collected for ~ 3 mins, with peak diameter typically being observed within that time. It should be noted however that different arteries, particularly lower limb arteries, may take up to ~ 6 mins to reach full dilation [222].

FMD is a technically challenging measure to perform and thus requires a high level of operator training and adherence to guidelines to ensure valid and reliable measures [160,218,223]. As a result, FMD is a less frequently used measure within prolonged sitting research but may still be a useful tool in understanding how prolonged sitting may affect the cardiovascular system. FMD can and has been performed in a number of arteries to assess the effects of prolonged sitting; for example studies have assessed the superficial femoral [38–41,224,225], popliteal [42,44,46,49–51,226], posterior tibial [212], and brachial arteries [39,50,224,227]. Whilst long-term clinical data suggests that chronic changes in brachial FMD may predict future CVD [192–195], the prognostic value of acute changes is unknown. Further, neither long- nor short-term data for the prognostic value of lower limb arteries exists. However, it is posited that FMD can be used as a tool to understand acute endothelial dysfunction in response to prolonged sitting as part of a broader picture sitting-induced cardiovascular dysfunction. Indeed, it is conceivable that repeated exposures to a dysfunctional phenotype may influence chronic changes in local endothelial function. Therefore, it is necessary to understand whether and the degree to which different arteries may be affected by acute bouts of prolonged sitting.

[As previously discussed](#), the primary stimulus for endothelial function is shear stress [228–233]. Prolonged uninterrupted sitting is posited to disturb shear stress and thus endothelial function in a number of ways. Firstly, a reduction in blood flow as a result of reduced stroke volume may contribute to a reduction in shear stress against the endothelium resulting in dysfunction [39–41]. Further, the increased hydrostatic pressure within the lower limb in particular may stimulate the myogenic response whereby blood flow is further reduced as arteries constrict to reduce blood flow and prevent further pooling [234]. The sitting posture also creates ‘bending’ in particular lower limb arteries that may disturb laminar flow, creating regions of disturbed, oscillating flow which has been shown to negatively impact endothelial function independent of changes in hydrostatic pressure [235]. Some researchers have suggested that frequent repeated exposure to this negative phenotype within lower limb arteries may explain the greater distribution of atherosclerosis within the lower limbs [210]. Others have posited that local endothelial dysfunction within lower limb arteries may

augment reflected pressure waves, disturbing normal arterial system function and contributing to increased myocardial burden [61].

Owing to the difficulty of FMD and the associated variability, prolonged sitting studies have typically recruited younger samples to control for age as a source of heterogeneity. This poses a particular issue as FMD responses in both upper and lower limb arteries have been shown to reduce with age [236,237]. Given that current model of sitting-induced dysfunction suggests that changes in lower limb artery endothelial function may contribute to increased arterial wave reflection and thus increased myocardial burden (**Figure 2.6**), the lack of evidence across age groups related to lower limb dysfunction is concerning. Research has focused on both interrupted and uninterrupted sitting identifying varied responses; therefore, it is pertinent to consolidate the existing evidence related to FMD in both interrupted and uninterrupted sitting whilst investigating potential sources of heterogeneity. This will be presented as a systematic review and meta-analysis in [Chapter 3](#).

2.4.4 Blood Pooling and Aortic Dysfunction

Reduced cardiac output as a consequence of reduced venous return has also been suggested as a mechanism for the observed increases in aortic stiffness in response to prolonged uninterrupted sitting. Aortic (or central) stiffness is typically measured using PWV. PWV assesses the time taken for a pulse wave to propagate from a proximal to distal point. The current gold standard of central PWV is carotid-femoral PWV (cfPWV) which has been shown to be a robust independent predictor of CVD and all-cause mortality in both general and clinical populations [134,238–241]. Whilst other measures of central arterial stiffness have been implemented, cfPWV continues to represent the gold standard measure as the arterial segment being assessed is primarily elastic arteries directly influencing left ventricular function [242,243]. By comparison, other measures of central arterial stiffness such as brachial-ankle and carotid-ankle encompass greater portions of both elastic and muscular arteries and thus are unable to delineate the dysfunctional portion of the arterial tree. Given the structural heterogeneity between elastic and muscular arteries it is possible that acute insults to the vascular system may present differently between different segments [244].

Longitudinal increases in cfPWV have been associated with increased risk of CVD and all-cause mortality, however, acute changes in cfPWV, as observed within prolonged sitting research, have unknown prognostic value. Further, longitudinal changes in cfPWV are likely a consequence of morphological changes in arterial structure whereas any acute changes are likely mediated by a combination of endothelial dysfunction within the aorta and increases in blood pressure. Endothelial dysfunction has been associated with increased PWV in peripheral arteries [161] however, it is not possible to assess aortic endothelial function non-invasively in the same way that FMD is used in peripheral arteries. Therefore, it is important to recognise that inferences regarding changes in cfPWV being a result of endothelial dysfunction is based on results from arteries of very different structure and function. It is also important to consider that PWV and blood pressure are intrinsically linked, whereby transient increases in one will present as an increase in the other [136]. As blood pressure, the force exerted by the blood against vessel walls, increases, artery diameter increases. As artery diameter increases, particularly in elastic arteries, more of the strain is borne by the stiffer collagen fibres of the extracellular matrix [137,245]. Subsequently, when assessing short term changes in cfPWV, it is necessary to statistically control for blood pressure to delineate whether either cfPWV or blood pressure has changed independently. Whilst blood pressure and cfPWV are intrinsically linked, cfPWV does not appear to display the same within- or between-day variability nor circadian fluctuations that peripheral blood pressure does [246–248]. As a result, cfPWV may still be a useful measure within prolonged sitting research and may identify different mechanisms of dysfunction.

Acute increases in aortic stiffness may augment the timing of wave reflections, which may present as increased systolic blood pressure and/or decreased diastolic blood pressure, inhibiting coronary perfusion. An increase in aortic stiffness may also disturb the stiffness gradient between central and peripheral arteries, resulting in increased pulsatile stress being transmitted to high blood flow end organs such as the kidneys and brain, potentially causing damage to the microcirculation [138,244,249]. To characterise how the relationship between elastic and muscular arteries may change, recent research has sought to express the differences between arterial segments as ratios. Of particular interest to prolonged sitting research, the recently proposed aortic-femoral stiffness gradient (af-SG) has been shown to be a reliable, blood pressure-independent tool for assessing haemodynamic integration [250,251].

Similarly to most cardiovascular measures within the prolonged sitting research, the prognostic value of acute changes are unknown however repeated prolonged exposures to a negative phenotype may help to explain longer term associations with CVD and all-cause mortality. Owing to the variety of arterial stiffness measures that have been implemented within prolonged sitting research and the associated heterogeneity that can be expected from comparing different metrics, it is necessary to consolidate the evidence in a systematic way. To achieve this, the literature regarding arterial stiffness and prolonged sitting is summarised as a systematic review within [Chapter 7](#).

2.4.5 Blood Pooling and Blood Pressure

Blood pooling within the lower limbs and the associated decrease in stroke volume is also posited to have detrimental effects on indices of peripheral blood pressure. It has been suggested that a reduction in stroke volume during prolonged sitting may result in decreased renal perfusion pressure and increased sympathetic nervous system activity [252]. The drop in renal perfusion pressure, sensed by juxtaglomerular cells, and the increase in sympathetic nervous system activity as a result of an upright posture, stimulates the release of renin from the kidneys, stimulating the renin-angiotensin-aldosterone system (RAAS) [253]. Renin then reacts with circulating angiotensinogen to form angiotensin I. Angiotensin I, via the action of angiotensin-converting enzyme, becomes the more biologically active angiotensin II. Angiotensin II subsequently binds with angiotensin type 1 receptors within the cardiovascular, renal, and adrenal systems and stimulates vasoconstriction, the release of aldosterone, epinephrine, and norepinephrine as well as several other actions forming a feedback loop whereby blood pressure and therefore renal perfusion pressure is increased [254]. Additionally, localised endothelial dysfunction in lower limb arteries and a concomitant increase in artery stiffness and tone [161,255,256] may increase total peripheral resistance, further increasing blood pressure. It is also conceivable that changes in peripheral artery tone may augment reflected pressure waves resulting in elevations of blood pressure.

Assessments of peripheral blood pressure remain one of the most established and widely used non-invasive measures of cardiovascular health. Blood pressure is a key marker of cardiovascular health due to the simplicity of measurement and its strong prognostic value. Epidemiological data shows that every 10 mmHg increase in peripheral blood pressure is associated with an 20 % increase in risk of CVD events and 13 % increase in

all-cause mortality [257,258]. Owing to the simplicity of assessments and strong prognostic value, peripheral blood pressure measures are the most frequently reported outcomes within prolonged sitting research, frequently reported as both primary and secondary outcomes. Oscillometric measures of blood pressure have been used in a number of prolonged sitting studies with a wide range of reported outcomes with some studies showing significant increases in systolic blood pressure and mean arterial pressure [47,48,56,57] and others showing little or change and even declines in blood pressure in response to bouts of uninterrupted sitting [259,260]. Equally, a range of responses have been observed when sitting bouts were regularly interrupted. The heterogeneity in observed responses may be the result of a number of factors including the means of assessing peripheral blood pressure and the age of the sample.

Whilst discrete oscillometric assessments remain the most common means of measuring peripheral blood pressure, in recent years, a minority of studies have also implemented ambulatory blood pressure assessments whereby a participant is fitted with a small automated sphygmomanometer which assesses blood pressure every 15 minutes throughout the day using traditional oscillometry [261,262]. Originally implemented as a means of evaluating blood pressure across the entire day rather than discrete snapshots, this technique has been shown to produce significantly different results than blood pressure assessments taken in a clinical setting [263–265]. Heralded as a truer reflection of individual patient blood pressure, these results may actually be the result of reduced control and reduced internal validity making their use within prolonged sitting research questionable. In prolonged sitting studies that have utilised ambulatory blood pressure assessments, participants are typically advised to temporarily cease what they are doing, straighten their arm and allow the measurement to be taken. This lack of control coupled with the inherent variability of blood pressure may produce erroneous results and may mask the true effects of prolonged sitting.

A further technique for blood pressure assessment that has been used is continuous blood pressure assessment via finger clamp plethysmography [47,48,266]. This technique takes pressure assessed at the fingers and estimates peripheral pressure using a height correction unit placed at the level of the heart. This technique provides higher temporal resolution than traditional oscillometry, however, the increased volume of data brings its own issues, principally how to analyse data. With beat-to-beat pressure

measures, a researcher is presented with issues of how to average the data to give a representative value. Previous prolonged sitting research using this technique has described averaging 5 min windows at pre-determined points during the protocol, however, these studies do not report how data is screened and how outliers within that period are controlled for [47,48]. For this reason, it is conceivable that averaged blood pressure measures may be influenced by outlying data points. Further, previous work has demonstrated that pressure waveforms in the arteries of the finger and the brachial artery are markedly different, likely as a result of a pressure gradient and changes in arterial wave reflections [267,268]. Whilst common devices that use the volume clamp method typically use a height correction unit that can be used in tandem with generalised transfer functions to account for differences between finger and brachial waveforms, these devices are typically designed for only slight differences in height between the two measurement sites and thus may be more prone to error if participants are in a seated position with the hands appreciably lower than heart level.

Previous work has demonstrated that regularly interrupting sitting may offset some of the deleterious effects observed with prolonged sitting. Interruption strategies may offset sitting-induced dysfunction by reducing venous pooling in the lower limbs and thus promoting venous return. As seen in **Figure 2.6**, venous pooling is thought to be the primary mechanism for a majority of the dysfunction observed with prolonged sitting and thus is likely to be an effective target for sitting interruptions. In an effort to offset sitting-induced dysfunction, studies have utilised a number of different interruption strategies including walking, running, standing, seated calf raises, and simple resistance activities. The varying physiological demands of each intervention and the subsequent haemodynamic stimulus is likely to differ greatly by interruption strategy and thus is likely a contributing factor in the apparent heterogeneity between studies. In order to inform future studies, and in time, public health policy, it is necessary to investigate what firstly, whether regularly interrupting sitting confers a robust protective effect and secondly, which interruption strategy is likely to be the most effective and practical.

The heterogeneity in blood pressure responses is likely further conflated by the wide range of ages that have been sampled. Studies employing peripheral blood pressure as an outcome measure have recruited participants ranging from ~19 [43] to ~71 [260] years. It is likely that age related changes to the cardiovascular system and its regulation in older

populations may augment the observed responses to prolonged sitting. Further, physical activity levels typically decrease with age which may also influence the response to prolonged sitting.

Owing to the variability of peripheral blood pressure outcomes reported in both uninterrupted and interrupting sitting studies, and the potential sources of heterogeneity commented on above, there is a need to effectively consolidate the existing evidence and explore sources of heterogeneity in a meaningful way. The amalgamation of the current evidence and exploration of heterogeneity are presented and explored as a systematic review and meta-analysis in [Chapter 5](#).

2.4.6 Central Blood Pressure

Whilst the previous section focused on peripheral blood pressure, an established and useful marker of cardiovascular health, this section will focus on indices of central blood pressure which more accurately reflect pressure within the aorta. Recent evidence has shown that augmentation index (AIx), central systolic blood pressure (cSBP), diastolic blood pressure (cDBP), and pulse pressure (cPP) are strong predictors of future cardiovascular events and may be more physiologically relevant to the progression of CVD [243,269,270]. These measures typically do not align with peripheral blood pressure values due to pulse wave propagation [141,270–272]. Central haemodynamics can be assessed by both invasive and non-invasive methods, however, due to the practicalities afforded by non-invasive methods, they will be the primary focus of this section. Central haemodynamics can be inferred using pulse wave analysis (PWA), whereby certain characteristics of the pressure waveform are “broken down” and analysed [143].

Non-invasive assessments of central haemodynamics via PWA can be performed using direct or indirect methods utilising applanation tonometry or oscillometric cuffs. The direct method typically employs applanation tonometry whereby the carotid artery, is assessed. This technique involves pressing a pressure transducer (tonometer) against the artery. This method works by principle that when a circular structure with a given internal pressure is compressed, the circumferential pressure will equalise, and the tonometer can accurately measure the pressure of that vessel [273]. This technique is described as the “direct” method as the common carotid and ascending aorta are in close proximity and the waveforms detected at the common carotid are acceptably similar to

the central aortic waveforms [274]. Therefore, analysis of these waveforms is proposed to accurately reflect the waveforms, and therefore haemodynamics, of the aorta [274].

The alternative “indirect” method works by assessing pressure waveforms at a peripheral vessel, typically the brachial or radial artery using either applanation tonometry or oscillometric cuffs. The waveforms detected at the peripheral site, in tandem with calibrating pressures assessed at the brachial artery, are then used to generate a central aortic waveform using a generalised transfer function [275]. Generalised transfer functions are mathematical functions which differ depending on the device being used and where the waveforms are detected. In general, these functions have been shown to be both valid and reliable with each new device being tested against existing validated devices or directly validated against central haemodynamics assessed using invasive catheters.

It should be noted that due to the assumptions made by generalised transfer functions and the inherent variability afforded by cuff-based assessments of blood pressure, there is a potential for error associated with these measurements [276]. Indeed, more recent guidelines now suggest that the practice of validating new devices against previously validated non-invasive devices is not appropriate and may result in spurious findings [277]. In several studies, PWA has been shown to slightly underestimate indices of central pressures, however, it has been suggested that these errors are no greater than the error associated with traditional cuff-based sphygmomanometry [278,279]. This may explain why only a limited number of studies have implemented central haemodynamics within prolonged sitting research [59,60,212,280].

Of the prolonged sitting studies that utilised PWA, none have found significant changes in cSBP, cDBP, or cPP [59,60,212,280]. Further, most have reported a decline in AIx in response to prolonged sitting. AIx relates to the augmentation pressure as a ratio of pulse pressure and is accepted as a means of assessing systemic arterial stiffness [136]. AIx is affected by changes in pulse wave reflections, whereby increases in stiffness cause the reflected wave to arrive sooner in systole causing increased augmentation pressure, thus increased AIx is an indication of increased arterial stiffness. Counterintuitively to the increases in cfPWV that have been observed in response to prolonged sitting [59,212], AIx has consistently decreased across studies [59,60,212,280]. It has been suggested that

venous pooling may dampen the reflected waves thus causing a reduction in Alx even in the presence of increased central arterial stiffness [212,281].

A further explanation for the lack of effect observed in PWA indices may be posture of assessments. Whilst evidence suggests that indices of central haemodynamics can be assessed reliably using oscillometric techniques, it is clear that both posture and arm position relative to the heart can influence the observed results [281–284]. Given that changes in central haemodynamics as a result of an acute bout of prolonged sitting are likely to be small, the trivial influence of posture or arm position may affect our ability to detect changes. This does not invalidate PWA as a tool to be used in prolonged sitting research, indeed insights into central haemodynamic responses are likely to provide important mechanistic understanding, however, a high level of control may be required by studies using this technique.

2.4.7 Prolonged Sitting and Metabolic Dysfunction

The previous section described principally haemodynamic and hormonal mechanisms by which an acute bout of prolonged sitting may present as cardiovascular dysfunction. However, a majority of prolonged sitting studies tightly control movement and food intake in order to maximise internal validity and identify potential mechanisms that may explain the longer-term associations between sedentary behaviour and CVD. By contrast, it is likely that in reality, prolonged sitting will cluster with other behaviours that may exacerbate or alleviate the detrimental effects of sitting. One such behaviour is the consumption of meals high in refined sugars and fat which are common components of the modern 'Westernised diet' [285].

2.4.8 Glucose Metabolism and Prolonged Sitting

In order to contextualise how consumption of different foods may interact with sitting behaviours, it is pertinent to understand how different energy substrates are metabolised and how that may interact with prolonged sitting to increase cardiovascular burden or cause dysfunction. Firstly, we will consider glucose metabolism. Glucose metabolism is primarily related to glucose uptake via insulin-mediated and contraction-mediated pathways.

Insulin-mediated glucose uptake is essential for glucose uptake in adipose tissues and skeletal muscle. Because cell membranes are impermeable to hydrophilic molecules such

as glucose, the transport of glucose into the cells is facilitated by the non-energy-dependent actions of glucose transporter proteins (GLUT) [253,286], of which, at least 13 have been identified [287]. Depending on the location in the body and the role of the tissues involved, different GLUTs within cells have varying affinities for glucose. In major insulin target organs such as skeletal muscle and adipose tissue, the predominant GLUT is GLUT4 which has a low affinity for glucose and is reliant on insulin to perform its actions. Circulating insulin binds to receptors, resulting in the translocation of GLUT4 to the cell surface and thus glucose uptake into the cell [253,286]. Working along similar signalling pathways, insulin can also facilitate increased NO production in endothelial cells [288] and has been shown to increase both capillary recruitment and dilation [289,290] as well as macrovascular flow [291,292]. It is thought that the synergistic effect of insulin on both endothelial and skeletal muscle cells facilitates increased glucose uptake [293,294].

Though incompletely understood, contraction-mediated glucose uptake appears to afford an insulin-independent pathway for GLUT4 translocation and subsequent glucose uptake [295,296]. During a bout of prolonged sitting, lack of muscle contractions and thus a decrease in contraction-mediated glucose uptake may be responsible for the associated metabolic dysfunction [297]. Thus it is perhaps not surprising that evidence suggests that regular physical activity interruptions to bouts of prolonged sitting appear to confer a protective effect [52,53,298,299]. A recent meta-analysis investigating the effects of interrupting prolonged sitting with physical activity interruptions identified a significant overall effect favouring interruptions (standardised mean difference [SMD] = -0.55, 95 % CI -0.73 to -0.37) [53]. This observation is supported by previous research identifying that breaking up prolonged sitting is associated with increased expression of genes related to the regulation of glucose uptake and metabolism [300].

Of interest, subgroup analysis from the aforementioned meta-analysis suggested that the effect of sitting interruptions was more pronounced in individuals who were less metabolically healthy (e.g., inactive, overweight, obese, or type II diabetics) compared to fit and active individuals (SMD = -0.62 vs -0.16) [53]. However, this observation should be treated with caution owing to the substantially different number of trials in each subgroup (unfit = 33 trials, fit = 6 trials). The imbalance in subgroup size is likely due to an increased interest in the effect of breaking up prolonged sitting in insulin-resistant

individuals compared to metabolically healthy individuals. As previously stated, contraction-mediated glucose uptake is insulin-independent and thus, in tandem with increased physical activity, may provide a novel target for interventions to improve whole-body glucose disposal.

The observed metabolic dysfunction observed in response to prolonged sitting is particularly problematic as both hyperglycaemia and associated insulin resistance are associated with acute vascular dysfunction and oxidative stress. Additionally, acute hyperglycaemia has been associated with increased peripheral artery stiffness, as assessed by pulse wave velocity in men [301–303], however, this effect has not been shown in a female population [304]. One study has also shown an increase in cfPWV during hyperglycaemia [305], however, in contrast to similar studies, the aforementioned trial utilised a mixed meal (80 g carbohydrate, 12 g fat, 18 g protein) rather than the typically employed glucose drink (70 g carbohydrate). Therefore, it is conceivable that the observed increase in cfPWV was not the result of hyperglycaemia in isolation but may also be related to fat intake which has been shown to acutely decrease vascular function [306,307]; a topic which will be discussed more fully in the following section.

Given that acute hyperglycaemia and prolonged sitting have both been shown to affect cardiovascular function independently, it is perhaps surprising that to date only one study has sought to investigate the potential effects of combining these behaviours [60]. In this randomised crossover trial, the changes in central, peripheral, and global (a composite measure of carotid-femoral, brachial-femoral, and femoral-ankle PWV) arterial stiffness following a bout of prolonged sitting were compared across conditions where participants consumed either a high or low glycaemic index drink. This study found that global PWV significantly increased in both trials ($0.29 \text{ m}\cdot\text{s}^{-1}$, $p < 0.001$), driven by increases in brachial-femoral and femoral-ankle PWV (0.36 and $0.55 \text{ m}\cdot\text{s}^{-1}$ respectively) with no condition ($p = 0.99$) or interaction ($p = 0.95$) effect. Given the findings of Williams et al., [304], the mixed sample of Kelsch et al., [60] (12 males, 6 females) may have influenced the results with females being less susceptible to hyperglycaemic increases in arterial stiffness, however, it is apparent from these findings that in a healthy population at least, hyperglycaemia induced by consumption of a high glycaemic index drink does not appear to augment sitting-induced dysfunction. Another factor to consider may be physical activity preceding the prolonged sitting trials. Whilst

Kelsch et al., [60] controlled for physical activity in the 24 hours preceding each trial, it is not known how much activity participants completed in the days prior. Previous work has demonstrated that greater habitual physical activity and cardiorespiratory fitness may prevent hyperglycaemia-induced increases in arterial stiffness [302,308,309]. Thus, it is conceivable that the recreationally active participants recruited by Kelsch et al. [60] may not have been as susceptible to hyperglycaemia and a different, less active sample may have produced a different outcome.

2.4.9 Triglycerides Metabolism and Prolonged Sitting

A further key component of the modern Westernised diet is food high in fat. Similarly to the previous section, it is pertinent to first discuss fat, or more specifically, triglyceride metabolism within the body before identifying how it may impact cardiovascular health and potentially interact with prolonged sitting to further increase cardiovascular burden.

Triglycerides are formed of three fatty acid chains bound to glycerol by ester bonds and are produced during the digestion of dietary lipids [310]. When lipids are digested in the intestine, following emulsification and transport through the enterocytes, they are transported through the lymphatic system as chylomicrons. Chylomicrons are composed of a triglyceride-rich and cholesterol core with a lipoprotein shell surrounding it. Apolipoproteins (Apo), non-covalently bound to the lipids, are also located on the outer surface [253]. During transport through the lymphatic system, high-density lipoproteins (HDL) donate Apo C and Apo E to the chylomicron before it is introduced into the circulation at the thoracic duct so it can be utilised by peripheral tissues [253]. In a separate but related process, the liver exports triglycerides in the cores of very-low density lipoproteins (VLDL). The delivery of fatty acids derived from the triglyceride cores of chylomicrons and VLDL to working tissues is facilitated through a common pathway, regulated by the rate-limiting enzyme, lipoprotein lipase (LPL) [311,312]. Lipoprotein lipase is found in the capillary bed of many tissues, including the heart, skeletal muscle, and adipose tissue. Apo C-II, originally donated by HDL, serves as a cofactor in the hydrolysis of triglyceride-rich cores by LPL [313].

Following hydrolysis by LPL, the triglyceride cores of chylomicrons and VLDL become depleted and lead to progressive decreases in size. The resultant particles are known as chylomicron and VLDL remnants, containing a higher relative cholesterol content as well

as Apo B and E. Chylomicron and VLDL remnants are removed from the blood by Apo B receptor-mediated endocytosis in the liver where the remaining cholesterol and apolipoproteins are absorbed and used in separate processes. VLDL remnants that are not taken up by the liver are transformed into low-density lipoproteins (LDL). Formation of LDL involves the removal of residual triglycerides by hepatic lipase, a reaction that is facilitated by Apo E [314]. Owing to the relatively longer half-life of LDL compared to other lipoproteins, LDL can accumulate in the blood and can play a major role in the development of atherosclerosis.

Atherogenic lipoproteins such as LDL are subject to oxidation by ROS and by macrophage-secreted lipoxygenases. Oxidised lipoproteins can cause impaired endothelial function and in prolonged inflammatory state can result in the secretion of adhesion molecules, facilitating the development of atherosclerosis [203]. The balance of LDL in the blood therefore is heavily influenced by the amount of VLDL present. As chylomicrons and VLDL share a common saturable metabolic pathway, i.e., hydrolysis by LPL, [315] postprandial lipemia, characterised by increased concentration of triglycerides, can occur after ingestion of meals containing fat [316]. Such elevations in circulating triglycerides can hasten the generation of atherogenic lipoproteins such as LDL [317].

Evidence from animal models suggests that acute bouts of sedentary behaviour and longer periods of physical inactivity can lead to a downregulation of LPL and thus reduced triglyceride uptake by skeletal muscle and adipose tissue [318,319]. Indeed, acute bouts of prolonged sitting in humans have been shown to augment triglyceride metabolism resulting in increased circulating triglycerides compared to when sitting is regularly interrupted [56,320–323]. This is particularly noteworthy as increases in circulating triglycerides have been shown to acutely impair vascular function and increase inflammation [306,324–326]. Taken together, it is conceivable that the combination of consuming a high-fat meal in tandem with prolonged uninterrupted sitting may produce a multiplicative effect on the cardiovascular system. Indeed, a randomised cross-over trial from our research group has demonstrated that the combination of prolonged uninterrupted sitting and consumption of a high-fat Westernised meal results in a greater increase in cfPWV (pre-post mean difference [MD] = 0.6 m·s⁻¹) than when sitting is combined with a low-fat meal (pre-post MD = 0.2 m·s⁻¹) [62]. Further research has

suggested that the combined deleterious effects of prolonged sitting and consumption of a high-fat meal may be offset by regularly interrupting sitting [63]. Cho et al., [63] employed a crossover design whereby participants consumed what the researchers describe as a high-fat meal before sitting for four hours with or without stair climbing interruptions. The results of this analysis showed that when sitting was regularly interrupted brachial FMD was maintained (baseline, 9.41 ± 2.61 % vs post, 10.34 ± 3.30 %, $p > 0.05$), whereas a significant reduction was observed in the uninterrupted sitting condition (baseline, 9.65 ± 2.63 % vs post, 7.84 ± 2.36 %, $p = 0.033$). Whilst brachial FMD has a wealth of epidemiological data to show that long term reductions in FMD are associated with increased risk of CVD events and all-cause mortality [192–195], the clinical utility of acute changes in brachial FMD are unknown. FMD in lower limb arteries may help to understand the acute changes in endothelial function that contribute to sitting associated CVD, however, it is unlikely that brachial FMD is a useful measure within prolonged sitting research as the upper limb arteries are not subjected to the same increases in hydrostatic pressure and venous pooling as lower limb arteries as ambulation is typically not reduced and hydrostatic pressure is unlikely to be increased to a similar degree. Also, of note within the findings of Cho et al., [63] was the changes in baseline diameters measured during the FMD protocol. Their results show that within the same group, resting baseline brachial diameter differed by ~ 4 % (interrupted sitting condition, 3.70 ± 0.59 mm vs uninterrupted sitting condition, 3.56 ± 0.62 mm). Given that the statistically significant change in FMD observed in this analysis was an absolute change of less than 2 %, these differences in baseline diameter highlight the issues associated with FMD as a measure (issues which will be discussed in greater detail in the [following Chapters](#)).

Further results from Cho et al. [63] suggest that popliteal artery blood flow and shear rate were improved in the interrupted sitting condition compared to the uninterrupted sitting condition. These results suggest, in line with the contemporary working model, that maintaining blood flow in the lower limbs prevents detrimental changes in cardiovascular function. However, these results should be treated with caution given the unusually high reported values. Cho et al., [63] report popliteal artery blood flow values in excess of $250 \text{ mL}\cdot\text{min}^{-1}$ following interrupted sitting and $\sim 150 \text{ mL}\cdot\text{min}^{-1}$ following uninterrupted sitting which are nearly double normative values reported by Thijssen et al., [222]. As such, despite the interesting findings of Cho et al., [63] a degree of caution

should be employed when considering their findings. Further, despite the promising evidence that regularly interrupting prolonged sitting may confer some protective effect against the combined effects of sitting and consumption of a high-fat Westernised meal, the interruption strategy employed is likely to be inaccessible for many individuals and impractical in most settings. The protocol employed by Cho et al., [63] involved participants ascending and descending six flights of stairs, twice, every 30 minutes representing vigorous exercise. Irrespective of any beneficial effects on cardiovascular function, this protocol is likely to have limited uptake owing to the time and physical demands. To ensure uptake and adherence to sitting interruption strategies they need to be widely accessible regardless of fitness level whilst being practical to use in most settings.

2.4.10 Physical Activity, Cardiorespiratory Fitness, and Prolonged Sitting

Physical activity is a key modifiable risk factor for CVD with those individuals maintaining higher activity levels reducing the risk of future CVD [116]. Physical activity can be broadly classified by intensity and guidelines exist worldwide for recommended minimum physical activity levels. It is important to consider however, that within the context of a 24-hour activity clock, achieving moderate intensity physical activity guidelines of 30 minutes per day represents only ~3 % of waking hours (assuming 8 hours of sleep). An important consideration for the remainder of the day is the amount of time spent in sedentary behaviours and light intensity physical activity (defined as physical activity with an energy expenditure of 1.5 – 3 METS) [15,61]. Indeed, the most recent physical activity guidelines from the World Health Organisation recommend reducing sedentary behaviour and replacing those behaviours with light-intensity physical activity which may include walking [15]. Evidence suggests that the patterns by which we engage in sedentary behaviour may be more important than the total time spent in such behaviours [327]. Shivgulam et al., [327] indicated that in a group of 98 healthy participants (19 – 77 years) increased time spent in sedentary bouts greater than one hour was significantly correlated with impaired resting popliteal artery FMD ($r = -0.304$, $p = 0.002$) whereas total daily sedentary time was not ($r = -0.057$, $p = 0.58$). Further, this work found that the number of breaks in sedentary behaviour (expressed as breaks per waking hour) were positively associated with resting popliteal artery FMD ($r = 0.214$, $p = 0.037$). These findings support the notion that sedentary behaviour may be

detrimental to the vascular system but also highlight the importance of regular interruptions with physical activity, whether that be light, moderate, or vigorous intensity.

A simple and effective means of quantifying light-intensity physical activity is the use of step-counts, whether they be with simple pedometers or more elaborate accelerometers. Previous reviews have shown that the use of step-count monitoring can significantly increase step-counts in both the short- and long-term [328]. Importantly, it has been shown that increases in step-count are associated with decreased risk of all-cause mortality [329–332] and CVD [333–336]. Whilst step counts initially were met with resistance as a means of quantifying physical activity, likely owing to the widely asserted but poorly supported target of 10,000 steps·day⁻¹, evidence now suggests that step counts are a viable tool [337].

It is conceivable that habitual physical activity, such as that measured by step counts, may influence haemodynamic and metabolic processes on both a short and long-term basis. Indeed, it has been shown that acute reductions in daily step count (reducing from $\geq 10,000$ steps·day⁻¹ to $> 5,000$ steps·day⁻¹) can impair both vascular function and glucose metabolism [308,338]. Further it has been suggested that acute reductions in physical activity may also augment triglyceride metabolism by means of reducing LPL availability [318]. Similarly to the prolonged sitting response, it has been suggested that a downregulation in skeletal muscle contraction-mediated glucose uptake combined with a more prolonged exposure to reduced blood flow and shear rate (as a result of reduced skeletal muscle activity) may also contribute to the observed dysfunction in response to reduced daily step counts [308,338]. Given the apparent crossover in the mechanisms by which dysfunction occurs, it is possible that habitual physical activity may influence the prolonged sitting response. To date, no studies have investigated the effect of habitual physical activity on vascular responses to prolonged sitting.

Intuitively, habitual physical activity may be related to cardiorespiratory fitness and indeed there is evidence to suggest that increased daily step counts are associated with improved components of cardiorespiratory fitness [339]. In line with habitual physical activity, it is possible that cardiorespiratory fitness may influence the cardiovascular response to sitting. However, it is currently unclear how cardiorespiratory fitness may influence the vascular response to prolonged sitting. Liu et al., [340] demonstrated a

significant negative correlation between cardiorespiratory fitness and change (pre vs post sitting) in popliteal FMD ($r = -0.51, p = 0.02$) suggesting that individuals with greater cardiorespiratory fitness may experience a greater level of vascular dysfunction in response to a bout of prolonged sitting. The findings of this research, however, are not consistent with previous work which has shown varied effects of cardiorespiratory fitness on the magnitude of prolonged sitting response. In direct contradiction, Morishima et al., [43] showed that individuals with a greater level of cardiorespiratory fitness maintained popliteal artery FMD following a bout of prolonged sitting compared to baseline values ($6.5 \% \pm 1.0 \%$ vs $6.0 \% \pm 1.0 \%$) whereas individuals with a lower level of cardiorespiratory fitness exhibited a significant decline in popliteal artery FMD ($4.4 \% \pm 1.2 \%$ vs $1.7 \% \pm 1.2 \%$). Further, Garten et al., [57] found no significant differences between cardiorespiratory fitness levels when assessing vascular function using the passive leg technique following a bout of prolonged uninterrupted sitting. The reason for these conflicting results is unclear, however, one factor may be the primary outcome measures employed.

