Interrupting prolonged sitting with intermittent physical activity in adults with abnormal glucose metabolism : Effects on vascular function

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STATEMENT OF AUTHORSHIP AND SOURCES

This thesis contains no material that has been extracted in whole or in part from a thesis that I have submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person's work has been used without due acknowledgement in the main text of the thesis.

The research procedures involving human data reported in the thesis received the approval of The Alfred Hospital Human Research Ethics Committee. Written informed consent was received and archived for all human research data reported in this thesis.

This thesis contains published work and/or work prepared for publication, some of which has been co-authored. The contributions to each publication from each co-author are acknowledged and approved.

[SIGNATURE REDACTED]

Frances Clare Taylor   28th April 2022

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If you are still reading this, thank you for sticking around. You've got about another 200 pages to go. So, pour yourself a wine, put up your feet and enjoy the read…

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PUBLICATIONS RELATED TO THESIS

1. **Taylor FC**, Dunstan DW, Fletcher E, Townsend MK, Larsen RN, Rickards K, Maniar N, Buman M, Dempsey PC, Joham AE, Cohen N, Owen N, Moran LJ, and Green DJ. (2020) **Interrupting Prolonged Sitting and Endothelial Function in Polycystic Ovary Syndrome**. *Medicine & Science in Sports & Exercise*. 53 (3), 479–486.

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OTHER RELEVANT PUBLICATIONS NOT DIRECTLY RELATED TO THESIS BUT COMPLETED DURING THE CANDIDATURE

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2. Homer AR, **Taylor FC**, Dempsey PC, Wheeler MJ, Sethi P, Townsend MK, Grace MS, Green DJ, Cohen ND, Larsen RN, Kingwell BA, Owen N and Dunstan DW. (2021) **Frequency of Interruptions to Sitting Time: Benefits for Postprandial Metabolism in Type 2 Diabetes.** *Diabetes Care.* 44(6), pp.1254-1263.

3. Homer AR, **Taylor FC**, Dempsey PC, Wheeler MJ, Sethi P, Grace MS, Green DJ, Cohen ND, Larsen RN, Kingwell BA, Owen N and Dunstan DW. (2021) **Different frequencies of active interruptions to sitting have distinct effects on 22 h glycemic control in type 2 diabetes.** *Nutrition, Metabolism and Cardiovascular Diseases.*

4. Loader J, **Taylor F**, Lampa E and Sundström J. (2022) **Renin-angiotensin aldosterone system inhibitors and COVID-19: A systematic review and meta-analysis revealing critical bias across a body of observational research.** *Journal of the American Heart Association.* (Accepted April 2022).

OTHER RELEVANT PUBLICATIONS IN PREPARATION FOR SUBMISSION NOT DIRECTLY RELATED TO THESIS

1. **Taylor FC,** Brakenridge CJ, Sethi P, Dempsey PC, Owen N, Head G, and Dunstan DW. **Associations of device measured sitting, standing and stepping time with 24-hr ambulatory blood pressure monitoring in Australian adults** *Manuscript under preparation*

CONFERENCE PRESENTATIONS RELATED TO THESIS

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INVITED PRESENTATIONS RELATED TO THESIS

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3. 3MT ACU Melbourne 2019 – Runner up
4. 3MT Australian Society for Medical Research 2019 – Runner up

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LIST OF ABBREVIATIONS AND NOMENCLATURE

AUC: Area under the curve

BMI: Body mass index

BP: Blood pressure

CVD: Cardiovascular disease

DM: Diabetes mellitus

ET-1: Endothelin-1

FFA: free fatty acids

FMD: Flow-mediated dilation

HR: Heart rate

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

L-NMMA: N^{G}monomethyl-L-arginine

MET: Metabolic equivalent of tasks

NO: Nitric oxide

OGTT: Oral glucose tolerance test

PA: Physical activity

PCOS: Polycystic ovary syndrome

ROI: Range of interest

RPE: Rating of Perceived Exertion

SD: Standard deviation

SEM: Standard error of the mean

SRA: Simple resistance activities

ROI: Region of interest

T2D: Type 2 diabetes

WHO: World Health Organization

95% CI: 95% confidence interval

ABSTRACT

Sedentary behaviours, defined as waking behaviours undertaken in a sitting/lying posture with low energy expenditure (i.e., ≤ 1.5 metabolic equivalent of tasks [METS]), are now recognised as being strongly associated with all-cause and cardiovascular disease (CVD)-related mortality. Specifically, acute experimental studies have reported prolonged uninterrupted sitting to exacerbate postprandial cardiometabolic risk biomarkers [1] and decrease vasodilatory function [2-4] People with abnormal glucose metabolism (which refers to a combination of clinical disorders that increase the risk for diabetes and cardiovascular disease) are disproportionally affected by the risks associated with prolonged uninterrupted sitting, owing partly to vascular dysfunction and consequent predisposition to atherosclerosis.

Lifestyle modification remains a cornerstone treatment for the prevention and management of CVD, and recent World Health Organization (WHO) guidelines have been expanded to include a recommendation to reduce and regularly replace sedentary time (sedentary behaviour) with activity. Despite this, relatively little is known about the effects of prolonged sitting on vascular function in those with abnormal glucose metabolism. Additionally, it is currently unknown whether reducing and interrupting sitting time with activity positively influences vascular function in these population groups. The primary aim of this Thesis was to examine the extent and nature of vascular impairment in response to prolonged sitting across the abnormal glucose spectrum; with a focus on reducing and interrupting time spent sitting with activity in clinical populations.

Study 1 aimed to determine the dose-response relationship between acute prolonged uninterrupted sitting and vascular function through a systematic review and meta-analysis. Additional sub-group analyses examined the effect of prolonged sitting in healthy adults relative to those with abnormal glucose metabolism. A secondary aim was to compare the acute effects of uninterrupted prolonged sitting to interventions involving acute light activity interruptions. The findings revealed that lower-limb vascular function is progressively impaired as time spent in prolonged sitting increases. Moreover, it was observed that prolonged sitting decreased lower-limb vascular function in healthy adults, who had higher *a priori* vascular function, but not in those with metabolic and vascular dysfunction. However, the limited number of studies in those with abnormal glucose metabolism make it difficult to draw conclusive findings. Additionally, while interrupting sitting with brief bouts

of physical activity improved vascular function, considerable heterogeneity was reported between trials, likely due to differing experimental design (mode, frequency, and duration of breaks).

Study 2 compared the acute effects of interrupting sitting with two different activity protocols of equivalent activity duration on vascular function in a clinical population with abnormal glucose metabolism - those with type two diabetes (T2D). Femoral flow-mediated dilation (FMD) averaged across 7h significantly increased when prolonged sitting was interrupted with 3 min of SRAs every 30 min. However, relative to prolonged sitting, interrupting sitting every 60 min with 6 min simple resistance activities (SRAs) did not result in significant changes in vascular function. Vascular shear rate and blood flow were also enhanced by interrupting sitting with SRAs, regardless of frequency. These findings suggest that more frequent, shorter interruptions may be more beneficial than longer, less frequent breaks for vascular improvement in those with T2D. Further, the study provides new insights into the frequency and duration of activity breaks that may be required to improve vascular function during prolonged sitting.

In addition to identifying a lack of studies assessing vascular function and sedentary behaviour in populations with metabolic dysfunction, study 2 also reported a lack of female participants in the trials conducted to date. Study 3 sought to address this gap by examining the effect of prolonged uninterrupted sitting on femoral vascular function in young women with polycystic ovary syndrome (PCOS). Relative to 3.5h prolonged sitting, interrupting sitting with 3 min of SRAs every 30 min significantly increased mean femoral resting shear and blood flow. However, no change was observed in FMD between conditions.

Collectively, this Thesis has contributed new knowledge to the sedentary behaviour and vascular function research field, specifically: by 1) highlighting the progressive impairment of lower-limb vascular function in response to prolonged uninterrupted sitting; 2) demonstrating that interrupting prolonged sitting with more frequent and shorter activity breaks may be more beneficial than longer, less frequent breaks, for vascular health, in those with T2D, and 3) demonstrating that interrupting prolonged sitting with activity breaks improves blood flow and shear rate for women with PCOS. Future research could build on these findings to focus on three key areas:

1. Obtaining a greater understanding of how vascular function changes over time in response to prolonged sitting. This includes free-living and longer-term studies, in addition to acute studies that measure vascular function at multiple time points across the day.

2. Assessing varying modes, duration, and frequency of interruptions in prolonged sitting to identify optimum activity interruptions.

3. Inclusion of female participants and older and clinical populations into clinical trials to enhance the generalisability of public health recommendations.

CHAPTER 1 – GENERAL THESIS INTRODUCTION

The increasing prevalence of those with abnormal glucose metabolism (inclusive of impaired fasting glucose [IFG], impaired glucose tolerance [IGT] and type 2 diabetes [T2D]) represents one of the greatest public health issues of the 21st century. Globally, the incidence of diabetes has quadrupled in recent decades, from 108 million in 1980 [5] to 462 million in 2017 [6]. With regard to global burden of disease, diabetes ranks as the 7th largest contributor [6]. Despite extensive public health interventions, developed countries, including Australia, show rising prevalence rates [6]. Current projections indicate that 70% of those with IGT and IFG will eventually develop T2D, with estimates suggesting that global diabetes incidence will rise to 693 million by 2045 [7].

People with pre-diabetes or T2D are disproportionally affected by cardiovascular disease (CVD), experiencing a two-fold increased risk of CVD mortality, respectively, compared to healthy individuals [8-10]. This is largely attributable to atherosclerosis, with increased risk of myocardial infarction, stroke, and microvascular diseases [11]. The importance of the vascular endothelium in maintaining artery function and health is now well accepted [12, 13]. Endothelial dysfunction is now recognised as an important and integral early event in atherogenesis and the progression of CVD, preceding IFG, IGT and T2D [14, 15]. It is associated with reduced vasodilation, platelet aggregation and adhesion, monocyte-derived macrophages, smooth muscle proliferation, increased leukocyte adhesion and superoxide anions [16]. In those with abnormal glucose metabolism, vascular reactivity is usually, but not always, reduced [17]. Consequently, interventions that improve arterial function can provide antiatherogenic benefit and may be an important tool for the management of abnormal glucose metabolism [2, 18, 19].

Lifestyle modification, including regular physical activity (PA) is considered a cornerstone for the prevention and management of CVD and T2D. However, despite the known cardiometabolic benefits of PA [20], meeting recommended levels (at least 150 min of moderate to vigorous activity weekly) continues to be challenging for those with abnormal glucose metabolism, with numerous barriers to exercise reported [21, 22]. Further, in the social and economic context of rapidly advancing technologies in workplaces, transportation, and home entertainment, fewer opportunities exist for incidental activity, creating many settings in daily life that promote prolonged sitting. Sedentary behaviours, defined as waking behaviours undertaken in a sitting/lying posture involving low energy

expenditure ≤ 1.5 metabolic equivalent of tasks (METs), are now recognised as strongly associated with all-cause and CVD-related mortality [23, 24]. In particular, the deleterious consequences of prolonged sitting have been highlighted, with acute experimental studies reporting prolonged uninterrupted sitting exacerbates postprandial cardiometabolic risk biomarkers [1] and may decrease vascular function [2-4].

Given that those who are older and with impaired glucose metabolism spend more time in sedentary behaviour, relative to those that are younger [25] and with normal glucose metabolism [26], a greater understanding of the effects of prolonged uninterrupted sitting on vascular function in populations with abnormal glucose metabolism is germane. Currently, many studies focus on measuring vascular function before and after an acute bout of prolonged sitting [27-29] but little is known about the dose-response relationship between prolonged sitting and vascular function [30, 31]. Charting the time course of vascular impairment in response to prolonged bouts of sitting could assist in addressing fundamental and pragmatic questions, such as: 1) what duration of sitting is considered harmful? 2) how often should people break up prolonged periods of sitting? and, importantly, 3) do these answers change depending on an individual's level of progression on the abnormal glucose metabolism spectrum?