All three of the aforementioned studies utilised ultrasound imaging of vessels, with two utilising popliteal artery FMD [43,340], and the final study assessing SFA peak hyperaemic response using the passive leg technique [57]. Whilst validated techniques, the potential for error using these assessments is large and whilst it is important to stress that the skill of such researchers should not be called into question, unknown error may be introduced [341]. As will be discussed in a [later Chapter](#), ultrasound derived measures whereby diameters are assessed can be prone to very small absolute errors that may drastically alter results. In **Figure 2.8**, reproduced from Morishima et al., [43], there are two apparent outliers within the “endurance-trained” group (highlighted). Using image analysis software [342], these data points can be estimated as baseline popliteal FMD% of 3.3 % and 1.2 %, which are over ~50 % lower than the group average of 6.5 %. Using the reported group level baseline and peak diameters reported in this study (0.529 cm and 0.564 cm, respectively), to achieve the unusual results of the two apparent outliers, raw peak diameter measurements would only need to differ by ~3-5 % (0.017-0.028 cm) from reported group level data. These fine margins of error on an individual level can have greater effects on the group level analysis. Indeed, repeating the analysis of Morishima et al., [43] utilising estimated data points from their published figures indicates that that change in FMD% between groups was not significantly different (as

assessed using an independent samples *t*-test) when the outlying data points were removed ($p = 0.108$). Whilst it should be acknowledged that this re-analysis using estimated data points is only speculative and introduces a greater margin of error and reduced precision than the original analysis, it does show that the fine margins of potential error with FMD can lead to varying conclusions.

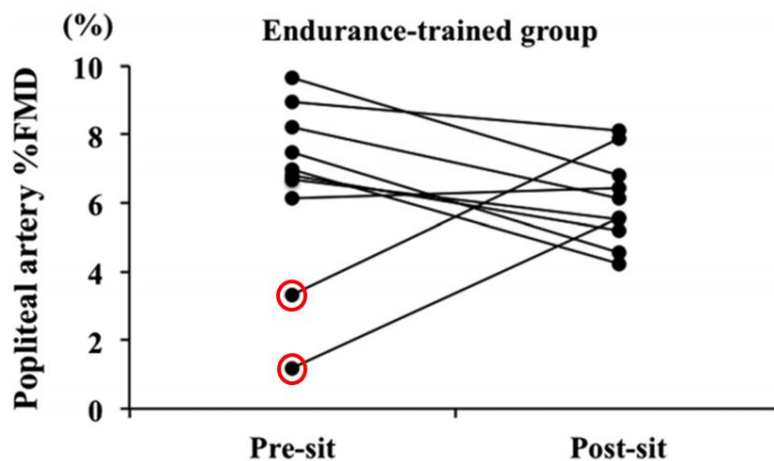


Figure 2.8 Reproduced from Morishima et al., [43]. Individual subject data for popliteal FMD% in the endurance-trained group with potential outliers highlighted with red circles.

The effect of habitual physical activity and sedentary behaviour, and cardiorespiratory fitness on the cardiovascular response to prolonged sitting is worthy of investigation and may yield interesting results, however, given the potential for error with ultrasound-derived measures, techniques such as FMD may not be the most appropriate. Presently, it is unclear how the aforementioned factors may influence the prolonged sitting response however, a different means of assessing cardiovascular function may be prudent.

3

The Effects of Acute Exposure to Prolonged Sitting, with and without Interruption, on Vascular Function Among Adults: A Systematic Review and Meta-Analysis

A modified version of this Chapter has been previously published
Paterson C, Fryer S, Zieff G, Stone K, Credeur DP, Barone Gibbs B, Padilla J, Parker JK, & Stoner L.
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3.1 Introduction

As previously stated, flow-mediated dilation (FMD) is considered the gold standard non-invasive assessment of endothelial health and a marker of vascular function [218]. Previous studies have demonstrated that bouts of prolonged uninterrupted sitting may result in transient reductions in vascular function following bouts of prolonged sitting [38–42,44–46,48–51,212,225–227,343]. It should be noted however that reductions in vascular dysfunction are not consistently observed, and the degree of dysfunction appears to vary by artery. Further, there are apparent methodological inconsistencies between studies which need to be acknowledged and addressed. As such, this Chapter will seek to consolidate the existing data relating to prolonged sitting-induced changes in FMD whilst highlighting issues that may need to be addressed in future research.

In response to research demonstrating that acute bouts of prolonged sitting may result in vascular dysfunction, a growing body of literature has investigated whether regular interruption strategies may offset the observed dysfunction [41,42,46,225,227,343]. Such studies have utilised a number of interruption strategies including leg fidgeting [46], simple resistance activities [227,343], cycling [42], walking [41,225], and standing [42]. Whilst some studies have shown a protective effect of regular interruptions, it remains unclear which mode of interruption strategy may be the most beneficial. Further, as alluded to previously, FMD is frequently assessed in multiple arteries, further complicating interpretations regarding what interruption strategy is the most efficacious. As such, and in line with the World Health Organization's recommendations for future research [35], this analysis will seek to identify whether an optimum sitting interruption strategy exists. Note that for the purposes of this Chapter, FMD will be referred to as a marker of vascular, rather than endothelial, function owing to no studies assessing endothelium-independent vasodilation.

3.1.2 Objectives

In order to inform future Chapters, the aims of this Chapter will be two-fold: (1) to conduct a meta-analysis to determine the effect of uninterrupted sitting on vascular function, with subgroup analysis to identify whether the artery assessed helped to explain heterogeneity in the analysis; and (2) to conduct a separate meta-analysis to

determine the effect of interrupted sitting on vascular function, with additional subgroup analysis of artery, and interruption strategy to explain heterogeneity.

3.2 Methods

This meta-analysis was reported in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [344], however it was not pre-registered.

3.2.2 Data Sources and Searches

Electronic databases (PubMed, Web of Science, SPORTDiscus, and Google Scholar) were searched by myself (CP) and another researcher (Gabriel Zieff [GZ]) utilising the keywords: (sitting OR prolonged sitting OR sedentary OR sedentary behaviour) AND (vascular function OR endothelial function OR endothelium function OR endothelial dysfunction OR endothelium dysfunction OR flow mediated dilation OR flow mediated vasodilation OR flow dependent dilation OR flow dependent vasodilation OR vascular reactivity OR FMD). The reference lists of all identified trials and relevant reviews were also examined. The search was limited to English Language studies published between inception and January 2020.

3.2.3 Article Selection

For the purpose of this meta-analysis, the terms 'article' and 'study' are used synonymously; 'trial' is the unit included in the meta-analysis. A given article may have resulted in more than one eligible trial if the article included more than one intervention group or FMD assessment site. Article titles and abstracts were screened for relevance, and duplicate studies were removed before obtaining the full text of potentially eligible articles to review for inclusion. The following criteria were used to select trials for inclusion in the review: (i) FMD was assessed pre- and post-sitting; (ii) studies were either randomised controlled, randomised crossover, or quasi-experimental pre- versus post-test trials; (iii) the prolonged sitting period was at least one hour; (iv) participants were non-smoking adults (≥ 18 years of age), not taking any vascular acting medication, and were considered healthy, having no major acute or chronic illness. To address the second objective of this review regarding sitting interruption strategies, further additional criteria were used; (i) if a strategy was employed to disrupt the effects of sitting, the strategy must have been during the sitting period; (ii) there must have been a

control (uninterrupted sitting) group or condition, and (iii) the interruption strategy must have involved the participants actively moving either the lower or upper limbs. If a study employed an intervention prior to or after the sitting period, only data from the control (uninterrupted sitting) trial was included in the analysis. Two researchers completed the study selection independently (CP and GZ).

3.2.4 Data Extraction and Quality Assessment

Data extracted for each eligible trial included bibliographic information (author, publication year), collected measures, sample characteristics (age, sex, body mass index, etc.), details of any interventions, arterial site, and uncorrected relative FMD values. Uncorrected FMD values were collected, as opposed to allometrically scaled or normalized to the shear rate stimulus, as there is a lack of consensus on the correction process and different approaches have been used. If these data were not included in the article, the investigators contacted the authors for further information. Data extraction was completed independently by two researchers (CP and GZ). Study quality was assessed using the Cochrane Risk of Bias Tool [345] and a modified Heyland Methodological Quality Score (HMQS) [346,347] with a maximum score of 10. Two additional levels were added to the standard HMQS, relating to the described FMD assessment. These extra levels addressed whether the measure was performed in the recommended and validated supine posture, and whether appropriate guidelines were followed [160,218,348]. Due to the technical aspects of assessing FMD, adherence to published guidelines are imperative to ensure reliable and reproducible results [218,349]. With respect to existing HMQS criteria, as blinding of participants is not feasible, blinding of the operator assessing FMD was considered a quality criterion for the HMQS as opposed to participant blinding. The HMQS criteria “extent of follow up”, “cointerventions”, and “outcomes” were removed from the current analysis as they are designed for longitudinal studies. Quality assessment was completed independently by two researchers (CP and Simon Fryer [SF]), with consultation from a third researcher (Lee Stoner [LS]) in the case of discrepancies.

3.2.5 Data Synthesis

For the outcome of interest, the pre- and post-intervention values (mean and standard deviation) as well as mean differences and associated standard deviations were entered into a spreadsheet. When data were not published, a request of the missing values was

made to the corresponding author and following non-response the values were estimated based on methods from the Cochrane Handbook for Systematic Reviews of Interventions [345]. For studies reporting multiple time points during the bout of sitting, only the pre-trial and final time point values were used in analysis. Aggregation and calculation of final results was conducted by two authors (CP and SF).

3.2.6 Data Analysis

All extracted data were entered into software specifically designed for meta-analyses (MetaXL, http://www.epigear.com/index_files/metaxl.html) with subsequent analysis performed using the *metafor* package [350] in R (version 4.0.3) [351]. Outcome measures (μ) were calculated as weighted mean differences (WMDs) as well as the standardised mean difference (SMD). The SMD was used to determine the magnitude of the effect, where <0.2, 0.2, 0.5, and 0.8 was defined as trivial, small, moderate, and large respectively [42]. Random-effects modelling, with the DerSimonian-Laird method, was used for both analyses as it allows for heterogeneity in experimental procedures and accounts for both within- and between-trial variance [352].

Subsequent to running the main analysis, the robustness of the pooled results, the potential for publication bias, the power of individual trials, and potential sources of heterogeneity were explored. The robustness of the pooled results were examined using studentised residuals and Cook's distances to identify potential outliers and/or influential trials respectively. In line with previous work, a trial was considered a potential outlier if the studentised residual was larger than the $100 \times (1 - 0.05/(2 \times k))$ th percentile of a standard normal distribution whilst trials with a Cook's distance greater than the median plus six times the interquartile range of the Cook's distance were considered potentially influential [353]. If a trial was identified as either a potential outlier or influential, the analysis was repeated with the trial omitted to test the robustness of the overall effect. The Luis Furuya-Kanamori (LFK) index in tandem with Doi plots was used as it is a means of identifying and quantifying asymmetry and potential small study bias, where <1 indicates no asymmetry, 1 to 2 suggests minor asymmetry, and >2 indicates major asymmetry [354]. Doi plots are reported to be more objective than traditional funnel plots which are typically assessed qualitatively and the LKF index has been shown to be a more sensitive measure of small-study (or publication) bias than Egger's regression [354]. The statistical heterogeneity across trials included in the meta-

analysis was assessed using the I^2 statistic, where <25 %, 25-75 %, and >75 % represent low, moderate, and considerable heterogeneity, respectively [355]. Finally, the statistical power of the meta-analysis and its composite trials was assessed using the methods described by Valentine et al., [356] and the *metaviz* package [357] Two authors (LS and CP) conducted the data analysis to ensure the accuracy of the analysis.

3.3 Results

3.3.1 Literature Search and Trial Selection

The literature search strategy is outlined in **Figure 3.1**. Initial database searches identified a total of 1797 potentially eligible articles with a further 5 identified through manual searches. Following screening of titles and abstracts, 1,769 articles were excluded because they did not meet inclusion criteria. The remaining 33 papers underwent full text screening and 16 further studies were excluded. The final analysis included 17 studies (22 trials) for objective (1), of which 6 (9 trials) were included in a separate analysis of interruptions to prolonged sitting for objective (2).

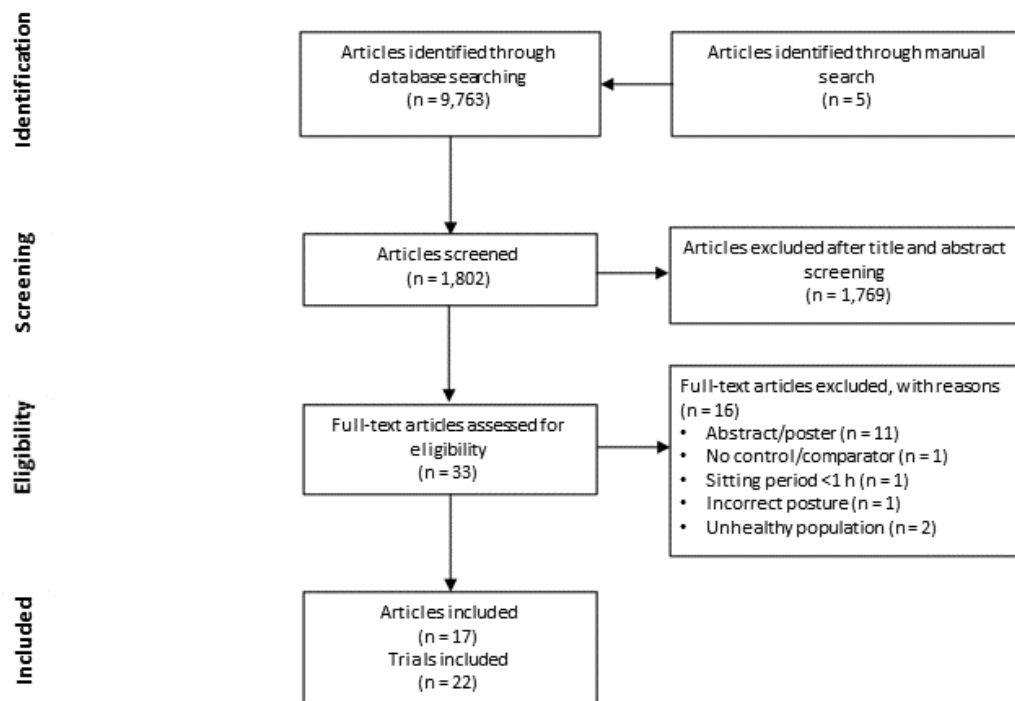


Figure 3.1 Flow chart of study selection

3.3.2 Characteristics of Included Studies

The trial characteristics are summarised in **Table 3.1**. The number of participants in each trial ranged from 8 [226] – 20 [212]. Of the 22 trials, 12 included only male participants [38–41,44,48–51,226], and 2 included only females [48,49], with 8 trials included both sexes [45,46,212,225,227,343]. Bouts of prolonged sitting ranged from 1.5 [227] to 6 hours [50], with a modal sitting duration of 3 [38–41,44–46,48,49,51,226]. Assessments of FMD were carried out predominantly in the lower limb, with only 4 of the 18 trials assessing brachial artery (BA) FMD [39,50,227,343]. Of the trials that assessed FMD in the lower limb, 6 assessed the superficial femoral artery (SFA) [38–41,225,343], and 11 assessed the popliteal artery (PA) [42,44–46,48–50,226], with 1 assessing the posterior tibial artery (PTA) [212]. Of the 18 trials, 9 included strategies to interrupt the bout of prolonged sitting [41,42,46,225,227,343]. These interruptions were categorised as aerobic [41,42,46,225], simple resistance activities [227,343], or standing [42].

3.3.3 Methodological Quality Assessment

The methodological assessment of included trials is summarised in **Table 3.1**. The quality of studies ranged from 3 to 8 out of a possible maximum of 10, with the median quality score being 7. All trials assessed and reported all collected data and reported any dropouts or unusable data. For blinding, 10 trials reported that offline analysis of FMD videos was performed by a blinded technician/researcher [38–41,48,226,343]. Ten trials reported the published FMD guidelines that they adhered to [39–41,45,48,212,225,227], and 8 trials performed FMD with participants in the suggested supine position [42,44–46,51,212,225,227].

Table 3.1 Characteristics of included trials

Ref	Quality	Sample [n (F); mean age, years (SD)]	FMD assessment site	Sitting duration (h)	Interruption strategy
Ballard et al. [38]	6	11 (0); 21.2 (1.9)	SFA	3	N/A
Carter et al. [225]	7	15 (5); 35.8 (10.2)	SFA	4	Aerobic
Carter et al. [227]	7	10 (4); 27.3 (8.1)	BA	1.5	SRA
Climie et al. [224]a	8	19 (8); 57 (12)	SFA	5	SRA
Climie et al. [224]b	8	19 (8); 57 (12)	BA	5	SRA
Credeur et al. [212]	6	20 (7); 26 (7)	PTA	3	N/A
Kruse et al. [42]	7	13 (3); 38 (3)	PA	4	Aerobic and Standing
Morishima et al. [44]	7	11 (4); 26 (1)	PA	3	Aerobic
Morishima et al. [45]	8	15 (5); 26.7 (0.5)	PA	3	N/A
Morishima et al. [46]	7	9 (0); 21.2 (2)	PA	3	N/A
O'Brien et al. [48]a	6	10 (0); 24 (2)	PA	3	N/A
O'Brien et al. [48]b	6	10 (10); 23 (2)	PA	3	N/A
Padilla et al. [226]	7	8 (0); 24 (1.7)	PA	3	N/A
Restaino et al. [50]a	4	11 (0); 27 (1)	PA	6	N/A
Restaino et al. [50]b	4	11 (0); 27 (1)	BA	6	N/A
Restaino et al. [51]	7	10 (0); 26 (1)	PA	3	N/A
Thosar et al. [40]	7	12 (0); 24.2 (4.2)	SFA	3	Aerobic
Thosar et al. [39]a	5	12 (0); 24.2 (4)	BA	3	N/A
Thosar et al. [39]b	5	12 (0); 24.2 (4)	SFA	3	N/A
Thosar et al. [41]	7	11 (0); 24.2 (4.4)	SFA	3	N/A
Vranish et al. [49]a	3	12 (12); 20 (0)	PA	3	N/A
Vranish et al. [49]b	3	8 (0); 22 (1)	PA	3	N/A

Abbreviations: F, females; SD, standard deviation; FMD, flow-mediated dilation; SFA, superficial femoral artery; BA, brachial artery; PTA, posterior tibial artery; PA, popliteal artery; SRA, simple resistance activities; N/A, not applicable. Quality was assessed using a modified Heyland Methodological Quality Score, with a maximum score of 10. Labels a and b denotes different trials from the same study.

3.4 Synthesis of the Results

3.3.4.1 Effects of Prolonged Sitting on Flow-Mediated Dilation

Prolonged sitting resulted in a moderate and significant decrease in FMD% ($\mu = -2.14$ %, 95 % Confidence Intervals (CI): -2.69 to -1.59, $p < 0.001$, SMD = -0.73) (**Figure 3.2**). The observed outcomes ranged from -5.0 % to 1.5 % with a majority of trials (86 %) showing a reduction in FMD% in response to an acute bout of prolonged sitting. Examination of the studentised residuals failed to identify any outliers, however, examination of the Cook's distances identified two potentially influential trials [39,225]. Removal of either study did not significantly influence the observed effect, however, removal of either study reduced overall heterogeneity (Carter et al., [225] $I^2 = 10.9$ %, $p = 0.32$, Thosar et al., [39] 11.7 %, $p = 0.31$). An LFK index of 1.45 did indicate minor asymmetry and the heterogeneity was moderate ($I^2 = 32$ %, $p = 0.08$), which may be partially explained by FMD being assessed on different arteries, including upper- and lower-limb arteries. Subgroup analysis revealed small and large significant decreases in SFA and PA FMD%, respectively (SFA, $\mu = -1.61$ %, SMD = -0.43; PA, $\mu = -2.57$ %, SMD = -1.41). There was a non-significant small decrease in PTA FMD% ($\mu = -5.00$ %, SMD = -0.37), and a non-significant trivial increase in BA FMD% ($\mu = 0.03$ %, SMD = -0.02) (**Table 3.2**). Post-hoc power analysis estimates that this analysis achieved 99 % power and thus should be able to accurately detect the true effect of an acute bout of prolonged sitting on FMD. Post-hoc power analysis on included trials identified a median power of 35 %, with 4 trials exceeding 80 % power [42,45,48] (**Figure 3.3**).

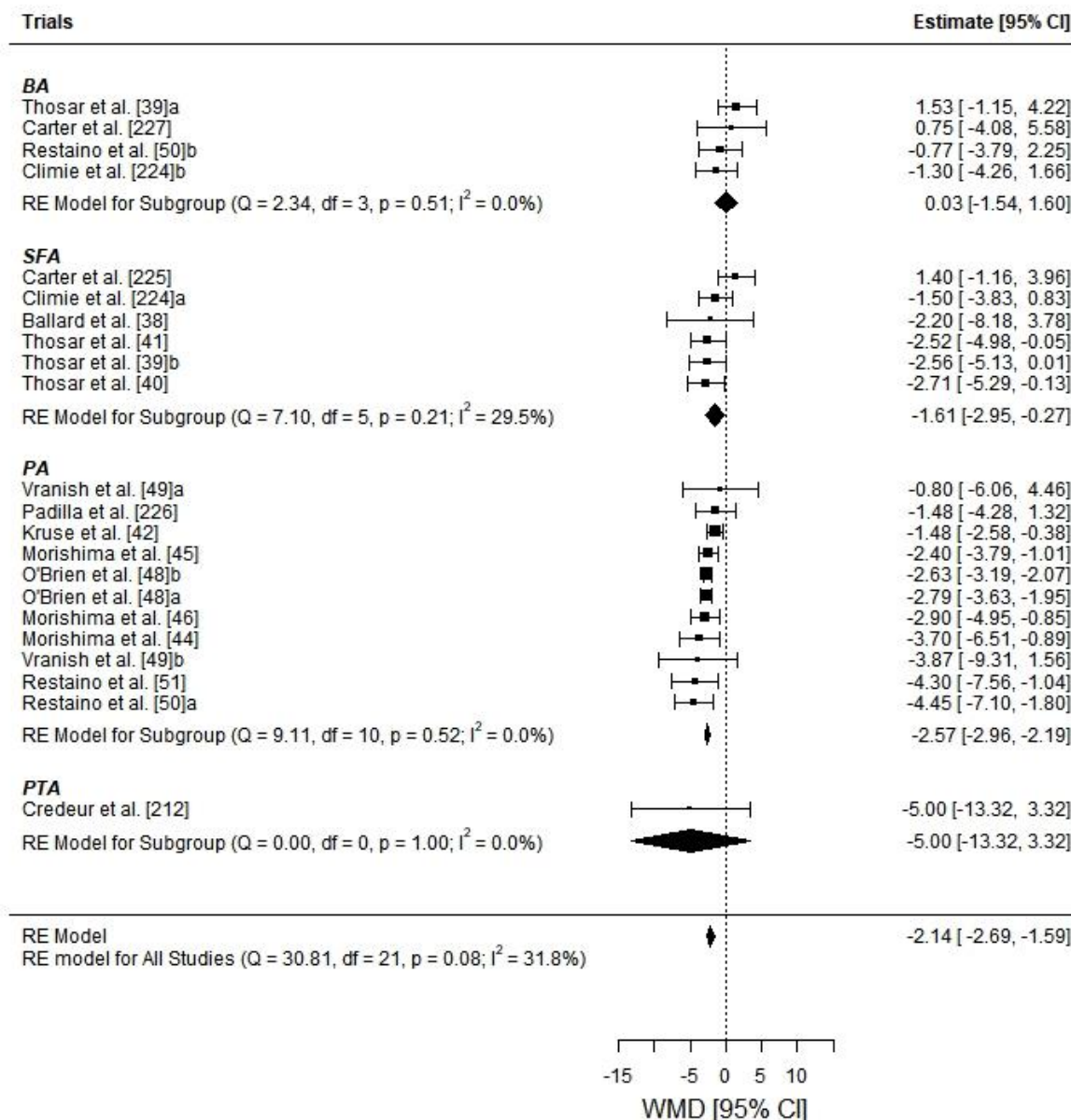


Figure 3.2 The effect of prolonged uninterrupted sitting on vascular function meta-analysis using a random-effects model grouped by artery.

Abbreviations: WMD, weighted mean difference; CI, confidence intervals; BA, brachial artery; SFA, superficial femoral artery; PA, popliteal artery; PTA, posterior tibial artery; RE, random effects. Labels a and b denotes different trials from the same study.

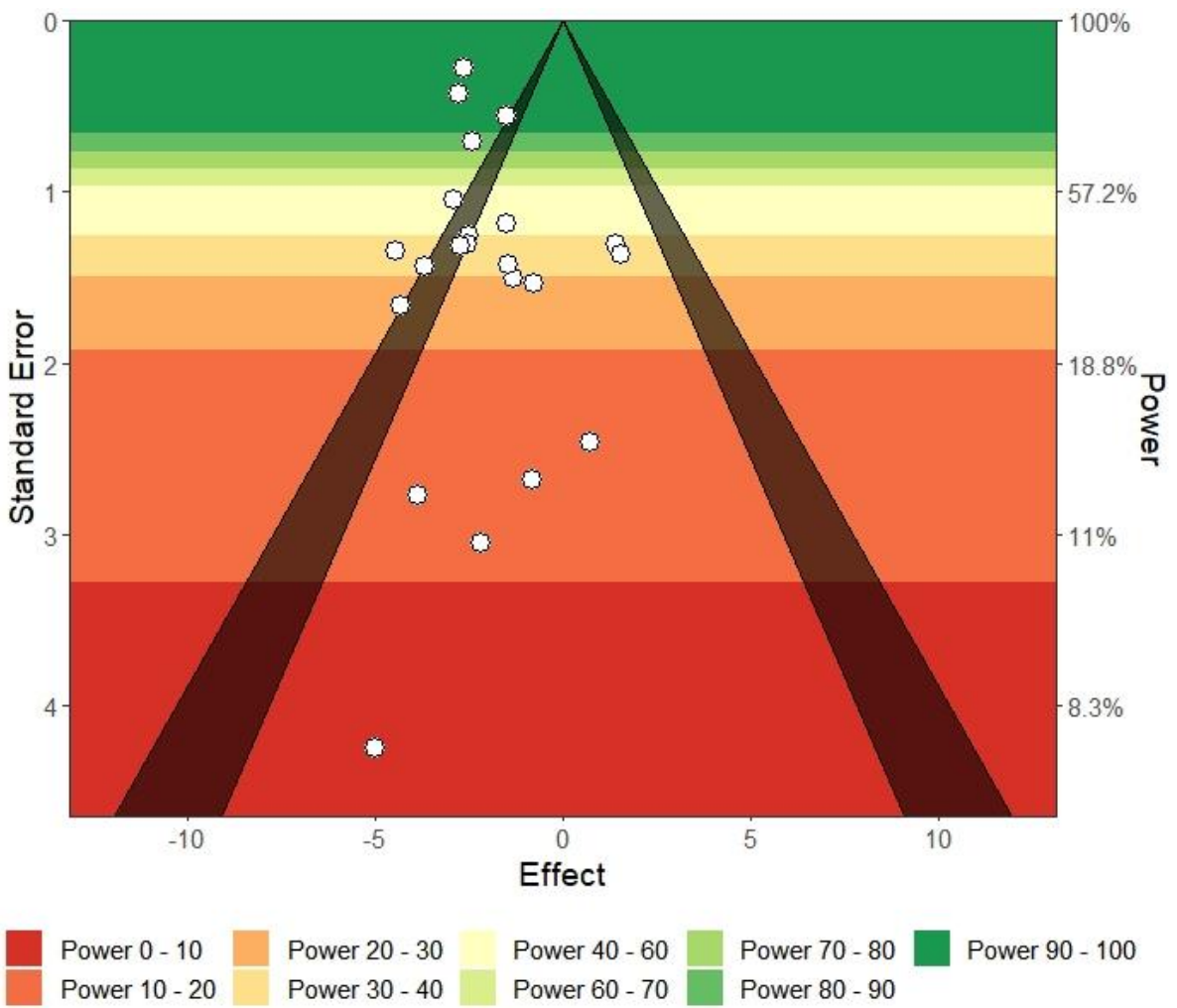


Figure 3.3 Power-enhanced funnel plot detailing power of individual trials within the uninterrupted sitting analysis

Table 3.2 Uninterrupted sitting with subgroup analysis by artery using a random-effects meta-analysis model

	Pooled Effect				Heterogeneity			Asymmetry				
	μ	LCI	UCI	P Value	SMD	Q	P Value	I ²	LFK	Quality	Trials	Sample
All	-2.14	-2.69	-1.59	<0.001	-0.73	30.8	0.08	30.8	1.45	6	22	269
Artery												
SFA	-1.61	-2.95	-0.27		-0.43	7.10	0.21	29.5		7	6	80
BA	0.03	-1.54	1.60		-0.02	2.34	0.51	0		6	4	52
PTA	-5.00	-13.32	-3.32		-0.37	N/A	N/A	N/A		6	1	20
PA	-2.57	-2.96	-2.19		-1.41	9.11	0.52	0		6	11	117

Abbreviations: LCI, lower confidence interval; UCI, upper confidence interval; SMD, standardised mean difference; LFK, Luis Furuya-Kanamori Index; SFA, superficial femoral artery; BA, brachial artery; PTA, posterior tibial artery; PA, popliteal artery; N/A, not applicable. SMD: Trivial, small, moderate and large effect sizes are defined as <0.2, 0.2, 0.5, and 0.8 respectively. LFK: <1 indicates no asymmetry, 1 to 2 suggests minor asymmetry, and >2 indicates major asymmetry. I²: 25 %, 50 %, and 75 % represent low, moderate, and high heterogeneity respectively.

3.3.4.2 Effects of Sitting Interruption on the Flow-Mediated Dilation Response to Prolonged Sitting

Across sitting interruption strategies there was a moderate, significantly greater FMD% for the experimental (interrupted) conditions compared to the control (uninterrupted) ($\mu = 1.91\%$, 95% CI: 0.40 to 3.42, $p = 0.01$, SMD = 0.57) (**Figure 3.4**). The observed outcomes ranged from -0.7% to 5.8% with a majority of trials (89%) showing a beneficial effect of regularly interrupting bouts of prolonged sitting on FMD%. Examination of the studentised residuals and Cook's distances failed to identify any potential outliers or influential trials. An LFK index of 3.36 indicated major asymmetry. The heterogeneity for this analysis was considerable ($I^2 = 79\%$, $p < 0.001$) and may be explained by the low number of trials, testing FMD on different arteries, and the use of varying interruption strategies. With respect to different arteries, subgroup analysis revealed non-significant effects on BA, SFA, and PA FMD% (**Table 3.3**). This analysis also revealed considerable heterogeneity in the SFA and PA subgroups ($I^2 = 77\%$ and 90% respectively). With regards to sitting interruption strategies, simple resistance activities and aerobic interruption strategies resulted in non-significant moderately greater FMD% compared to control conditions (**Table 3.3**). This subgroup analysis also revealed considerable heterogeneity for the aerobic subgroup ($I^2 = 86\%$) and moderate heterogeneity for the simple resistance activities subgroup ($I^2 = 47\%$). Finally, only one trial included standing as an interruption strategy [42], reporting a non-significant difference in FMD% between conditions ($\mu = 0.24$, 95% CI: -0.90 to 1.38) (**Table 3.3**). Post-hoc power analysis estimates that this analysis achieved 50% power whilst post-hoc power analysis of included trials identified a median power of 33%, with two trials from one study exceeding 80% power [42] (**Figure 3.5**).

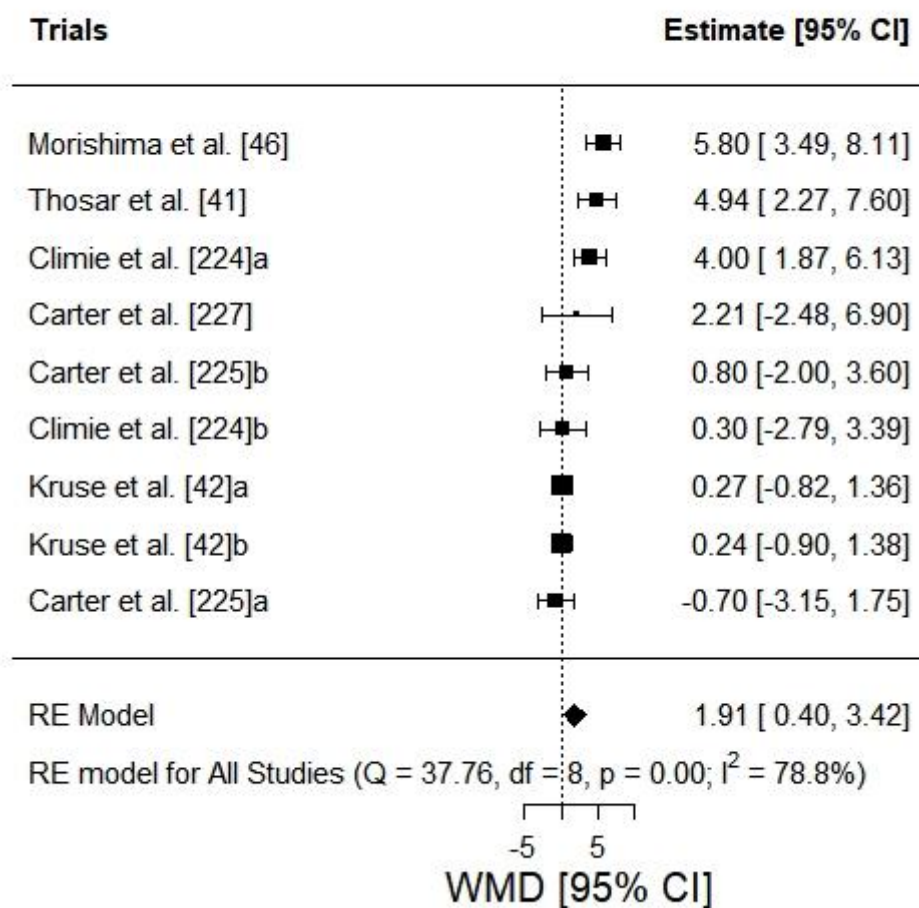


Figure 3.4 The effect of interrupted prolonged sitting on vascular function meta-analysis using a random-effects model

Abbreviations: WMD, weighted mean difference; CI, confidence intervals; RE, random effects. Labels a and b denotes different trials from the same study.

Table 3.3 Interrupted sitting with subgroup analysis by artery and interruption strategy using a random-effects meta-analysis model

	Pooled Effect				Heterogeneity			Asymmetry				
	μ	LCI	UCI	P Value	SMD	Q	P Value	I ²	LFK	Quality	Trials	Sample
All	1.91	0.40	3.42	0.01	0.57	37.8	<0.001	78.8	-3.36	7	9	127
Artery												
SFA	2.28	-0.32	4.88		0.65	13.1	<0.001	77		8	4	31
BA	0.88	-1.70	3.46		0.18	0.44	0.51	0		8	2	29
PA	1.86	-0.68	4.40		0.76	19.8	<0.001	90		7	3	37
Interruption Strategy												
SRA	2.40	-0.08	4.88		0.55	3.78	0.15	47		8	3	48
Aerobic	2.17	-0.34	4.67		0.69	27.8	<0.001	86		7	5	66
Standing	0.24	-0.90	1.38		0.16	N/A	N/A	N/A		7	1	13

Abbreviations: LCI, lower confidence interval; UCI, upper confidence interval; SMD, standardised mean difference; LFK, Luis Furuya-Kanamori Index; SFA, superficial femoral artery; BA, brachial artery; PA, popliteal artery; SRA, simple resistance activities; N/A, not applicable. SMD: Trivial, small, moderate and large effect sizes are defined as <0.2, 0.2, 0.5, and 0.8 respectively. LFK: <1 indicates no asymmetry, 1 to 2 suggests minor asymmetry, and >2 indicates major asymmetry. I²: 25 %, 50 %, and 75 % represent low, moderate, and high heterogeneity respectively.

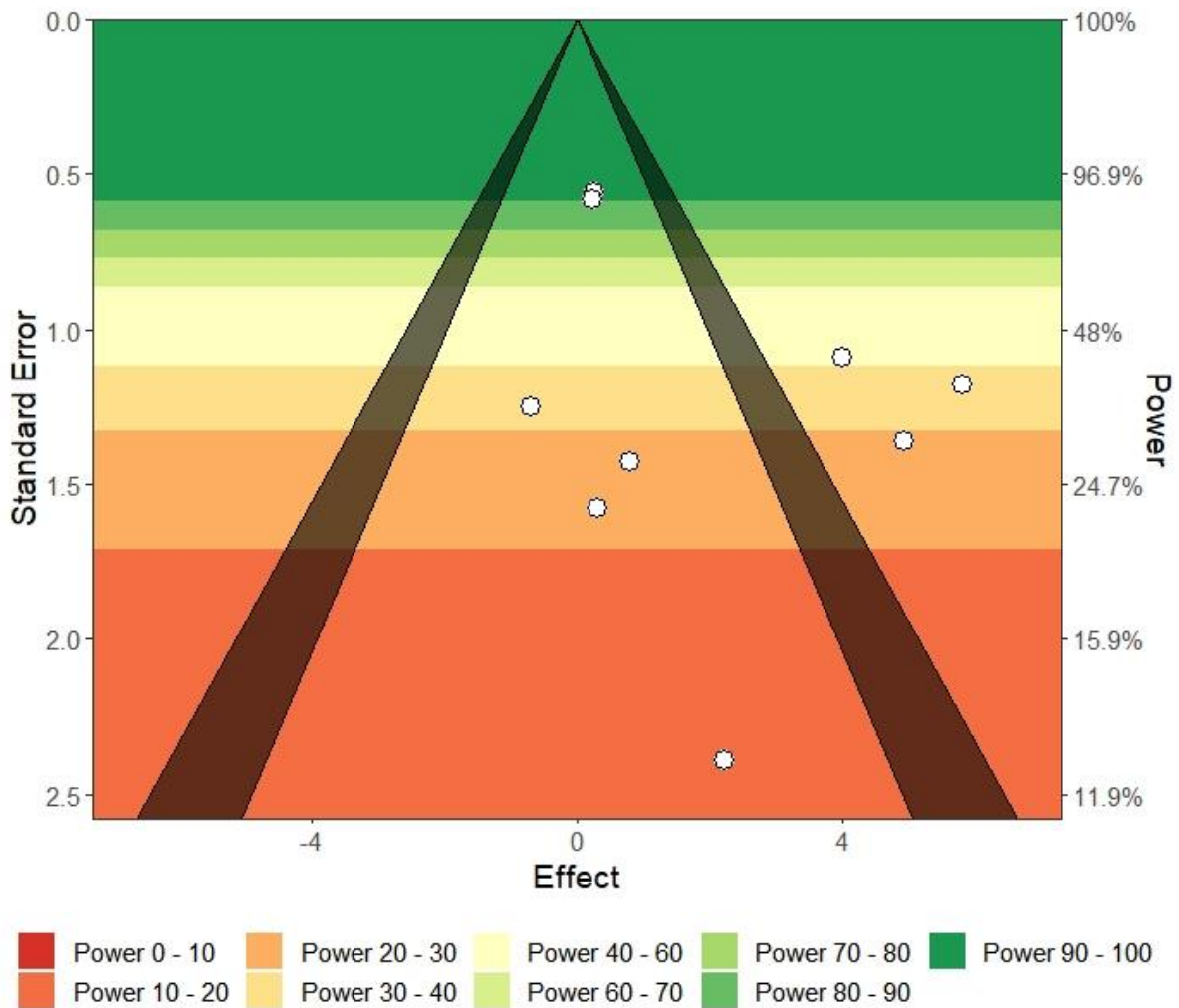


Figure 3.5. Power-enhanced funnel plot detailing power of individual trials within the uninterrupted sitting analysis

3.4 Discussion

The aim of this meta-analysis was to synthesise existing data with respect to the effects of prolonged sitting (>1 hr), with and without interruption, on vascular function in adults. The main findings were that: (1) prolonged uninterrupted sitting resulted in a significant decrease (detrimental) in FMD% ($\mu = -2.14\%$, 95% CI: -2.69 to -1.59, SMD = 0.73), with these effects occurring in the lower limbs (SFA, PA, and PTA), but not in the upper limb (BA); and (2) regular interruptions to sitting appear to confer a protective effect against vascular dysfunction, however, the optimum interruption strategy cannot yet be identified at this time due to the limited number of trials.

3.4.2 Strengths and Limitations

Whilst this meta-analysis has produced meaningful information, several potential limitations should be acknowledged when interpreting the results of this analysis. Firstly,

there was a limited number of eligible trials and the sample sizes were small (range = 8 – 20, median = 12). Additionally, only 4 trials reported sample size calculation for the FMD% outcome [38,40,41,51] none of which, based on this analysis, reached pre-determined power. However, it should be noted that the analysis investigating uninterrupted sitting achieved 99 % power and thus is unlikely to be affected by the underpowered trials. Further, this is the first meta-analysis looking at the effects of prolonged sitting with and without interruption and some important methodological insights for future studies are noted. Second, our analysis considered change in FMD%. FMD% is a ratio calculated by dividing the maximum change in artery diameter in response to reactive hyperaemia by the resting artery diameter. This approach has been criticised as the change in diameter is inversely proportional to baseline diameter and thus a ratio is statistically unsuitable [358]. Allometric scaling has been offered as a means of controlling for the influence of baseline diameter, however this approach may not be able to adequately correct FMD% in different vascular beds or at an individual level, i.e. it can only be applied to group means [218,359,360]. An alternate approach has been to correct FMD% by using shear rate as a covariate [361,362], though this approach has also been suggested to have limitations [363]. These points, taken together, indicate that there is a current lack of consensus about the best statistical strategy for controlling for factors that influence FMD. Consequently, current FMD guidelines still suggest reporting FMD% irrespective of any further analysis [160,348]. Subsequently, these data are readily available within the literature and, whilst certain limitations of the metric are acknowledged, FMD% served as an appropriate metric for this analysis. Thirdly, this study has highlighted a sex-specific void in the research. Specifically, 75 % of the overall sample were male, and of the 10 trials which included females, 6 studied females in the follicular phase of the menstrual cycle [42,48,49,212,225,227], 2 did not control for menstrual cycle [45,46], and 2 studied menopausal women [343]. Given the potential influence of the menstrual cycle on FMD% [307,364–367], and the small number of females sampled, generalising the findings of this meta-analysis to females is difficult. Lastly, this analysis only considered the difference between baseline and final FMD%, so any inferences regarding the time course of vascular dysfunction during uninterrupted sitting cannot be made. This practice was based on ~70 % of trials only implementing pre- and post-sitting FMD assessments [42,44–46,48–51,212,225–227] and the indication by current expert guidelines that participants should be supine for FMD

assessments [160,218]. Consequently, any posture transitions to facilitate repeated assessments would not constitute uninterrupted sitting.

3.4.3 Prolonged Sitting

This meta-analysis demonstrated that prolonged uninterrupted sitting leads to a moderate and significant decline in FMD% (**Table 3.2**), specifically in the lower-limbs. The lower-limb specific findings may be explained by several factors. Firstly, the reported differences in the reduction of shear stress during sitting between upper and lower arteries may explain some of the results [38,41,42,44–46,48,50,51,212,226,343]. Shear stress is considered to be a primary regulator of endothelial function [160]. Indeed, multiple lines of evidence demonstrate that reduced shear stress impairs endothelial function [228–233]. Additionally, changes in shear patterns, specifically increases in retrograde shear in the absence of increased antegrade shear, have been shown to blunt FMD responses [162]. Whilst a majority of trials in this analysis only reported mean shear, the limited trials that reported shear patterns consistently found that retrograde shear did not significantly increase in SFA during prolonged sitting [39–41,225]. Instead, the observed reduction in mean shear appears to be the result of decreased antegrade shear [39–41] and overall blood flow, however more research is required across different arteries to confirm this. As a result of low muscle activity in the lower limbs during prolonged sitting, blood flow, and subsequently shear stress, are likely reduced [210]. In contrast, during trials that sampled the BA, participants were allowed to perform desk-based activities throughout the sitting period [39,50,227,343]. Subsequently, reductions in shear stress may not have occurred to the same extent, explaining, in part, the maintenance of BA FMD. Furthermore, by allowing participants to perform desk-based activities, it is unlikely that significant increases in hydrostatic pressure would have occurred within the upper limbs. Conversely, the increased hydrostatic pressure likely experienced by lower limb arteries [234], compounded by the loss of any muscle pump action, may have resulted in blood pooling [210] and activation of the myogenic response, thereby further reducing blood flow-induced shear stress [234].

Secondly, arterial bending created by flexion at the hip and knee joints during sitting may have also impacted vascular function in arteries of the lower limbs. Arterial tortuosity alone has been shown to significantly reduce blood flow and shear stress independent of changes in hydrostatic pressure, whilst also creating an area of turbulent blood flow

immediately downstream, and thus resulting in an impaired FMD% [45,235]. The greater decline in FMD% seen at the PA compared to the SFA (**Table 3.2**) may be explained by increased turbulent flow, as the assessment site is located close to the knee joint, a site of increased tortuosity. Finally, there is a negative correlation between resting diameters and FMD% [222], and so the greater decline in FMD% in the lower limbs may be explained by arterial location given that arteries further down the vascular tree become narrower [222]. This is likely true of the present findings whereby the PTA (smallest resting diameter) showed the greatest reduction in FMD% as a consequence of prolonged sitting (**Table 3.2**). More studies are required to further investigate this phenomenon, as only one trial assessing the PTA was included in the present analysis. Nevertheless, these data, in tandem with previous work suggesting impaired cerebrovascular endothelial function as a result of prolonged sitting [266], indicate that it is highly conceivable that sitting-induced vascular dysfunction is not solely restricted to larger conduit arteries.

3.4.4 Sitting Interruption

Despite growing evidence demonstrating leg vascular dysfunction following an acute bout of prolonged sitting, research investigating practical sitting interruption strategies is limited. Our analysis, which included 9 trials and a sample size of 127, demonstrated a significantly ($p = 0.01$) greater FMD% ($\mu = 1.91$ %, 95 % CI: 0.40 to 3.42, SMD = 0.57) when sitting was regularly interrupted compared to uninterrupted sitting. However, it should be noted that this analysis was underpowered and thus the results should be interpreted with caution.

One of the challenges when investigating the effect of interrupting sitting is the variety of arteries assessed. It is apparent that lower limb arteries are more affected by uninterrupted sitting, and therefore perhaps also more amenable to the effects of interrupting sitting periods. Indeed, subgroup analysis demonstrated that BA FMD% was the least affected by interruption. Conversely, whilst failing to reach significance, sitting interruption had a moderate effect on SFA FMD% and PA FMD% (**Table 3.3**). The greater FMD% observed in lower limb arteries following interruption is likely the result of preserved blood flow and subsequently shear stress as a product of greater lower-extremity activity [46]. In order to identify optimum interruption strategies to preserve vascular function, separate subgroup analysis was performed.

Findings from the interruption subgroup analysis indicated that both simple resistance activities and aerobic interruption strategies resulted in moderate non-significant differences in FMD% between the experimental and control conditions (**Table 3.3**). The failure of any of the subgroups to reach significance may be a product of the limited number of trials, or the observed heterogeneity present within each subgroup as a product of differing FMD assessment locations or experimental designs. Indeed, this is apparent in the simple resistance activities subgroup which only consisted of 3 trials, 2 of which assessed BA FMD% [227,343] and the third assessed SFA FMD% [343]. It is plausible that simple resistance activities may preserve vascular function, however more trials assessing lower limb arteries are necessary.

With respect to the aerobic subgroup, whilst failing to reach statistical significance, it is likely that this modality is a viable interruption strategy. Indeed, of the 5 trials within the subgroup 4 trials reported an improvement in FMD% from baseline [41,46,225] and 3 reported improvements between conditions [41,46,225]. The considerable heterogeneity within this subgroup ($I^2 = 86\%$) likely contributed to the lack of statistical significance and may be a result of key methodological differences between trials. Of particular note are the findings by Carter et al. [225], which shows that the aerobic interruption strategy utilised resulted in a poorer FMD% outcome than the control condition (**Figure 3.4**). However, this may be a result of uncontrolled lower limb movement during the control condition. In an attempt to improve ecological validity, Carter et al. [225] was the only trial to not restrict lower limb movement during the control condition and may explain why it is the only trial to show improved FMD% in a lower limb artery in response to prolonged sitting (**Figure 3.2**). Subsequently, whilst the original data from this trial shows that the aerobic interruption strategy preserved vascular function, it is masked in this analysis by the elevated control FMD%. Further supporting the notion that aerobic interruption strategies may be beneficial in preventing sitting-induced vascular dysfunction, McManus et al. [368] demonstrated that aerobic interruptions could prevent sitting-induced leg vascular dysfunction in 9-year old girls. As the inclusion criteria for the current meta-analysis was adults, these data were not included in the current analysis. However, this finding, in combination with the data from the present meta-analysis, indicates that aerobic interruption strategies may prevent sitting-induced vascular dysfunction.

Finally, whilst standing has been suggested as a viable sitting interruption strategy and can prevent a decline in central arterial health during bouts of prolonged sitting [369], the present meta-analysis revealed a non-significant trivial difference in FMD% in the lower limbs ($\mu = 0.24\%$, 95 % CI: -0.91 to 1.38, SMD = 0.16) between conditions. It is possible that standing breaks are an insufficient stimulus to increase shear stress and thus prevent sitting-induced vascular dysfunction. However, it is noteworthy that when sitting is fully substituted by standing for 3 hours, leg vascular function is effectively preserved [45]. Accordingly, it appears that while standing breaks may not be sufficient to prevent sitting-induced leg vascular dysfunction, replacing sitting for standing could be a viable strategy to retain vascular function; yet further research is needed to support this conclusion.

3.4.5 Methodological concerns

Determining the effect of interrupting sitting is made challenging by the differences in the experimental design and protocols used by the included trials. This may also be the cause of the high heterogeneity present in the separate subgroup analyses (**Table 3.3**). For example, the considerable heterogeneity across PA trials ($I^2 = 90\%$) may be explained by key differences in the time between the end of the final interruption and the final FMD assessment, which ranged from ~ 10 [46] to ~ 60 [42] mins. Given that shear stress, the principal driver of changes in FMD, has been shown to significantly decrease following as little as 10 minutes of sitting [370], extended periods of inactivity (i.e., 60 minutes) prior to post-sitting FMD assessments will likely mask the true effect of interruption strategies. Conversely, by assessing FMD within 10 minutes of the final interruption [46] it could be argued that the subsequent elevation in shear stress as a consequence of the interruption will likely mask the true effect of prolonged sitting [38]. Whilst it is beyond the scope of this Chapter and indeed this thesis to suggest an optimum methodology, it is clear that the differences between trials seeking to answer similar questions make drawing conclusions challenging. The development and implementation of standardised guidelines may facilitate a better understanding of this research area.

Additionally, there appears to be a discourse in the methodologies employed across prolonged sitting studies using FMD as an outcome measure. One of the most pressing being the posture in which FMD is assessed. Current FMD guidelines state that assessments are performed with participants supine [218]. However, 13 of the 22 trials

in this analysis assessed FMD with participants seated or semi-recumbent [38–41,48–50,343]. To date, there is no evidence that this is either an accurate (valid) or reliable measure compared to the recommended supine position. Additionally, in trials that conducted FMD assessments with participants in a supine position, some trials have reported performing FMD assessments immediately [46], whereas others imply slightly longer rest periods [212]. These divergent practices, post-sitting transition are likely to increase the risk of under- or over-estimation of FMD. The development and implementation of standardised guidelines may improve the congruency of future research. Additionally, the validation of seated FMD assessments would allow researchers to confidently chart the time course of vascular dysfunction as a result of prolonged uninterrupted sitting and whether a dose-response curve exists. Currently, trials that have performed seated SFA FMD assessments multiple times throughout a bout of uninterrupted sitting have all demonstrated significant declines within the first hour of sitting [38–41]. However, some have proceeded to continue a gradual decline as sitting time increases [38], whereas others have shown an upwards trend past the 1 hour point [39–41,343]. Without a validated means of assessing vascular function with participants in a seated position and standardised guidelines, understanding the time course of dysfunction and the mechanisms responsible remain challenging.

3.5 Conclusions

Epidemiological literature has established a detrimental association between sedentary behaviours, such as prolonged sitting, and CVD incidence and all-cause mortality. Vascular dysfunction may be a key mechanism in explaining this association. This meta-analysis is the first to amalgamate the existing data of the effect of prolonged sitting on vascular function. The results of this analysis indicate that (1) periods of prolonged uninterrupted sitting in excess of 1 hour may lead to a meaningful decrease in vascular function in lower limb arteries, and that, (2) this dysfunction can be avoided by regularly interrupting sitting, particularly with aerobic interruptions or simple resistance activities. In order to identify optimum interruption strategies, future research, utilising synergistic experimental methodologies is required. This future research should aim to determine the optimal dose, duration, and intensity of sitting interruption. Within the context of this thesis, the results of this review suggest that if FMD is to be used as means of assessing vascular function, any assessments should be made in a lower limb artery.

However, questions remain about the suitability of FMD as a measure in prolonged sitting research which will be discussed further in the following Chapter.

4

Joining Chapter

Prior to Chapter 3, growing evidence suggested that bouts of prolonged sitting may negatively impact vascular function and that regular interruptions to sitting may offset that effect. However, the magnitude of the effect of sitting on vascular function and whether effects differed across arteries was unclear. Additionally, whilst various studies had investigated potential interruption strategies, it remained uncertain how different arteries were affected and whether an optimum interruption strategy may exist. The data presented in Chapter 3 are the first to robustly synthesise the existing data related to vascular function and prolonged sitting with and without interruption. The results of Chapter 3 indicate that bouts of prolonged sitting (≥ 1 hr) result in significant, moderate to large declines in FMD% in lower limb arteries (superficial femoral, popliteal, and posterior tibial arteries), but not in the brachial artery. Further to this, in response to the call by the World Health Organization to investigate the optimum sitting interruption [35], the effect of regularly interrupting sitting was investigated with subgroup analysis to identify the likely effect of different interruption strategies. Current data suggests that regularly interrupting bouts of prolonged sitting confers a protective effect against vascular dysfunction, likely as a result of increased shear stress within the artery, however, it is not possible to identify an optimum interruption strategy at this point. The findings of Chapter 3 are the first to demonstrate a robust decline in lower limb arteries and as such provide evidence for the contemporary working model of sitting-induced cardiovascular dysfunction. Additionally, the finding that regularly interrupting sitting may confer some protective effect against prolonged sitting via maintenance of lower limb blood flow and shear rate provides further support for the contemporary working model.

Several additional methodological concerns were also highlighted related to inconsistencies between studies and the urgent need for standardised guidelines that are specific to prolonged sitting research. These guidelines may reduce the chance of error in future measurements. Indeed, previous research has shown that greater adherence to FMD guidelines related to resting brachial artery FMD is inversely associated with typical error estimates [223]. Currently, typical reported coefficients of variation for repeated FMD assessments range from 11.6 – 17.5 % [371–373]. Given that a change in FMD of 1% is purported to be clinically meaningful, such variation in results may bring the suitability of FMD into question given the wide variety of responses reported within Chapter 3. Indeed, upon close inspection of several studies included in the analysis of Chapter 3,

there are potential causes for concern. Examining reported baseline diameters, five trials report increases in resting diameter pre-sitting vs post-sitting of ~3 – 12 % with no obvious physiological explanation [44–46,224]. In the absence of physiological underpinnings, it is not unreasonable to conclude that these differences are, at least in part, due to measurement error associated with the difficulties inherent to FMD assessments and analysis. Given that the pooled estimate of sitting-induced dysfunction in Chapter 3 was found to be $\mu = -2.14\%$, and the greatest observed change in any trial was 5 %, such a large potential for measurement error is concerning. Consequently, whilst FMD may provide some useful mechanistic data in the pursuit of developing a biologically plausible model for the association between sedentary time and cardiovascular disease, the potential for error highlights that it may not be a suitable tool in many circumstances. As such, alternative tests of cardiovascular health and function may be necessary for future trials and later Chapters within this thesis. These tools should seek to be less prone to error whilst also having established prognostic and clinical value. Two such measures may be peripheral blood pressure and pulse wave velocity (PWV). Peripheral blood pressure can be readily assessed in many situations by trained or minimally trained individuals and arguably is a simple metric for clinicians to assess, epidemiological studies to utilise, and for the public to understand. Whilst PWV is less readily translatable to the public, it is a relatively easy measure to perform (compared to measures such as FMD) and has clear clinical implications. In order to understand the current evidence as it pertains to blood pressure and prolonged sitting, the following Chapter will consolidate the existing evidence using meta-analysis before the evidence related to pulse wave velocity is consolidated in [Chapter 7](#).

5

The Effects of Acute Exposure to Prolonged Sitting, with and without Interruption, on Peripheral Blood Pressures Among Adults: A Systematic Review and Meta-Analysis

A modified version of this Chapter has been previously published

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<https://doi.org/10.1007/s40279-021-01614-7>

5.1 Introduction

The previous Chapters have identified that prolonged uninterrupted sitting can negatively affect vascular function, particularly in the lower limbs, however, owing to the technical challenges, associated error, and methodological inconsistencies between trials using FMD, it may not be an appropriate tool for prolonged sitting research. An alternative, well-established marker of cardiovascular health is peripheral blood pressure. Multiple studies have demonstrated that an increase in peripheral blood pressure over the course of a single bout of prolonged uninterrupted sitting [43–45,47–49,57,59,261,266,321,369,374,375]. However, to date, there has been no effective synthesis of the existing literature.

Sedentary behaviours, particularly prolonged sitting, comprise a large portion of the day for people across many societies [21–23,25,26,28,30–32]. As such, it is critical that the physiological consequences of this ubiquitous behaviour on the cardiovascular system are better understood. This understanding is required to not only guide intervention development, but also to establish biological plausibility and inform policy [376]. Blood pressure reflects the amount of force exerted on vessel walls by the blood, whilst also indicating the acute burden on vital end-organs such as the brain and kidneys [377–379]. As a function of sedentary behaviour, it is conceivable that many individuals are repeatedly exposed to prolonged elevations in blood pressure both daily and cumulatively. This is of particular concern as epidemiological data suggests that long term increases in blood pressure are associated with increased incidence of CVD [380], though it should be noted that the prognostic value of sitting-induced elevation in blood pressure are as yet unknown. It is also plausible that the blood pressure response to prolonged sitting is moderated by demographic factors, including aging. Different age groups may experience varying effects of prolonged uninterrupted sitting as a function of the age-related changes to the vascular system and blood pressure regulation [146,381].

Previous reviews and [Chapter 3](#) have demonstrated that regularly interrupting bouts of prolonged sitting with aerobic interruption strategies, such as walking, can offset some of the deleterious effects on cardiometabolic health [52,53,298,299]. It is therefore plausible that similar strategies may help to offset any detrimental effects on peripheral blood pressure. In line with the World Health Organization's suggestion [35] and the

findings of Chapter 3, it is important to distinguish which physical activity interruption strategies are most efficacious. Understanding which strategies may be most effective in preventing sitting-induced blood pressure elevations is an important step in informing future policy and guidelines as well as informing subsequent Chapters. Therefore, it is also necessary to consolidate the existing literature investigating how regularly interrupting bouts of prolonged sitting may affect peripheral blood pressure.

5.1.2 Objectives

The objectives of this Chapter are two-fold: (1) determine the effect of uninterrupted sitting on peripheral blood pressure, with subgroup analysis to identify whether age of participants helped to explain heterogeneity; and (2) to conduct a separate meta-analysis to determine the effect of sitting interruption strategies on peripheral blood pressure, with subgroup analysis of sample age and interruption strategy type.

5.2 Methods

This systematic review and meta-analysis was reported in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [382], however it was not pre-registered.

5.2.2 Data Sources and Searches

Electronic databases (PubMed, Web of Science, and SportDiscus) were searched by two authors (myself [CP] and Lee Stoner [LS]). As peripheral blood pressure is frequently assessed as a secondary, as well as primary outcome measure the search terms utilised reflected the need to capture all relevant literature related to prolonged sitting, cardiovascular and cardiometabolic health. The reference lists of all relevant articles and reviews were also examined. The search was limited to English language studies published between inception and March 2021. Full details of the search strategy were as followed: (sitting OR prolonged sitting OR sedentary OR sedentary behaviour) AND (blood pressure OR flow-mediated dilation OR cardiometabolic risk OR cardiovascular OR hemodynamic OR haemodynamic OR pulse wave velocity OR vascular OR arterial stiffness OR heart rate OR hypertension OR endothelial function OR blood flow OR metabolic OR lipids OR cholesterol OR triglycerides OR glucose OR insulin).

5.2.3 Article Selection

For this meta-analysis, the terms 'study' and 'article' are used interchangeably, whereas 'trial' refers to the unit included in the meta-analysis. As such, a given study can comprise multiple eligible trials, for example separate independent samples within a study (for example, males vs females). After the primary searches were complete, duplicate studies were removed and study titles and abstracts were screened for relevance. After this initial screening, the full texts of potentially eligible articles were reviewed for inclusion. The following criteria were used to select trials for inclusion: (i) peripheral blood pressure (systolic [SBP], diastolic [DBP], or mean arterial pressure [MAP]) was measured non-invasively in the upper limb pre- and post-sitting; (ii) studies were either randomised controlled, randomised crossover, or quasi-experimental pre- versus post-test trials; (iii) the prolonged sitting period was at least 60 mins; (iv) pre- and post-sitting blood pressure assessments were performed in the same posture; (v) participants were adults (≥ 18 years), free of autonomic or neuromuscular dysfunction and any other known chronic illness. It should be noted that trials were still included in the analysis for objective (1) if restroom and water breaks were permitted during the sitting period as these were deemed to not represent sitting interruption strategies.

To address the second objective of this review, further additional inclusion criteria were used; (i) if a strategy was employed to disrupt the effects of sitting, the strategy must have been during the sitting period; (ii) there must have been a control (uninterrupted sitting) group or condition, and (iii) the interruption strategy must have involved the participants actively moving their limbs. If a study employed an intervention prior to or after the sitting period, only data from the control (uninterrupted sitting) trial was included in the analysis. Study selection was completed independently by two researchers (CP and LS).

5.2.4 Data Extraction and Quality Assessment

Data extracted for each eligible trial included bibliographic information (author, publication year), collected measures, sample characteristics (age, sex, body mass index [BMI], etc.), details of any interventions, and pre- and post-sitting peripheral blood pressure values. If data were presented as figures, values were extracted using ImageJ image analysis software [342]. This method of extracting data inevitably introduces a degree of error. To quantify the likely amount of measurement error introduced, those researchers extracting data (CP and Gabriel Zieff [GZ]) completed tests of reliability and

validity on existing known data sets from previous projects conducted by our group. Researchers extracted data three times from four published figures, closing and recalibrating extraction software each time. Researchers completed these extractions independently and were blinded to the true data values and each other's extracted values throughout. Comparison of extracted values to known data values showed excellent validity and reliability for both researchers (CP, intraclass correlation coefficient [ICC] = 0.99, $r = 0.99$, and GZ, ICC = 0.99, $r = 0.99$) [383]. Inter-rater reliability was then assessed using a two-way mixed, absolute agreement, average-measures ICC to assess the degree of consistency between researchers using *irr* package [384] in R [351]. The ICC (0.99) indicated that minimal measurement error was likely to have been introduced [383]. Data extraction was completed independently by two researchers (CP and GZ) and checked for agreement.

Study quality was assessed using a modified Heyland Methodological Quality Score (HMQS) [346,347] with a maximum score of 9. The HMQS criteria "blinding", "extent of follow up", and "outcomes" were not considered for the current analysis as these criteria are for longitudinal study designs. Quality assessment was conducted independently by two researchers (CP and Keeron Stone), with a third researcher (Simon Fryer) acting as an adjudicator in the event of a lack of consensus.

5.2.5 Data Synthesis

For the primary outcomes, the pre- and post-intervention values (mean and standard deviation) as well as mean difference and associated standard deviations were entered into a spreadsheet. When data were not published, a request for missing data was made to the corresponding author and following a non-response, the values were estimated based on methods described within the Cochrane Handbook for Systemic Reviews of Interventions.[345] For trials reporting multiple time points, only pre- and post-sitting values were used in analysis. Aggregation and calculation of final results was conducted by two authors (CP and LS).

5.2.6 Data Analysis

Data was analysed using the *metafor* package (version 2.4.0) [350] in R (version 4.0.3) [351]. Outcome measures (μ) were expressed as weighted mean difference (WMD) and standardised mean difference (SMD), expressed as Cohen's d [385]. The SMD was used to

assess the magnitude of effect, where < 0.2 , 0.2 , 0.5 , and 0.8 was defined as trivial, small, moderate, and large respectively [385]. Data were pooled using the inverse variance heterogeneity (IVhet) model of meta-analysis to account for potential heterogeneity within and between studies. This method has been shown to be a suitable alternative to the more traditional random-effects model, which has been suggested to produce overconfident estimates when using heterogeneous data [386]. Corresponding forest plots for each analysis were also generated.

Subsequent to running the IVhet models, we examined the robustness of the pooled results, the potential for publication bias, and explored potential sources of heterogeneity. Potential outliers or influential trials were examined using studentised residuals and Cook's distances with the same thresholds identified in [Chapter 3](#) [353]. If a trial was identified as influential or a potential outlier, the model was repeated with the trial omitted in order to test the robustness of the overall effect. Small-study bias was adjudicated using the Luis Furuya Kanamori (LFK) indexes in tandem with Doi plots [354]. Doi plots are reported to be more objective than traditional funnel plots, which are assessed qualitatively, and the LFK index has been shown to be a more sensitive measure of small-study bias than the traditional Egger's regression [354]. Using this method, $< \pm 1$ indicates no asymmetry, ± 1 to ± 2 suggests minor asymmetry, and $> \pm 2$ indicates major asymmetry [354]. Last, statistical heterogeneity was assessed using the I^2 statistic, where $< 25\%$, 50% , and 75% represent low, moderate, and considerable heterogeneity, respectively [355]. Heterogeneity $\geq 25\%$ was assumed to indicate that effect sizes could not be treated as estimates of one common effect size, justifying *a priori* determined subgroup analysis. Finally, the statistical power of each analysis and its composite trials was examined using the methods described in [Chapter 3](#). Data analysis was conducted by two authors (CP and LS) to ensure accuracy of the analysis.

5.3 Results

5.3.1 Literature Search and Trial Selection

The literature search strategy and outcomes are outlined in **Figure 5.1**. Initial database searches identified a total of 9,763 potentially eligible articles with no additional ones identified through manual searches. Following removal of duplicates and initial screening of titles and abstracts, 9,680 articles were excluded as they failed to meet all inclusion

criteria. The remaining 83 articles were subjected to full text screening and 50 further studies were excluded, bringing the total to 33 individual articles. The final analyses for objective (1) included 24 trials (21 articles) for SBP, 22 trials (20 articles) for DBP, and 26 trials (20 articles) for MAP. The final analyses for objective (2) included 31 trials (17 articles) for SBP and 27 trials (16 articles) for DBP. Fourteen trials (9 articles) reported MAP data, however, owing to the limited number of articles, meta-analysis was not performed.

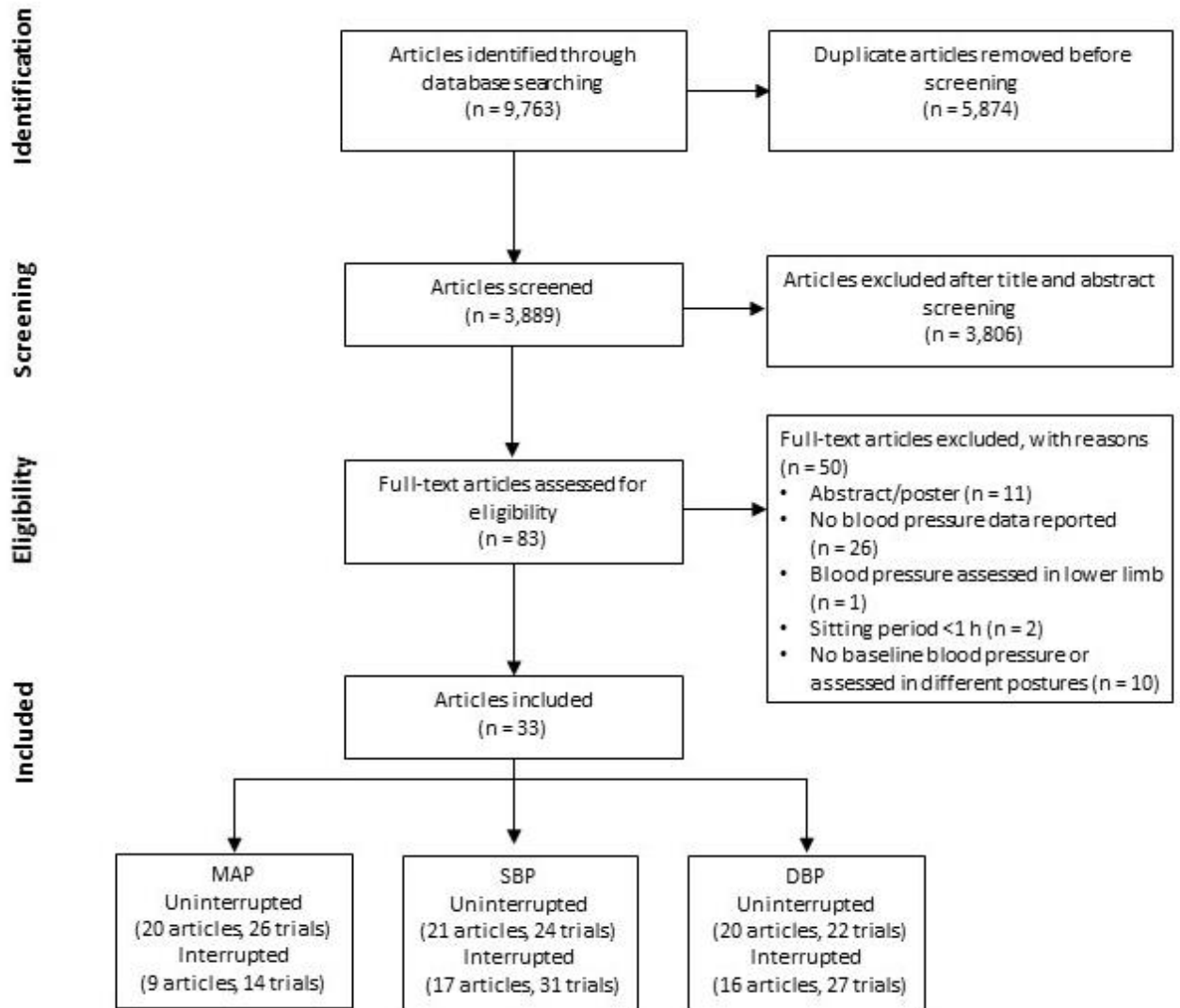


Figure 5.1 Flow chart of study selection

5.3.2 Characteristics of Included Studies

Trial characteristics are summarised in **Table 5.1**.