It is currently challenging to answer these questions, given that most of the acute experimental studies examining the effects of sedentary behaviour on vascular function have recruited young, healthy, male participants [31]. Indeed, there is little research that currently investigates the impact of sedentary behaviour on vascular function in women, older adults, and adults with abnormal glucose metabolism. This can lead to a "one size fits all" approach for an area that has inevitable complexities. Studies that assess vascular function in females, older individuals and participants with abnormal glucose metabolism are needed to develop specific sedentary behaviour recommendations that can cater to an individual's cardiovascular risk profile. Additionally, the optimal frequency and duration of interruptions that are required to elicit benefits for individuals across the abnormal glucose metabolism spectrum remains unclear. Such evidence is required to inform larger randomised, controlled trials and produce more specific public health guidelines pertaining to the optimal timing and duration of breaks in sedentary behaviour.

Cognisant of the aforementioned gaps in the literature, Chapter 3 of this thesis provides a detailed review of sedentary behaviour and vascular function across the abnormal glucose metabolism spectrum. The findings of this review will then further inform studies to follow in Chapters 4, 5 and 6. Finally, a discussion and summary in Chapter 7 addresses the overarching implications of the findings reported in the thesis.

Thesis Questions

1. What is the dose-response relationship between acute uninterrupted sitting and vascular function?

2. Does prolonged sitting affect the vascular function of individuals across the abnormal glucose metabolism spectrum differently?

3. What is the optimal frequency and duration at which sitting should be interrupted to improve vascular function in those with metabolic disturbance?

CHAPTER 2 - LITERATURE REVIEW

This chapter reviews empirical evidence that informed the scientific rationale for the aims of this thesis: understanding the impact of prolonged sitting and intermittent physical activity on vascular function across states of abnormal glucose metabolism in adults.

Abnormal Glucose Metabolism, Diabetes Mellitus and Cardiovascular Risk

Diabetes mellitus (DM) and its complications represent one of the most challenging public health issues for the 21st century, accounting for 1.6 million deaths in the year 2015 [32]. Moreover, the incidence of DM is steadily increasing and in the last three decades has nearly quadrupled from ~108 million adults worldwide in 1980, to ~ 422 million in 2014 [5], with T2D accounting for the majority [33]. Abnormalities in glucose metabolism such as IGT and IFG are also considered clinically important. While the transition from early glucose abnormalities to T2D can take many years, current estimates indicate that 70% of individuals with IGT and IFG will eventually develop DM [34]. Further, 2.2 million deaths were attributable to high blood glucose in 2015 [32]. The rise is abnormal glucose metabolism and diabetes is rapid and alarming. Current projections indicate high blood glucose to rise to 471 million by 2035, and diabetes to 693 million by 2045[7].

Persons with IGT, IFG and diabetes also display increased risk for CVD. Part of this group includes women with PCOS, who have been identified as a population group at heightened risk of developing T2D [35]. Cardiovascular diseases account for 65-75% of all deaths among people with DM [36]. While the cardiovascular complications associated with abnormal glucose metabolism may be partly attributed to traditional risk factors, such as hypertension and obesity, research has demonstrated that vascular endothelial dysfunction following chronic sustained hyperglycaemia may contribute to the initial and ongoing development of diabetes and CVD [37]. More recently, evidence of progressive vascular impairment across the spectrum of abnormal glucose metabolism has been reported with chronic vascular dysfunction evident in those considered overweight, long before the clinical onset of IFG, IGT or T2D [15]. Indeed, vascular dysfunction is a precursor to atherosclerotic change in the vasculature such as platelet aggregation and adhesion, smooth muscle proliferation and increased leukocyte adhesion [16, 18, 38, 39]. These findings highlight the importance of developing strategies

that target vascular health in the prevention and management of dysmetabolism and consequent CVD.

Normal Vascular Structure and Function

Blood vessels typically (with the exception of capillaries) comprise three distinct layers or "tunics": the tunica adventitia, tunica media and tunica intima (Figure 2.1) [40, 41]. The intima consists of a squamous monolayer of endothelial cells that produce paracrine hormones that have abluminal impacts of platelets, monocytes and clotting, and luminal impacts of sub-intimal oxidative stress and smooth muscle cell contraction and transmigration. An important physiological stimulus to the production of endothelial hormones is arterial shear stress, the dragging force exerted by the passage of blood across the endothelial cell lining, which projects glycoproteins into the lumen that are mechanosensitive and trigger intra-cellular biochemical events including the activation of enzymes that trigger production of substances such as nitric oxide (NO) and prostacyclin [42, 43]. The tunica media contains vascular smooth muscle cells which are responsible for controlling arterial diameter, or tone, and hence resistance to blood flow and its consequent distribution around the circulation [41, 44]. Finally, the tunica adventitia composed primarily of fibro-elastic connective tissue which plays a key role in structural support of the vessel and its connection to surrounding tissues [41, 44].

Vascular "tone" refers to the balance of constrictor versus dilator factors that influence the contractile state of vascular smooth muscle and hence arterial resistance to blood flow. Multiple factors contribute to vasomotor tone, including shear stress, byproducts of tissue metabolism, and neural control [45-47]. Shear stress, the frictional force exerted on the endothelial cell lining of the internal arterial wall, is a key physiological stimulus responsible for maintaining vascular health [39]. In synergy with other agonists such as insulin, acetylcholine, bradykinin, and adenosine triphosphate, shear stress stimulates the production of vasodilators (nitric oxide, EDHF and PGI2) and some vasoconstrictors (endothelin-1, angiotensin II and prostanoids) that diffuse into vascular smooth muscle cells to control the balance vasodilation and vasoconstriction [43, 48, 49]. The net result of an increase in shear stress, for example in response to exercise, is arterial vasodilation, mediated primarily by NO. Repeated episodic bouts of shear stress, which occur as a result of exercise training or increases in physical activity, upregulate the NO-dilator system and decrease atherosclerosis. In contrast, prolonged periods of inactivity, and diminished shear stress, are associated with diminished

NO and a pro-atherogenic milieu. Shear stress and endothelial cell function are critical in promoting vascular health through regulation of platelet aggregation and adhesion, pro-inflammatory cytokines, angiogenesis, leukocyte adhesion and blood pressure regulation [50, 51].
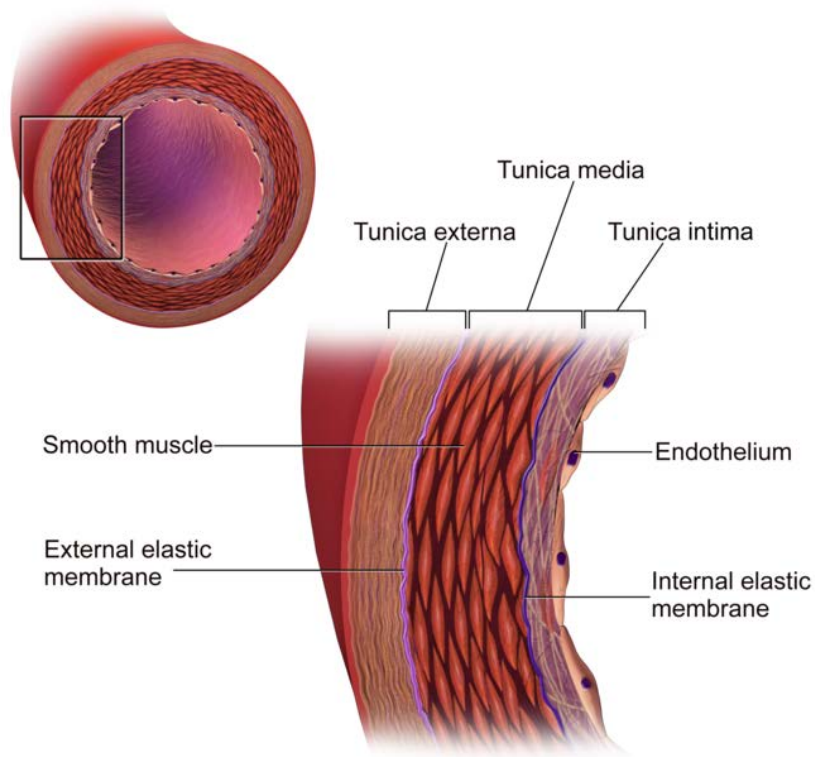
Figure 2.1. Structure of a blood vessel artery. Lumen Learning (2017)

Measurement of Vascular Function

Given that vascular dysfunction is a precursor to atherosclerotic change and the pathogenesis of CVD [43], evaluation of vascular dys/function may provide a prognostic marker of the progression of CVD and cardiovascular health. Flow-mediated dilation (FMD), in assessed via high resolution ultrasound measurement of conduit arteries, is a recognised surrogate measure of endothelium- and NO mediated vascular function and health in humans [52]. Chapter 3 contains the detailed FMD methods used in this Thesis. However, a brief explanation will be included here.

Flow-mediated dilation compares the artery diameter at rest (baseline), relative to the diameter following a period of post-occlusion hyperaemia. Occlusion is typically achieved by inflating a

sphygmomanometer cuff on the forearm of leg, distal to the ultrasound imaged site, for 5 minutes. The cuff is then rapidly deflated to induce and increase in luminal blood flow, and consequently internal-wall shear stress [52, 53]. Shear stress represents an important stimulus for arteries to dilate and adapt, and it is commonly quantified in conjunction with artery diameter to determine the stimulus to diameter increase via enhanced NO-bioavailability [39, 48]. The relative percentage change from baseline diameter, in the post-occlusive period is considered the FMD response [52, 53]. The magnitude of the response is dependent on the size of the artery being assessed (smaller arteries exhibit larger %FMD) and the shear stress stimulus. Structural characteristics of the artery wall and the transduction of the vasodilator response to smooth muscle, along with the response of the smooth muscle to endothelial production of NO, are all determinants of the %FMD [53].

Shear Stress and Flow-mediated Dilation

Shear stress is a key stimulus that induces acute changes in arterial function, along with chronic changes in arterial structure [39, 48]. However, distinct testing conditions (e.g. cuff position, occlusion duration, vasodilator infusion [i.e. acetylcholine], and cooling/heating), induce different shear stress profiles [48]. For example, increasing shear stress via hand warming or the intra-arterial infusion of acetylcholine is consistent with a gradual shear stress enhancement, whereas reactive hyperaemia following occlusive cuff release is a large and transient stimulus [48] (Figure 2.2).
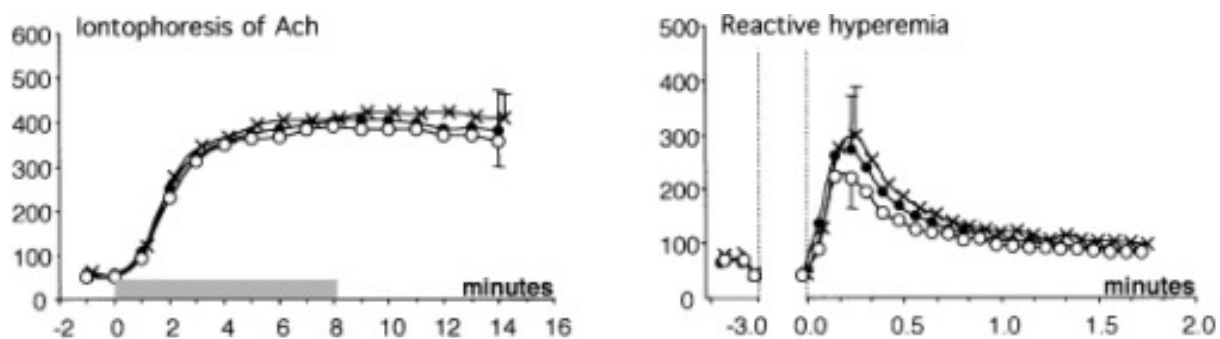
Figure 2.2 Different shear stress profiles. Iontophoresis of acetylcholine (left) versus reactive hyperaemia (right). Sourced from Feihl et al. (2003).