5.3.2.2 Systolic Blood Pressure

A total of 24 trials (21 articles) were included in this analysis. The number of participants in each trial ranged from 6 [262] to 67 [387]. Of the 24 trials, 19 included both male and female participants [369,262,321,224,56,259,59,388,280,375,42,374,389–391,387,260,261], 4 included only females [47,48,392], and one included only males [48]. Bouts of prolonged sitting ranged from 2.5 [280] to 10 hours [388], with a modal duration of three hours. Average ages ranged from 21.7 [59] to 71 [260] years and BMI ranged from 23 [259] to 33 [56] kg.m².

Of the 21 articles that reported SBP outcomes, 17 (31 trials) included sitting interruption strategies [42,56,59,224,259–262,321,369,374,375,388–392]. These interruption strategies included walking or running [56,260–262,321,374,388,389,391,392], cycling [42,259,261], standing breaks [42,260,261,369,392], seated calf-raises [59], and simple resistance activities [56,224,375,390]. For subgroup analysis, all sitting interruption strategies including walking or running, and cycling were grouped together as an ‘aerobic interruption strategy’ subgroup. Further, simple resistance activities and seated calf raises were group together as a “simple resistance activities” subgroup.

Table 5.1 Characteristics of included trials

Ref	Quality	Sample [n (F); mean age, years (SD)]	Body Mass Index [kg.m²] Mean (SD)	Reported BP outcomes	Sitting duration (h)	Interruption strategy
Ballard et al. [38]	6	11 (0); 21.2 (1.9)	24.7 (1.0)	MAP	3	N/A
Barone Gibbs et al. [389]	8	25 (9); 42 (12)	31.9 (5.0)	SBP, DBP, MAP	7	Standing
Bhammar et al. [262]	5	6 (2); 32 (5)	30.3 (4.6)	SBP, DBP, MAP	9	Aerobic
Carter et al. [266]	7	15 (5); 35.8 (10.2)	25.5 (3.2)	MAP	4	Aerobic
Carter et al. [227]	7	10 (4); 27.3 (8.1)	NR	MAP	1.5	SRA
Champion et al. [321]	8	24 (12); 35.8 (14.7)	25.7 (4.8)	SBP, DBP	6.5	Aerobic
Charlett et al. [393]	8	12 (7); 25 (6)	24.7 (4.9)	MAP	5	SRA
Climie et al. [224]	9	19 (8); 57 (12)	30.3 (3.4)	SBP, DBP	5	SRA
Credeur et al. [212]	5	20 (7); 26 (7)	30.0 (7.0)	MAP	3	N/A
Decker et al. [58]a	6	12 (0); 25 (4)	22.0 (2.0)	MAP	1.5	N/A
Decker et al. [58]b	6	14 (14); 23 (3)	26.0 (3.0)	MAP	1.5	N/A
Dempsey et al. [56]	9	24 (10); 62 (6)	33.0 (3.4)	SBP, DBP	8	Aerobic and SRA
Dogra et al. [259]	5	10 (5); 24.7 (3)	23.0 (2.1)	SBP, DBP	4	Aerobic
Evans et al. [59]	8	20 (14); 21.7 (2.5)	25.5 (6.1)	SBP, DBP, MAP	3	Aerobic
Freire et al. [388]	7	25 (15); 24.4 (3.8)	26.1 (3.4)	SBP, DBP, MAP	10	Aerobic
Garten et al. [57]a	5	10 (2); 25 (3.2)	23.0 (6.3)	MAP	3	N/A
Garten et al. [57]b	5	10 (2); 25 (3.2)	25.0 (3.2)	MAP	3	N/A
Hartman et al. [394]	7	24 (15); 65 (5)	29.8 (3.9)	MAP	3	Aerobic
Headid et al. [280]	3	12 (6); 22.3 (2)	23.9 (3.0)	SBP, DBP, MAP	2.5	N/A
Kerr et al. [392]	8	10 (10); 66 (9)	30.6 (4.2)	SBP, DBP	5	Aerobic and Standing
Kowalsky et al. [375]	8	14 (12); 53.4 (9.5)	30.9 (4.8)	SBP, DBP	4	SRA
Kruse et al. [42]	8	13 (3); 38 (3)	29.7 (2.0)	SBP, DBP	4	Aerobic and Standing
Larsen et al. [374]	9	19 (8); 53.8 (4.8)	31.2 (3.9)	SBP, DBP, MAP	5	Aerobic
Morishima et al. [45]	6	15 (5); 26.7 (0.5)	25.6 (0.5)	MAP	3	N/A
Morishima et al. [43]a	6	10 (0); 19.7 (0.6)	22.5 (2.3)	MAP	3	N/A

Morishima et al. [43]b	6	9 (0); 21.1 (1.8)	24.8 (1.5)	MAP	3	N/A
Morishima et al. [44]	7	9 (0); 21.2 (2)	22.0 (3.0)	MAP	3	N/A
O'Brien et al. [47]a	7	9 (9); 23 (3)	24.5 (3.0)	SBP, DBP, MAP	3	N/A
O'Brien et al. [47]b	7	9 (9); 23 (3)	23.6 (2.8)	SBP, DBP, MAP	3	N/A
O'Brien et al. [48]a	6	10 (10); 23 (2)	24.2 (3.2)	SBP, DBP, MAP	3	N/A
O'Brien et al. [48]b	6	10 (0); 24 (2)	26.6 (2.0)	SBP, DBP, MAP	3	N/A
Peddie et al. [389]	9	18 (7); 23.5 (5)	23.7 (2.6)	SBP, DBP	6	Aerobic
Taylor et al. [390]	9	24 (11); 61.5 (7.8)	32.6 (3.5)	SBP, DBP	7	SRA
Vranish et al. [49]a	6	12 (12); 20 (0)	24.0 (2.8)	MAP	3	N/A
Vranish et al. [49]b	6	8 (0); 22 (1)	25.7 (2.6)	MAP	3	N/A
Wennberg et al. [391]	9	19 (9); 59.7 (8.1)	31.5 (4.7)	SBP, DBP	7	Aerobic
Wheeler et al. [387]	9	67 (35); 67 (7)	31.2 (4.1)	SBP, DBP	8	Aerobic
Yates et al. [260]a	9	30 (15); 69 (6.7)	26.7 (4.3)	SBP	7.5	Aerobic and Standing
Yates et al. [260]b	9	30 (14) ; 71 (6.7)	26.5 (2.4)	SBP	7.5	Aerobic and Standing
Zeigler et al. [261]	6	9 (7) ; 30 (15)	28.7 (2.7)	SBP, DBP	8	Aerobic and Standing

Abbreviations: F, females; SD, standard deviation; BP, blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; SRA, simple resistance activities; NR, not reported; N/A, not applicable, i.e. no interruption strategy. Quality was assessed using a modified Heyland Methodological Quality Score, with a maximum score of 9. Labels a and b denotes different trials from the same study.

5.3.2.3 Diastolic Blood Pressure

A total of 22 trials (20 articles) were included in this analysis. The number of participants in each trial ranged from 6 [262] to 67 [387]. Of the 22 trials, 17 included both male and female participants [42,56,59,224,259,261,262,280,321,369,374,375,387–391], 4 included only females [47,48,392], and one included only males [48]. Bouts of prolonged uninterrupted sitting ranged from 2.5 [280] to 10 hours [388], with a modal duration of 3 hours. Average age ranged from 21.7 [59] to 67 [387] years and BMI from 23 [259] to 33 [56] kg.m².

Of the 20 articles that reported DBP, 16 (27 trials) assessed sitting interruption strategies. These interruption strategies included various intensities and durations of walking or running [56,261,262,321,374,388,389,391,392], cycling [42,259,261], simple resistance activities [56,224,375,390], seated calf-raises [59], and standing breaks [42,261,369,392]. For subgroup analysis, all sitting interruption strategies including walking or running, and cycling were grouped together as an ‘aerobic interruption strategy’ subgroup. Further, simple resistance activities and seated calf raises were group together as a “simple resistance activities” subgroup.

5.3.2.4 Mean Arterial Pressure

A total of 26 trials (20 articles) were included in this analysis. The number of participants in each trial ranged from 6 [262] to 25 [369]. Of the 25 trials, 16 included both male and female participants [43,45,57,59,212,227,262,266,280,369,374,388,393,394], 5 included only females [47–49,58], and 5 included only males [38,44,48,49,58]. Bouts of prolonged uninterrupted sitting ranged from 1.5 [58,227] to 10 [388] hours, with a modal duration of 3 hours. Average age ranged from 19.7 [43] to 65 [394] years and BMI ranged from 22 [44,58] to 31.9 [369] kg.m².

Of the 20 articles that reported MAP, 9 (14 trials) assessed sitting interruption strategies. Sitting interruption strategies included seated calf-raises [59], standing breaks [369], simple resistance activities [227,393], and walking [262,266,374,388,394].

5.3.3 Methodological Quality Assessment

The methodological quality assessment of included trials is summarised in **Table 5.1**. The quality in studies ranged from 3 to 9, with a maximum score of 9 available. The average

methodological quality score across all trials was 7, with a modal score of 8, indicating that most trials were of moderate to high methodological quality.

5.3.4 Synthesis of the Results

5.3.4.1 *Sitting without interruption*

Prolonged uninterrupted sitting resulted in a trivial significant increase in SBP ($\mu = 3.2$ mmHg, 95 % Confidence Intervals [95 % CI]: 0.6 to 5.8, $p = 0.016$, SMD = 0.14) (**Figure 5.2**). Three trials were identified as potentially influential [47,48,387], though omission of each of these trials did not significantly affect the observed result. Visual inspection of Doi plot and an LFK index of -0.21 indicated no asymmetry. The heterogeneity was moderate ($I^2 = 60\%$, $p < 0.001$), justifying subgroup analysis. Subgroup analysis by age is presented in **Table 5.2**. Post-hoc power analysis estimates that this analysis achieved 98 % power. Post-hoc power analysis on included trials identified a median power of 11.8 %.

Prolonged uninterrupted sitting resulted in a trivial non-significant increase in DBP ($\mu = 0.1$ mmHg, 95 % CI: -1.3 to 1.6, $p = 0.864$, SMD = 0.00) (**Figure 5.3**). No trials were identified as a potential outlier or influential, and visual inspection of Doi plot and an LFK index of -0.44 indicated no asymmetry. The heterogeneity in this analysis was low ($I^2 = 24\%$, $p = 0.150$). Post-hoc power analysis estimates that this analysis achieved 0.03 % power. Post-hoc power analysis on included trials identified a median power of 5 %.

Prolonged uninterrupted sitting resulted in a small and significant increase in MAP ($\mu = 3.3$ mmHg, 95 % CI: 2.2 to 4.4, $p < 0.001$, SMD = 0.37) (**Figure 5.4**). Two trials were identified as potentially influential [47,280], though omission of each of these trials did not significantly affect the observed result. Visual inspection of Doi plot and an LFK index of -0.75 indicated no asymmetry. The heterogeneity in this analysis was low ($I^2 = 0\%$, $p = 0.669$). Subgroup analysis by age is presented in **Table 5.2**. Post-hoc power analysis estimates that this analysis achieved 31% power. Post-hoc power analysis on included trials identified a median power of 20.7 %.

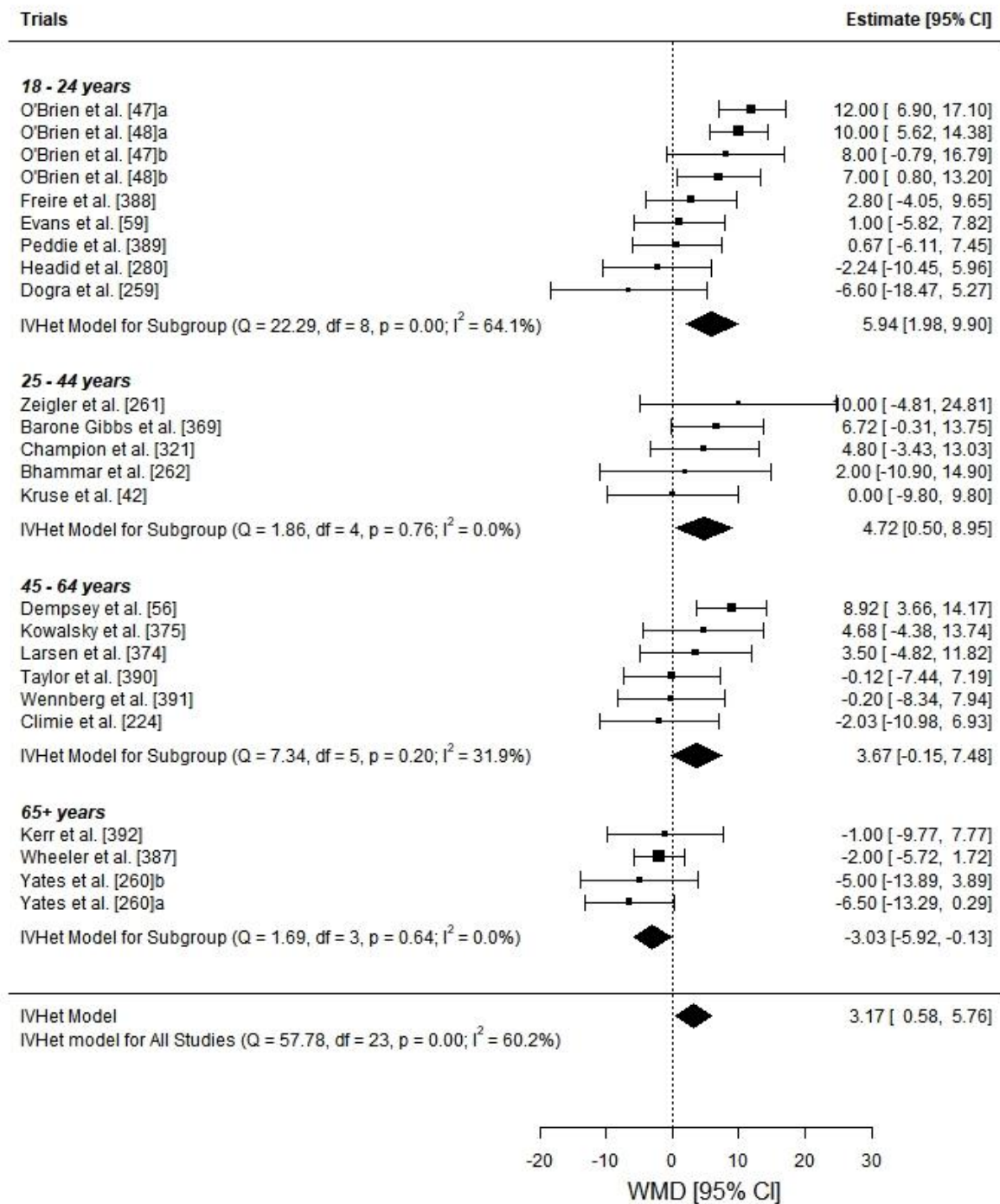


Figure 5.2 The effect of prolonged uninterrupted sitting on systolic blood pressure meta-analysis using an inverse heterogeneity model grouped by participant age. Abbreviations: WMD, weighted mean difference; CI, confidence intervals; IVHet, inverse heterogeneity.

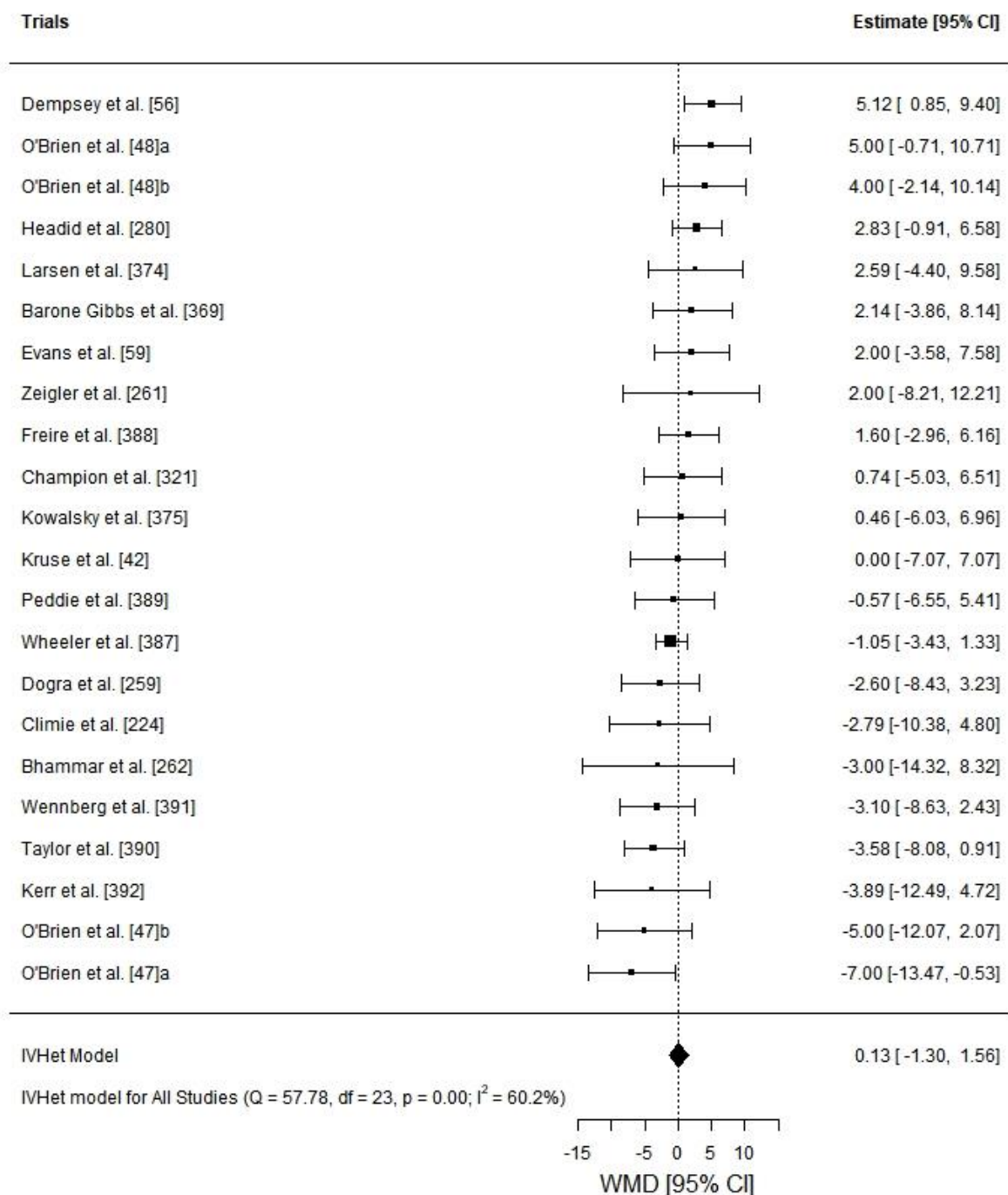


Figure 5.3 The effect of prolonged uninterrupted sitting on diastolic blood pressure meta-analysis using an inverse heterogeneity model.
Abbreviations: WMD, weighted mean difference; CI, confidence intervals; IVHet, inverse heterogeneity.

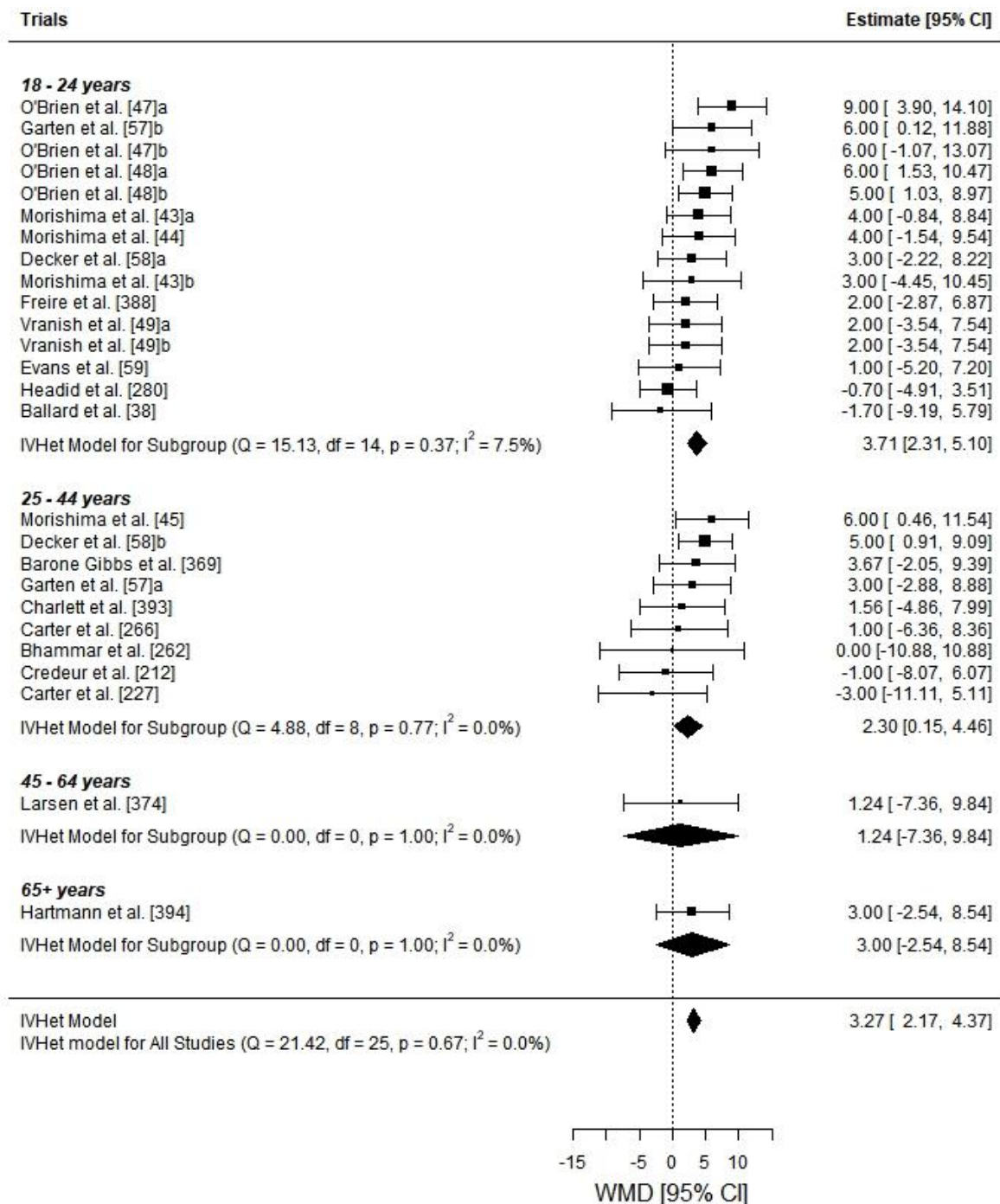


Figure 5.4 The effect of prolonged uninterrupted sitting on mean arterial pressure meta-analysis using an inverse heterogeneity model grouped by participant age. Abbreviations: WMD, weighted mean difference; CI, confidence intervals; IVHet, inverse heterogeneity.

Table 5.2 Meta-analysis of the effect of uninterrupted sitting on systolic blood pressure and mean arterial pressure with subgroup analysis by age

	Pooled Effect				Heterogeneity			Asymmetry		Quality	Trials	Sample
	μ	LCI	UCI	P Value	SMD	Q	P Value	I ²	LFK			
SBP	3.17	0.58	5.76	0.016	0.14	57.8	<0.001	60	-0.21	8	24	450
Age (years)												
18-24	5.94	1.98	9.90		0.38	22.3	<0.001	64		7	9	117
25-44	4.72	0.50	8.95		0.36	1.86	0.76	0		8	5	77
45-64	4.45	0.11	8.79		0.30	6.09	0.19	34		9	6	95
65+	-2.63	-5.32	0.06		-0.23	2.21	0.70	0		9	4	161
MAP	3.27	2.17	4.37	<0.001	0.37	21.4	0.669	0	-0.75	6	26	331
Age (years)												
18-24	3.71	2.31	5.10		0.50	15.1	0.37	7.5		6	15	173
25-44	2.30	0.15	4.46		0.23	4.88	0.77	0		6	9	125
45-64	1.24	-7.36	9.84		0.09	N/A	N/A	N/A		9	1	19
65+	3.00	-2.54	8.54		0.40	N/A	N/A	N/A		7	1	14

Abbreviations: SBP, systolic blood pressure; MAP, mean arterial pressure; LCI, lower confidence interval; UCI, upper confidence interval; SMD, standardised mean difference; LFK, Luis Furuya-Kanamori Index. SMD: Trivial, small, moderate and large effect sizes are defined as <0.2, 0.2, 0.5, and 0.8 respectively. LFK: <±1 indicates no asymmetry, ±1 to ±2 suggests minor asymmetry, and >±2 indicates major asymmetry. I²: 25 %, 50 %, and 75 % represent low, moderate, and high heterogeneity respectively. Quality is median quality score of included trials assessed using Heyland Methodological Quality Score, with a maximum score of 9

5.3.4.2 Sitting with interruption

Across sitting interruption strategies, SBP was significantly lower for the interruption compared to non-interruption condition ($\mu = -4.4$ mmHg, 95 % CI: -7.4 to -1.5, $p = 0.003$, SMD = 0.26) (**Figure 5.5**). Two trials from the same study were identified as potential outliers and potentially influential [56]. The Doi plot and an LFK index of -0.44 indicated no asymmetry. The heterogeneity in this analysis was moderate ($I^2 = 70$ %, $p < 0.001$). Subgroup analysis by interruption strategy is presented in **Table 5.3**. Omission of each of the potentially influential/outlier trials individually did not affect statistical significance of the model but did have a substantial effect on the observed outcome and heterogeneity ($\mu = -3.1$ mmHg 95 % CI: -5.4 to -0.8, $p = 0.009$, $I^2 = 52$ %, and $\mu = -3.2$ mmHg, 95 % CI -5.6 to -0.8, $p = 0.010$, $I^2 = 54$ %). In the interests of rigour, the analysis was repeated with the omission of both trials and the pooled effect was reduced to a trivial significant difference between interrupted and uninterrupted sitting conditions ($\mu = -1.6$ mmHg, 95 % CI: -3.2 to -0.01, $p = 0.049$, SMD = -0.14, $I^2 = 0$ %). Subgroup analysis by interruption strategy was repeated with the omission of the influential trials (**Table 5.3**). Post-hoc power analysis estimates that this analysis achieved 88 % power and 50 % when potential outliers were excluded. Post-hoc power analysis on included trials identified a median power of 12.5 %.

Across sitting interruption strategies, DBP was significantly lower for the interruption compared to non-interruption condition ($\mu = -2.4$ mmHg, 95 % CI: -4.5 to -0.3, $p = 0.022$, SMD = -0.19) (**Figure 5.6**). Two trials from the same study were identified as potential outliers and potentially influential [56]. Doi plot and an LFK index of 0.93 indicated no asymmetry. The heterogeneity in this analysis was moderate ($I^2 = 55$ %, $p < 0.001$). Subgroup analysis by interruption strategy is presented in **Table 5.4**. Omission of each of the potentially influential/outlier trials individually resulted in a loss of overall statistical significance and reduced the observed effect ($\mu = -1.6$ mmHg, $p = 0.069$, and $\mu = -1.55$, $p = 0.053$). In the interests of rigour, the analysis was repeated with the omission of both trials, resulting in a non-significant difference between interrupted and uninterrupted sitting conditions ($\mu = -0.6$ mmHg, 95 % CI: -2.0 to 0.7, $p = 0.359$, SMD = 0.06, $I^2 = 0$ %). Subgroup analysis by interruption strategy was repeated with the omission of the influential trials (**Table 5.4**). Due to the limited number of trials available, a meta-analysis assessing MAP responses to interrupted sitting was not performed. Post-hoc power analysis estimates that this analysis achieved 57 % power and 12 % when

potential outliers were excluded. Post-hoc power analysis on included trials identified a median power of 7.6 %.

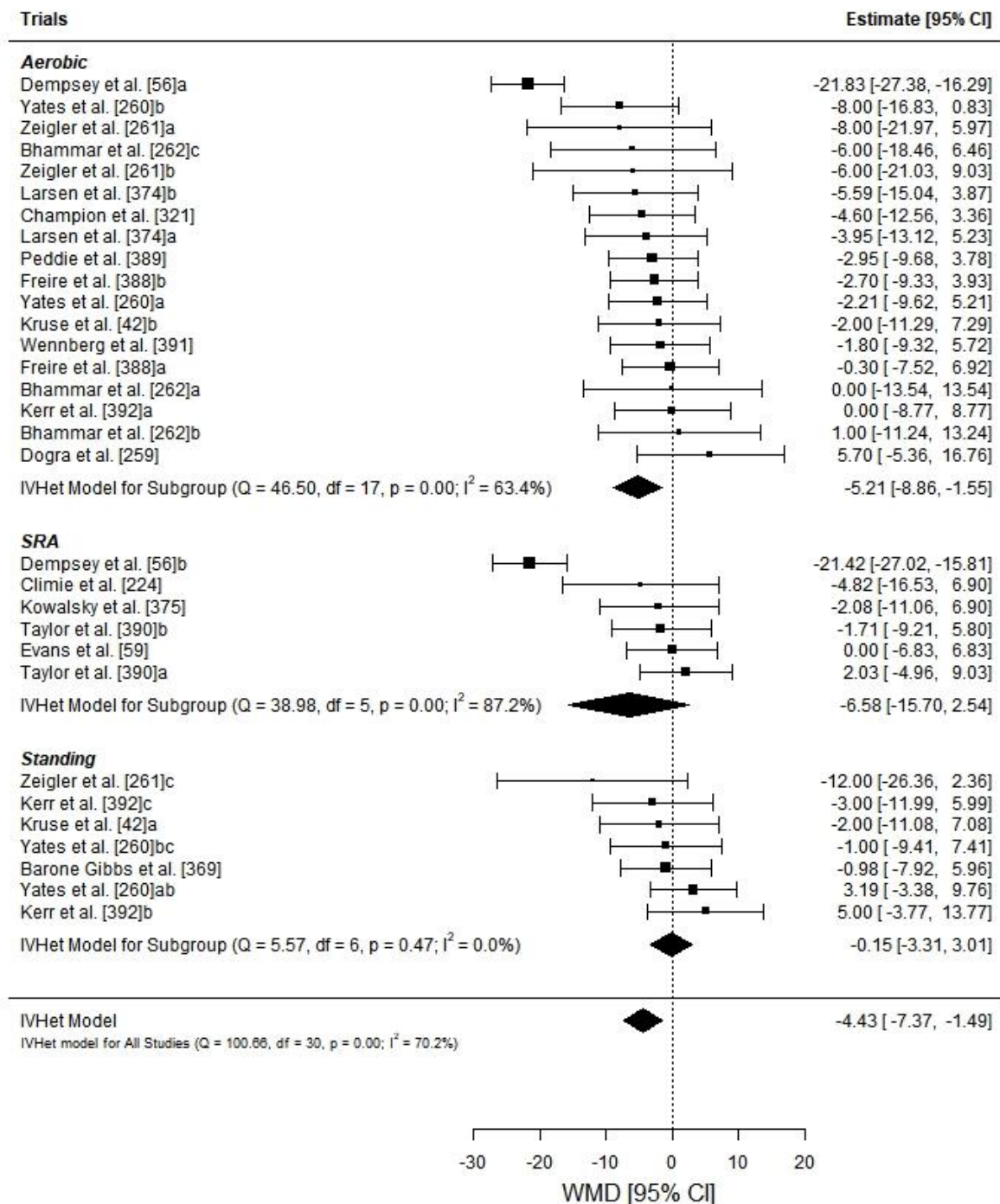


Figure 5.5 The effect of interrupted prolonged sitting on systolic blood pressure meta-analysis using an inverse heterogeneity model grouped by interruption strategy
 Abbreviations: WMD, weighted mean difference; CI, confidence intervals; IVHet, inverse heterogeneity; SRA, simple resistance activities. Labels a and b denotes different trials from the same study.

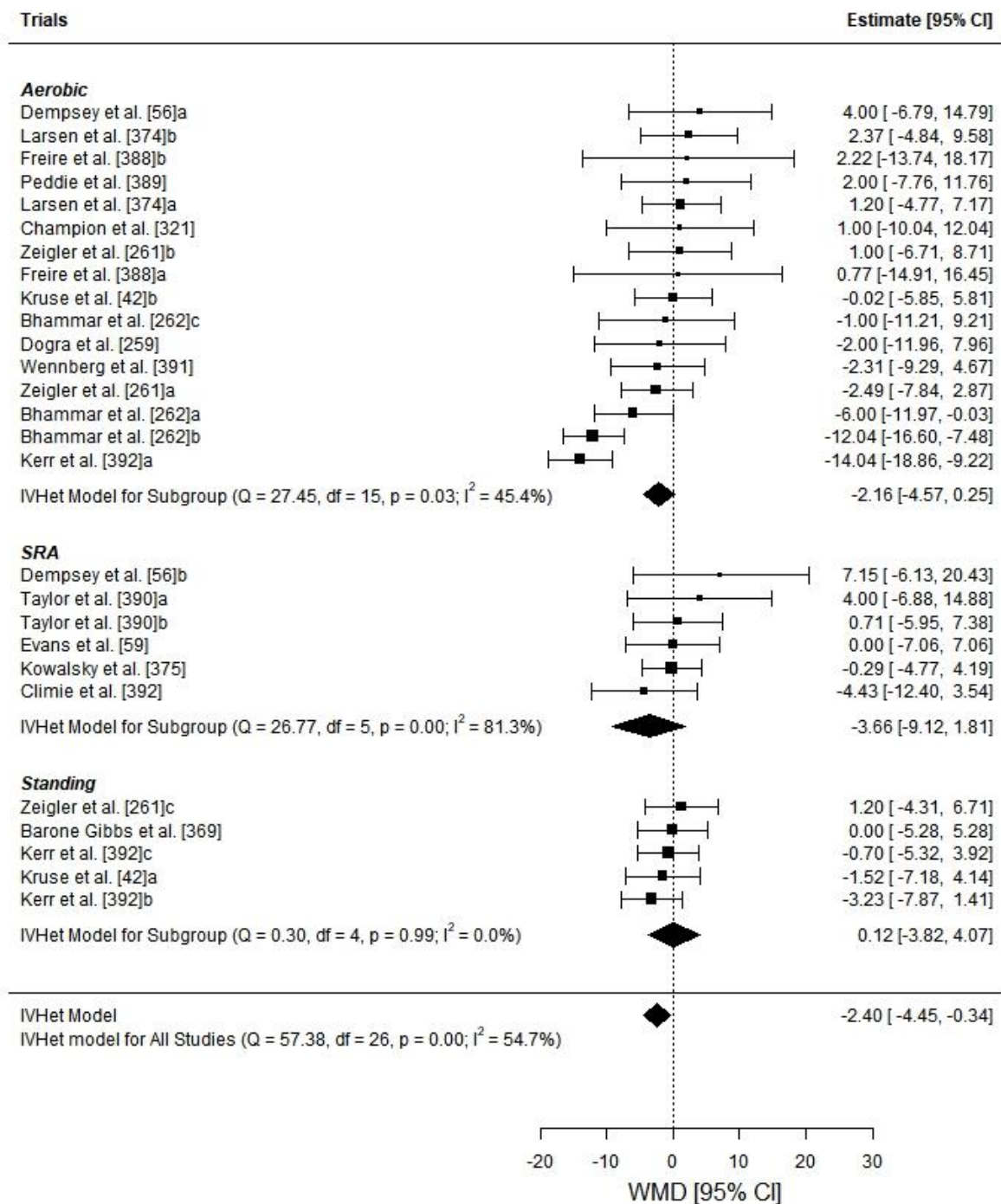


Figure 5.6 The effect of interrupted prolonged sitting on diastolic blood pressure meta-analysis using an inverse heterogeneity model grouped by interruption strategy
 Abbreviations: WMD, weighted mean difference; CI, confidence intervals; IVHet, inverse heterogeneity; SRA, simple resistance activities. Labels a and b denotes different trials from the same study.

Table 5.3 Meta-analysis of the effect of interrupted sitting on systolic blood pressure with and without influential trials. Subgroup analysis by sitting interruption strategy

	Pooled Effect				Heterogeneity			Asymmetry			
	μ	LCI	UCI	P Value	SMD	Q	P Value	I ²	LFK	Quality	Trials
All	-4.43	-7.37	-1.49	0.003	-0.26	100.7	<0.001	70	-0.44	8	31
Interruption Strategy											
Aerobic	-5.21	-8.86	-1.55		-0.33	46.5	<0.001	63		8	18
SRA	-6.58	-15.7	2.54		-0.33	39.0	<0.001	87		9	6
Stand	-0.15	-3.31	3.01		-0.02	5.57	0.47	0		8	7
Analysis excluding influential trials											
All	-1.61	-3.20	-0.01	0.049	-0.14	15.5	0.97	0	-0.72	8	29
Interruption Strategy											
Aerobic	-2.66	-4.83	-0.49		-0.22	6.65	0.98	0		8	17
SRA	-0.63	-4.18	2.92		-0.06	1.26	0.87	0		9	5
Stand	-0.15	-3.31	3.01		-0.02	5.57	0.47	0		8	7

Abbreviations: LCI, lower confidence interval; UCI, upper confidence interval; SMD, standardised mean difference; LFK, Luis Furuya-Kanamori Index; SRA; simple resistance activities; N/A, not applicable. SMD: Trivial, small, moderate and large effect sizes are defined as <0.2, 0.2, 0.5, and 0.8 respectively. LFK: <±1 indicates no asymmetry, ±1 to ±2 suggests minor asymmetry, and >±2 indicates major asymmetry. I²: 25 %, 50 %, and 75 % represent low, moderate, and high heterogeneity respectively. Quality is median quality score of included trials assessed using Heyland Methodological Quality Score, with a maximum score of 9.

Table 5.4 Meta-analysis of the effect of interrupted sitting on diastolic blood pressure with and without influential trials. Subgroup analysis by sitting interruption strategy

	Pooled Effect				Heterogeneity			Asymmetry			
	μ	LCI	UCI	P Value	SMD	Q	P Value	I ²	LFK	Quality	Trials
All	-2.40	-4.45	-0.34	0.022	-0.19	57.4	<0.001	55	0.93	8	27
Interruption Strategy											
Aerobic	-1.97	-4.20	0.26		-0.17	28.0	0.03	43		8	17
SRA	-4.46	-11.0	2.1		-0.39	24.52	<0.001	84		9	5
Stand	0.12	-3.82	4.07		0.02	0.30	0.99	0		8	5
Analysis excluding influential trials											
All	-0.63	-1.98	0.72	0.359	-0.06	11.2	0.99	0		8	25
Interruption Strategy											
Aerobic	-0.64	-2.30	1.02		-0.06	6.82	0.96	0		8	16
SRA	-1.01	-4.39	2.36		-0.11	3.88	0.27	23		9	4
Stand	0.12	-3.82	4.07		0.02	0.30	0.99	0		8	5

Abbreviations: LCI, lower confidence interval; UCI, upper confidence interval; SMD, standardised mean difference; LFK, Luis Furuya-Kanamori Index; SRA, simple resistance activities; N/A, not applicable. SMD: Trivial, small, moderate and large effect sizes are defined as <0.2, 0.2, 0.5, and 0.8 respectively. LFK: <±1 indicates no asymmetry, ±1 to ±2 suggests minor asymmetry, and >±2 indicates major asymmetry. I²: 25 %, 50 %, and 75 % represent low, moderate, and high heterogeneity respectively. Quality is median quality score of included trials assessed using Heyland Methodological Quality Score, with a maximum score of 9.

5.4 Discussion

The aim of this meta-analysis was to consolidate the existing data relating to the effect of prolonged sitting (>1 hr), with and without interruption, on peripheral blood pressure in adults. The main findings were that; (1) prolonged uninterrupted sitting resulted in significant small and trivial increases in SBP and MAP respectively, particularly in younger age groups. Conversely, DBP appeared to be unaffected by prolonged sitting; and (2) regular interruptions to sitting may confer a protective effect against increases in SBP, particularly when aerobic interruption strategies are employed.

5.4.2 Strengths and Limitations

Whilst the results of this meta-analysis provide novel insights, in order to fully contextualise the results, several potential limitations should be recognised. Firstly, there is a possibility of incongruency between trials utilising different methods of assessing peripheral blood pressure. Most trials utilised discrete oscillometric devices, whereas a limited subgroup utilised a mixture of ambulatory oscillometric [261,262] and photoplethysmography techniques [47,48,266]. Multiple reviews suggest that discrete and ambulatory pressure measurements can differ when implemented in a clinical setting [263–265]. However, in the interests of capturing all available data, trials utilising varying modes of peripheral blood pressure assessment were included with subgroup analysis performed to explore any differences. These subgroup analyses (Appendix 3) revealed that studies utilising continuous blood pressure measures typically reported higher values for MAP and SBP, but this did not explain any excess significant heterogeneity. A further limitation of this analysis was the inability to effectively explore how sitting duration may influence the observed results. Currently it is unknown how sitting duration affects sitting-induced dysfunction, however, it was not possible to properly investigate this within this analysis given that trials employing longer sitting periods (> 4 hours [median split]) typically recruited older samples than trials utilising shorter sitting periods (\leq 4 hours) (48.7 years vs 27.9 years, respectively). Another potential limitation is the use of tightly controlled periods of prolonged uninterrupted sitting as a comparison for objective (2). Extended periods of uninterrupted sitting may lack external validity and provide an unfair comparison producing inflated results in favour of interrupting sitting. Of note, it is important to acknowledge the potential confounding effect of diurnal variations in blood pressure whereby blood pressure

typically increases from the early hours of the morning until the early afternoon [395,396]. Diurnal variation is influenced by both intrinsic and extrinsic factors and whilst intrinsic factors are important, evidence suggests that extrinsic factors may be more influential [397,398]. Importantly, most studies in this analysis controlled for extrinsic factors known to affect diurnal variations in blood pressure, specifically food, alcohol, and caffeine intake, and physical activity, prior to experimental visits. Encouragingly, the results from objective (2) suggest that the observed increases in blood pressure are avoidable and thus still lend evidence in support of regularly interrupting bouts of prolonged sitting. Finally, only two studies performed *a priori* power calculations with peripheral blood pressure as the primary outcome measure [261,262] with neither study achieving the necessary power to detect the effect observed in these analyses. To this end, it is evident that many of the included trials were underpowered to detect the true effect of prolonged sitting, with and without interruption, on peripheral blood pressures. However, by conducting this meta-analysis, statistical power has been increased and an indication of the likely effect has been calculated.

5.4.3 Prolonged Uninterrupted Sitting

The results of this meta-analysis demonstrated that an acute bout of prolonged uninterrupted sitting resulted in small and trivial increases in MAP and SBP, respectively. Conversely DBP appears to be relatively unaffected by an acute bout of prolonged uninterrupted sitting, showing no significant change. The increases in MAP and SBP are likely mediated by a complex interaction of mechanisms. One such mechanism may be a consequence of the increased blood pooling observed in the lower limbs during sitting [46,48–50,59,212,252,399]. This would induce a reduction in venous return, which has been suggested to decrease renal perfusion pressure, stimulating the renin-angiotensin aldosterone system (RAAS), ultimately driving an increase in blood pressure [252,400]. Total peripheral resistance, a key moderator of blood pressure, may also increase due to localised endothelial dysfunction in lower-limb arteries [401] and a concomitant increase in stiffness and vascular tone, again elevating blood pressure. Whilst the intrinsic co-dependency of blood pressure and arterial stiffness prevent the identification of the primary antecedent, it is also conceivable that observed increases in central [59,212,369] and peripheral arterial stiffness may augment pulse wave propagation throughout the arterial tree causing a transient increase in SBP [138,141]. The reasons for the lack of

change in DBP are unclear. The major determinants of changes in DBP are arterial compliance and total peripheral resistance [402]. It is possible that the trivial to small changes in arterial stiffness (and thus compliance) and total peripheral resistance observed in previous studies are not sufficient to significantly influence DBP but may of sufficient magnitude to explain the trivial to small increases in SBP and MAP observed in this analysis.

Age-related structural changes in elastic arteries and the subsequent concomitant alterations to peripheral blood pressure may help to explain the observed differences in MAP and SBP responses to sitting across age groups (**Table 5.2**). It is understood that with increasing age, there is typically a concomitant increase in aortic stiffness which is associated with increased resting SBP [146], thus the small-moderate insult posed by prolonged sitting may be masked by already elevated resting values. Additionally, the lack of a detrimental response with increasing age may be the result of diminished RAAS activity which is associated with increased age [381,403,404]. Age-related differences may also be explained in part by differences in the habitual physical activity of samples. Typically, trials that recruited middle-aged (45-64 years) and aged (65-79 years) participants, appear to have purposefully recruited more sedentary individuals and excluded those who were physically active [260,374,375,391,392]. In contrast, trials involving young adults, aged between 18-24 years, typically reported that participants were healthy and recreationally active [38,44,48,49,57]. Currently, there are limited and conflicting data exploring the effect of habitual physical activity and fitness on the cardiovascular responses to prolonged uninterrupted sitting. Of the three articles that have assessed the effect of fitness status on sitting-induced vascular dysfunction [43,57,340], none have identified statistically-significant differences in MAP between groups. However, it should be noted that as MAP was not the primary outcome, these studies may not have been adequately powered to detect significant changes in peripheral blood pressure. Nonetheless, future research should aim to understand the potential confounding effect of cardiorespiratory fitness, habitual physical activity and sedentary behaviour patterns on sitting-induced changes to the vascular system.

5.4.4 Sitting Interruption

Due to the limited number of trials assessing MAP responses to interrupted sitting, meta-analysis was only performed for SBP and DBP. Meta-analysis of DBP demonstrated a

trivial significant decrease (beneficial) when sitting was interrupted compared to control ($\mu = -2.40$ mmHg, 95 % CI: -4.45 to -0.34, SMD = -0.19), however, sensitivity analysis showed that two trials from one study [56] unduly influenced this result and that the removal of either resulted in a loss of overall statistical significance. The reason for this undue influence is unclear. However, it may be the case that the novel sample, i.e., participants with the highest BMI (33 ± 3.4 kg·m²) and type II diabetes mellitus, influenced the results. Regardless, due to the limited robustness of this analysis, any inferences about DBP responses to bouts of interrupted prolonged sitting should be made with caution. The same trials were also deemed to unduly influence the meta-analysis of SBP responses to interrupted sitting, however, sensitivity analysis suggests that the overall effect is more robust. Analysis of 31 trials, including two potentially influential trials from one study [56], demonstrated a small significant beneficial effect on SBP when prolonged sitting was interrupted ($\mu = -4.4$ mmHg, 95 % CI: -7.4 to -1.5, SMD = -0.26). Re-analysis of 29 trials, omitting influential trials, revealed a significant trivial beneficial effect on SBP ($\mu = -1.6$ mmHg, 95 % CI: -3.2 to -0.01, SMD = -0.14).

Subgroup analysis was conducted to investigate whether an optimum interruption strategy existed. As shown in **Table 5.3**, aerobic interruption strategies produced a significant small effect for SBP in both models, with and without influential trials. Conversely, simple resistance activities and standing breaks produced non-significant small and trivial effects respectively, with both becoming trivial in the model omitting influential trials (**Table 5.3**). It should be noted, however, that both the simple resistance activities and standing breaks subgroups consisted of far fewer trials compared to the aerobic interruption strategy subgroup. Thusly, further research is needed before firm conclusions can be made regarding the efficacy of either simple resistance activities or standing breaks as sitting interruption strategies.

The varying efficacy of different interruption strategies may be due to differences in the circulatory stimulus each type of intervention created. The majority of aerobic interruption strategies consisted of walking or running breaks [56,260–262,321,374,388,389,391,392], and three trials implemented cycling interruptions [42,259,261]. Within the aerobic interruption strategy subgroup, the greatest differences in SBP and MAP between control and experimental conditions were observed in trials that typically used longer (≥ 5 minutes) [260,262], more frequent [321,374], or higher

intensity interruption strategies [262]. Conversely, trials that implemented shorter interruptions (≤ 5 minutes) typically showed more trivial effects [262,392]. Longer interruptions or those of a higher intensity are likely to result in a greater mechanical “muscle pump” in the lower limbs, promoting venous return [405,406], potentially offsetting the blood pooling associated with prolonged sitting. In turn, this may prevent the previously discussed activation of the RAAS and therefore avert increases in blood pressure. Alternatively, it is conceivable that longer bouts, particularly the 30 minutes of moderate intensity exercise implemented by one trial [262], may result in transient post-exercise hypotension [405,406]. Previous work has demonstrated that frequent short bouts of exercise (3 x 10 minutes) can result in similar or indeed greater transient reductions in SBP compared to longer exercise bouts of the same intensity [407,408]. Whilst none of the frequent interruption strategies investigated in this analysis were as long as 10 minutes, it is conceivable that differing magnitudes of post-exercise hypotension may explain the greater effect of longer interruption strategies.

5.5 Conclusions

Existing epidemiological research has highlighted an association between sedentary behaviours, such as prolonged sitting, and CVD incidence and all-cause mortality. Subsequent research has identified that bouts of prolonged sitting negatively impact a number of cardiometabolic outcomes, including peripheral blood pressure. Repeated acute increases in blood pressure may expose the vascular system and critical end-organs to excessive stress. Thus, understanding the implications of blood pressure responses to bouts of prolonged sitting is prudent. This meta-analysis is the first to consolidate the existing data regarding peripheral blood pressure responses to prolonged uninterrupted and interrupted sitting. The results of this analysis show that (1) acute bouts of prolonged sitting (≥ 1 hr) result in statistically significant increases in MAP and SBP, but not DBP, particularly in young individuals, and that, (2) interrupting sitting, especially with aerobic interruption strategies such as walking breaks, may prevent these deleterious effects.

6

Joining Chapter

Prior to the meta-analysis presented in Chapter 5, mounting evidence suggested that bouts of prolonged uninterrupted sitting may negatively impact markers of cardiometabolic and vascular health including peripheral blood pressure. However, despite the ease with which peripheral blood pressure can be measured and the strong prognostic value it provides, the existing data was yet to be consolidated. As a result, it was unclear to what degree bouts of prolonged uninterrupted sitting affected peripheral blood pressure in adults and whether regular interruption strategies may prevent any deleterious effects. Additionally, while sedentary behaviours such as prolonged sitting are common across age demographics, it was not known whether age-associated changes in cardiovascular physiology may influence sitting-induced responses. The data in Chapter 5 are the first to robustly synthesise the existing data related to peripheral blood pressure and prolonged sitting with and without interruption. The results of Chapter 5 found that bouts of prolonged sitting resulted in a small but significant increase in MAP driven by an increase in SBP. Further, bouts of prolonged sitting appear to have a greater effect on peripheral blood pressure in younger demographics compared to older individuals. Finally, it appears that interrupting prolonged sitting, particularly with aerobic interruption strategies, may confer a protective effect. These findings provide support for the contemporary working model of sitting-induced dysfunction as well as helping to answer the call by the World Health Organization to identify the most efficacious modality of sitting interruption strategy.

It should be noted however that the observed results from Chapter 5, whilst statistically significant, represent very small absolute changes. Given the potential for error with both oscillometric and continuous devices [264,409,410], these small changes (~3 mmHg) may not represent meaningful changes in blood pressure. Consequently, it is pertinent to explore an additional measure of cardiovascular health, arterial stiffness as assessed by pulse wave velocity. Whilst arterial stiffness and blood pressure are co-dependent, previous work suggests that PWV may provide additional prognostic information over and above peripheral blood pressure and thus is worthy of further investigation. To date several prolonged sitting studies have utilised measures of PWV, however, there has been consolidation of the existing literature. Additionally, whilst Chapter 5 indicates that age may augment the magnitude of sitting-induced increases in blood pressure, these results may be confounded by the fact that studies that recruited younger participants aimed for healthy, active individuals whilst studies recruiting older participants typically aimed to

recruit more sedentary individuals. As such, it is still unclear to what degree age augments sitting-induced changes in blood pressure compared to habitual physical activity and overall cardiorespiratory fitness. As such, further research is still required.

7

The Effects of Acute Exposure to Prolonged Sitting, with and without Interruption, on Central and Peripheral Pulse Wave Velocity Among Adults: A Systematic Review

7.1 Introduction

The previous Chapters have identified that both vascular function, as measured by flow-mediated dilation (FMD), and peripheral blood pressure are worsened by acute bouts of prolonged sitting, but regular interruptions may confer some protection against such deleterious effects. Although FMD may provide mechanistically important information, the requirement for high operator skill and the propensity for subsequent high measurement error inherently makes FMD unsuitable for future large-scale trials. Whilst peripheral blood pressure is clinically widespread and easily translatable, additive mechanistic and prognostic information may be gained through the use of novel cardiovascular health biomarkers, one of which is arterial stiffness. Arterial stiffness can be assessed simply, accurately, and reliably using pulse wave velocity (PWV) [243] and has been shown to be a predictor of future cardiovascular disease and all-cause mortality [238,239,411,412] as well as providing insights into acute vascular dysfunction [161,413]. Additionally increased aortic stiffness serves as a subclinical endpoint for the contemporary model of sitting-induced cardiovascular dysfunction [61]. However, at present little is known as to whether PWV can be used to help better understand the consequences of prolonged sitting and whether it is impacted by activity interruption.

Typically, PWV is measured over an arterial segment by dividing the time taken for an arterial pulse to propagate from a proximal to distal site by the estimated distance between those points [414,415]. Assessments of PWV can be classed as central (concerning principally central, large elastic arteries such as the aorta) and peripheral measures (concerning principally peripheral muscular arteries such as those of the upper or lower limb). Whilst estimates of central arterial stiffness have been shown to be predictors of future cardiovascular disease and all-cause mortality over and above traditional risk factors [238,239,411,412], acute changes in central PWV are likely to be largely driven by changes in blood pressure owing to their intrinsic co-dependency [416]. As such, the observed increases in mean arterial pressure observed in Chapter 5 may also present as increases in central arterial stiffness during a bout of prolonged sitting. By contrast, peripheral estimates of arterial stiffness are more limited in their prognostic value compared to central estimates [244], however, they may provide novel insights into changes in vascular function over arterial segments [161,413]. Owing to the greater concentration of vascular smooth muscle cells in the walls of peripheral arteries, they

may be more prone to acute detrimental changes in endothelial function [244], as observed in Chapter 3, which may in turn, influence local and systemic cardiovascular function. Thus, understanding these segment-specific changes in stiffness may improve mechanistic understanding of sitting-induced dysfunction. As such, several recent prolonged sitting studies have utilised segmental measures of PWV [59,60,212,280,369,375], however, to date, there has been no synthesis of the existing data.

The previous Chapters have identified that regularly interrupting bouts of prolonged sitting may confer some protective effect on vascular function and blood pressure. Owing to the co-dependency of blood pressure and arterial stiffness, the protective effect of regularly interrupting sitting on blood pressure observed in Chapter 5 may also influence changes in arterial stiffness. Further, as changes peripheral PWV are likely mediated by changes in endothelial function, the beneficial effect of sitting interruptions observed in Chapter 3 may also protect against increases in PWV by maintaining shear stress and reducing venous pooling. As such, it is prudent to explore how regularly interrupting bouts of prolonged sitting may affect central and peripheral PWV.

7.1.2 Objectives

The objectives of this Chapter are two-fold: (1) consolidate the existing evidence regarding PWV and prolonged uninterrupted sitting; and (2) consolidate the existing evidence of how regularly interrupting bouts of prolonged sitting may affect measures of PWV.

7.2 Methods

This systematic review was reported in accordance with published guidelines, PRISMA (Preferred Reporting Items for Systematic Reviews) [344], however, it was not pre-registered.

7.2.2 Data Sources and Searches

Three electronic databases (Web of Science, SportDiscus, and PubMed) were searched by two authors (myself [CP] and Lee Stoner [LS]) utilising the keywords: (((("arterial stiff*[Title/Abstract])) OR ("aortic stiff*[Title/Abstract])) OR ("PWV"[Title/Abstract])) OR ("pulse wave velocity"[Title/Abstract]))) AND

((("prolonged sitting"[Title/Abstract]) OR ("sedentary behaviour"[Title/Abstract])) OR ("sitting"))). Following the search, backward and forward citation searching was performed for all included trials as well as relevant reviews. The search was limited to English language studies published between inception and August 2021.

7.2.3 Article Selection

Initially, duplicate studies were removed, and the remaining article titles and abstracts were then screened for relevance. Following this, full-text screening of the remaining potentially eligible studies was conducted using the following criteria: (i) any segmental measure of PWV was assessed; (ii) PWV was assessed pre- and post-sitting in the same posture; (iii) studies were either randomised-controlled, randomised-crossover, or quasi-experimental pre- versus post-test trials; (iv) the prolonged sitting period was at least 1h; and (v) participants were adults (≥ 18 years), free of autonomic or neuromuscular dysfunction and any other known chronic illness.

To address the second objective of this review, further additional inclusion criteria were used; (i) if a strategy was employed to disrupt the effects of sitting, the strategy must have been during the sitting period; (ii) there must have been a control (uninterrupted sitting) group or condition, and (iii) the interruption strategy must have involved the participants actively moving their limbs. It should be noted that this review was primarily concerned with segmental PWV. As such, studies that reported composite measures of PWV that incorporate large sections of both central and peripheral arteries, such as carotid-ankle PWV, were excluded. Additionally, studies that reported single point measures of arterial stiffness were not included as such measures are only indicative of the small area of assessment rather than an arterial segment. Study selection was completed independently by two researchers (CP and LS).

7.2.4 Data Extraction and Quality Assessment

Bibliographic information, collected measures, sample characteristics, and procedural information was extracted from all relevant studies. Methodological quality was assessed using a modified Heyland Methodological Quality Score (HMQS) with a maximum score of 9. The HMQS criteria "blinding", "extent of follow up", and "outcomes" were not considered for the current analysis as these criteria are for longitudinal study designs. Quality assessment was conducted independently by two researchers (CP and Keeron

Stone), with a third researcher (Simon Fryer) acting as an adjudicator in the event of a lack of consensus.

7.3 Results

The literature search yielded 208 potentially relevant studies with no additional studies identified by forward or backward citation searching (**Figure 7.1**). After removal of duplicates, 123 titles and abstracts were screened, resulting in 10 potentially eligible articles for full-text evaluation. Full-text evaluation resulted in 6 eligible studies for review [59,60,212,280,369,375].

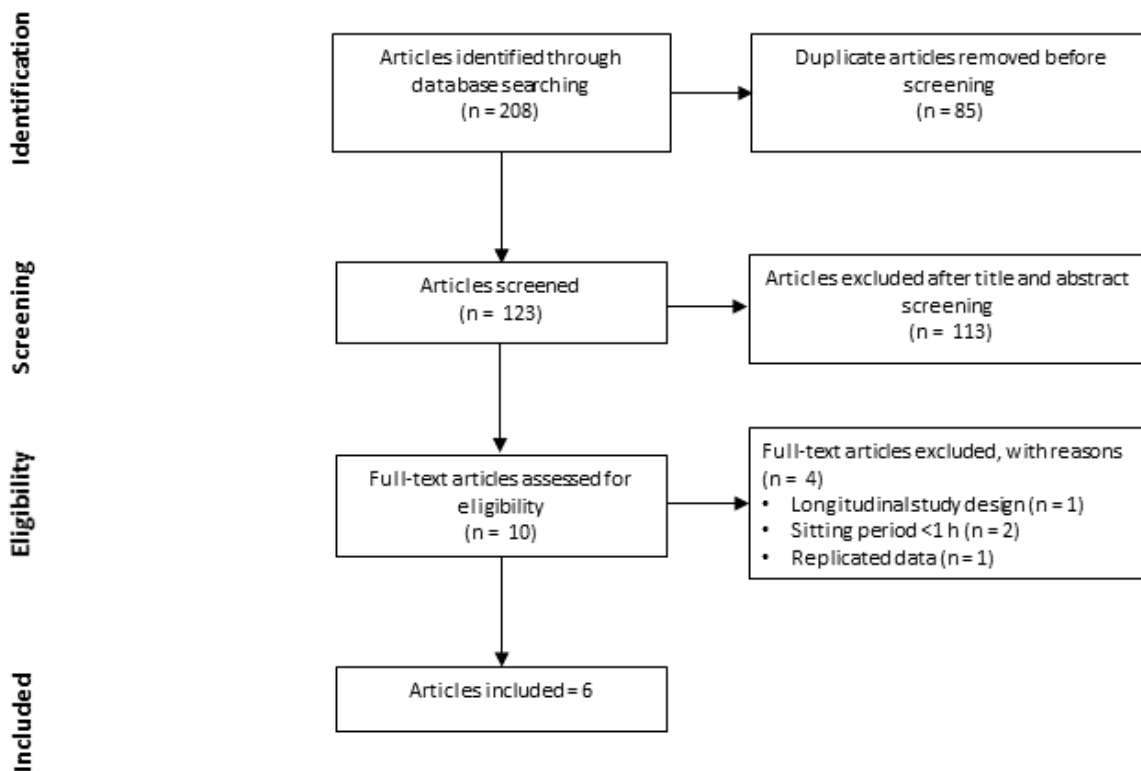


Figure 7.1 Flow chart of study selection

7.3.2 Characteristics of Included Studies

Study characteristics and outcomes are presented in **Table 7.1**. The number of participants in each study ranged from 12 [280] to 25 [369]. All studies assessed both males and females with an average split of 45 % males and 55 % females. Bouts of prolonged sitting ranged from 2.5 [280] to 7 h [369], with a modal sitting duration of 3 h.

Three of the identified studies explored the effects of breaking up bouts of prolonged sitting. The strategies employed were standing breaks [369], simple resistance activities [375], and seated calf raises (fidgeting) [59].

7.3.3 Effect of Prolonged Sitting with and without Interruption

All 6 studies assessed cfPWV, of which, 2 studies identified statistically significant increases [59,212] amounting to increases of $\sim 0.3 \text{ m}\cdot\text{s}^{-1}$ [59] and $\sim 0.4 \text{ m}\cdot\text{s}^{-1}$ [212]. The remaining 4 studies did not identify significant changes in cfPWV [60,280,369,375]. Of the 6 studies, 3 assessed carotid-radial PWV (crPWV) and found no significant changes in response to prolonged uninterrupted sitting [280,369,375]. One study assessed brachial-femoral (bfPWV) and femoral-ankle (faPWV) and found a significant increases of $0.36 \text{ m}\cdot\text{s}^{-1}$ and $0.55 \text{ m}\cdot\text{s}^{-1}$, respectively [60]. The 3 studies which assessed sitting interruption strategies all assessed cfPWV [59,369,375], of which 2 also assessed crPWV [369,375]. No studies identified significant effects on cfPWV or crPWV.

Table 7.1 Characteristics of included studies

Study	Quality	Sample [n (F); age (y, mean (SD or range)]	BMI [kg/m ² , mean (SD)]	Reported PWV outcomes	Sitting duration (h)	Effect of sitting	Interruption strategy	Effect of interruption
Barone Gibbs et al. [389]	8	25 (9); 42 (12)	31.9 (5.0)	Cf, cr	7	No significant effects	Standing	No significant difference
Credeur et al. [212]	5	20 (7); 26 (7)	30.0 (7.0)	Cf	3	Significant increase in males (0.4 m·s ⁻¹). No sig increase in females (0.1 m·s ⁻¹)	N/A	N/A
Evans et al. [59]	8	20 (14); 21.7 (2.5)	25.5 (6.1)	Cf	3	Significant increase (0.3 m·s ⁻¹)	Fidgeting	No significant difference
Kelsch et al. [60]	7	18 (6); 22.6 (3.1)	24.3 (3.7)	Cf, fa, bf	3	Bf - Significant increase (0.36 m·s ⁻¹). Fa - Significant increase (0.55 m·s ⁻¹). Cf - No significant effect	N/A	N/A
Kowalsky et al. [375]	8	14 (12); 53.4 (9.5)	30.9 (4.8)	Cf, cr	4	No sig effects	SRA	No significant difference
Headid III et al. [280]	3	12(6); 22.3 (2)	23.9 (3.0)	Cf, cr	2.5	No sig effects	N/A	N/A

Abbreviations: SD, standard deviation; BMI, body mass index; cf, carotid-femoral; cr, carotid-radial; fa, femoral-ankle; bf, brachial-femoral; N/A, not applicable.

7.4 Discussion

The aim of this Chapter was to assess the effect of prolonged sitting, with and without interruption, on central and peripheral measures of PWV. This review found that currently PWV is an underutilised measure of cardiovascular function within prolonged sitting research and studies that have utilised PWV as an outcome measure have assessed several different arterial segments with equivocal results. As such, it is currently unclear how prolonged sitting, with and without interruption, may influence measures of central and peripheral PWV, however, several important considerations have been highlighted that may serve to help researchers better utilise these measures in future prolonged sitting studies.

7.4.2 Strengths and Limitations

Whilst this review has served to consolidate the existing literature pertaining to measures of PWV in the context of prolonged sitting, there are several potential limitations that should be considered in order to fully contextualise the findings. Firstly, the limited number of trials in this review and the broad range of arterial segments assessed makes drawing conclusions regarding the effect of prolonged sitting, with and without interruption, on central and peripheral PWV challenging. The lack of trials also meant that meta-analysis of the existing literature would have been inappropriate and potentially lead to erroneous conclusions. Additionally, despite the known interdependence of mean arterial pressure and PWV measures [416], only two of the included trials in this analysis statistically controlled for MAP [60, 389]. By not statistically controlling for MAP, it is unclear whether any observed changes in PWV are reflective of anything more than change in blood pressure. However, by consolidating the existing evidence a number of important insights have been made and considerations highlighted which will aid further Chapters in this thesis and potentially researchers seeking to implement these techniques in future studies. The previous review Chapters have each highlighted methodological concerns with the measure in question, issues which are compounded by the number of studies that have already been undertaken. The hope is that by addressing any methodological concerns with measures of central and peripheral PWV at this point, whilst they are still underutilised modalities, future ambiguity and incongruency between studies can be avoided leading to stronger conclusions.

7.4.3 Central measures of pulse wave velocity

Central measures of PWV estimate the stiffness of large elastic arteries such as the aorta. This segment of the arterial tree provides the largest buffering capacity in the entire arterial system, changes in which can lead to a number of adverse health consequences [243,417]. Owing to the importance of central arterial stiffness, it is perhaps unsurprising that all trials in this review reported cfPWV as a primary outcome measure and one further study also assessed bfPWV [60]. Of the six studies in this review, two identified significant increases in cfPWV of ~ 0.3 [59] and $\sim 0.4 \text{ m}\cdot\text{s}^{-1}$ [212] whilst the remaining four reported no significant change [60,280,369,375]. Additionally, the one study that reported bfPWV identified a significant increase of $0.36 \text{ m}\cdot\text{s}^{-1}$ in response to a bout of prolonged uninterrupted sitting [60]. Changes in central arterial stiffness are likely driven by several mechanisms, however, the greatest determinant is likely to be changes in blood pressure. As discussed in Chapter 5, during a bout of prolonged uninterrupted sitting, blood pooling within the lower limbs and the subsequent reduction in venous return and thus stroke volume may result in decreased renal perfusion pressure, which stimulates the renin-angiotensin-aldosterone system (RAAS). The stimulation of RAAS in turn may result in increased blood pressure. However, a further consideration may be that the reduction in stroke volume within the aorta as a consequence of reduced venous return may reduce the shear stress against the endothelium of the aorta thus resulting in acute endothelial dysfunction which may present as increased stiffness [161]. It should be noted however that whilst endothelial function/dysfunction may augment aortic stiffness to some degree, the relatively fewer vascular smooth muscle cells within the wall of the aorta (compared to peripheral muscular arteries) means that endothelial function may not have as prominent an effect as changes in blood pressure [244].