Current consensus guidelines stipulate cuff placement distal to the imaged artery with an occlusion period of 5-minutes [52, 53]. When studies adopt this approach, the majority of dilation is mediated by NO [54]. Koojiman *et al.* [55] found that 5-minutes of ischemia, but not 10-minutes, produced a

predominantly NO-dependent dilation, while Doshi *et al.* [56] found the FMD response had considerably less NO-dependency (35%) following proximal cuff occlusion compared to distal cuff occlusion (98%) following infusion of NO blocker N^Gmonomethyl-L-arginine (L-NMMA) [54]. Similarly, when a prolonged, plateau-like shear stress response was created by hand warming or acetylcholine infusion, the FMD response was unaffected by L-NMMA [57]. These findings suggest that NO significantly contributes to the FMD response when cuff placement is distal to the imaged artery and an appropriate occlusion period (5-minutes) is implemented. It is crucial to follow appropriate guidelines when assessing and interpreting FMD response [52, 53].

Shear Stress Normalization

Given that shear stress influences the magnitude of the FMD response, it has been suggested that the shear stress stimulus be considered when interpreting FMD responses. Shear rate (four times the velocity divided by diameter) provides an appropriate surrogate measure for shear stress and may be used to normalise the FMD response [48, 58, 59]. By equating an individual's response to the degree of shear stress, the probability increases that between-population or treatment differences in FMD response actually represent differences in biological variability in endothelial function, rather than between-subject scaling factors [53, 58]. For example, in studies comparing populations where different endothelial function would be expected (e.g. young children vs older adults), the relationship between shear rate area-under-the-curve and FMD may be weaker [60]. This highlights the biological variability in the FMD response to shear, demonstrating that different individuals may experience different shear stress stimuli despite presenting with the same FMD. Based on the above, recent guidelines recommend measuring and reporting the shear stimulus responsible for the magnitude of FMD observed [52, 53].

The ongoing advancement and improved understanding of FMD over the last three decades has made it a valuable tool for the measure of artery function and health. Indeed, FMD in peripheral arteries independently predicts cardiac events, in individuals with CVD or risk factors, with a 1% decrease in FMD% associated with a 13% increase in CVD risk [14]. By performing and reporting FMD in a manner consistent with the current consensus guidelines, FMD measurement can provide independent prognostic information and indicate the effect of varying treatment strategies on artery health. In clinical studies, the FMD test provides important physiological insights into the influence

of risk factors and occult cardiovascular disease on artery function [53]. Indeed, it is often used as an easily accessible measure and early marker of vascular dysfunction [52].

Vascular Dysfunction and Abnormal Glucose Metabolism

Vascular dysfunction, whether transient or chronic, reduces the capacity of blood vessels to respond to stimuli (e.g., increased shear stress on the arterial wall). Although historically atherosclerosis was assumed to result from injury to the endothelial lining and consequent infiltration of lipids and other cells that ultimately resulted in plaque formation, contemporary theories favour a "response to dysfunction" hypothesis, whereby endothelial cells that fail to produce anti-atherogenic hormones predispose to excessive sub-intimal oxidative stress and inflammation [16]. In addition to contributing to increased atherosclerosis and cardiovascular risk, vascular dysfunction plays an important role in the progression of conditions further along the abnormal glucose metabolism spectrum including those associated with IGT (i.e. polycystic ovary syndrome [PCOS]) and T2D [61, 62]. Recent evidence demonstrates a continuum of impairment where the degree of vascular dysfunction progressively increases across the abnormal glucose metabolism spectrum [15]. For those with PCOS and diabetes, this vascular impairment leads to three to eight fold increase in cardiovascular risk [63]. Changes in behavioural and dietary factors (e.g. increased sedentary behaviour and poor dietary habits) can contribute to an endothelial dysfunction and an atherogenic pattern of risk factors for vascular dysfunction including hyperglycaemia, insulin resistance and low-grade inflammation [4, 47, 64].

Role of Hyperglycaemia

Hyperglycaemia is linked to an increased risk of developing T2D, metabolic syndrome and obesity [47, 65, 66]. Vascular dysfunction is also implicated in this increased risk [65]. A recent meta-analysis provided evidence that endothelial function was decreased following acute hyperglycaemia in both healthy people and those with cardiometabolic disease [65], further supporting suggestions that the pathogenesis of CVD may begin long before manifestation of morbidities such as obesity, hypertension or T2D [15]. Increased oxidative stress has been proposed as a key trigger for vascular impairment owing to its multifaceted role in decreasing nitric oxide bioavailability [67]. Indeed, when blood glucose concentrations rise, oxidative metabolism initiates a pro-oxidative environment where reactive oxygen species are produced at a rate beyond suppressive capacities, consequently,

impact upon NO bioavailability and impairing vascular reactivity [11, 67]. In turn, the impact of excessive oxidative stress (especially production of superoxide anions) induced by hyperglycaemia leads to the production of a potent oxidative species, peroxinitrite radicals (ONOO-), in the presence of counter-regulatory induction of elevated levels of NO [68-70]. A vicious cycle ensues, whereby each compounds the increase of production of potent oxidative stress and endothelial dysfunction promotes this [67].

Role of Insulin Resistance

Vascular function is adversely impacted by insulin resistance, a defining characteristic of T2D. While postprandial release of insulin plays an integral role in the vasodilation that is critical for glucose uptake and delivery, impaired insulin signaling disrupts the normal uptake of glucose, contributing to the cycle of chronic hyperglycaemia [64, 71]. Insulin resistance-induced vasoconstriction, which is caused by an over-production of endothelin-1, decreases bioavailability of NO and disrupts the endothelium's ability to vasodilate in response to stimuli [72, 73]. These prolonged disruptions in vasodilatory mechanisms also lead to adverse structural adaptations, including increased intima-medial thickness due to pre-clinical atheroma and the decreased sensitivity of vascular smooth muscle cells to vasodilating substances [74]. Subsequently vascular stiffness is increased, alongside atherosclerotic progression in the artery which thereby contribute to increased cardiovascular risk [75].
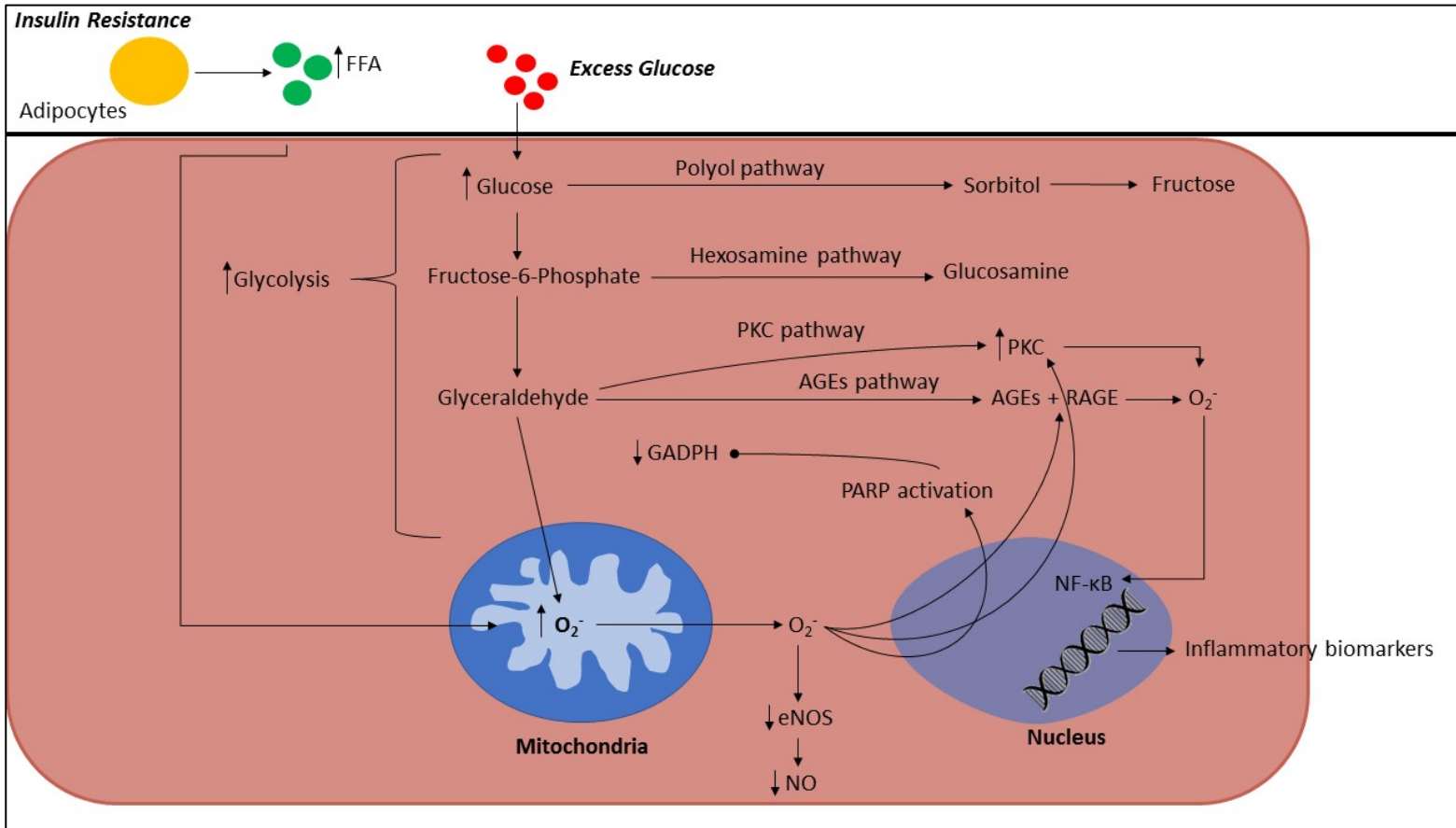
Figure 2.3. Pathways involved in vascular dysfunction. The four primary pathways through which abnormally high blood glucose levels reduce vascular function: increased polyol pathway flux and aldose reductase; protein kinase C (PKC); increased intracellular advanced glycation end products (AGEs); increased expression of the receptor for advanced glycation end products; and increased hexosamine pathway flux. These pathways are unified by a single hyperglycemic-induced process, whereby the mitochondrial electron-transport chain leads to the overproduction of superoxide. Insulin resistance additionally impairs vascular function via reduced NO bioavailability. AGE; intracellular advanced glycation end product, FFA; Free fatty acids, eNOS; endothelial nitric oxide synthase, GADPH; NF- κB; nuclear factor- κB, NO; nitric oxide, O^{2-}; oxide ion, PARP; Poly (ADP-ribose) polymerase, PKC; protein kinase C, RAGE; receptor for advanced glycation end products. Adapted from Madamanchi and Runge (2007) [76] and Medina et al., (2015) [77]

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Role of Low-grade Inflammation

It is well established that low-grade inflammation predisposes to vascular dysfunction and promotes atherosclerotic changes that progress the clinical manifestation of CVD [78]. Indeed, both insulin resistance and hyperglycaemia induce a chronic state of vascular inflammation in obesity and cardiometabolic disease [79, 80]. Accordingly, tests of inflammatory biomarkers are often used as surrogate measures of vascular risk to track cardiovascular health. Increased expression of vasoconstrictors such as cellular adhesion molecules mediate the attachment of leukocytes to the endothelium, an important early event in atherogenesis [81]. Cellular adhesion molecules may also be released from the endothelium in a soluble form that circulates via blood plasma. Thus, soluble cellular adhesion molecules are considered to reflect an atherosclerotic milieu [82, 83]. Pro-inflammatory molecules such as C-reactive protein and tumor necrosis factor-*a* may additionally activate endothelial cells to express an atherogenic phenotype [84, 85]. These inflammatory biomarkers, in addition to several pro-thrombotic and anti-fibrinolytic molecules, are often commonly used to provide prognostic information about the progression and outcome of CVD in diabetic populations [80].

As mentioned above, changes in behavioural factors impact vascular function. Indeed, the relationship between vascular function and physical activity is a well-explored topic [86, 87]. The majority of studies have shown PA positively impacts vascular function. More recently, attention has been directed towards understanding the effects of sedentary behaviour (put simply, too much sitting) on vascular function.

Sedentary Behaviour and Chronic Disease Risk

Sedentary behaviour is defined as waking behaviours undertaken in a sitting/lying posture involving low energy expenditure ≤ 1.5 METs [24]. A growing body of epidemiological evidence links excessive time spent sitting to all-cause mortality, cardiovascular disease incidence and mortality, and incident diabetes [23]. Recent data indicates the adult population spends 55-70% of their waking hours partaking in sedentary behaviour [88]. Indeed, with the introduction of smart phones, electronic-gaming and unlimited TV availability (e.g., streaming services), the opportunities to spend time sedentary are increasing. Certainly, the advances in technology have resulted in working adults spending the majority of their working day at desk- and screen-based jobs where minimal movement is required [25, 64, 89, 90].

Over the past decade sedentary behaviour has become an additional focus of health research and there is increasing recognition regarding the detrimental biological impacts of prolonged uninterrupted sitting. Government and health agencies, including WHO, the American Diabetes Association and the Australian Government, now identify sedentary behaviours as a modifiable risk factor to reduce all-cause and cardiovascular mortality [91-93].

For the vast majority of adults, even when the influence of moderate-vigorous physical activity is controlled for, the risks associated with sedentary time appear to persist [94], suggesting that those meeting the minimum physical activity levels within the guidelines (> 150 mins/week) may still be at increased risk for poor cardiovascular health [95]. However, for those participating in high levels of physical activity (60-75 min/day moderate intensity), the mortality risks associated with high total sitting time are eliminated [95]. Of particular concern, is the sedentary behaviour profile of individuals with abnormal glucose metabolism. In addition to low physical activity levels, individuals with abnormal glucose metabolism, report higher volumes of sedentary time compared to those with normal glucose metabolism [26]. Given this population already reports increased cardiovascular risk [10, 96, 97], there is a need to better understand the degree to which sedentary behaviour impacts cardiovascular risk in this population group.

In addition to the health risks associated with the total volume of sedentary behaviour, the evidence suggests that the accumulation patterns of sedentary time may also be associated with an increased risk of all-cause mortality [64, 89]. Accumulating sitting in longer bouts (thus having infrequent interruptions) may be differentially associated with cardiometabolic biomarkers [98]. Indeed, observational studies and acute laboratory research links high volumes and prolonged periods of sitting with increased waist circumference and BMI, with modest associations present for postprandial glucose and insulin [98-100]. This may render the vasculature more vulnerable to disease, which manifests when additional risk factors (i.e., IFG, IGT and T2D) are also present. Consistent with this, epidemiological evidence indicates that sedentary time is associated with higher odds of peripheral arterial diseases, independent of moderate-vigorous physical activity and traditional peripheral arterial disease risk factors [101, 102]. This is noteworthy as excessive sitting could plausibly have negative impacts on the lower limbs and invariably increase the risk of leg macrovasculature disease [4]. Indeed, peripheral artery disease primarily manifests in the lower extremities.

Prolonged Sitting and Abnormal Glucose Metabolism

It is hypothesised that the degree to which prolonged sitting impacts insulinemia and postprandial hyperglycaemia may be dependent on the underlying metabolic health of the study population [64]. However, it is currently unknown if the same is true for vascular function. Recent meta-analyses investigating the link between vascular function and prolonged sitting have not generated conclusive findings [30, 103]. This may be due, in part, to the lack of research investigating the relationship between vascular function and sitting in individuals with abnormal glucose metabolism. Several studies have examined the impacts of prolonged sitting on glucose metabolism in adults with T2D showing increased glucose and insulin incremental area under the curve, compared to prolonged sitting with activity interruptions [104, 105]. However, currently no evidence exists on the impact of prolonged sitting on vascular function in those with T2D. Further little evidence exists on the impacts of sedentary behaviour on specific population groups where abnormal glucose metabolism is a hallmark of the health condition, such as PCOS [106].

Prolonged Sitting and Polycystic Ovary Syndrome

Women with PCOS have been identified as a population group at heightened risk for developing T2D [35]. Further, they are 2 to 4 more times likely to develop CVD compared to non-affected women [107]. This is likely due to women with PCOS displaying multiple risk factors for T2D and CVD including vascular dysfunction, dyslipidemia, oxidative stress, and inflammation [108]. Given up to 13% women globally are diagnosed with PCOS [109], it is critical to identify effective approaches for the prevention and management of T2D and CVD.

Growing evidence indicates that vascular dysfunction is inherent in this population, irrespective of obesity and visceral adiposity [110, 111]. Additionally, observational studies indicate women with PCOS are more sedentary [106], and less active [112], spending an additional half an hour in sedentary time per day, compared to those without PCOS. Given excessive sedentary behaviour – or "sitting time" – has been independently linked to reduced vascular function, and increased T2D risk, it is possible that increased sedentary time may be further compounding pre-existing cardiovascular risk for this population.

Little is currently known about the role of sedentary behaviour and vascular function in women with PCOS. To date, only two studies have examined associations of 'sitting time' in women

with PCOS [106, 113] and neither of these studies assessed vascular function. Therefore, additional research is needed to examine whether prolonged sitting is associated with vascular dysfunction in women with PCOS.

Prolonged Sitting and Sedentary Physiology

In an extension to the observational findings, a body of experimental research has emerged in recent times to better understand the mechanistic pathways linking sitting time to various health outcomes. This increased attention to understanding "sedentary physiology" is distinct from traditional "exercise physiology" [114, 115]. For those who frequently sit uninterrupted for long periods, the cycle of reduced contraction-mediated glucose uptake and insulin-resistance induced oxidative stress, contributes to the progression of vascular impairment [47, 64]. Recent research indicates the magnitude of insulin response to prolonged sitting is associated with the degree of underlying insulin resistance [116]. Accordingly, for those who are further along the abnormal glucose metabolism spectrum (i.e. those with T2D or PCOS), prolonged sitting may contribute to impaired insulin responses, compounding pre-existing vascular dysfunction [4, 30]. Elevated inflammatory biomarkers may also be associated with prolonged sitting. Reports indicate that television viewing time (a common leisure-time sedentary behaviour) and self-reported sitting time are adversely associated with soluble adhesion molecules and C-reactive protein, respectively [117, 118]. However, when this relationship is adjusted for BMI and waist circumference, a loss of association is reported suggesting adiposity may mediate this relationship [100, 117].

Prolonged Sitting and Vascular Function

Traditionally, many studies examining the impact of inactivity on vascular function investigated space-travel, bed rest or immobilization via injury [119]. Accumulating evidence now suggests that prolonged sitting impairs vascular function. Experimental studies of prolonged bouts of sitting have reported that distinct vascular mechanisms may be affected, including reduced shear stress, associated with blood pooling, and increased hydrostatic pressure [3, 120-122]. Importantly, sitting time for up to 6 hours leads to reductions in lower limb shear stress and blood flow, resulting in conduit artery vascular dysfunction [2, 29, 123, 124]. Prolonged sitting-induced vascular dysfunction is typically specific to the lower-limb vasculature. Indeed, popliteal, but not brachial artery, FMD has reported to be blunted following 6-hours of prolonged uninterrupted sitting in health young men [29]. Similar results

have been observed in older and overweight adults. Following 5-hours of prolonged uninterrupted sitting, a significant decline was observed in femoral FMD, whereas no change was observed in brachial FMD [2].

Prolonged Sitting and Shear Stress

An important and consistent finding of arising from prolonged uninterrupted sitting research, is reduced shear stress [4]. Given that shear stress represents a key stimulus for maintaining vascular health [43], a reduction in shear may stimulate a proatherogenic environment [120, 124, 125]. Accordingly, researchers have investigated whether modifying shear stress in the leg vasculature is a factor mediating leg endothelial function and/or dysfunction [124]. In healthy young men, local heating has been used to maintain blood flow and consequently shear stress in one leg, whereas the contralateral leg remained served as an unheated control. Vascular function was maintained in the heated leg, suggesting preservation of shear is imperative to prevent vascular impairment [124]. Morishima *et al.* [123] used fidgeting to prevent the marked reduction of shear stress during prolonged sitting. In healthy young subjects, one leg was subjected to intermittent fidgeting while the contralateral leg served as the control. Following three hours of sitting, popliteal FMD was reduced in the control leg but improved in the fidgeting leg.

However, it should be noted that while reduced blood flow and shear are often observed in prolonged sitting studies, it does not always lead to a corresponding decline in lower-limb FMD. In healthy, young men and women, no change was observed in popliteal artery FMD during three hours of sitting [120, 126]. Likewise, Carter *et al.*, [127] observed no change in superficial femoral artery FMD following four hours of prolonged uninterrupted sitting in healthy desk workers. Nonetheless, all three studies observed a decline in blood flow and shear stress. It is possible that a more protracted stimulus (i.e. longer reduction in blood flow and shear) is needed to mediate a corresponding decline in lower-limb FMD [127]. The threshold and magnitude of reduction in blood flow and shear stress needed to induce a significant decline in FMD is currently unknown.

Prolonged Sitting, Vascular Function and Endothelin-1

Increased production of the endothelium-derived vasoconstrictor endothelin-1 (ET-1), induced by lowering shear stress, may also be implicated in the pathophysiology of sedentary

physiology [4, 128]. Upregulation of ET-1 has been reported in the inactive lower extremities of spinal cord injury patients who are chronically subjected to low vascular shear stress in the lower extremities [129]. More recently, plasma ET-1 total area under the curve has been reported to increase over 5h of prolonged sitting in overweight and obese adults [2]. Elevated ET-1 may also be a marker of microvascular complications in those with T2D [130], with evidence suggesting that endothelin receptor blockers reduce blood pressure and protect against renal events in those with T2D [131, 132]. Similar results have been observed in women with PCOS, with increased ET-1 production levels in women with PCOS linked to microvascular endothelial dysfunction [133, 134]. Thus, for those with abnormal glucose metabolism, high levels of time spent in sedentary behaviour [26] and consequently reduced shear stress, may lead to vascular dysfunction via excessive ET-1 production [4].

Prolonged Sitting, Vascular Function and Methodological Concerns

Lower-limb leg movement during sitting has been put forward as a possible reason for the lack of FMD decline in some studies [127]. As mentioned above, a movement as small as fidgeting has been reported to prevent a decrease in endothelial function following prolonged sitting [123]. The variance in results may also be explained by differing methodologies. Many studies assessing prolonged sitting and vascular function report significant differences in methodologies. These include different postures adopted during FMD assessment (supine vs seated) and different arterial assessment sites (popliteal, femoral, tibial, brachial) [30]. Indeed, the methodological inconsistencies between studies make it difficult to draw definitive conclusions from the existing literature.