The reason for the divergent effects of prolonged sitting on central arterial stiffness may be due in part to differences in sample demographics. The three studies that found significant increases in central arterial stiffness recruited young, healthy participants (**Table 7.1**). By contrast the remaining studies recruited slightly older participants (**Table 7.1**). As stated in Chapter 5, RAAS activity decreases with age and thus may not stimulate the same sitting-induced increase in blood pressure as observed in younger adults. Additionally, with increasing age, central elastic arteries naturally become stiffer owing to structural changes in the artery wall. As such, it may be the case that the small

effect that a single bout of prolonged sitting may impose is insufficient to elicit a meaningful response. A further source of variance between trials may be the timing of assessments between studies relative to changes in posture. Similarly to the concerns flagged in Chapter 3, there is a discourse between the timing of supine measures following a posture change at the of a prolonged sitting bout. In the current review, one study fails to report this time period [280], one suggests that the measurements were performed as soon as possible [212], three report a 10 min wait [60,369,375], and the final study reports a 20 minute wait [59]. By performing assessments immediately post-posture change, it is possible that the true effect of sitting is masked by acute hypotension in response to the change in posture. Alternatively, the longer delays (~20 mins) between posture change and final measures may result in an underestimation of the sitting response as the primary stimuli for sitting-induced dysfunction returns to homeostasis.

7.4.4 Peripheral Measures of Pulse Wave Velocity

In contrast to central measures, peripheral measures of PWV may carry limited prognostic value [418], however, owing to the greater density of vascular smooth muscle cells in the walls of peripheral arteries, they may be more prone to acute functional detriments driven by acute endothelial dysfunction [161,244]. As such, peripheral measures of PWV may provide novel insight to local dysfunction throughout the arterial system in response to prolonged sitting. Further, the stiffness of peripheral arteries and changes therein may augment pulse wave propagation within the arterial tree. Of the studies in this review, three assessed an upper-limb measure of arterial stiffness, crPWV [280,369,375], and one assessed a lower-limb measure, faPWV [60]. Given that increases in peripheral artery stiffness are likely the result of acute endothelial dysfunction, it is perhaps unsurprising that studies assessing crPWV did not report any significant changes in response to prolonged sitting. In line with the results of Chapter 3, upper limb arteries are likely to be relatively unaffected by a bout of prolonged sitting as studies rarely control upper limb movement coupled with the reduced hydrostatic pressure compared to lower limb arteries. Without reductions in movement, there is unlikely to be a reduction in shear stress within the artery and thus endothelial dysfunction is unlikely to occur. Further, the absence of increased hydrostatic pressure means that venous pooling or a compensatory myogenic response is unlikely to occur. By contrast, the one study that assessed faPWV found a significant increase in PWV ($0.5 \text{ m}\cdot\text{s}^{-1}$) in response to prolonged

sitting which, again, is in line with the results of Chapter 3 whereby lower limb artery function is impaired by a bout of prolonged uninterrupted sitting likely due to decreased shear and increased venous pooling.

7.4.5 Sitting Interruptions

Only three of the included trials investigated the effect of sitting interruption strategies on measures of PWV [59,369,375]. The strategies employed included; standing breaks [369], seated leg fidgeting [59], and simple resistance activities [375]. Of these studies, none found a significant effect of regularly interrupting bouts of prolonged sitting. Due to the limited number of trials and the range of interruption strategies employed, it is not possible to make firm conclusions regarding the efficacy of sitting interruption strategies and how they may influence measures of central and peripheral PWV. However, given the results of Chapters 3 and 5 coupled with evidence to support the positive effects of exercise on both central and peripheral PWV [419,420], further investigation is warranted.

7.4.6 Methodological Concerns

Pulse wave velocity, particularly cfPWV, is a useful clinical measure and may be a useful tool in future prolonged sitting research, however, in line with the observations of previous Chapters, there are methodological considerations that should be addressed within the research area going forward. Firstly, is the timing of posture changes in relation to post-sitting measures. As noted above and in previous Chapters, it is possible that the timing between posture changes and measurements may obscure the true effects of prolonged sitting. Inconsistencies between the timing of post-sitting measures may make the results of studies incongruent and explain divergent findings between studies. This issue highlights the importance of standardised procedures for this research area if the area is to continue to develop.

Following from the broader issue of standardised guidelines, and a particular issue for studies using indices of PWV, is the statistical correction for blood pressure. As mentioned previously, blood pressure and arterial stiffness are interdependent [416]. As mean arterial pressure (MAP), the vessel distending pressure, increases more stress is borne by the collagen fibres of the arterial wall increasing the functional stiffness of the vessel [136]. One proposed solution is to statistically correct for MAP during analysis in

the same way that FMD is frequently corrected for shear rate during analysis. Indeed, two of the included trials in this review report such corrections [60,369]. Without statistical corrections for MAP, increases in any measure of PWV need to be interpreted with caution. However, this approach suffers from similar shortcomings as FMD analyses, principally that statistical correction occurs at a group rather than individual level. As such, statistical correction may not fully account for individual differences in PWV-pressure relationships potentially leading to erroneous results [421]. Whilst it is beyond the scope of this review to address these more fundamental issue with the use of PWV measures, researchers may consider reporting both “raw” and “corrected” PWV results in a similar fashion to current FMD guidelines [218].

7.5 Conclusions

This Chapter sought to consolidate the existing evidence regarding PWV as a measure of cardiovascular function in response to prolonged sitting. This Chapter identified that PWV has not been widely used in this research area but the most common, and perhaps most clinically significant measure, is cfPWV, a measure of central arterial stiffness. This Chapter also identified that some measures of peripheral arterial stiffness such as faPWV may also convey useful information pertaining to the integration of the entire arterial system. Whilst this Chapter was unable to identify a likely effect of prolonged sitting on various measures of PWV, it has highlighted important methodological considerations that will inform subsequent Chapters. However, given the ease of measurement and mechanistic information segmental measures of PWV may provide, the use of PWV in prolonged sitting research should not be ruled out.

8

Joining Chapter

Chapters 3 and 5 have identified that acute bouts of prolonged uninterrupted sitting negatively impact both vascular function and peripheral blood pressure, important markers of cardiovascular health. However, owing to the observed effects relative to the potential for error with both measures, investigation of other markers of cardiovascular health was necessary. One such marker is segmental (central and peripheral) arterial stiffness as expressed by pulse wave velocity. Prior to Chapter 7, several prolonged sitting studies had utilised various segmental measures of PWV, however, there had been no consolidation of the existing literature. Chapter 7 broadly identified that all studies that have used PWV as an outcome measure have assessed the gold standard measure of central arterial stiffness, carotid-femoral PWV, plus various other central and peripheral markers with equivocal results. As such, it is still unclear how prolonged sitting, with and without interruption, may affect measures of central and peripheral arterial stiffness. Despite the equivocal results of the previous Chapter, markers of central and peripheral arterial stiffness may still yield important clinical and mechanistic insights into sitting-induced cardiovascular dysfunction. As such, the further use of PWV within prolonged sitting studies should be encouraged and thus, the subsequent experimental trials will feature measures of central and lower limb arterial stiffness.

The preceding Chapters have identified that acute bouts of prolonged uninterrupted sitting produce robust detriments in vascular function and peripheral blood pressure whilst also identifying that central and peripheral arterial stiffness may also be affected. Further, the results of the preceding Chapters demonstrate the regularly interrupting bouts of prolonged sitting confers a protective effect against such detriments. These results collectively support the contemporary working model of sitting-induced cardiovascular dysfunction. However, despite the novel findings and value added by the preceding Chapters, a majority of studies included within the reviews and meta-analyses focused on bouts of prolonged sitting as an independent behaviour. Given that sitting represents an omnipresent behaviour in modernised economies, it is highly likely that other facets of modern life and the associated behaviours may interact with prolonged sitting to influence and observed cardiovascular dysfunction. The following chapters will seek to investigate several prominent lifestyle factors.

9

Leg fidgeting prevents increases in central arterial stiffness following prolonged uninterrupted sitting combined with a high-fat Westernised meal

9.1 Introduction

As identified in [Chapter 2](#), there is an association between increased sedentary behaviour and both cardiovascular disease (CVD) and all-cause mortality [18,20,34]. To understand the mechanisms for this association, a number of acute prolonged sitting studies now exist which have been reviewed in Chapters 3, 5, and 7. The preceding Chapters have consolidated the existing evidence base as it relates to three markers of cardiovascular health, and potential mechanisms; flow-mediated dilation (FMD), peripheral blood pressure (BP), and pulse wave velocity (PWV). These Chapters conclude that prolonged uninterrupted sitting is likely to have detrimental effects on each marker of cardiovascular health and that regular interruptions may offset those effects. However, whilst these reviews have consolidated the evidence as it pertains to acute bouts of prolonged uninterrupted and interrupted sitting, in reality, it is likely that prolonged sitting clusters with other lifestyle behaviours that may augment how the cardiovascular system responds.

One such behaviour that is of interest is the consumption of a meal indicative of a Westernised diet, that is one high in saturated fat and carbohydrates. Previous work from our research group has shown that the consumption of high glycaemic index (GI) foods alone, indicative of a typical Westernised meal, do not appear to have an additive effect on prolonged sitting-induced changes in peripheral blood pressure or arterial stiffness compared to a low GI control [60]. By contrast, further work from our group has demonstrated that a Westernised meal high in fat does have an additive effect on sitting-induced dysfunction [62]. The additive effect of consuming a high-fat meal is thought to be a result of i) the oversaturation of the lipoprotein lipase (LPL) metabolic pathway which facilitates triglyceride uptake into working tissues coupled with ii) reduced LPL activity as a consequence of reduced muscle activity [315,316,318]. Despite the worsening response when sitting is combined with a high-fat meal, it is plausible that physical activity interruptions may still confer a protective effect. For example, regular physical activity interruptions, and the associated muscle activity, may prevent LPL downregulation as well as preventing venous pooling in the lower limbs, the proposed primary mechanism for sitting-induced detriments in cardiovascular dysfunction. By preventing both LPL downregulation and venous pooling in the lower limb, it is possible

that the combined deleterious effects of prolonged sitting and consumption of a high-fat meal may be offset.

Cho et al., [63] demonstrated that regularly interrupting a bout of prolonged sitting with high intensity stair climbing may offset the combined effects of sitting and consumption of a high-fat meal. However, the decision to use a high intensity physical activity interruption may limit the wider implementation of this interruption strategy making it unsuitable for everyday use. To ensure the uptake of strategies to reduce sedentary behaviour, it is necessary to ensure that such interruptions are practical and broadly accessible, independent of fitness level. One such strategy may be regular high cadence leg fidgeting as described in Morishima et al., [46]. Morishima et al., [46] demonstrated that interrupting a bout of prolonged sitting with 1 min of high cadence leg fidgeting every four minutes was sufficient to maintain lower limb shear stress and vascular function. Indeed, the results of Chapter 3 suggest that the protocol implemented by Morishima et al., [46] produced the greatest improvement in lower limb vascular function of all the sitting interruption strategies included in the analysis (mean difference [MD] = 5.80 %). It is conceivable that the same strategy may facilitate the maintenance of LPL activity, preventing the additive effect of a high-fat meal, whilst also being a more easily accessible and translatable sitting interruption strategy. However, it is currently unknown whether light intensity leg fidgeting is sufficient to offset the combined deleterious effects of prolonged sitting and consumption of a high-fat Westernised meal.

9.1.2 Objectives

The objective of this Chapter is to determine whether a simple desk-based leg fidgeting strategy is sufficient to confer a protective effect against the combined detrimental effects of a high-fat Westernised meal and prolonged sitting on cardiovascular health.

9.2 Methods

This randomised, controlled cross-over trial is reported in accordance with CONSORT (Consolidated standards of Reporting Trials) guidelines [422]. This Chapter includes data that has previously been published exploring the effects of prolonged uninterrupted sitting combined with either a high-fat or low-fat Westernised meal [62]. This Chapter builds on the previously published data by including a physical activity intervention but includes the previously published data as a comparison. It should be noted that any

differences in the results presented in this Chapter compared to the previously published data are likely to be due to the different statistical models employed within this Chapter.

9.2.2 Participants

Thirteen young (18-35 years), apparently healthy male participants were recruited from a university student population. Participants were non-smokers, free of illness at the time of testing, and were free of any known metabolic diseases, nor were they taking any known vascular-acting medication. Institutional ethics approval was obtained prior to recruitment and all participants provided written informed consent.

9.2.3 Study Design

This randomised crossover trial was undertaken at the University of Gloucestershire with all data collection taking place in an environmentally controlled laboratory (21 °C). Participants attended the laboratory on four separate occasions: a familiarisation session, and three trial conditions (low-fat Westernised meal [LF], high-fat Westernised meal [HF], and high-fat Westernised meal with fidgeting [HFF]) in a randomised order. Trial conditions were separated by at least 48-hours and all visits were completed within a 10-day period. Trial condition order was randomised by a third-party using an online tool (www.randomizer.org) with participants blinded to the condition until the start of each trial.

9.2.4 Experimental Protocol

The initial familiarisation visit introduced participants to all equipment and experimental procedures. This was followed by assessment of stature, mass, and screening for any food allergies or intolerances that may interfere with their participation. On trial days, participants arrived between 0830 and 0900 following an overnight fast, consuming only water and having refrained from caffeine for 12 hours. Participants were also asked to refrain from alcohol and strenuous exercise for 24 hours prior to each trial. To ensure consistency, prior to the first visit, participants were asked to record their evening meal and to replicate that meal on the evening preceding each subsequent trial.

For each trial visit, participants were asked to void their bladder completely prior to laying on a test bed in a supine position for 20 minutes. During this time, the participants were fitted with an oscillometric blood pressure cuff (SphygmoCor XCEL, Atcor Medical, Sydney, Australia) over the left brachial artery to determine central and peripheral blood

pressures. Thigh and ankle blood pressure cuffs were placed on the left side of the body for determination of carotid-femoral pulse wave velocity (cfPWV) and femoral-ankle pulse wave velocity (faPWV). A continuous-wave near-infrared spectroscopy (NIRS) device (Artinis Portalite, Artinis Medical Systems, BV Zetten, Netherlands) was placed over the belly of the gastrocnemius on the participant's right leg to determine changes in blood volume over the sitting period to estimate blood pooling in the gastrocnemius in line with previous research [59,60,399].

A schematic detailing the protocol for the experimental visit is presented below (**Figure 9.1**). All conditions (HF, HFF, and LF) were identical with the exception of the HFF condition whereby participants were instructed to tap their heels and bounce their knees at a natural tempo of approximately 240 taps per minute for 1 minute every 4 minutes over the 180-min sitting period. Following 20 minutes of supine rest, baseline measures of central and peripheral blood pressure were collected using the SphygmoCor XCEL device. Immediately following these measures, cfPWV and faPWV measures were collected. All cardiovascular measures were collected in triplicate and the average of the closest two taken. Participants were passively moved into a seated position using an electronic three-way tilt table (Plinth 2000, Plinth Medical, Suffolk, UK). Once seated, calf circumference was measured at the point of greatest girth on participants' dominant leg and marked for post-sitting measurements. Participants sat quietly for 5 minutes prior to repeat assessments of central and peripheral blood pressures and cfPWV. Following these measures, baseline measures of blood triglycerides and high-density lipoprotein (HDL) were taken via a fingertip capillary sample. Participants were then given 10 minutes to consume their meal, after which the prolonged sitting period of 180 minutes commenced. During the prolonged sitting period, participants were asked not to move their lower limbs aside from those movements prescribed in the HFF condition. In line with previous literature, a non-stimulating television program was used to maintain the wakefulness of participants [60]. The choice to use a standardised television program was to control for and minimise the potential for mental stress that may have come from participants working throughout the sitting period. Further blood samples were collected at 30, 60, 120, and 170 minutes, with central and peripheral blood pressures and cfPWV collected at 60, 120, and 170 minutes. Following 180 minutes of sitting, post-sitting calf circumference was measured. Participants were then moved back to the supine position

where they rested for 10 minutes before post-trial measures of cfPWV, faPWV, and central and peripheral blood pressure were repeated.

9.2.5 Experimental Procedures

9.2.5.1 Meal Type

In line with previous research that has identified vascular impairment following consumption of a high-fat meal [63,306,326,423], participants consumed a McDonald's Corporation breakfast meal which consisted of a double sausage and egg McMuffin, two hash browns, and a regular sized hot chocolate with added double cream (1066 kcal, fat 61 g [of which 20 g saturated fat], carbohydrates 86 g, protein 40 g, and salt 5 g) for the HF and HFF conditions. For the LF condition, participants consumed 200 ml skimmed milk with 22 g of unflavoured whey protein powder (MyProtein), and two large English crumpets (Kingsmill Inc.) each with 10 g of low-fat spread (Tesco PLC.) and 5 g of Marmite (601 kcal, fat 10 g [of which 3 g saturated fat], carbohydrate 86 g, protein 40 g, salt 5 g). Importantly, the HF and LF meals were closely matched for glycaemic index and macronutrient content except for a 50 g difference in fat content to ensure that any observed differences were likely to be the effect of fat consumption.

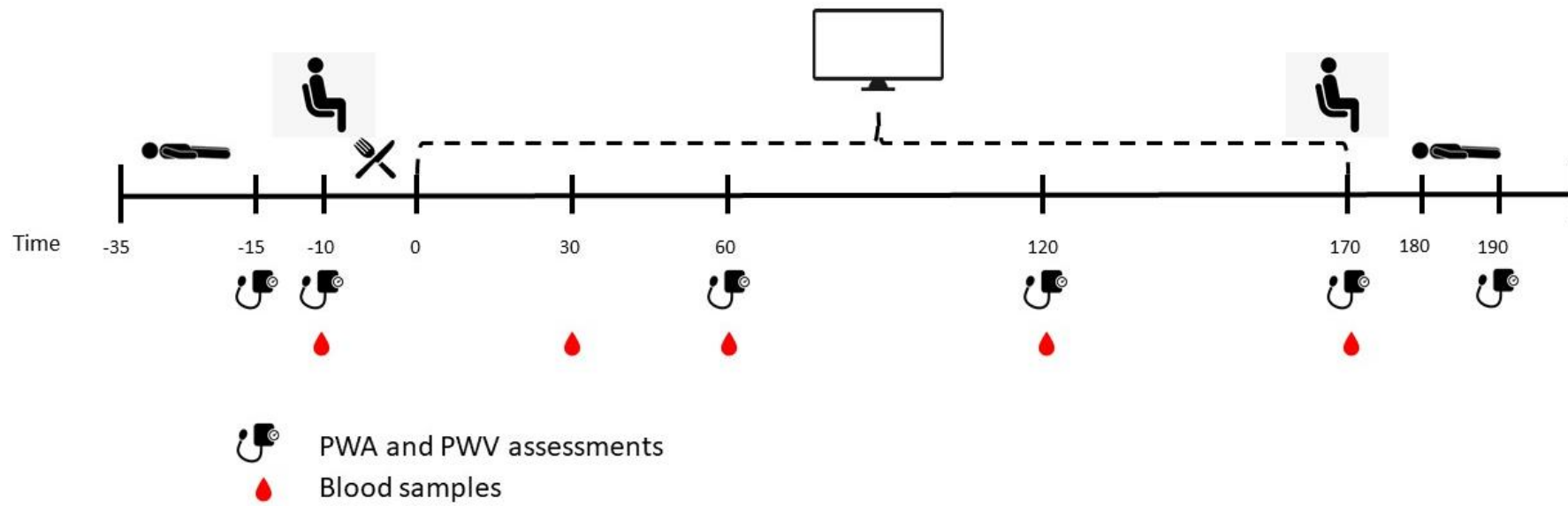


Figure 9.1 Schematic of experimental protocol.

Abbreviations: PWA, pulse wave analysis; PWV, pulse wave velocity

9.5.5.2 Stimulus: Bloods and Venous Pooling

Blood samples were collected via finger-prick capillary sampling at every sampling interval (0, 30, 60, 120, and 170 mins). All samples were collected using 32 μ L lithium heparin capilliettes (Sarstedt Aktiengesellschaft & Co, Germany) and immediately extracted onto Reflotron test strips (Hoffman-La Roche LTD) for determination of triglyceride and HDL concentration using a Reflotron Plus (Hoffman-La Roche LTD). Blood glucose was analysed separately using Biosen C-Line Clinic (EKF Diagnostics, United Kingdom).

In line with previous studies, venous pooling was estimated using cw-NIRS to determine blood volume change in the gastrocnemius [59,211,399]. The Artinis Portalite device is a single wired optode consisting of three light-emitting diodes positioned 30, 35, and 40 mm from a single receiver. The light emitting diodes transmit near-infrared light at wavelengths of 760 and 850 nm and allows the determination of oxy- (Hb) and deoxyhaemoglobin (HHb), the sum of which is total haemoglobin (tHb). An appropriate pathlength correction factor, specific to the calf, was employed to account for scattering and absorption of light within the tissue. Signals were allowed to stabilise in the final 10 mins of supine rest. Values were normalised to baseline in each trial rather than being reported as the absolute change owing to the potential for participant or trial variability. Positioning of the NIRS device was confirmed via ultrasound (Terrason T3300, Burlington, MA, USA).

9.2.5.3 Primary Outcomes: Pulse Wave Analysis and Regional Pulse Wave Velocity

Pulse wave analysis (PWA) and regional pulse wave velocity (cfPWV and faPWV) were obtained using SphygmoCor XCEL (AtCor, Sydney, Australia). For PWA, oscillometric pressure waveforms were recorded at the brachial artery as well as peripheral blood pressure. A corresponding aortic pressure waveform was generated using a validated transfer function from which central blood pressure was estimated [424]. Additionally, the generated aortic waveform was analysed to assess augmentation index (AIx), forward aortic pressure (Pf), and backward aortic pressure (Pb).

Pulse wave velocity was determined by dividing pulse transit time (PTT) by arterial path length. PTT was assessed by simultaneously capturing arterial pressure waveforms at proximal and distal sites using applanation tonometry and oscillometric cuffs, respectively. For cfPWV, the tonometer was placed on the left carotid artery at the point

of greatest pulsation and the oscillometric cuff was placed on the left thigh at the level of the femoral artery, following manufacturer guidelines. Arterial path length was estimated in line with current guidelines whereby the distance between suprasternal notch and point of greatest carotid pulsation was subtracted from the distance between suprasternal notch and the top of the femoral oscillometric cuff [414]. For faPWV, the tonometer was placed at the point of greatest pulsation at the level of the left superficial femoral artery, whilst an oscillometric cuff was positioned immediately proximal to the left medial malleolus. Arterial path length was estimated by measuring the linear distance between the point of greatest pulsation and the top of the ankle cuff. Femoral-ankle PTT was then corrected as previously described prior to calculation of PWV [425].

9.2.6 Sample Size

Using the small effect observed for mean arterial pressure (MAP) (SMD = 0.36) in the preceding meta-analysis of blood pressure responses to prolonged uninterrupted sitting, an alpha level of 0.05, power of 0.8, and an assumed correlation between measurements of 0.7, power analysis estimated that 12 participants would be required [426]. This study aimed to recruit 13 participants to account for any data errors or participant attrition.

9.2.7 Statistical Analysis

Statistical analyses were performed using Jamovi (Version 1.8) [427], a graphical front-end to the R programming language [351], using the General analyses for linear models (GAMLj) module [428]. Raw data are presented as mean (standard deviation [SD]) and mixed model data are presented as mean (95 % confidence interval [CI]). The alpha level for main and interaction effects was set at ≤ 0.05 . Follow up analyses were controlled using Bonferroni corrections of the observed p value.

All analyses used linear mixed models with random effects of participants and fixed effects of time and condition. Owing to the interdependence of blood pressure and PWV, models assessing cfPWV and faPWV were adjusted for MAP [369]. Data was checked for normality using Shapiro-Wilk test and visual inspection of histograms and Q-Q plots of residuals. Effect sizes were expressed as Cohen's d , where ≤ 0.2 , 0.2, 0.5, and 0.8 were defined as trivial, small, moderate, and large, respectively [385]. For the linear mixed models, Cohen's d was calculated by dividing the beta estimate by SD as previously reported [60].

9.3 Results

9.3.1 Participants

Participant characteristics are presented in **Table 9.1**. Thirteen Caucasian male participants were recruited and successfully completed all three trials.

Table 9.1. Characteristics of included participants

Characteristic	Mean (SD)
Age (y)	21.5 (1.79)
Stature (m)	1.78 (0.06)
Mass (kg)	76.7 (9.21)
BMI (kg.m ²)	24.2 (2.72)

Abbreviations: SD, standard deviation; BMI, body mass index.

9.3.2 Stimulus: Bloods and Venous Pooling

9.3.2.1 Triglyceride Concentration

Figure 9.2 illustrates the change in triglyceride concentration across all conditions. Mixed model analysis identified a significant group*time interaction effect ($p < 0.001$). Follow up analysis identified a significant effect of time in HF and HFF conditions (both $p < 0.001$) but not in the LF condition ($p = 0.57$). In the HF condition, triglyceride concentration significantly increased from pre (0 min) to 120 min (MD = 55 mg·dL⁻¹, 95 % CI: 36 to 74, $p < 0.001$, $d = 1.61$) and pre to 170 min (MD = 65 mg·dL⁻¹, 95 % CI: 47 to 84, $p < 0.001$, $d = 1.91$). In the HFF, triglyceride concentration significantly increased from pre (0 min) to 120 min (MD = 55 mg·dL⁻¹, 95 % CI: 36 to 75, $p < 0.001$, $d = 1.57$) and pre to 170 min (MD = 39 mg·dL⁻¹, 95 % CI: 20 to 58, $p < 0.001$, $d = 1.12$). Triglyceride concentrations at 170 min were not significantly different between HF and HFF ($p = 0.884$).

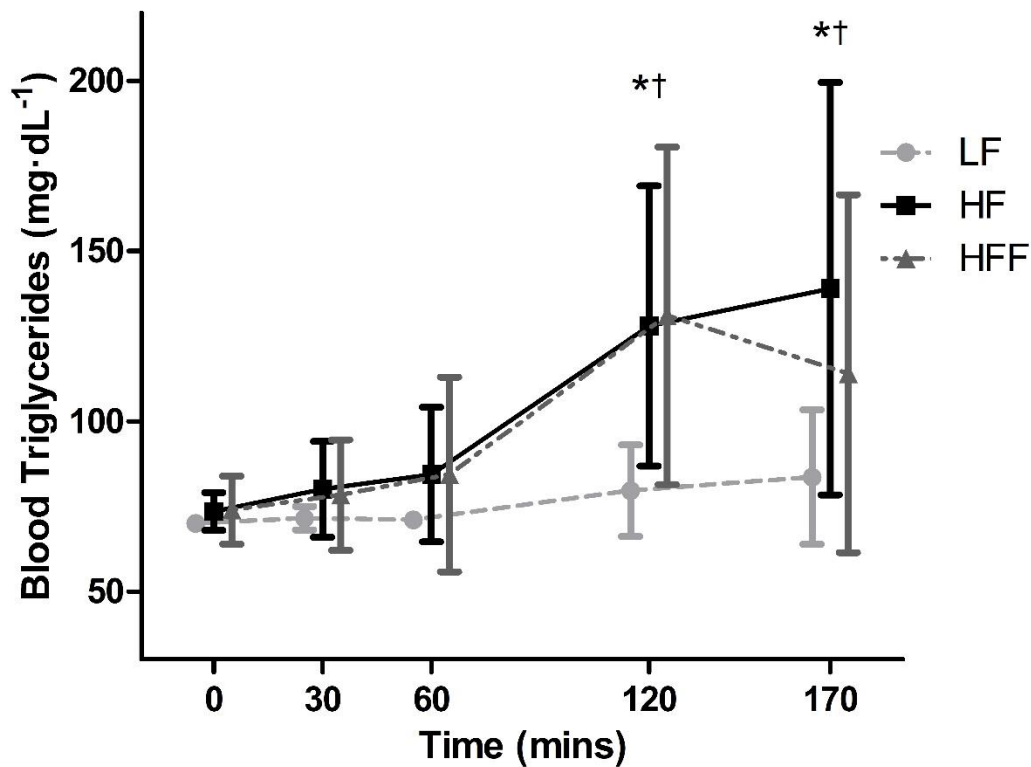


Figure 9.2 Mean (\pm standard deviations) blood triglyceride concentrations ($\text{mg}\cdot\text{dL}^{-1}$) during prolonged sitting.

Abbreviations: LF, low-fat condition; HF, high-fat condition; HFF, high-fat fidget condition. * denotes HF significantly different from baseline ($p < 0.05$), † denotes HFF significantly different from baseline ($p < 0.05$). Note; data at each time point has been staggered along X axis to provide clarity, it should be noted that all blood samples were collected at the same time points.

9.3.2.2 Glucose Concentration

Figure 9.3 illustrates the change in glucose concentration across all conditions. Mixed model analysis identified a significant group*time interaction effect ($p = 0.002$). Follow up analysis identified a significant effect of time in all conditions (all $p < 0.001$). In the LF condition, glucose remained significantly elevated from baseline for all subsequent time points (all $p < 0.05$). In the HF condition, glucose was significantly higher than baseline at 30 mins only (MD = $1.54 \text{ mmol}\cdot\text{L}^{-1}$, 95 % CI: 1.02 to 2.06, $p < 0.001$, $d = 1.64$). In the HFF condition, glucose was significantly elevated from baseline at 30 (MD = 1.66, 95 % CI: 1.15 to 2.18, $p < 0.001$, $d = 1.76$) and 60 mins (MD = 0.56, 95 % CI: 0.04 to 1.07, $p = 0.034$, $d = 0.59$).

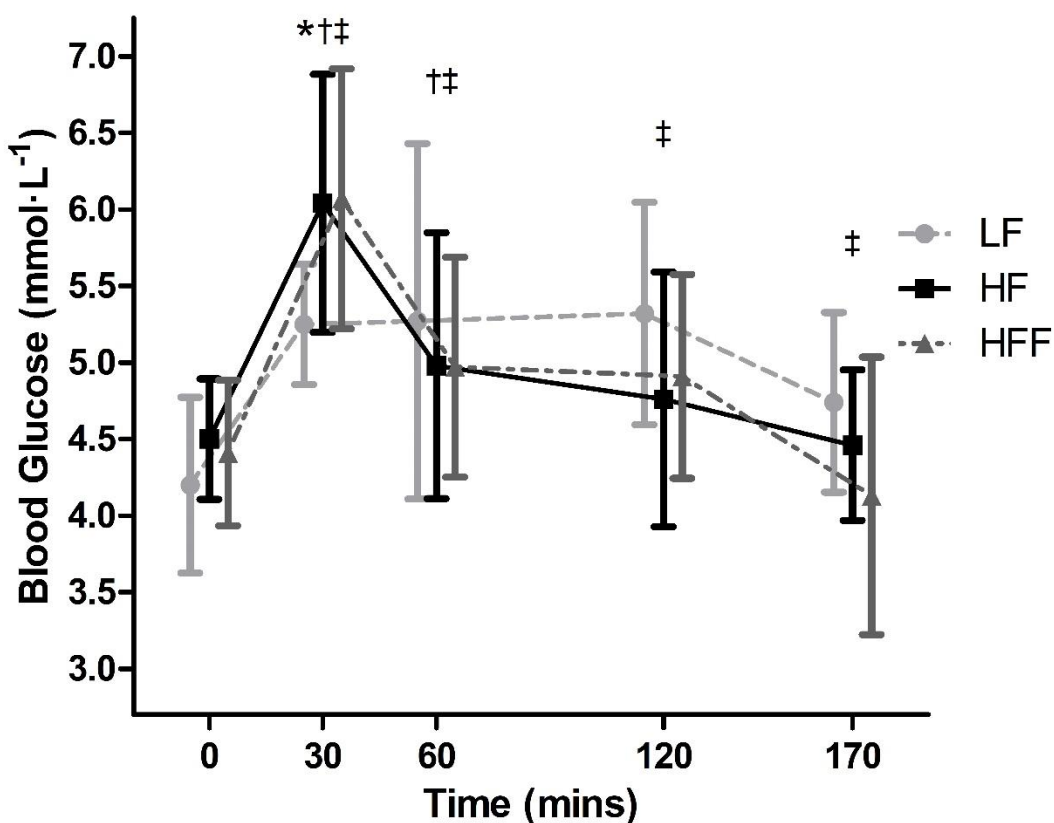


Figure 9.3 Mean (\pm standard deviations) blood glucose concentrations ($\text{mmol}\cdot\text{L}^{-1}$) during prolonged sitting.

Abbreviations: LF, low-fat condition; HF, high-fat condition; HFF, high-fat fidget condition. * denotes HF significantly different from baseline ($p < 0.05$), † denotes HFF significantly different from baseline ($p < 0.05$), ‡ denotes LF significantly different from baseline ($p < 0.05$). Note; data at each time point has been staggered along X axis to provide clarity, it should be noted that all blood samples were collected at the same time points.

9.3.2.3 Cholesterol

Mixed model analysis revealed no significant interaction effect or main effects of time or group for total cholesterol ($p > 0.05$). Similarly, there were no significant effects for HDL ($p > 0.05$).

9.3.2.4 Near-Infrared Spectroscopy and Calf Circumference

Analysis of pre vs post values identified a significant interaction effect for HHb ($p = 0.002$) but not tHb ($p = 0.259$). Follow up analysis identified that HHb significantly increased from baseline in LF (MD = $6.29 \mu\text{mol}$, 95 % CI: 3.37 to 9.21, $p < 0.001$, $d = 1.20$) and HF (MD = $4.31 \mu\text{mol}$, 95 % CI: 1.45 to 7.17, $p = 0.004$, $d = 0.84$) conditions but did not significantly change in HFF (MD = $-1.04 \mu\text{mol}$, 95 % CI: -3.82 to 1.74, $p = 0.457$, $d = 0.21$). Analysis of total area under the curve (tAUC) was performed to characterise changes in

tHb and HHb across the entire sitting period. tAUC for tHb identified a non-significant difference between groups ($p = 0.142$), however, there was a significant difference between groups for HHb ($p = 0.007$). Follow up analysis tAUC was significantly higher in LF compared to HFF (MD = 68.9, 95 % CI: 30.7 to 107.1, $p = 0.007$, $d = 0.98$). The difference between HF and HFF was non-significant (MD = 46.1, 95 % CI: 8.08 to 84.1, $p = 0.086$, $d = 0.66$). tAUC for HF and LF were not significantly different (MD = 22.8, 95 % CI: -15.4 to 61.0, $p = 0.773$, $d = 0.32$).

There was a significant interaction effect for calf circumference ($p = 0.012$). Follow up analyses showed that calf circumference significantly increased in all conditions. Increases in calf circumference were greatest in the HF condition (MD = 1.66 cm, 95 % CI: 1.22 to 2.09, $p < 0.001$, $d = 2.13$), followed by the LF condition (MD = 1.42 cm, 95 % CI: 1.04 to 1.79, $p < 0.001$, $d = 2.10$). Calf circumference increased by the smallest increment in the HFF condition (MD = 0.84 cm, 95 % CI: 0.47 to 1.20, $p < 0.001$, $d = 1.29$).