Prolonged Sitting and Vascular Resistance

Whilst reduced leg blood flow induced-shear stress is the likely mediator of reduced vascular function [48], other related mechanisms contributing to leg vascular resistance have also been proposed. Increased hydrostatic pressure within the leg vasculature may contribute to blood pooling, which in turn impacts on reductions in blood flow and shear stress [29]. In contrast to supine positions, sitting has been associated with increased calf circumference, calf pooling and decreased thigh blood flow [47, 122, 126, 135]. Other mechanistic contributions to the effect of prolonged sitting on arterial function include increased blood viscosity and increased muscle sympathetic nerve activity, both of which may also result in reduced shear stress and endothelial function [28, 136, 137].

Prolonged Sitting and Upper-limb Vascular Function

Several studies indicate that prolonged sitting is associated with acute impairment of vascular function in the lower, but not upper, limb arteries [30]. However, findings from the exercise training literature suggest vascular changes may occur in the upper limbs. In response to lower-limb cycle training, significant vascular functional changes have been observed in untrained upper limbs, likely due to the effects of large muscle group exercise on systemic haemodynamics and shear stress [138, 139]. The lack of acute change in upper-limb vascular function after prolonged sitting may reflect the relatively modest effects on shear rate associated with sitting. Future studies that measure vascular function more frequently, across weeks and months, are needed to provide greater insight into the time course of vascular change and adaptation in response to prolonged sitting in both the upper and the lower limbs.

Current Gaps in the Evidence

Many acute experimental studies have focused on the effect of prolonged uninterrupted sitting in young and healthy male populations [30], with little attention given to females, older adults and individuals with abnormal glucose metabolism [31]. Consequently, it is difficult to understand the impact of prolonged sitting across the full spectrum of sex, age, and metabolic function. A greater understanding of the effects of prolonged uninterrupted sitting on vascular function in these underrepresented populations is necessary. Continued failure to recruit females, older adults and individuals with abnormal glucose metabolism not only widens the existing gap in the literature but makes it difficult to provide recommendations for a large portion of the population.

Reducing Sedentary Behaviour and Vascular Function

Given the link between vascular dysfunction and cardiovascular risk, the importance of vascular preservation is now well accepted [12, 13]. Interventions that improve and/or maintain vasodilator function can provide antiatherogenic benefit and are considered an integral tool for the management of cardiometabolic disease [2, 18, 19]. Lifestyle modification, including regular physical activity is considered a cornerstone for the prevention and management of CVD. However, with concerns about low prevalence of physical activity, attention has recently been directed towards understanding the effects of reducing and interrupting sedentary behaviour to broaden non-communicable disease prevention and management initiatives. It is

now identified as an area for recommendations that should be disseminated across key audiences and multiple settings [140]. Consequently, there has been an accumulation of evidence investigating the effect of reducing and interrupting sedentary behaviour on vascular function [2, 30, 123]. Further, World Health Organization (WHO) guidelines have incorporated this evidence to make evidence-based recommendations on sedentary behaviour for adults signifying the importance of sedentary behaviour research [93]. However, currently there is insufficient evidence to provide specific recommendations regarding sedentary behaviour thresholds with the consensus for people to 'sit less and move more.' Specifically, there is insufficient evidence to set quantified time periods on 'sitting time'. This is partly due to the variations in how sedentary behaviour is assessed (e.g., self-reported sitting time, accelerometer) and reported in studies. Further, the pattern at which sedentary behaviour should be interrupted (frequency and duration) for health benefits is currently unknown [93]. Indeed, there are important evidence and practice gaps that warrant further attention including: 1) a consensus on thresholds for sedentary time and disease risk, and 2) specific recommendations on how and how often to break up sitting with activity [140].

Reducing and Interrupting Prolonged Sitting - Acute Experimental Studies

Experimental studies have explored countermeasure strategies to reduce and interrupt prolonged sitting that may be used to improve vascular function. A number of studies have explored countermeasures to stimulate vascular function including simple resistance activities [2], walking and standing. However, study results are typically varied with evidence reporting improved FMD [2, 28], no change in FMD [127, 141] or decreased FMD [142] in response to brief activity breaks. It is likely that the mixed results are due to the wide-range of methodologies employed to assess FMD, including arterial measurement site, posture adopted during FMD assessment, frequency of activity interruption and length of protocol [30, 143]. Nevertheless, shear stress responses appear more consistent with a recent meta-analysis reporting lower-limb shear to increase in response to sedentary behaviour interventions, compared to prolonged sitting [143]. Given shear stress is considered a key physiological stimulus in maintaining vascular health, increasing shear stress by interrupting sitting may still be considered beneficial for vascular health [27, 43, 144].

Reducing and Interrupting Prolonged Sitting - Interventions

In a recent systematic review and meta-analysis, Zheng *et al*. (2021) examined the effects of

sedentary behaviour interventions on haemodynamic alterations in the vasculature [143]. Of the 21 studies included in the meta-analysis, 15 assessed FMD, 11 assessed shear rate and 5 assessed arterial stiffness. Results from this meta-analysis revealed that interrupting sitting increased FMD by 1.5% (95% CI 1 – 1.99) for short-term studies and 0.93% (95% CI 0.25 – 1.62) for long-term studies. Shear rate also increased but no significant pooled effect was reported for arterial stiffness.

Despite the rapid development of the evidence base, the limited research investigating practical sitting interruption strategies precludes the development of quantitative guidelines for sedentary behaviour. While a small number of studies have started to investigate potentially optimal interruption strategies to preserve vascular function [127, 145], the limited number of trials and different methodologies have prevented firm conclusions. For example, when investigating interruption strategies in healthy, young adults on lower-limb vascular function one study reported no significant main effect for the change in FMD for 2-min walking every hour [127], whilst another study with similar interruption strategies reported a vascular preservation compared to the effects of prolonged sitting [146]. It has been suggested the standardised of methodology guidelines may facilitate a better understanding of this research area [30]. Moreover, given the limited number of studies assessing the impact of interrupting sitting on vascular function, there is inadequate representation of individuals with clinical conditions pertinent to cardiovascular complications including women, older adults and those with metabolic disturbances [47]. Further research is needed to determine the optimal frequency and duration of interruptions in sitting to preserve and/or improve vascular function.

Summary

As the research into sedentary behaviour and vascular function continues to expand, it is important that gaps in the literature are addressed within well-controlled research studies. As mentioned above, there is currently a lack of evidence assessing the impact of sedentary behaviour on vascular function in: females, older adults, and individuals with abnormal glucose metabolism. Research that focuses on these populations is needed to provide scope for the future development of specific sedentary behaviour recommendations that can cater to an individual's cardiovascular risk profile. Additionally, the optimal duration and frequency of interrupting sitting to provide benefits for individuals across the abnormal glucose metabolism spectrum remains unclear. This evidence is required to inform larger, randomised controlled

trials and provide more specific recommendations around the optimal timing and duration of interruptions to sitting.

Since impaired vascular function is considered an early precursor to the pathogenesis of cardiovascular disease, and sedentary behaviour potentiates this, research into the effects of interrupting and reducing sedentary behaviour is crucial. Indeed, this thesis provides evidence into the impact of prolonged sitting and interrupting prolonged sitting, on vascular function across states of abnormal glucose metabolism. Additionally, recommendations are provided on next steps to continue to advance this area of research.

CHAPTER 3 - METHODOLOGY

The following section provides a detailed description of the methodology for the assessment of vascular function, which was measured utilising flow-mediated dilation (FMD). Subsequently, Chapters 4, 5 and 6 contain the specific methods used in studies 1, 2, and 3, respectively, which are presented according to guidelines provided by the journals that these manuscripts are either published in or being prepared for submission to.

2.1 Vascular Function

All vascular function assessments were performed in a quiet, darkened, temperature-controlled (22°C –24°C) room. Participants rested in the seated position for ~15 minutes prior to assessment. Heart rate and BP were determined from an average of three measurements on the arm contralateral to the cannulated arm. Participants were asked to rest both heels flat on the floor, shoulder-width apart. Placement of the probe was marked and recorded on the first scan at the first visit and replicated for corresponding vascular measurements. To avoid any transient effects of simple resistance activities (SRA), all FMD measures occurred prior to each SRA bout.

Femoral artery flow mediated dilation

The superficial femoral artery was measured in the right leg. Endothelium dependent endothelial function was assessed using a 10-Mhz multi frequency linear array probe in conjunction with a high-resolution duplex ultrasound (Terason t3200, Teratech, Burlington, MA) machine at an isonation angle of 60°. A rapid inflatable cuff (SC-12-D, D.E. Hokanson Inc., Bellevue, WA) was placed around the thigh, distal to the femur. Once an optimal image of the artery was obtained, a 1-min recording of continuous resting vessel diameter and blood velocity was measured (live duplex mode). The cuff was then inflated (~220mmHg) for 5 minutes. Following 5 min of inflation, the cuff was released to induce reactive hyperaemia, and continuous duplex ultrasound recording continued for a further 3 min to observe the post-deflation diameter and peak response.

Femoral artery diameter and blood flow velocity analysis

Analysis of femoral artery diameter and blood velocity was performed using offline, automated edge detection and wall tracking software by one scanner [147]. Analysis of ultrasound recordings were performed using automated edge detection and wall tracking software. This

software has previously been demonstrated to overcome multiple methodological issues, reproducing diameter measurements that significantly reduce observer error with an intra-observer CV of 6.7% [147]. Three regions of interest (ROI) were selected for each ultrasound recording to automatically calibrate the arterial diameter and velocity;

     i)   Calibration ROI – conversion of image size on the computer to the diameter of the artery (mm).

     ii)   Vessel ROI – automated calibration of the artery diameter on the B-mode image
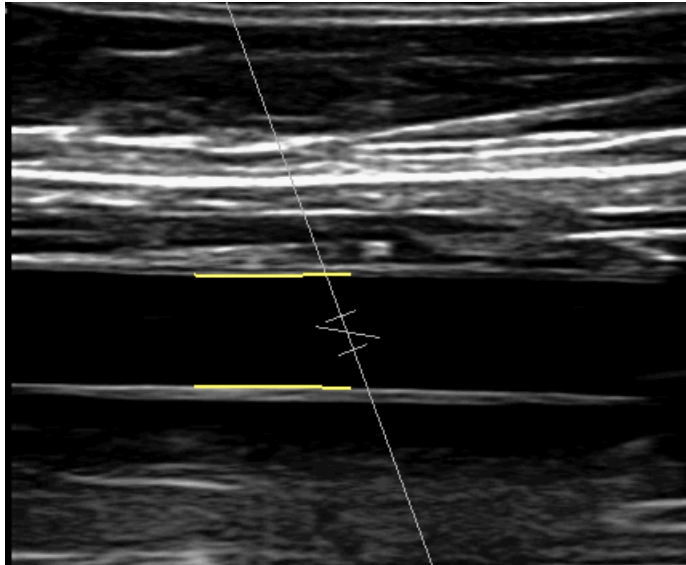
Figure 3.1. Wall tracking software to perform (semi) automated analysis

     iii)   ECG ROI – drawn around the Doppler waveform to detect each R wave
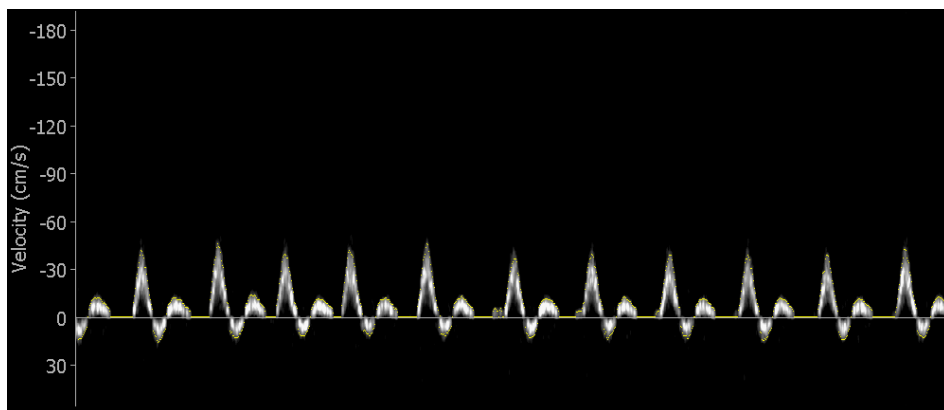
Figure 3.2. Doppler blood flow velocity range of interest.

The synchronisation of vessel ROI and ECG ROI facilitated the calculation of blood flow (the product of lumen cross-sectional area and Doppler velocity) and shear rate (4 x velocity in cm per second/diameter in cm) at 30Hz [148].

Vascular data analysis

Baseline diameter, flow and shear rate were calculated as the mean of the data acquired during the 1-min recording of continuous resting vessel diameter. Peak diameter was automatically determined following cuff deflation according to an algorithm that identifies the peak of the post-deflation artery diameter curve [147]. This algorithm is based on a smoothing routine that calculates the median value of 100 consecutive samples in a data bracket, shared with 20% overlap of the preceding bracket. The peak of the diameter curve is then calculated based on maximum value of all calculated median values. Flow mediated dilation was calculated as the percentage increase in peak diameter from the resting baseline diameter. Time to peak diameter (sec) was calculated as the point of cuff deflation to peak diameter. Shear rate (s^{-1}), derived from blood velocity and diameter, was used as an estimate of shear stress on the artery wall. Shear rate area under the curve (AUC) was defined as the time of cuff release to peak dilation, using the sum of trapezoids method [148].

CHAPTER 4 – THE ACUTE EFFECTS OF PROLONGED UNINTERRUPTED SITTING ON VASCULAR FUNCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Publication statement:

This chapter is comprised of the following paper published in *Medicine & Science in Sports & Exercise.*

4.1 Chapter 4 Introduction

As outlined in Chapter 3, laboratory-based experimental studies have reported impaired vascular function following a bout of acute prolonged sitting. However, the majority of studies have focused on young, healthy males, with limited evidence in females, older adults or adults with metabolic disturbances. It is also currently unknown whether a minimum "amount" of uninterrupted sitting exists as a threshold for clinically relevant improvement in vascular function and health. Considering this, this Chapter aimed to address gaps in sedentary behaviour research by assessing whether a dose-response relationship exists between acute prolonged uninterrupted sitting and vascular function. Additional sub-group analyses were performed to address the acute effect of prolonged sitting in (1) healthy adults relative to adults with metabolic dysfunction, (2) older individuals relative to younger, (3) female participants compared to males, and (4) whether posture influences vascular responses to prolonged sitting.

The Acute Effects of Prolonged Uninterrupted Sitting on Vascular Function: A Systematic Review and Meta-analysis

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[1]Baker Heart and Diabetes Institute, Melbourne, VIC, AUSTRALIA; [2]Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, VIC, AUSTRALIA; [3]Applied Physiology and Nutrition Research Group, Laboratory of Assessment and Conditioning in Rheumatology, School of Physical Education and Sport, Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, BRAZIL; [4]School of Behavioural and Health Sciences, Australian Catholic University, Melbourne, VIC, AUSTRALIA; [5]Sports Performance, Recovery, Injury and New Technologies (SPRINT) Research Centre, Australian Catholic University, Fitzroy, Victoria, AUSTRALIA; and [6]Department of Exercise and Sport Science, School of Human Sciences, The University of Western Australia, Perth, AUSTRALIA

ABSTRACT

TAYLOR, F. C., A. J. PINTO, N. MANIAR, D. W. DUNSTAN, and D. J. GREEN. The Acute Effects of Prolonged Uninterrupted Sitting on Vascular Function: A Systematic Review and Meta-analysis. *Med. Sci. Sports Exerc.*, Vol. 54, No. 1, pp. 67–76, 2022. **Objective:** This study aimed to determine the dose–response relationship between prolonged sitting and vascular function in healthy individuals and those with metabolic disturbances and to investigate the acute effects, on vascular function, of interventions that target interrupting prolonged sitting. **Design:** This is a systematic review with meta-analysis. **Data Sources:** Ovid Embase, Ovid Medline, PubMed, and CINAHL were searched from inception to 4 December 2020. **Eligibility Criteria:** Randomized crossover trials, quasi-randomized trials, and parallel group trials where vascular function (flow-mediated dilation [FMD]) was assessed before and after an acute period of sedentary behavior was used in this study. **Results:** Prolonged sitting resulted in a significant decrease in the standardized mean change (SMC) for lower-limb FMD at the 120-min (SMC = −0.85, 95% confidence interval [CI] = −1.32 to −0.38) and 180-min (SMC = −1.18, 95% CI = −1.69 to −0.66) time points. A similar pattern was observed for lower-limb shear rate. No significant changes were observed for any outcomes in the upper limb. Subgroup analysis indicated that prolonged sitting decreased lower-limb FMD in healthy adults (SMC = −1.33, 95% CI = −1.89 to −0.78) who had higher *a priori* vascular endothelial function, but not in those with metabolic and vascular dysfunction (SMC = −0.51, 95% CI = −1.18 to 0.15). Interrupting sitting with active interruptions increased the standardized mean difference for FMD, relative to prolonged sitting, but it was not statistically significant (0.13, 95% CI = −0.20 to 0.45). **Conclusions:** Lower-limb vascular function is progressively impaired as a consequence of prolonged sitting, up to 180 min. A similar trend was not observed in upper-limb vascular function. Subgroup analysis indicated that prolonged sitting negatively affects healthy populations, a finding not observed in those with metabolic disturbances. Regularly interrupting sitting with activity may be beneficial for those with metabolic disturbances. **Key Words:** ARTERIES, BLOOD FLOW, SEDENTARY BEHAVIOR

Excessive time spent in sedentary behaviors, defined as seated, reclined, or lying posture with low energy expenditure ≤1.5 METs (1), is an independent risk factor for all-cause and cardiovascular disease–related mortality (2,3).

Acute experimental studies have reported that prolonged periods of time spent sitting exacerbate postprandial cardiometabolic risk factors (4–6) and may result in transient vascular dysfunction (7–10). Impaired vascular function is an early and integral atherogenic event preceding morphological changes in the artery wall (11,12). To date, two meta-analyses (8,13) have summarized evidence on acute exposures to prolonged sitting, suggesting that sitting leads to a significant decline in vascular function in healthy adults. However, neither examined the time course of vascular impairment, and it is presently unknown whether there is a minimum amount of uninterrupted sitting, which results in clinically relevant changes in vascular function and health.

Determining the time course of vascular dysfunction is particularly important for clinical populations because impaired vascular reactivity represents a key stage in the pathogenesis of dysmetabolism (11). However, many acute experimental studies have focused on the effect of prolonged uninterrupted

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sitting in young and healthy populations (8) rather than older populations with metabolic disturbances. As a result, previous meta-analyses have only included healthy populations (8,13) with only one meta-analysis reporting exposure to prolonged sitting leads to lower-limb vascular function (8). Given that those with metabolic disturbances spend a greater proportion of time in sedentary behavior (14,15), a greater understanding of the effects of prolonged uninterrupted sitting on vascular function in clinical populations is germane.

The World Health Organization has recently highlighted the need to quantify thresholds of prolonged sitting to determine the frequency and duration of activity interruptions (16). Although studies indicate interrupting prolonged sitting can episodically improve vascular function (7,17,18), interruption strategies have reported mixed results, likely because of the wide range of methodologies used when assessing flow-mediated dilation (FMD), the standard for vascular assessment (19). Other factors include different arterial sites and postures adopted during FMD assessments (8) and a lack of female participants (8,10,17,20–26). Establishing the dose–relationship between vascular impairment and prolonged sitting across the dysmetabolism spectrum, and whether active interruptions in sitting are able to counteract sitting-related vascular impairment, is fundamental to informing larger trials and producing quantifiable sedentary behavior public health recommendations.

The current systematic review, with meta-analysis, aimed to determine the dose–response relationship between acute prolonged uninterrupted sitting across multiple hours on upper- and lower-limb vascular function, with additional subgroup analyses on the acute effect of prolonged sitting in 1) healthy adults relative to adults with metabolic disturbances, 2) older individuals relative to younger, 3) female participants compared with males, and 4) whether posture assessment influences vascular responses to prolonged sitting. A further aim was to compare prolonged uninterrupted sitting to episodic interventions involving brief activity interruptions.

METHODS

This systematic review with meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (PROSPERO trial registration no. CRD42020171394) (27).

Search strategy and study selection. Four electronic databases (Ovid Embase, Ovid Medline, PubMed, and CINAHL) were searched from inception to June 23, 2021 (December 4, 2019; September 23, 2020; and June 23, 2021). The search strategy combined the terms in the following domains: exposure (sedentary OR "physical inactiv*" OR sitting OR "low physical activ*" OR "seated-rest" OR "bed-rest" OR "bed rest" OR lying OR supine OR inactiv*), outcome ("flow-mediated dilation" OR FMD OR "nitrate-mediated dilation" OR NMD OR "brachial artery ultrasound" OR "femoral artery ultrasound" OR "reactive hyper*" OR vasodilation OR "vascular function" OR "endothelial function"), and population (obes* OR

"pre-diabet*" OR prediabet* OR "metabolic syndrome" OR metS OR diabet* OR T2D OR health* OR "cardio-metabolic" OR cardiometabolic OR dysmetaboli* or IGT or "impaired glucose tolerance" or IFG or "impaired fasting glucose" or overweight). The reference lists of all identified trials and relevant reviews were also examined.

Studies were imported into Endnote software (Clarivate Analytics, Philadelphia, PA), and duplicates were removed. Titles and abstracts of all identified records were screened, and relevant full-text articles were retrieved and reviewed by two independent reviewers (F.C.T. and A.J.P.). Discrepancies in inclusion or exclusion were resolved by consensus or through consultation with a third reviewer (D.W.D.).

Eligibility criteria. For the primary aim, manuscripts were eligible if they met the following criteria:

- Study design: randomized crossover trials and quasi-experimental.
- Population: adults and older adults (≥18 yr) that represent key stages in the pathogenesis of type 2 diabetes (T2D): healthy, overweight (28), obese (28), impaired fasting glucose (29), impaired glucose tolerance (29), metabolic syndrome (30), and T2D (29).
- Exposure: prolonged, uninterrupted sedentary behavior (1) period >30 min, but <24 h.
- Outcome: FMD was assessed pre- and post-prolonged sedentary behavior and complied with standardized FMD protocol to ensure our protocol was comparable between studies (19). Although we acknowledge that current FMD guidelines typically stipulate assessments be performed in the supine position (19), movement between seating and supine would necessitate muscular activity that may affect FMD measures. For reasons of ecological validity in the context of the current analysis, studies of prolonged sitting that performed FMD measurements in the seated position were included.
- FMD time points were 30, 60, 120, 180, and ≥240 min of prolonged, uninterrupted sedentary behavior. The following secondary outcomes were assessed before and after at 180 min of prolonged sedentary behavior: blood flow, shear rate area under the curve (SRAUC), and mean arterial pressure (MAP).