9.3.3 Primary Outcomes: Regional Pulse Wave Velocity

Interaction and main effects for supine cfPWV and faPWV are presented in **Table 9.2**. Post-hoc analysis identified that cfPWV significantly increased from pre-post in HF (MD) = 0.62 m·s⁻¹, 95 % CI: 0.35 to 0.89, $p < 0.001$, $d = 1.28$) whereas there were no significant differences in LF (MD = 0.13 m·s⁻¹, 95 % CI: -0.14 to 0.40, $p = 0.338$) or HFF (MD = 0.04 m·s⁻¹, 95 % CI: -0.23 to 0.31, $p = 0.752$). There was a non-significant effect of time ($p = 0.352$), group ($p = 0.544$), or interaction ($p = 0.808$) for faPWV. Interaction and main effects for supine PWA are presented in **Table 9.3**. Post-hoc analysis of supine cPP identified a borderline significant increase in the HF condition (MD = 3.0 mmHg, 95 % CI: -0.05 to 6.04, $p = 0.053$, $d = 0.55$) and no significant changes in either LF or HFF conditions ($p > 0.05$).

Table 9.2. Supine pulse wave velocity results from all conditions. Data are presented as means (\pm standard deviations)

		cfPWV (m·s ⁻¹)	faPWV (m·s ⁻¹)
Time			
Low-fat	Pre	5.82 (0.71)	9.29 (1.05)
	Post	5.96 (0.53)	9.11 (1.07)
High-fat	Pre	5.54 (0.51)	9.10 (1.04)
	Post	6.13 (0.56)	9.06 (1.08)
High-fat fidget	Pre	5.65 (0.68)	9.02 (0.98)
	Post	5.68 (0.46)	8.87 (1.09)
Group effect	<i>p</i>	0.275	0.544
Time effect	<i>p</i>	0.001*	0.352
Interaction effect	<i>p</i>	0.007*	0.808

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; faPWV, femoral-ankle pulse wave velocity. * denotes statistical significance ($p < 0.05$)

Table 9.3. Supine pulse wave analysis results from all conditions. Data are presented as means (\pm standard deviations)

		HR	SBP	DBP	MAP	cSBP	cPP	AIx	Pf	Pb
Time										
Low-fat	Pre	56.7 (6.4)	113.6 (10.3)	65.6 (5.1)	77.9 (5.6)	99.4 (7.5)	33.1 (6.2)	3.0 (9.1)	24.5 (4.6)	13.6 (9.2)
	Post	59.5 (6.4)	116.3 (7.6)	64.7 (3.4)	76.2 (7.9)	100.5 (5.6)	35.5 (7.6)	2.7 (6.7)	27.0 (3.3)	12.3 (1.4)
High-fat	Pre	58.7 (9.2)	118.0 (7.3)	64.5 (4.1)	78.2 (5.1)	101.9 (6.0)	37.6 (6.7)	6.5 (8.8)	28.0 (3.5)	12.7 (2.4)
	Post	60.9 (9.9)	116.6 (9.5)	64.0 (6.5)	77.3 (5.7)	99.5 (6.8)	34.6 (7.7)	0.4 (7.3)	27.9 (4.8)	11.5 (2.3)
High-fat fidget	Pre	56.8 (6.9)	113.0 (9.9)	63.6 (5.2)	76.3 (5.5)	98.9 (7.3)	34.0 (6.8)	7.0 (10.7)	26.2 (4.6)	12.1 (2.5)
	Post	62.1 (8.7)	114.3 (8.9)	63.8 (9.2)	75.8 (6.8)	97.4 (6.6)	34.2 (6.0)	1.4 (10.5)	27.5 (4.4)	12.2 (2.3)
Group effect	<i>p</i>	0.329	0.047*	0.482	0.444	0.094	0.122	0.630	0.050*	0.658
Time effect	<i>p</i>	<0.001*	0.478	0.684	0.352	0.320	0.873	<0.001*	0.079	0.361
Interaction effect	<i>p</i>	0.384	0.375	0.881	0.890	0.294	0.049*	0.092	0.325	0.775

Abbreviations: HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; cSBP, central systolic blood pressure; cPP, central pulse pressure; AIx, augmentation index; Pf, forward pressure; Pb, backward pressure. * denotes statistical significance ($p < 0.05$)

9.4 Discussion

The aim of this Chapter was to determine whether a simple desk-based leg fidgeting strategy was sufficient to offset the combined deleterious effects of prolonged sitting and consumption of a high-fat Westernised meal on markers of cardiovascular health. The main findings of this study were that leg fidgeting was sufficient to attenuate peak blood triglyceride concentrations and venous pooling which appears to offer a protective effect against significant increases in cfPWV and cPP.

9.4.2 Strengths and Limitations

In order to fully contextualise the findings of this study, it is important to acknowledge the strengths and weaknesses of the methods employed. Firstly, this study solely recruited healthy physically-active male participants in an effort to control for potential variability induced by age, as observed in the prior meta-analysis, or sex. As such, the results of this study are not widely generalisable beyond this limited population. Secondly, the fat content of each meal was not prescribed by body mass and thus some participants may have consumed a greater relative amount of fat than others thus producing a more exaggerated response. However, previous research has shown that consumption of 48-50 g of fat impairs vascular function in males, regardless of participants' body mass [306,325]. Given the relatively small standard deviation of participants' body mass in the current study and the within-subject design of this study, the impact of not correcting fat intake for body mass is unlikely to have influenced these results. Thirdly, whilst participants were asked to consume the same pre-trial meal the night before each experimental visit, there is no guarantee that this was done. However, post-hoc analysis identified that baseline triglyceride and glucose concentrations were not significantly different between conditions ($p = 1.00$) and thus any variations in pre-trial meals are unlikely to have influenced the observed results. Fourth, owing to budgetary constraints, it was not possible to measure the habitual physical activity of participants prior to or between trials. Given that acute reductions in habitual physical activity has been shown to impair lower limb vascular function [338] as well as augment glucose and lipid metabolism [308,429], this is an important consideration. Participants were explicitly asked to maintain current physical activity levels, however, there is no guarantee of adherence. It is hoped that the randomised allocation to condition sequence

and the within-subject design in this study may offset any influence of changes in physical activity levels, but it is not possible to confirm this.

9.4.3 Comparison to Literature

This study found that supine cfPWV significantly increased following a bout of prolonged uninterrupted sitting coupled with consumption of a high-fat meal (MD = 0.62 m·s⁻¹, 95 % CI: 0.35 to 0.89, $p < 0.001$, $d = 1.28$) but not when sitting was paired with consumption of a low-fat control (MD = 0.13 m·s⁻¹, 95 % CI: -0.14 to 0.40, $p = 0.338$, $d = 0.27$). The increase in cfPWV was coupled with a borderline significant increase in supine cPP in the HF condition (MD = 3.0 mmHg, 95 % CI: -0.05 to 6.04, $p = 0.053$, $d = 0.55$) where no effect was observed in other conditions. Pulse pressure represents the pulsatile component of blood pressure and reflects the cushioning capacity of elastic vessels. In instances of increased arterial stiffness, reflected waves return sooner in a cardiac cycle, augmenting the pressure wave, increasing SBP and decreasing diastolic blood pressure (DBP), reflected by increased PP [430]. These findings are likely the result of the significantly elevated triglyceride concentration observed in the HF condition compared to no significant increases in the LF condition (**Figure 9.2**). Previous research has identified that consumption of a triglyceride rich meal similar to the one employed in this study can impair vascular function, as indicated by flow-mediated dilation, in healthy male participants for up to 4 hours [306,325]. Further, the increase in cfPWV in this study is greater than those observed in the studies summarised in Chapter 7, suggesting that the consumption of the high-fat meal likely contributed to the observed effect. Previous prolonged sitting studies that have employed cfPWV as an outcome measure have typically reported increases of ~0.4 m·s⁻¹ [59,212] in contrast to the increase of 0.62 m·s⁻¹ observed in this study, suggesting that the increased circulating triglycerides may augment the prolonged sitting response leading to greater dysfunction.

These differences occurred despite similar increases in blood pooling indicated by both NIRS (HHb pre vs post) and calf circumference in HF and LF conditions. The contemporary model explaining prolonged sitting-induced increases in cfPWV suggests that blood pooling in the lower limb reduces venous return to the heart, thereby reducing cardiac output (**Figure 2.6**). The reduction in cardiac output in turn results in reduced shear stress in the aorta, resulting in endothelial dysfunction indicated by increased stiffness (i.e., cfPWV). Given there were similar increases in blood pooling but divergent

changes in cfPWV, it is likely that the higher circulating triglyceride concentrations played a considerable role in the observed dysfunction.

Conversely, when sitting was interrupted with frequent leg fidgeting, cfPWV did not significantly change (MD = 0.04 m·s⁻¹, 95 % CI: -0.23 to 0.31, *p* = 0.752). Equally, HHb showed a non-significant decrease post sitting in the HFF condition compared to significant increases in both HF and LF conditions. Further, the increase in calf circumference measured in HFF, whilst significant, was the smallest increase across all conditions. These results indicate that leg fidgeting may be sufficient to prevent excessive blood pooling in the lower limb and thus offset the combined effect of sitting and a high-fat meal. Limited studies have investigated the use of calf fidgeting as a sitting interruption strategy and no other studies have investigated it as a means of offsetting the additional burden of a high-fat meal. Previous work has shown that a similar leg fidgeting strategy (1 min on/4 min off) is sufficient to maintain popliteal vascular function via increased blood flow and shear rate [46]. Work in animal models also suggests a limited amount of muscle activity may also preserve the function of lipoprotein lipase, facilitating the metabolism and clearance of blood triglycerides [319], which in turn may reduce the deleterious effects of elevated blood triglyceride concentrations [431]. Indeed, **Figure 9.2** shows blood triglyceride concentrations dropping at 170 mins in the HFF condition compared to the apparently stable concentrations in HF. Given that a typical postprandial triglyceride response is 2-4 hours, it is possible that we were unable to fully elucidate the triglyceride response, however, the change in trend in the HFF condition may indicate that fidgeting could be a sufficient stimulus to attenuate the triglyceride response. In contrast with our results, Evans et al., [59] identified no positive effect of using leg fidgeting as a sitting interruption when assessing cfPWV. Contrary to the present study and that of Morishima et al., [46], the work by Evans et al., [59] used a slower, less-frequent leg fidgeting protocol (10 calf raises every 10 minutes). Whilst the protocol utilised by Evans et al., [59] attenuated blood pooling, the reduced and less frequent stimulus may not have been sufficient to facilitate venous return and thus maintain cardiac output and aortic function. Subsequently, it appears that leg fidgeting may be sufficient to offset deleterious effects on cfPWV, however, only when the fidgeting cadence is sufficiently high and fidgeting bouts are frequent.

In addition to cfPWV, this study also assessed peripheral faPWV and found no significant effects of time or condition in contradiction to the findings of Chapter 7. Given the results of [Chapter 3](#) which showed that vascular function is significantly decreased in lower limb arteries, it is perhaps surprising that lower limb PWV did not noticeably change in any condition. Previous work has shown that acute decreases in endothelial function are associated with increases in PWV [161], however, this work was conducted in the upper limb and given the differences in vessel size [222] and structure, between upper and lower limb arteries, it is conceivable that there may be differing effects of endothelial dysfunction on PWV. A further consideration is the posture and timing of assessments. faPWV has only been validated in the supine posture [425], thus we assessed faPWV pre- and post-sitting in the supine posture. However, faPWV was the final measure taken, following a 10-min post-tilt rest period, PWA, and cfPWV measures. Consequently, participants were supine for ~20 minutes before faPWV was assessed. It is possible that in this time, blood flow and subsequently shear stress, would increase in lower limb arteries, restoring any endothelial dysfunction and therefore masking any effects of prolonged sitting on faPWV.

Interestingly, there were no significant interaction effects for SBP, DBP, or MAP despite the study initially being powered to detect changes in MAP (**Table 9.3**). The reasons for this are unclear, however, it is conceivable that differences in baseline fitness and/or habitual physical activity may have played a role. It has previously been shown that acute changes in habitual physical activity (i.e., reducing daily step counts from >10,000 to $\leq 5,000$ steps \cdot day $^{-1}$) can significantly impair resting vascular function [338], however it was not possible to accurately measure participant's habitual physical activity prior to or during the experimental period in this study. To that end it is possible that differences between and within participants may not have been adequately controlled and thus the resulting heterogeneity may have masked any changes in blood pressure indices created by sitting. This notion is perhaps best shown by the significant group effect observed for supine SBP (**Table 9.3**) where baseline values for the HF condition were noticeably higher than other conditions, though not statistically significant.

Consistent with previous research that has assessed AIx responses to prolonged sitting, this study found a consistent, counter-intuitive decrease in AIx in all conditions and postures (**Table 9.3**). AIx represents the augmentation pressure in the pressure wave as

a ratio of pulse pressure and in instances of increased stiffness would be expected to increase. However, this study and previous prolonged sitting studies have consistently observed decreases in AIx [59,60,212,280]. As discussed in [Chapter 2](#), this is likely the effect of pressure waves being dampened by blood pooling in the lower limbs.

9.5 Conclusions

The current findings provide evidence that regularly interrupting a bout of prolonged sitting with seated high cadence leg fidgeting may confer a protective effect against the combined deleterious effects of prolonged sitting and consumption of a high-fat Westernised meal. Further work is needed to understand whether this effect is consistent across different populations. Additionally, future work should ensure habitual physical activity is controlled for.

10

Joining Chapter

Prior to the previous Chapter, there was evidence to suggest that consumption of a high-fat Westernised meal may have an additive effect on sitting-induced cardiovascular dysfunction. However, previous attempts to investigate the effect of sitting interruption strategies had utilised high intensity physical activity that may not be easily adopted in normal working environments. To address this, the previous Chapter investigated whether a simple low-intensity leg fidgeting interruption strategy found to be highly effective at maintaining lower limb vascular function in Chapter 3 was sufficient to offset the combined deleterious effect of prolonged sitting and consumption of a high-fat Westernised meal. The previous Chapter subsequently identified that a simple high-cadence leg fidgeting interruption strategy was sufficient to offset detrimental increases in carotid-femoral pulse wave velocity likely via decreases in venous pooling within the lower limbs. These results show for the first time that the offsetting of lower limb venous pooling via a low intensity, practical sitting interruption strategy that can be widely adopted may offset not only sitting-induced cardiovascular dysfunction, but also the additive effect of consuming a high-fat Westernised meal.

Despite the encouraging findings, there were several limitations with the previous study, detailed above. One of the most pertinent being the lack of control of habitual physical activity of participants. Without accurately assessing physical activity between conditions, it is impossible to know the level of potential confounding. A further major limitation, not detailed within the limitations section, is the speculative nature of the mechanisms responsible for the observed dysfunction. As described in earlier Chapters, the primary stimulus for sitting-induced dysfunction is believed to be venous pooling within the lower limb contributing to reduced venous return and subsequently reduced stroke volume. However, whilst the previous study was able to estimate blood pooling via changes in tHb, HHb, and calf circumference, it was unable to delineate the effect of these variables on stroke volume and thus the degree to which blood pooling may have influenced the observed results.

To address each of these limitations the purchase of new equipment was necessary. With great thanks to the Health, Life Sciences, Sport and Wellbeing Research Priority Area at the University of Gloucestershire, it was possible to secure the acquisition of several PAL Technologies ActivPal 4 inclinometers to quantify both habitual physical activity and sedentary behaviour as well as the acquisition of AD Instruments Non-Invasive Blood

Pressure Nano device for the estimation of beat-to-beat changes in both blood pressure and stroke volume. The purchase of these items has subsequently facilitated completion of the following Chapter and allowed the final objective of this thesis to be addressed, principally, to identify the effects of habitual physical activity and cardiorespiratory fitness on cardiovascular responses to acute bouts of prolonged sitting.

11

The Effect of Cardiorespiratory Fitness and Habitual
Physical Activity on Cardiovascular Responses to
Prolonged Uninterrupted Sitting

11.1 Introduction

The preceding Chapters have identified the detrimental effects of prolonged uninterrupted sitting on cardiovascular and cardiometabolic health. However, as noted in **Chapter 9**, it is expected that other modifiable lifestyle factors may concomitantly impact cardiovascular responses to prolonged sitting. Two important, and likely intertwined [432,433], factors may be cardiorespiratory fitness and habitual physical activity. Both increased cardiorespiratory fitness and habitual physical activity are independently associated with reduced blood pressure and arterial stiffness, as well as improved vascular function via reductions in systemic inflammation and repeated exposure to increased shear stress [434–438]. Further, both increased cardiorespiratory fitness and physical activity have been shown to offset the long term associations between sedentary behaviour and cardiovascular disease [18,133,439]. Cardiorespiratory fitness is known to influence autonomic function and cardiovascular regulation and thus may impact how the cardiovascular system is affected by an acute bout of prolonged sitting. Thus, understanding the influence of cardiorespiratory fitness may provide important mechanistic information as well as highlight cardiorespiratory fitness as a potential therapeutic target to offset sitting-induced dysfunction. However, the impact of both cardiorespiratory fitness and habitual physical activity on cardiovascular responses to acute bouts of prolonged sitting remains unclear. As such, the preferential recruitment of physically active, healthy individuals (as noted in **Chapter 5**) or their opposites may obfuscate the conclusions that can be made about the effects of prolonged sitting on the vascular system. Understanding the interactions of cardiorespiratory fitness, habitual physical activity, and cardiovascular responses to prolonged sitting is necessary in order to guide and improve recruitment strategies in future studies. Improved recruitment strategies, in turn, will ensure the generalisability of findings and the formation of informed public health guidelines.

Previous research has reached equivocal conclusions regarding the effect of cardiorespiratory fitness on cardiovascular responses to prolonged sitting. Morishima et al., [43] found that vascular function was better maintained in individuals with a higher aerobic capacity ($\Delta = -0.5\%$) compared to individuals with a lower aerobic capacity ($\Delta = -2.7\%$) following 180 mins of prolonged sitting, however, as discussed in **Chapter 2**, this study appears to suffer from clear outliers that may impact any inferences that can be

made. Further Garten et al., [57] concluded that cardiorespiratory fitness had no effect on lower limb microvascular function. In contrast, Liu et al., [340] found a positive correlation between cardiorespiratory fitness and sitting-induced reductions in popliteal flow-mediated dilation (FMD) ($r = 0.51$, $p = 0.02$) suggesting that those with a greater aerobic capacity may be acutely more susceptible to the deleterious effects of prolonged sitting. It should be noted however, that this observation, and that of Morishima et al., is based on popliteal artery FMD, which as stated previously within this thesis, is a technically-challenging measure to undertake, with a high proclivity for error (see [Chapter 4](#) for details). Further, the use of FMD in lower limb arteries has no established clinical or prognostic value. If the aim of prolonged sitting research is to begin to inform public health guidelines, it is necessary to use markers of cardiovascular health which are established intermediate outcomes that have causal links with overall health outcomes [376,440]. Such measures include peripheral blood pressure and carotid-femoral PWV (cfPWV), both of which have established causal links with health outcomes (i.e., cardiovascular disease, all-cause mortality) in general and clinical populations [238,239,411,412,441]. As such, it is necessary to build on the work of Liu et al., [340] and establish whether the observed association between cardiorespiratory fitness and the magnitude of sitting response is present when cardiovascular health is assessed using markers with more established links to cardiovascular health outcomes, principally blood pressure and cfPWV.

11.1.2 Objectives

The aim of this Chapter is to investigate the association between cardiorespiratory fitness and habitual physical activity versus the changes in established markers of cardiovascular health; blood pressure and cfPWV.

11.2 Methods

11.2.1 Participants

22 young (25 – 44 years), apparently healthy participants were recruited from a university population. Participants were non-smokers, free of illness or any known metabolic disorders at the time of testing, nor were any taking known vascular-acting medications. Institutional ethics approval was granted prior to recruitment and all participants provided written informed consent. As this study was not designed to

distinguish sex differences, nor the effect of the menstrual cycle on cardiovascular responses to prolonged sitting, the menstrual cycle phase of female participants was not considered to be a problem.

11.2.2 Study Design

Participants completed two laboratory visits, a familiarisation session with a maximal aerobic capacity test ($\dot{V}O_{2max}$), and a sitting trial which occurred within 10 days of the familiarisation visit. The first visit comprised a familiarisation of the equipment involved, as well as an initial screening and completion of informed consent. Anthropometric measurements (stature and body mass) were also conducted. After which participants completed a treadmill based $\dot{V}O_{2max}$ test to determine cardiorespiratory fitness. Following completion of the $\dot{V}O_{2max}$ test, participants were fitted with an activity monitor in line with manufacturer guidelines facilitating physical activity and sedentary behaviour measurement for the 7 days immediately preceding their second laboratory visit. On the second laboratory visit, participants completed a 2-hour uninterrupted prolonged sitting trial.

11.2.3 Experimental Protocol

In line with the protocol described in Chapter 9, participants arrived at 0830 following an overnight fast, consuming only water and having refrained from caffeine for 12 hours. Participants were also asked to refrain from alcohol and strenuous exercise for 24 hours prior. Upon arrival, participants were asked to void their bladder completely prior to laying on the test bed in a supine position for 20 minutes. During this period, and in line with protocols described in Chapter 9, participants were fitted with oscillometric blood pressure cuffs over the left brachial artery, left thigh, and left ankle for assessment of central and peripheral blood pressures, carotid-femoral pulse wave velocity (cfPWV), and femoral-ankle PWV (faPWV) (SphygomoCor XCEL, Atcor Medical, Australia). A continuous-wave near-infrared spectroscopy (NIRS) device was placed over the belly of the gastrocnemius on the participant's right leg to determine changes in blood volume over the sitting period to estimate blood pooling in the gastrocnemius [399]. Additionally, participants were fitted with a three-lead electrocardiogram as well as beat-by beat finger blood pressure monitors to estimate stroke volume.

Following 20 minutes of supine rest, baseline measures of central and peripheral blood pressure were collected using the SphygmoCor XCEL device. Immediately following these measures, cfPWV and faPWV measures were collected. All measures of central and peripheral pressure and velocities were collected in triplicate and the average of the closest two were used in analyses. Heart rate and estimated stroke volume were collected continuously throughout the sitting period. Following completion of baseline measures, participants were moved to an adjacent comfortable sitting chair. Once seated, calf circumference was measured at the point of greatest girth on participants' dominant leg and marked for post-sitting measures. Participants sat quietly for 5 minutes prior to repeat assessments of central and peripheral blood pressures and cfPWV. Following these measures, participants sat uninterrupted for 120 minutes. During this time, participants were reminded to keep their lower limbs as still as possible but were allowed to work on their laptops or read a book. Seated measurements of central and peripheral blood pressure were repeated at 60 mins. It should be noted that the decision to change the sitting period from 180 minutes as utilised in Chapter 9, to 120 minutes was informed by subsequent evidence to suggest that the most ecologically valid sitting duration may be 2 hours [442].

Following 120 minutes of sitting, post-sitting measures of calf circumference, central and peripheral blood pressures, and cfPWV were taken before participants were manoeuvred back to a supine position on the test bed. Following 20 minutes of supine rest, all pre-sitting measures were repeated in the same order.

11.2.4 Experimental Procedures

11.2.4.1 Habitual Physical Activity and Sedentary Behaviour Monitoring

Participants wore an activPAL monitor (ActivPAL4, Pal Technologies Ltd, Glasgow, United Kingdom) 24 h·day⁻¹ concurrently for 7 days immediately prior to the prolonged sitting test visit. To avoid any potential confounding, during the data collection period, the $\dot{V}O_{2\max}$ was performed at least 8-10 days prior to the sitting visit. A minimum of 5 days data (including 1 weekend day) was required [443]. In line with manufacturers guidelines, the activPAL was waterproofed and secured to the midline of the right thigh, one-third of the way between the hip and the knee using transparent waterproof dressing (Tegaderm, 3M). Participants were provided with a standardised diary with which to report daily wake and sleep times and to report any instances of the device being

removed during the data collection period in line with previous recommendations [444]. Data was analysed using PALanalysis software (Pal Technologies Ltd, Glasgow, United Kingdom).

11.2.4.2 Cardiorespiratory Fitness Test

To assess cardiorespiratory fitness, participants completed a ramp-style maximal exercise test on a motorised treadmill (Pro XL, Woodway Inc., Waukesha, USA). Maximal oxygen consumption ($\dot{V}O_{2\max}$) was measured using a breath-by-breath gas and volume analyser (Cortex Metamax 3B, Leipzig, Germany) which was calibrated prior to each testing session. All testing took place in an environmentally-controlled chamber maintained at a constant temperature and humidity (18 °C and 50 %rh, respectively). The protocol consisted of a 2 min warm-up performed at 6 km·h⁻¹ before gradually increasing speed in a continuous ramp fashion at a rate of 1 km·h⁻¹ every minute until volitional exhaustion occurred. Ramp protocols, as opposed to step protocols, have been shown to be better tolerated by a broader demographics as well as those with lower exercise capacities [445–447]. As this study sought to recruit individuals at a range of cardiorespiratory fitness levels, a ramp protocol was therefore utilised.

11.2.4.3 Primary Outcomes: Pulse Wave Analysis and Regional Pulse Wave Velocity

Please refer to [Chapter 9](#)

11.2.4.4 Near-Infrared Spectroscopy

Please refer to [Chapter 9](#)

11.2.4.5 Beat-to-beat Haemodynamics

Heart rate was measured continuously using 3-lead electrocardiogram (ADInstruments, Colorado Springs, United States of America [USA]) and beat-to-beat stroke volume was estimated using pressure waves collected via finger volume-clamp photoplethysmography (Human NIBP Nano, ADInstruments, Colorado Springs, USA) and the ModelFlow method [213]. Heart rate and stroke volume were sampled at 1000 Hz and 200 Hz respectively using a PowerLab data acquisition system (PL3508 PowerLab 8/53, ADInstruments, Colorado Springs, USA). For participant comfort, finger blood pressure cuffs were fitted on both index and middle fingers of the participant's right hand and inflation alternated every 30 mins. For analysis, data was averaged over a 5 minute period at each time point of interest. Each 5 minute period was screened for erroneous values and signal quality and, where possible, data from the same finger cuff was analysed

to ensure consistency. All analysis was performed using LabChart software (ADInstruments, Colorado Springs, USA).

11.2.6 Sample Size

Using the observed correlation of $r = 0.51$ observed in previous research investigating the relationship between cardiorespiratory fitness and sitting-induced change in popliteal artery flow-mediated dilation [340], an alpha level of 0.05, and a power of 0.8, power analysis estimated that 22 participants would be required [426].

11.2.7 Statistical Analysis

Statistical analyses were performed using Jamovi (Version 1.8) [427], a graphical front end to the R programming language [351]. Raw data are presented as mean (standard deviation [SD]). Data was checked for normality using Shapiro-Wilk test and visual inspection of histograms and found to be normally distributed. To test whether the 120 mins prolonged sitting period elicited an effect on markers of cardiovascular health, supine and seated measures of pulse wave analysis (PWA) and central and peripheral PWV were analysed using paired samples *t*-tests. Further, to assess the degree of venous pooling in the gastrocnemius, calf circumference and 5 minute averages of NIRS-derived Hb and tHb, were compared pre versus post sitting using paired samples *t*-tests. Cohen's *d* was used as a measure of effect with < 0.2, 0.2, 0.5, and 0.8 representing a trivial, small, moderate, and large effect, respectively. The alpha level was set a priori at 0.05.

To address the primary objective of this Chapter, Pearson correlation analyses were performed to determine the relationships between cardiorespiratory fitness and habitual physical activity versus sitting-induced changes in measures of cfPWV, faPWV, SBP, DBP, MAP, cSBP, and cPP. Owing to the interdependence of arterial stiffness and blood pressure, additional partial Pearson correlation analysis was also performed to investigate the relationship between sitting-induced changes in cfPWV versus cardiorespiratory fitness and habitual physical activity whilst controlling for sitting-induced changes in mean arterial pressure (MAP) using the *ppcor* package in R [448]. Further, to investigate the proposed mechanism of sitting-induced dysfunction, intra-individual associations between tHb and Hb with heart rate and estimated stroke volume were analysed using repeated measures correlations using the *rmcorr* package in R [449].

11.3 Results

11.3.1 Participants

Participant characteristics are presented in **Table 11.1**. Twenty-one Caucasian individuals (16 males, 5 females) were recruited and completed both study visits.

Table 11.1 Characteristics of included participants

Characteristic	Mean (SD)
Age (y)	33.2 (5.62)
Stature (m)	1.75 (0.08)
Mass (kg)	79.8 (12.6)
Body Mass Index (kg.m ²)	26.1 (2.91)
Absolute $\dot{V}O_{2max}$ (L·min ⁻¹)	4.08 (0.80)
Relative $\dot{V}O_{2max}$ (mL·kg·min ⁻¹)	51.3 (8.15)
Daily step count (steps·day ⁻¹)	10,088 (3,137)

11.3.2 Stimulus: Prolonged Sitting

Results for supine measures of PWA and segmental measures of PWV are presented in **Table 11.2**.

Table 11.2 Pulse wave analysis and pulse wave velocity responses to 120 mins prolonged uninterrupted sitting

Measure	Pre	Post	<i>p</i>	<i>d</i>
cfPWV (m·s ⁻¹)	6.08 (0.55)	6.25 (0.50)	0.007*	0.65
faPWV (m·s ⁻¹)	9.95 (0.78)	10.3 (0.74)	0.011*	0.65
SBP (mmHg)	112.7 (11.4)	114.9 (8.04)	0.222	0.27
DBP (mmHg)	65.8 (6.71)	68.2 (6.16)	0.074	0.41
MAP (mmHg)	78.2 (7.41)	80.1 (6.24)	0.150	0.33
cSBP (mmHg)	100.5 (9.61)	100.8 (6.85)	0.814	0.05
cPP (mmHg)	33.1 (6.66)	31.8 (5.69)	0.121	0.35
AIx (AU)	10.6 (11.8)	3.26 (11.2)	<0.001*	1.37
Calf circumference (cm)	38.7 (2.47)	39.7 (2.71)	<0.001*	1.97
HHb (μmol)	16.3 (6.34)	18.1 (7.58)	0.012*	0.62
tHB (μmol)	59.8 (19.4)	59.4 (18.8)	0.810	0.05

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; faPWV, femoral-ankle pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; cSBP, central systolic blood pressure; cPP, central pulse pressure; AIx, augmentation index; AU, arbitrary units; HHb, deoxygenated haemoglobin; tHB, total haemoglobin. * denotes statistical significance.

Repeated measures correlations identified a significant inverse association between HHb and estimated stroke volume ($r = -0.409$, 95 % CI: -0.653 to -0.087, $p = 0.012$). Further repeated measures correlations identified significant positive correlations between heart rate versus HHb ($r = 0.454$, 95 % CI: 0.143 to 0.684, $p = 0.004$) and tHb ($r = 0.456$, 95 % CI: 0.145 to 0.685, $p = 0.004$). There was no significant association between tHb and estimated stroke volume ($r = 0.086$, 95 % CI: -0.255 to 0.407, $p = 0.615$) (**Figure 11.1**).

11.3.3 Primary Outcomes: Associations Between Cardiorespiratory Fitness and Habitual Physical Activity Versus Change in Central and Peripheral Measures

Partial correlation analysis found no significant associations between cardiorespiratory fitness and habitual physical activity versus change in cfPWV whilst controlling for MAP ($r = 0.158$, $p = 0.505$ and $r = -0.272$, $p = 0.247$, respectively). Further there were no significant partial correlations between cardiorespiratory fitness and physical activity versus change in faPWV whilst controlling for MAP ($r = -0.201$, $p = 0.423$ and $r = -0.348$, $p = 0.157$, respectively). Results from Pearson correlation analyses are presented in

Table 11.3.

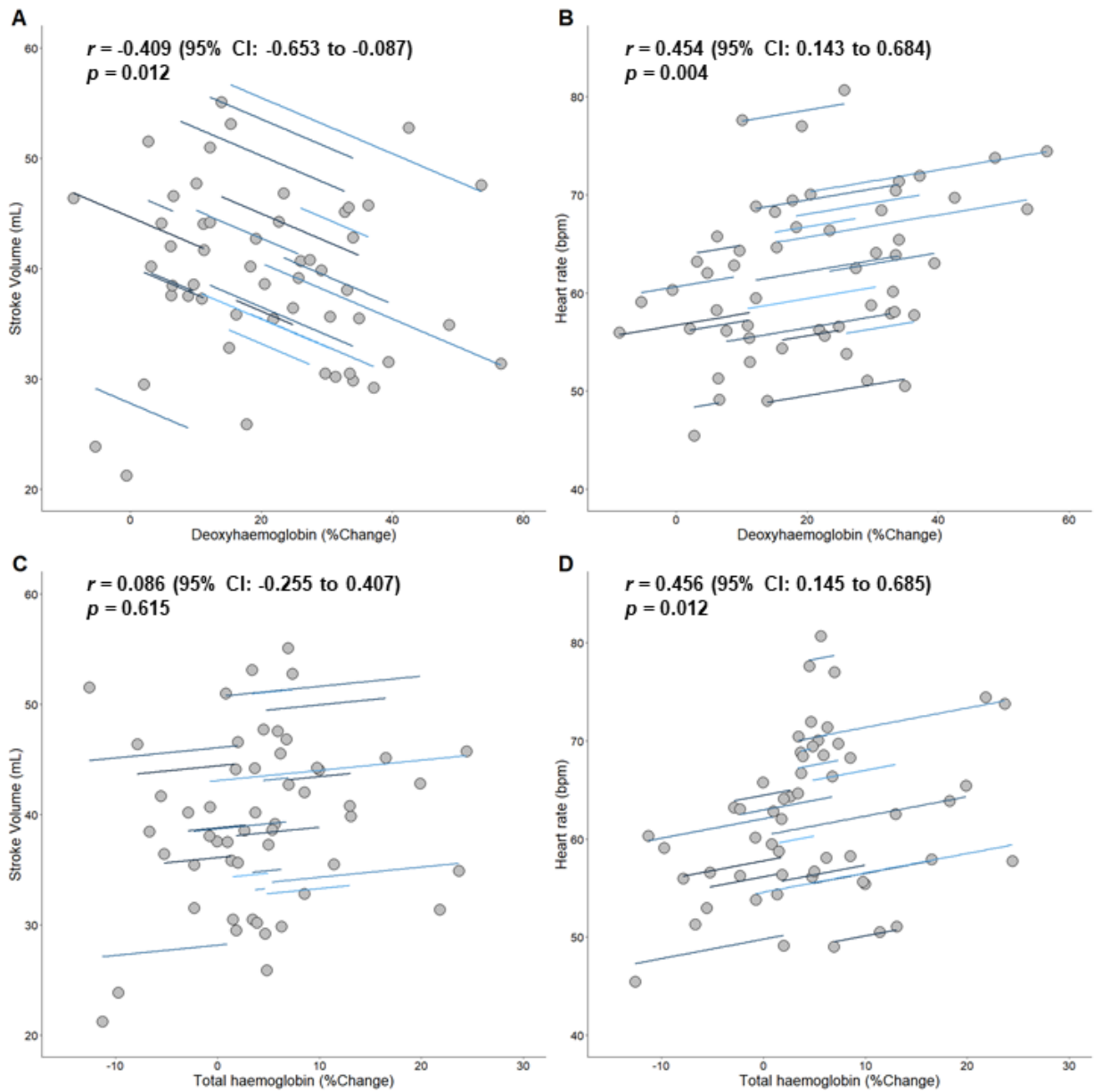


Figure 11.1 Intra-individual associations between deoxyhaemoglobin with (A) stroke volume and (B) heart rate, and between total haemoglobin with (C) stroke volume and (D) heart rate.