For the secondary aim, studies were included if they additionally met the following criteria:

- Intervention: any light-intensity or moderate- to vigorous-intensity physical activity that targeted interrupting sitting across multiple hours. To ensure that protocols were sufficiently homogeneous for comparison, only interventions with activity interruptions <10 min in duration were included in meta-analyses.

Studies were excluded if participants consumed high-fat or high-carbohydrate meals before and/or during the trial.

Data extraction and quality assessment. The same reviewers carried out independent data extraction. Data were

extracted relating to population characteristics (age, sex, body composition, and health status), exposure to sedentary behavior (position and duration), FMD assessment (arterial site and method of collection), outcomes (FMD%, blood flow, SRAUC, and MAP), and details of any intervention aimed at interrupting sitting. Uncorrected FMD values were collected, as opposed to allometrically scaled values or those normalized to shear rate. If the data were unclear or were not available in the published manuscripts, the corresponding author or the first author was contacted by e-mail to request this information.

Study quality was assessed using the Cochrane Risk of Bias Tool (31), which evaluates six domains of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias. Each component was rated as "high risk" or "low risk." If details for a particular domain were insufficient, the risk of bias was assessed as "unclear." Two reviewers (F.C.T. and A.J.P.) scored studies accordingly. In case of disagreements, a third reviewer (D.W.D.) evaluated the article.

Meta-analysis. All statistical analyses were performed using selected packages on R statistical software (version 3.6.1) (32,33). Pre-to-post comparisons were calculated as the standardized mean change (SMC) using pre- and post-prolonged sedentary behavior data, pre-prolonged sedentary behavior standard deviation (SD), sample size, and pre-to-post correlation. A random-effects model was used to determine the pooled effect estimate of all studies within the variable or subgroup as appropriate, with variance estimated through a restricted maximum likelihood model. The first analysis compared the effects of prolonged sedentary behavior (30, 60, 120, 180, and ≥240 min) on FMD%, blood flow, SRAUC, and MAP response in upper- and lower-limb arteries. For studies that had multiple time points at ≥240 min, the time point closest to 240 min was used. A negative mean change indicated that vascular function was impaired at that time point when compared with baseline (0 min). Subsequently, a subgroup analysis was performed at the ≥180 min time point to investigate the potential influence of 1) health status (healthy adults relative to adults with metabolic disturbances) and 2) posture in which vascular function was assessed. Meta-regressions were performed to examine the association between age and sex with SMC for lower-limb FMD at the ≥180 min time point. Subgroup analyses were only performed at ≥180 min as it contained the greatest number of studies ($n = 16$), and therefore the risk of reporting an exaggerated treatment effect was reduced (34). To assess the effects of interrupting prolonged sedentary behavior, between-group comparisons (prolonged sedentary behavior vs activity interruptions) were calculated as the effect size difference (standardized mean difference) using post-intervention (FMD%), pre-intervention SD, sample size, and pre- to postcorrelation for each group. Provided that none of the studies included in the meta-analysis presented pre-to-post correlation, this was estimated with data from our group (7). Where multiple arms involved different types of activity interruptions, we combined the sample size, mean, and SD of both arms (35). Heterogeneity was measured using Higgin's I^2 test and was interpreted by the following thresholds: 0%–40%, might not be important; 30%–60%, may represent moderate

heterogeneity; 50%–90%, may represent substantial heterogeneity; and 75%–100%, considerable heterogeneity. A sensitivity analysis was carried out to identify the presence of highly influential studies by removing one study at a time and then examining its effect on pre-to-post comparisons and between-group comparisons. Studies were considered as influential if removal resulted in a change of the SMC significance or magnitude. Publication bias was evaluated by visual inspection of the Begg's funnel plot when at least eight trials were included in the meta-analysis. Significance was set at $P < 0.05$ (two-tailed). Data are presented as SMC or standardized mean difference and 95% confidence interval (CI).

RESULTS

Systematic Review

Study inclusion. Figure 1 shows the PRISMA flow diagram. The systematic search resulted in the inclusion of 6203 potential articles. Most were removed at abstract screening with 72 articles screened at full text. Most articles were excluded on the basis that they did not comply with standardized FMD protocol ($n = 16$; among them, 5 reported that the cuff was inflated for <5 min, 5 reported that diameter was not continuously monitored, 5 reported that the postdeflation diameter was monitored for <3 min, and 1 did not report the complete FMD protocol) and did not include a control experimental condition ($n = 9$). Thirty-one studies were included in the systematic review.

Characteristics of included studies. The population and design of each study are reported in Supplementary Table 1 (see Table, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). From the 31 studies included in the analyses, vascular function was assessed in a total of 484 participants: healthy, 322 (9,10,17,18,20–26,36–49); overweight or obesity, 121 (7,50–52); metabolic syndrome, 5 (53); and T2D, 36 (42,54). No participants from any of the included studies were described as having impaired fasting glucose or impaired glucose tolerance. Sample sizes were usually <20 (median, 12; range, 5 [53] to 56 [52]), and most participants were male ($n = 263/395$; 67%). One study did not report the sex of participants [52]. Typically, studies recruited young participants <30 yr (median, 26 yr; range, 20.0 [10] to 61.5 [54]), whereas populations with chronic disease risk factors or clinical conditions were likely to be older (median, 47.9; range, 32.2 yr [50] to 61.5 [54]).

Bouts of prolonged sitting ranged from 0.5 h (44,46) to 8.5 h (54), with the median duration of 3.0 h. The majority of study protocols assessed the effect of prolonged sitting on vascular function; however, three studies utilized prolonged laying down (44,47,50) and two studies used undefined sedentary behavior (40,52). Assessments of FMD were mostly performed in the supine position (9,10,18,20–23,26,37,39,41,42,44,46–51), with only eight studies performing (49) FMD in the seated position (7,17,24,25,38,45,53,54) and one study in a semirecumbent position (36). Vascular function was predominantly assessed in the lower limb ($n = 23$; superficial femoral artery, 8 studies
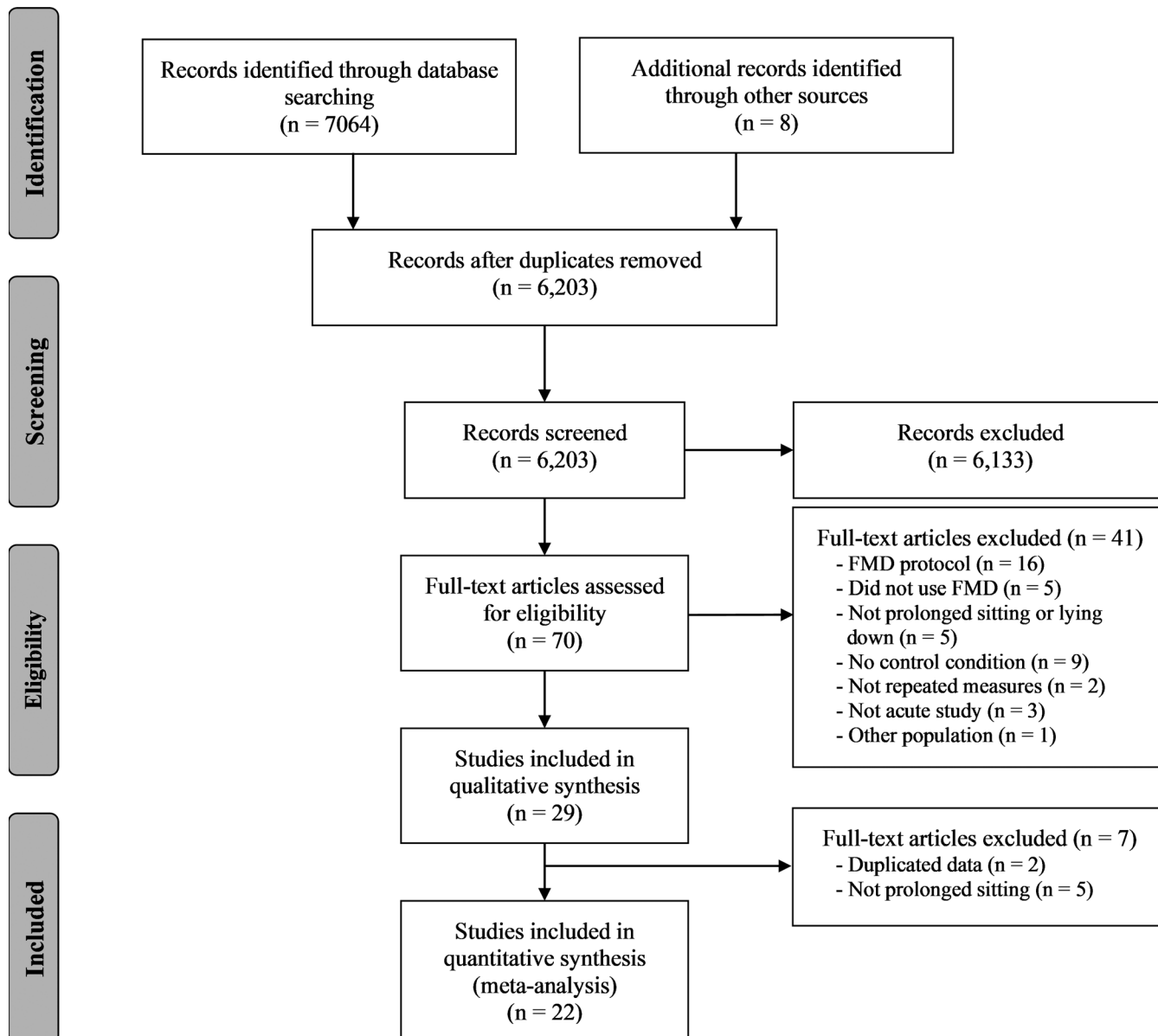
FIGURE 1—Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of the literature search results.

[7,17,18,24,25,38,46,54]; popliteal artery, 14 studies [9,10, 20–23,26,36,43,45,47–49,51]; posterior tibial artery, 1 study [41]), with only 12 trials assessing the brachial artery (7,23,25,37,39,40,42–44,50,52,53). Assessments for FMD were recorded at 30 min (3 studies), 60 min (12 studies), 120 min (10 studies), 180 min (17 studies), and ≥240 min (10 studies).

Seven studies interrupted prolonged sitting via strategies including simple resistance activities (*n* = 2) (7,54), walking (*n* = 3) (17,18,48), stair sprints (49), and calisthenics (*n* = 1) (37). Experimental protocols lasted between 1.4 h (37) and 8.5 h (49). Duration and frequency of interruptions in sitting ranged from 2 min of active interruption every 20 min of sitting (37) to 6 min every 60 min (54). From the seven studies included in the analyses, vascular function was assessed in a total of 108 participants that were considered healthy (*n* = 65)

(17,18,37,48,49), overweight or obese (*n* = 19) (7), or type 2 diabetic (*n* = 21) (54). Sample sizes were <20, except Taylor et al. (2020) (54), and most included participants were male (*n* = 73).

Study quality. The quality score and the risk of bias for each study are reported in Supplementary Figure 1 (see Figure, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Most studies included in the systematic review were randomized, except seven studies that were quasi-experimental (22,23,25,26,36,41,45). All included studies were classified as low risk of bias for selective report. Only five studies provided details regarding allocation concealment (7,21,40,48,54). Most studies, except two studies (21,40), did not report any form of blinding. Only 10 out of the 31 studies reported blinding of the outcome assessor (7,17,21,24,25,40, 45,48,50,54). Most studies were classified as low risk of bias

for incomplete outcome data. Finally, one study reported not controlling participants leg movement (18), and one study reported being underpowered to assess FMD% (37).