Table 11.3 Results of Pearson correlations between cardiorespiratory fitness and habitual physical activity versus sitting-induced changes in measures of central and peripheral markers of cardiovascular health

Measure	Cardiorespiratory fitness		Habitual physical activity	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
cfPWV	-0.058	0.803	-0.337	0.135
faPWV	-0.212	0.385	-0.355	0.135
SBP	-0.198	0.389	-0.166	0.472
DBP	-0.283	0.214	-0.147	0.524
MAP	-0.265	0.246	-0.209	0.364
cSBP	-0.374	0.095	-0.192	0.406
cPP	-0.222	0.333	0.000	0.999

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; faPWV, femoral-ankle pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; cSBP, central systolic blood pressure; cPP, central pulse pressure.

11.4 Discussion

This study sought to investigate the associations between cardiorespiratory fitness and habitual physical activity versus central and peripheral markers of cardiovascular health, specifically cfPWV, faPWV, and central and peripheral blood pressure. This study found that 120 mins elicited significant increases in cfPWV and faPWV, however, neither cardiorespiratory fitness nor habitual physical activity was significantly associated with any measure of central or peripheral cardiovascular health. As such, these results suggest that neither cardiorespiratory fitness nor habitual physical activity impact cardiovascular responses to prolonged uninterrupted sitting.

11.4.2 Strengths and Limitations

In order to fully contextualise the findings of this study, it is first important to acknowledge potential limitations. Firstly, despite efforts to recruit participants of a broad range of cardiorespiratory fitness levels, the current sample represents an above average level of aerobic capacity based on normative values for age and sex [450]. Indeed, the lowest $\dot{V}O_{2max}$ of any participant was 39 mL·kg⁻¹·min⁻¹ which, according to normative data for age and sex, remains in the 50th percentile and is considered average [450]. As such, it is still unclear how individuals with a below average $\dot{V}O_{2max}$ may influence the

relationships explored in this study. Further work is needed to fully explore the relationship between cardiorespiratory fitness and cardiovascular responses to prolonged uninterrupted sitting and thus improve generalisability.

A further limitation is the use of daily step count as a measure of habitual physical activity. Whilst step counts are a useful measure of habitual physical activity [328] with clear links to cardiovascular disease and all-cause mortality [451,452] and easy translation to public health, they are unable to effectively delineate the effect of physical activity intensity. For example, it is conceivable that individuals who recorded low step counts may have completed shorter, more vigorous bouts of physical activity on any given day or conversely, individuals with higher step counts may have not participated in any moderate-vigorous intensity physical activity, potentially confounding results. As such, whilst this study cannot delineate the effect the different intensities of physical activity, these findings do suggest that step count alone may not be a specific enough measure for future studies. Consequently, future studies may consider combining ActiVpal devices, which can characterise habitual sedentary behaviour, with other accelerometers which are better suited to characterising physical activity intensity.

A further potential limitation of this study is the relatively small sample size. Whilst the power calculation for this study was based on previous research following a similar design and sample size ($n = 21$) [340], the aforementioned study investigated popliteal artery FMD rather than central and peripheral measures of cardiovascular health. As such, it is still possible that there may be an effect of cardiorespiratory fitness or habitual physical activity on central and peripheral measures, but it may be smaller and thus a larger sample would be required to detect it. Finally, whilst every attempt was made to balance the study sample, it is largely comprised of male participants (76 % male, 24 % female) and menstrual cycle phase was not considered. Whilst previous research suggests that sex [48], menstrual cycle, and oral contraceptive pill phases [47] do not influence sitting-induced changes in popliteal endothelial function, it is still possible that these factors may be sensitive to differences in cardiorespiratory fitness and habitual physical activity.

11.4.3 Comparison to Literature

This study found a significant increase in cfPWV following a 120 min bout of prolonged uninterrupted sitting. However, this increase does not appear to be influenced by either cardiorespiratory fitness or habitual physical activity. The moderate increase observed in this study is similar in magnitude to that observed in the control (low-fat Westernised meal) condition in Chapter 9, whilst being slightly lower than the increases reported in Chapter 7. The less pronounced increase in cfPWV observed in this study is likely the result of the shorter sitting period employed (120 mins) compared to the typically employed 180 mins [38–41,43–51,57,59,60,212,226,394]. Differences in the degree of venous pooling are likely to influence the magnitude of change in cfPWV. Increased venous pooling in the lower limb is thought to result in reduced venous return, and in turn, reduced stroke volume, leading to endothelial dysfunction in the aorta, presenting as increased cfPWV [61]. Therefore, it is conceivable that the shorter sitting duration may have resulted in less venous pooling. Indeed, when comparing change in HHb in the current study (MD = 1.78 μ mol, 95 % CI: 0.44 to 3.13, p = 0.012, d = 0.62) to the low-fat condition in Chapter 9 (MD = 6.29 μ mol, 95 % CI: 3.37 to 9.21, p < 0.001, d = 1.20) and other papers reporting 180 mins of sitting [60] (MD = 4.5 μ mol, 95 % CI: -1.64 to 10.6, p = 0.08, d = 0.55) the degree of pooling in the current study appears to be lower. This is further supported by calf circumference data whereby the increase in circumference in the current study (MD = 0.94 cm, 95 % CI: 0.71 to 1.15, p < 0.001, d = 1.97) is less than reported in the low-fat condition in Chapter 9 (MD = 1.42 cm, 95 % CI: 1.04 to 1.79, p < 0.001, d = 0.48) and previous research employing 180 mins of sitting [212] (MD = 2.0 cm, p < 0.001, d = 0.1). Despite these differences, the associations between HHb versus estimated stroke volume and heart rate (**Figure 11.1**) are similar to those observed in previous research [211]. As such, whilst the same mechanisms appear to be in play, the apparently reduced venous pooling in the current study, as a function of the reduced sitting period, may partially explain differences in sitting-induced increases in cfPWV. These results, taken together with previous research [211], may provide evidence of an important mechanism within a biologically plausible model of sitting-induced cardiovascular dysfunction, thus progressing towards achieving the aims set out by the World Health Organization [35,36].

The significant increase in faPWV observed in this study ($\Delta = 0.32 \text{ m}\cdot\text{s}^{-1}$, 95 % CI: 0.08 to 0.56, $p = 0.011$, $d = 0.65$) is likely to share similar mechanisms to decreases in lower limb artery FMD summarised in Chapter 3. Owing to the high concentration of vascular smooth muscle cells present in peripheral artery walls, whose function is mediated by endothelium-derived vasodilators [453], peripheral arteries may be more prone to acute detriments in endothelial function which may present as increased arterial stiffness [161]. During a bout of prolonged sitting, reduced blood flow in the lower limbs, and therefore shear stress [51], coupled with increased hydrostatic pressure [226] and arterial bending [235] lead to decreased vasodilator availability and therefore impaired endothelial function. Given the interaction between central and peripheral arteries and the effect on central haemodynamics, understanding the effect of prolonged sitting on lower limb arterial stiffness is an important step. As discussed previously, pressure wave reflection sites occur at bifurcations and points at which arterial geometry changes and contribute to normal function. However, in the presence of increased peripheral artery stiffness, reflection points move closer to the heart which results in wave reflections arriving earlier, in systole, rather than diastole [454]. These disturbed wave reflections contribute to increased SBP and PP and inhibit coronary perfusion [137]. Further, there is evidence to suggest that this increased pressure can be transmitted to the microcirculation, causing damage [145,378]. As such faPWV may provide useful insight into the interaction between the central and peripheral vasculature as well as an indication of changes in endothelial function within the lower limb.

Given the interdependence of blood pressure and PWV, it is perhaps surprising to observe an increase in cfPWV in the absence of a significant change in any metric of blood pressure (**Table 11.2**). The magnitude of change observed for SBP and MAP in the current study (MD = 2.26 mmHg and MD = 1.88 mmHg, respectively) are similar to the summary effects from Chapter 5 ($\mu = 3.2 \text{ mmHg}$, and $\mu = 3.3 \text{ mmHg}$, respectively) but failed to reach statistical significance. As noted in Chapter 6, such small changes in blood pressure may be difficult to accurately detect owing to the associated error with oscillometric devices [410], thus the need for additional measures of cardiovascular health and function such as cfPWV which are less prone to error. Alternatively, it is important to note that changes in cfPWV can be influenced by sympathetic nervous system (SNS) activity, independent of changes in blood pressure [455]. As such, the lack of association between cardiorespiratory fitness and habitual physical activity versus

cfPWV and indices of blood pressure is perhaps surprising given evidence to suggest that physical activity levels can influence SNS outflow, whereby less active individuals may have impaired SNS regulation which can contribute to increased blood pressure [64,65]. Physical inactivity may contribute to increased sensitivity to excitation in brain regions related to the regulation of blood pressure [64,65]. As such, one might expect that those of a lower cardiorespiratory fitness or habitual physical activity level may be more prone to sitting-induced changes in blood pressure, and consequently, arterial stiffness. However, as highlighted previously, it may be a case that an effect does exist, but a larger sample is required to detect it. The significant reduction in Aix (**Table 11.2**), whilst counterintuitive, is consistent with previous literature [59,60,212,280]. Aix, an indication of systemic arterial stiffness, would typically be expected to increase in the presence of increased central and peripheral arterial stiffness as observed in the present study. However, this reduction in Aix should be interpreted with caution owing to the observed increase in HR (**Figure 11.1**). Increased HR effectively shortens the ejection duration and thus the timing of the reflected wave [136]. This shortened cardiac cycle moves the timing of the reflected wave later into diastole, thus the reflected wave has less of an augmenting effect on the forward travelling wave, and in turn Aix, presenting instead as an attenuated Aix value [136].

Previous research investigating the effect of cardiorespiratory fitness on sitting-induced changes in cardiovascular health and function have principally focused on lower limb vascular function [43,57,340]. Specifically, macrovascular function via flow-mediated dilation in the popliteal artery [43,340] and microvascular function using the passive leg technique [57]. Owing to the associations between endothelial dysfunction and increased arterial stiffness [161], faPWV is perhaps the closest measure for comparison from the current study. In line with Garten et al., [57] the present study found no association between cardiorespiratory fitness and sitting-induced dysfunction. However, it should be noted that research in this area has reached equivocal conclusions. Morishima et al., [43] concluded that cardiorespiratory fitness conferred a protective effect against sitting-induced dysfunction in the popliteal artery, whereas Liu et al., [340] concluded that increased cardiorespiratory fitness was associated with increased sitting-induced popliteal artery dysfunction. As previously mentioned, the reason for these divergent findings is unclear but is likely the product of the high proclivity for error associated with FMD measures. Additionally, FMD assessments are arguably only indicative of the local

area being analysed. By contrast, faPWV provides an indication of a greater portion of lower limb vasculature. It is conceivable that fitness related changes in artery geometry and structure may have some influence on local sitting-induced dysfunction, as assessed by FMD, without such changes representing vascular function within the entire lower limb.

The long-term benefits of cardiorespiratory fitness and habitual physical activity are thought to be the product of reduced systemic inflammation and exposure to regular bouts of increased blood flow, and therefore shear stress, facilitating endothelial function [434–438]. From the results of this study, it does not appear that the benefits associated with increased cardiorespiratory fitness and habitual physical activity carry over to acute bouts of sedentary behaviour. However, previous research has shown that physical activity immediately prior to bouts of prolonged sitting confer a protective effect [38,45]. Therefore, the results of this study should not be interpreted as suggesting that cardiorespiratory fitness and habitual physical activity are not important targets for individuals. Indeed, previous research has demonstrated the negative effects of acutely reduced physical activity on vascular function [338] as well as the beneficial effects of acute increases in physical activity [456]. Instead, these results indicate that the cardiorespiratory fitness and habitual physical activity levels of participants may not be a major consideration when recruiting for larger studies in the future, however, this assertion should be investigated in a larger sample before being implemented.

11.5 Conclusions

This Chapter sought to investigate whether there was an association between cardiorespiratory fitness and habitual physical activity versus the sitting-induced changes in central and peripheral markers of cardiovascular health and function. The results of this Chapter suggest whilst significant increases in cfPWV and faPWV were observed following 120 mins of uninterrupted sitting, neither cardiorespiratory fitness nor habitual physical activity, as assessed via daily step counts, influenced such increases. Further work is needed to investigate these associations in larger cohorts before the findings can be used to inform future recruitment strategies for large scale trials.

12

Summary and Future Directions

Sedentary behaviours represent a large portion of the waking hours of individuals in many modernised economies. This is particularly concerning as increased time spent in sedentary behaviours has been associated with increased risk of cardiovascular disease and all-cause mortality. In response to this mounting evidence, national and international health agencies have introduced recommendations for reducing sedentary behaviour. Perhaps the most prominent sedentary behaviour is sitting; a behaviour which is ubiquitous in modernised economies, forming a large part of many individuals' days. In an effort to develop a biologically plausible model for how bouts of prolonged sitting may contribute to the incidence of cardiovascular disease, many experimental trials now exist, however, to date there has been no synthesis of the existing literature or data. Further, investigating prolonged sitting is particularly problematic as it is likely to cluster with other lifestyle behaviours, such as diet and physical activity. Understanding the interaction of these lifestyle factors is likely to aid the understanding of how sedentary behaviour may lead to CVD and may aid the development of a biologically plausible model. Therefore, the aims of this thesis and the major findings are summarised below:

1a) to determine the effect of uninterrupted sitting on vascular function, with subgroup analysis to identify whether responses differed by artery,

The results of Chapter 3 suggest that an acute bout of prolonged uninterrupted sitting results in a significant moderate reduction in vascular function ($\mu = 2.12\%$, 95 % CI: -2.68 to -1.55, $p < 0.001$, $d = 0.79$). Subgroup analysis identified that reductions were most pronounced in lower limb arteries (superficial femoral artery, $\mu = -1.61\%$, 95 % CI: -2.95 to -0.27, $d = -0.43$; popliteal artery, $\mu = -2.51\%$, 95 % CI: -3.06 to -1.97, $d = -1.41$; posterior tibial artery, $\mu = -5.00\%$, 95 % CI: -13.32 to -3.32, $d = -0.37$), with no significant reductions in brachial artery vascular function ($\mu = 0.03\%$, 95 % CI: -1.54 to 1.60, $d = -0.02$).

1b) to determine the effect of regularly interrupting sitting on vascular function, with subgroup analysis to determine whether responses differed by interruption strategy.

Regular interruptions to bouts of prolonged sitting appear to confer a protective effect against decrements in vascular function ($\mu = 1.91\%$, 95 % CI: 0.40 to 3.42, $p = 0.01$, $d = 0.57$). An optimum interruption strategy cannot yet be inferred owing to the limited number of trials, however, both aerobic and simple resistance activities appear to confer

a protective effect (aerobic, $\mu = 2.17\%$, 95 % CI: -0.34 to 4.67, $d = 0.69$; simple resistance activities, $\mu = 2.40\%$, 95 % CI: -0.08 to 4.88, $d = 0.55$).

2a) to determine the effect of uninterrupted sitting on peripheral blood pressure, with subgroup analysis to determine whether age of participants influenced the observed result.

The results of Chapter 5 suggest that an acute bout of prolonged uninterrupted sitting results in significant trivial to small increases in systolic blood pressure ($\mu = 3.17$ mmHg, 95 % CI: 0.58 to 5.76, $p = 0.016$, $d = 0.14$) and mean arterial pressure ($\mu = 3.27$ mmHg, 95 % CI: 2.17 to 4.37, $p < 0.001$, $d = 0.37$), with these responses appearing to be more pronounced in younger age group. Further, prolonged uninterrupted sitting appears to have no significant effect on diastolic blood pressure ($\mu = 0.13$ mmHg, 95 % CI: -1.30 to 1.56, $p = 0.864$, $d = 0.00$).

2b) to determine the effect of regularly interrupting sitting on peripheral blood pressure, with subgroup analysis to determine whether responses differed by interruption strategy.

Regular interruptions to bouts of prolonged sitting appear to confer a protective effect against sitting-induced increases in blood pressure ($\mu = -4.43$, 95 % CI: -7.37 to -1.49, $p = 0.003$, $d = -0.26$). Subgroup analysis, after sensitivity analysis, suggests that aerobic interruption strategies may confer the most robust protective effect ($\mu = -2.66$ mmHg, 95 % CI: -4.83 to -0.49, $d = 0.22$).

3) consolidate the existing evidence regarding PWV and prolonged sitting with and without interruption

The results of Chapter 7 identified that regional measures of PWV are underutilised in prolonged sitting research with only 6 eligible trials being identified. As such, drawing firm conclusions regarding the effect of prolonged uninterrupted sitting on measures of segmental PWV is not yet possible. Limited evidence suggests that both carotid-femoral and femoral-ankle PWV may increase in response to prolonged sitting ($\Delta \approx 0.3$ m·s⁻¹ and 0.5 m·s⁻¹, respectively), however, it is unclear how regular interruptions may affect such responses.

4) to determine whether a simple desk-based leg fidgeting strategy is sufficient to confer a protective effect against the combined detrimental effects of a high-fat Westernised meal and prolonged sitting on cardiovascular health.

The results of Chapter 9 suggest simple desk-based leg fidgeting may be sufficient to attenuate peak blood triglyceride concentrations and venous pooling compared to prolonged sitting combined with a high-fat Westernised meal, thus preventing an increase in carotid-femoral PWV ($\Delta = 0.62 \text{ m}\cdot\text{s}^{-1}$ versus $\Delta = 0.04 \text{ m}\cdot\text{s}^{-1}$).

5) to investigate the association between cardiorespiratory fitness and habitual physical activity versus the changes in established markers of cardiovascular health; blood pressure and cfPWV.

The results of Chapter 11 suggest that whilst 120 mins was sufficient to significantly increase cfPWV ($\Delta = 0.17 \text{ m}\cdot\text{s}^{-1}$, $p = 0.007$) and faPWV ($\Delta = 0.32 \text{ m}\cdot\text{s}^{-1}$, $p = 0.011$), these increases were not influenced by cardiorespiratory fitness ($r = 0.158$, $p = 0.51$) or habitual physical activity ($r = -0.201$, $p = 0.42$).

12.2 Strengths and Limitations

In order to fully contextualise the findings of this thesis, it is first important to acknowledge potential limitations. Firstly, the meta-analyses presented in Chapters 3 and 5 were comprised of principally small experimental studies with a median sample size of between 11 and 18 for different analyses. Meta-analyses and the results they produce are only as good as the studies that are included within the analysis [457]. As such, the typically small sample sizes may be a limitation to the generalisability and accuracy of the observed outcomes. However, it should be noted that generally the included studies were assessed using the Heyland Methodological Quality Score [347] and Cochrane Risk of Bias tool [345] were found to be of a moderate to high methodological quality despite the small samples. Further, both Chapter 3 and 5 report no apparent effect of small study bias, as indicated by Luis Furuya-Kanamori Index and Doi plot assessment, a more objective and sensitive measure of small study bias than traditional funnel plots [354], and extensive sensitivity analysis of all analyses were performed. Taken together, despite the small samples in the included studies, the observed results appear to be robust.

Secondly, building on the previous point related to the quality of included studies governing the quality of the overall meta-analysis, inconsistencies in methods between

studies may obscure the true effect of sitting. As noted in **Chapters 3, 5, and 7**, there are clear methodological incongruencies between studies seeking to answer similar questions. These differences, the most pressing of which being the posture in which assessments were taken and the timing of cardiovascular measures relative to changes in posture, may obfuscate the true effect of sitting when data is amalgamated. However, despite this potential limitation, it is hoped that the identification of these issues now, whilst the field is in its relative infancy, may lay the foundation for more congruent and robust study designs in future research.

Thirdly, whilst the contemporary model of sitting-induced cardiovascular dysfunction suggests multiple pathways by which prolonged sitting increases myocardial burden (**Figure 2.11**), specifically; haemodynamic, autonomic/hormonal, and metabolic, this model remains relatively underdeveloped. For example, whilst the influence of the renin-angiotensin-aldosterone system cannot be ignored, there is currently no direct evidence of its stimulation in response to sitting. Instead, researchers, this author included, have induced that it may be one of the pathways contributing to sitting-induced dysfunction. As such, further work is still needed to fully elucidate the mechanisms contributing to sitting-induced changes in cardiovascular health and function. Finally, the mechanisms by which acute bouts of sedentary behaviour may contribute to future cardiovascular disease risk remains speculative. As noted by the World Health Organization (WHO), the need for a biologically plausible model which can then be tested with larger observational studies and RCTs remains [35,36]. However, this thesis and the studies contained within have served to consolidate the likely effects of acute bouts of sedentary behaviour whilst identifying areas for improvement related to study design which will aid in the development of a biologically plausible model which can subsequently be tested in larger trials in the future.

12.3 Key Outcomes

The Chapters contained within this thesis consistently demonstrate that acute bouts of prolonged sitting result in detriments in markers of cardiovascular health and function. Chapter 3 demonstrated that an acute bout of sitting is likely to result in a significant reduction in vascular function ($\mu = 2.12\%$), particularly in lower limb arteries. Further, Chapter 5 identified that an acute bout of sitting is likely to result in small but significant increases in systolic blood pressure ($\mu = 3.17\text{ mmHg}$) and mean arterial pressure ($\mu =$

3.27 mmHg). Whilst Chapter 7 was unable to effectively conclude the effect of an acute bout of prolonged sitting on segmental measures of PWV, evidence exists to suggest that prolonged sitting may result in increased central and peripheral arterial stiffness. Subsequently, Chapter 11 showed that an acute bout of 120 mins prolonged sitting resulted in a significant increase in cfPWV ($\Delta = 0.17 \text{ m}\cdot\text{s}^{-1}$) and faPWV ($\Delta = 0.32 \text{ m}\cdot\text{s}^{-1}$). Relating back to the contemporary model of how sedentary behaviour may contribute to myocardial burden, and in turn, CVD, it is likely that the observed results throughout this thesis are the consequence of increased venous pooling within the lower limbs, a phenomena present in both Chapters 9 and 11.

Chapters 9 and 11 demonstrate that acute bouts of prolonged sitting result in significant increases in venous pooling within the lower limb using both simplistic measures, such as calf circumference, and more comprehensive measures, such as changes in deoxyhaemoglobin within the gastrocnemius. This increase in venous pooling is likely to be the effect of reduced muscle activity and subsequently reduced activation of the muscle pump. This pooling is thought to result in a subsequent reduction in venous return, and in turn reduced stroke volume via the Frank-Starling mechanism. Prior to this thesis, only one study [211] had provided evidence to support this mechanism by demonstrating significant correlations between changes in deoxyhaemoglobin and estimated stroke volume ($r = -0.59$). The results of Chapter 11 lend further support to this purported mechanism and showed similar associations between deoxyhaemoglobin and estimated stroke volume ($r = -0.41$). Interestingly, the degree of venous pooling observed in Chapter 11 appears to be lower than that observed in Chapter 9 and other previous studies utilising longer 180 mins sitting bouts [60]. In line with the purported mechanism, the lower degree of pooling appears to result in a lesser increase in cfPWV compared to sitting studies which used a longer sitting period. Further, results from Chapter 9 demonstrated that a simple seated leg fidgeting interruption strategy was sufficient to reduce venous pooling and in turn, offset the combined deleterious effects of prolonged sitting combined with a high-fat meal. The results of these Chapters provide support for the notion that venous pooling may drive sitting-induced decrements in cardiovascular function, but importantly, add new insight in to the effect of differing degrees of venous pooling and how this pooling may directly impact the amount of sitting-induced dysfunction observed.

The novel findings of Chapters 9 and 11 provide a clear contributing mechanism by which the protective effect of physical activity interruptions observed in Chapters 3 and 5 may be explained. Chapters 3 and 5, demonstrated that regularly interrupting sitting with physical activity breaks appears to confer a protective effect against sitting-induced detriments in vascular function and peripheral blood pressure. These regular interruptions are likely to increase muscle activity and in turn reduce venous pooling, thus preventing dysfunction. These findings provide more evidence to suggest the efficacy of the current sedentary behaviour guidelines recommending that people regularly interrupt their sedentary time [15]. Additionally, Chapters 3 and 5 are the first consolidation of the existing data regarding the effect of interrupting sitting with physical activity. Subsequently they provided the first platform to address the call by the WHO to investigate whether aerobic or simple resistance activities are likely to be the most efficacious strategy [35]. By consolidating the existing data, the meta-analyses presented in Chapters 3 and 5 suggest that aerobic interruptions may confer the most robust protective effect against sitting-induced changes in vascular function and peripheral blood pressure. The beneficial effects of aerobic interruption strategies may be related to a greater overall number of muscular contractions compared to simple resistance activities, however, further research is required to corroborate this notion.

12.4 Implications and Future Directions

Prior to this thesis, sedentary behaviour had been identified as a novel, independent risk factor for CVD and all-cause mortality [18,20,34,133] and consequently, both national and international health agencies advise reducing time spent in sedentary behaviours [15,114,115]. However, the current advice from many health agencies regarding sedentary behaviour remains vague and ambiguous. To address this issue and move closer to more specific guidelines, the WHO issued a call to researchers to investigate a biologically plausible model whereby acute bouts of sedentary behaviour may contribute to future CVD [35]. Prior to this call, many experimental studies had been conducted investigating several key markers of cardiovascular health, principally flow-mediated dilation, peripheral blood pressure, and segmental measures of pulse wave velocity, producing equivocal results. In line with the novelty of this field, methodological practice between studies lacked cohesion which may have contributed to heterogeneity between findings. As such, despite the earlier experimental work in this field, it was unclear what

the likely effect of acute bouts of prolonged sitting, an experimental surrogate for acute bouts of sedentary behaviour, had on markers of cardiovascular health and function. Further, in the absence of a biologically plausible model for how acute bouts of sedentary behaviour may contribute to future cardiovascular disease, it remained unclear how the findings of the catalogue of experimental findings translated to a bigger picture.

In support of the contemporary model of sitting-induced dysfunction leading to increased cardiovascular burden, meta-analysis of the existing evidence contained in Chapters 3 and 5 demonstrated that acute bouts of uninterrupted sitting resulted in significant detriments in vascular function and increases in peripheral blood pressure, whereas regularly interrupting prolonged sitting appears to confer a protective effect. Further Chapter 9 provided evidence in support of this model by demonstrating that a low intensity seated leg fidgeting interruption strategy, that reduced venous pooling, conferred a protective effect against the combined deleterious effects of prolonged sitting and consumption of a high-fat meal. Finally, Chapter 11 identified that reduced venous pooling, as a consequence of a shorter bout of prolonged sitting, showed similar mechanistic associations between the degree of venous pooling versus changes in estimated stroke volume as longer sitting durations, whilst resulting in a lower increase in cfPWV than longer sitting bouts [211].

The findings of this thesis also identified several factors that will be important considerations for larger observational studies and randomised controlled trials in the future. Chapter 11 identified that despite the independent associations between increased cardiorespiratory fitness and habitual physical activity on reduced CVD risk, neither factor appears to influence cardiovascular responses to acute bouts of prolonged sitting. As such, neither cardiorespiratory fitness nor habitual physical activity may be important confounders for consideration when recruiting participants for large trials in the future. The results of Chapter 11 also indicate that the differences between age groups observed in Chapter 5 are more likely to be the result of age-related differences than differences in physical activity levels. As such, large trials in the future should carefully consider the age of participants and the implications for generalisability.

The systematic reviews and meta-analyses presented in Chapters 3, 5, and 7 also identified several methodological inconsistencies between studies seeking to answer similar questions. As noted throughout this thesis, the development of methodological

guidelines specific to this research area are a necessary next step. A common theme throughout Chapters 3, 5, and 7 was the incongruity between experimental studies, in particular, inconsistencies between the rest period between posture transitions and subsequent cardiovascular measures. As noted in Chapters 3, 5, and 7, these post posture transition rest periods range from 0 to 20 minutes and thus serve to obscure the likely effect of sitting and make consolidating evidence more challenging. Measurements taken immediately post-posture transition may be influenced by a hypotensive response to the posture change, whereas longer rest periods may afford too much time for the apparent primary stimulus for sitting-induced dysfunction, venous pooling, to normalise and thus underestimate the effect of sitting on cardiovascular measures. The development of these guidelines will aid in making the results of future studies more readily comparable and thus aid in answering the call of the WHO to develop and refine a biologically plausible model of how acute bouts of sedentary behaviour may contribute to future CVD [35]. However, the development of these guidelines will take further experimental work which may not directly contribute to the overall goal of developing a biologically plausible model, therefore it is necessary to ensure that the effort spent is wisely invested. As such, it is likely more prudent to invest future efforts in to refining the use of cardiovascular measures with established causal associations with cardiovascular disease and/or all-cause mortality. Future public health policy will be informed by large randomised controlled trials and observational studies focusing directly on health outcomes (CVD, all-cause mortality) or intermediate outcomes (measures with causal associations with health outcomes, such as peripheral blood pressure) [376,440]. As such, future guidelines, and indeed future research should focus on measures that have established causal associations with health outcomes but are also practical to undertake on a large scale. Therefore, an important next step for this field is to investigate the effect of different post-posture transition rest periods following a bout of prolonged sitting and to develop future guidelines.

12.5 Conclusions

To conclude, the findings of this thesis provide robust evidence to show that acute bouts of prolonged uninterrupted sitting lead to detriments in several key markers of cardiovascular health and function, specifically, flow-mediated dilation, peripheral blood pressure, and segmental measures of pulse wave velocity. Further, it appears that whilst

habitual physical activity and cardiorespiratory fitness does not confer a protective effect against acute bouts of prolonged sitting, regular sitting interruptions should be encouraged. The results of this thesis show that regularly interrupting sitting bouts with physical activity confers a protective effect against not only the effects of sitting, but also the combined deleterious effects of prolonged sitting and consumption of a high-fat meal via a reduction in venous pooling. Additionally, the findings of this thesis identify that sitting duration may influence the degree of venous pooling that occurs which in turn moderates the degree of aortic dysfunction observed. Taken together, the findings of this thesis provide robust evidence for the contemporary working model of sitting-induced cardiovascular dysfunction. Further, whilst future work is required to fully elucidate all pathways proposed within the contemporary working model, this work will be aided by addressing the broader methodological issues identified in prolonged sitting research identified within this thesis.

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Appendices

Appendix 1: Chapter 9 Ethics Approval



Via email

Dr Simon Fryer

06/04/22

Dr Robin Bown
Research Ethics Committee Vice-Chair
Senior Lecturer in Marketing Interpretation
Oxstalls Campus
Longlevens, Gloucester, GL2 9HW

Tel: +44 (0)1242
Email:

Dear Simon

I am pleased to confirm that you received ethical clearance in 2018 for your research following ethical review by the University of Gloucestershire's Research Ethics Committee.

Please keep a record of this letter as a confirmation of your ethical approval.

Project Title:	'The effects of prolonged sitting, a high fat meal and physical activity on vascular function, cognitive performance and blood lipid profile.'
Start Date:	20/04/2018
Completion Date:	20/04/2023
REC Approval Code:	REC.18.30.2

If you have any questions about ethical clearance please feel free to contact me. Please use your REC Approval Code in any future correspondence regarding this study.

Best of luck with your research project.

Regards,

Dr Robin Bown
Vice-Chair of Research Ethics Committee



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Appendix 2: Chapter 11 Ethics Approval



Dear Craig,

Thank you for your application for ethical approval.

I am pleased to confirm ethical clearance for your research following ethical review by the School of Sport and Exercise - Research Ethics Panel (SSE-REP).

Please keep a record of this letter as a confirmation of your ethical approval.

Project Title: The effect of cardiorespiratory fitness and habitual physical activity on vascular responses to prolonged sitting

Start Date: June 2021

Projected Completion Date: October 2022

SSE-REP Clearance code: PATERSON20-21

If you have any questions about ethical clearance, please feel free to contact me. Please use your SSE-REP clearance code in any future correspondence regarding this study.

Best wishes

Stephen C. How PhD
Chair of School of Sport and Exercise - Research Ethics Panel

School of Sport and Exercise - Research Ethics Panel (SSE-REP)
University of Gloucestershire, Oxstalls Campus, Oxstalls Lane, Gloucester, GL2 9HW
Tel: 01242
Email:

Appendix 3: Subgroup analysis of mean arterial pressure, systolic, and diastolic blood pressure by assessment type for Chapter 5

	Pooled Effect				Heterogeneity				Asymmetry		Trials	Sample
	WMD	LCI	UCI	P Value	SMD	Q	P Value	I ²	LFK	Quality		
MAP												
Overall	3.27	2.17	4.37	<0.001	0.37	21.4	0.67	0	-0.75	6	26	331
Discrete	2.52	1.22	3.82		0.29	11.9	0.86	0			19	
Continuous	5.18	3.10	7.26		0.65	5.05	0.54	0			7	
SBP												
Overall	3.17	0.58	7.76	0.016	0.14	57.4	<0.001	60	-0.21	8	24	450
Discrete	0.72	-1.47	2.92		0.03	25.2	0.09	33			18	
Continuous	9.46	6.79	12.1		1.07	3.01	0.70	0			6	
DBP												
Overall	0.13	-1.30	1.56	0.864	0.00	27.6	0.15	24	-0.43	8	22	390
Discrete	0.17	-1.14	1.47		0.01	16.2	0.37	7			16	
Continuous	-0.10	-4.76	4.56		-0.02	11.4	0.04	56			6	

Abbreviations: WMD, weighted mean difference; LCI, lower confidence interval; UCI, upper confidence interval; SMD, standardised mean difference; LFK, Luis Furuya-Kanamori Index. SMD: Trivial, small, moderate and large effect sizes are defined as <0.2, 0.2, 0.5, and 0.8 respectively. LFK: <1 indicates no asymmetry, 1 to 2 suggests minor asymmetry, and >2 indicates major asymmetry. I²: 25%, 50%, and 75% represent low, moderate, and high heterogeneity respectively.