Meta-analysis

Study inclusion. Of the 31 studies, 7 studies were excluded from the meta-analysis, as 5 did not impose prolonged sitting (40,44,47,50,52) and 2 reported duplicated data (17,24). Twenty-four studies were included in the meta-analysis to determine the dose–response relationship between acute prolonged uninterrupted sitting and upper- and lower-limb vascular function. Seven studies were included in the meta-analysis to compare uninterrupted prolonged sitting to any acute physical activity intervention that targeted interrupting sitting.

Evaluation of the effects of prolonged sitting on vascular function. Prolonged sitting resulted in a significant decrease in lower-limb FMD at the 120-min (-0.85, 95% CI = -1.32 to -0.38; see Fig. 2; Supplementary Table 2, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407) and 180-min (-1.18, 95% CI = -1.69 to -0.66; Fig. 2; Supplementary Table 2, http://links.lww.com/MSS/C407) time points. Although no significant differences were observed at the 30-, 60-, or ≥240-min time points ($P > 0.31$ for all; Supplementary Table 2, http://links.lww.com/MSS/C407), there was a trend for lower-limb FMD to decrease as time spent sitting increased, from 30 to 180 min (see Supplementary Fig. 2, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Sensitivity analysis indicated that none of the time points unduly influenced the observed outcome, except for the 240-min time point, where removing a trial in healthy adults (48) significantly reduced lower-limb FMD (-0.40, 95% CI = -0.92 to -0.12; Supplementary Table 2, http://links.lww.com/MSS/C407). Lower-limb shear rate was significantly reduced at 30 min (-0.52, 95% CI = -0.87 to -0.16), 180 min (-0.77, 95% CI = -1.01 to -0.54), and 240 min (-0.24, 95% CI = -0.43 to -0.05), but no significant changes were observed at the 60- or 120-min time point (see Supplementary Table 2; Supplementary Fig. 3, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Sensitivity analysis at the 240-min time point indicated that the removal of the least beneficial study (23) failed to elicit changes in shear rate. No significant changes were observed for lower-limb blood flow ($P > 0.49$ for all), except for the 180-min time point (-1.00, 95% CI = -1.61 to -0.39) (Supplementary Table 2, http://links.lww.com/MSS/C407). Sensitivity analysis indicated that none of the studies unduly influenced blood flow at any time points. MAP did not significantly change across any of the time points ($P > 0.14$ for all). Sensitivity analysis indicated that none of studies unduly influenced MAP at any time points. For all upper-limb FMD measurements, no significant changes were observed at any of the time points ($P > 0.10$ for all), with the largest pooled effect observed at the 240-min time point (-0.33, 95% CI = -0.74 to 0.07; Supplementary Fig. 2, http://links.lww.com/MSS/C407).

Subgroup analyses for FMD assessment were only performed on lower-limb FMD results at the ≥180-min time point. Subgroup analysis indicated that prolonged sitting resulted in a significant decrease in lower-limb FMD in healthy adults (-1.16, 95% CI = -1.75 to -0.58), but not in adults with metabolic disturbances (-0.51, 95% CI = -1.18 to 0.15; see Supplementary Fig. 4, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Prolonged sitting resulted in a significant decrease in lower-limb FMD irrespective of the position in which FMD was assessed (seated position, -1.25; 95% CI = -2.23 to -0.26; and supine position, -0.82, 95% CI = -1.27 to -0.37; see Supplementary Fig. 5, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Meta-regression indicated a significant positive association between age and SMC for lower-limb FMD ($\beta = 0.04$, 95% CI = 0.01 to 0.07, $P = 0.02$; see Supplementary Fig. 6, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). However, there was no influence of sex ($\beta = -0.01$, 95% CI = -0.02 to 0.01, $P = 0.39$; see Supplementary Fig. 7, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407).

Evaluation of the effects of active interruptions in sitting on vascular function. Interrupting sitting with activity increased FMD, relative to prolonged uninterrupted sitting across multiple hours (0.13, 95% CI = -0.02 to 0.45;
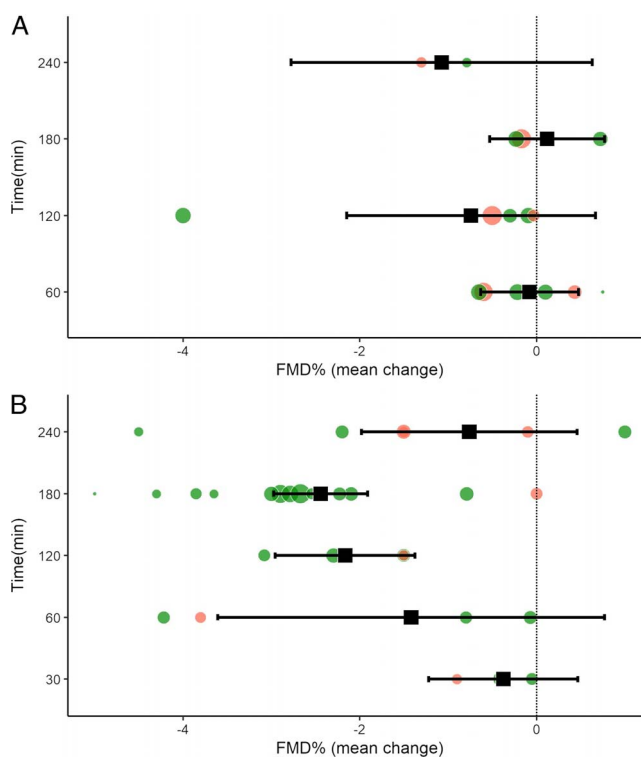
FIGURE 2—Mean time course change for raw FMD% after 30, 60, 120, 180, and ≥240 min of prolonged uninterrupted sitting. A, Changes in FMD% in the upper limb. B, Changes in FMD% in the lower limb. *Black square* and *error bars* are presented as mean change (95% CI). *Dots* represent the mean change for each individual study; *green dots*, healthy adults; *red dots*, adults with metabolic disturbances. Dots sizes are proportional to the weight of each study in the analysis. There was no upper-limb FMD data reported at the 30 min.

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Fig. 3), but the result was nonsignificant. Sensitivity analysis indicated that none of the studies were influential to the analysis. A nonsignificant increase was observed in both lower-limb FMD (0.12, 95% CI = −0.33 to 0.56) and upper-limb FMD (0.13, 95% CI = −0.38 to 0.65). Subgroup analysis for health status reported no change in FMD for healthy (0.00, 95% CI = −0.49 to 0.49) and a nonsignificant increase for metabolic disturbances (0.29, 95% CI = −0.12 to 0.69). Because of the low number of studies interrupting sitting, subgroup analysis for health status contained both upper- and lower-limb FMD results.

Publication bias and heterogeneity. Visual inspection of the funnel plot at ≥240 min revealed no asymmetry (see Supplementary Fig. 9, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407); however, 180 min revealed some asymmetry (see Supplementary Fig. 8, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). However, trim and fill analysis showed that imputing missing studies to reduce asymmetry did not significantly change the effect size. The heterogeneity was moderate to substantial for time points 60 to 240 min, which may be partially explained by varying methodology (arterial sites measured and posture transitions) and population groups (healthy, metabolic syndrome, and T2D). For lower-limb shear rate, there was considerable heterogeneity at the 60- and 120-min mark, which may reflect the small number of studies reporting shear rate (Supplementary Table 2, http://links.lww.com/MSS/C407). For lower-limb blood flow, heterogeneity was considerable for all time points, which again may reflect the small number of studies reporting blood flow. Visual inspection of the funnel plots at the 180 min for shear rate, blood flow, and MAP did not reveal any substantial asymmetry.

DISCUSSION

The aim of this systematic review was to determine the dose–response relationship of prolonged uninterrupted sitting (>30 min) with upper- and lower-limb vascular function across multiple hours during the day. Prolonged uninterrupted sitting for 120 and 180 min significantly decreased lower-limb vascular function but did not affect upper-limb function. Although no statistically significant decrease was observed at the 30 and 60 min, there was a clear trend for lower-limb vascular function to decline as time spent sitting increased. A similar pattern was observed for lower-limb shear rate. Subgroup analysis indicated that prolonged sitting was detrimental to young healthy individuals, with this negative effect being less pronounced in adults with metabolic disturbances and older adults. Finally, interrupting sitting with activity resulted in a small nonsignificant increase in FMD, relative to prolonged sitting, for those with metabolic disturbances.

Time course to vascular impairment. This is the first meta-analysis to assess the time course of impairment in vascular function. A major implication is that these findings suggest that 120 min of continuous prolonged sitting may represent a critical threshold for lower-limb vascular susceptibility. There are several reasons why this decline in lower-limb vascular function may be observed. Notably, a corresponding decrease in shear rate was observed. Shear stress is the frictional force exerted on the arterial wall (55), and it is recognized as the key physiological stimulus in maintaining endothelial health (56). The progressive reduction in lower-limb shear stress from 30 to 180 min may be partly responsible for the corresponding decline in FMD%. It was recently suggested that changing shear patterns may be responsible for the reduced FMD% following prolonged sitting (8). Although our meta-analysis did not address changes in the patterns of shear, the absence of antegrade shear in the presence of increased retrograde shear has been shown to decrease FMD responses (55,57,58). Reduced muscle activity and increased pressure in the back of the thighs are also likely contributors (59). Indeed, lower-limb shear rate decreased at all time points relative to presitting in the same stepwise manner as FMD%.

In contrast to our lower-limb findings, there were no significant changes in upper-limb FMD responses at any time points.

Study	SMD	[95% CI]	Weight
a) Upper			
Carter et al. 2017	0.31	[-0.57; 1.19]	9.7%
Climie et al. 2018	0.04	[-0.59; 0.68]	14.6%
Total (95% CI)	0.13	[-0.38; 0.65]	24.3%
Heterogeneity: Tau^2 = 0; Chi^2 = 0.23, df = 1 (P = 0.63); I^2 = 0%			
b) Lower			
Carter et al. 2019	0.01	[-0.71; 0.72]	12.7%
Climie et al. 2018	0.72	[0.06; 1.38]	14.1%
Taylor et al. 2020	0.13	[-0.49; 0.75]	15.0%
Thosar et al. 2015	0.83	[0.00; 1.66]	10.5%
Caldwell et al. 2020	-0.55	[-1.45; 0.34]	9.5%
Peddie et al. 2021	-0.48	[-1.14; 0.18]	13.9%
Total (95% CI)	0.12	[-0.33; 0.56]	75.7%
Heterogeneity: Tau^2 = 0.17; Chi^2 = 11.42, df = 5 (P = 0.04); I^2 = 56%			
Total (95% CI)	**0.13**	**[-0.20; 0.45]**	**100.0%**
Heterogeneity: Tau^2 = 0.09; Chi^2 = 11.65, df = 7 (P = 0.11); I^2 = 40%			

FIGURE 3—Effects of active interruptions on vascular function (FMD) meta-analysis using a random-effects model grouped by upper- and lower-limb assessment. SMD, standardized mean difference.

Meta-analyses have observed similar findings in healthy populations after 180 min of sitting (8). Maintenance of shear stress by continuation of desk-based activities while sitting has been postulated to contribute to the preservation of brachial artery FMD (8). Indeed, a lack of upper-limb shear stress reduction relative to lower-limb FMD was observed at all time points. Although studies examining the vascular effect of upper-limb inactivity during sitting are needed to clarify this hypothesis, the exercise training literature may provide additional insights. In response to lower-limb cycle training, significant vascular functional changes have been observed in untrained upper limbs, likely due to the relatively large systemic effects of large muscle group exercise on systemic hemodynamics and shear stress (60,61). The lack of change in upper-limb vascular function after prolonged sitting may reflect the relatively modest effects on shear rate associated with sitting. Reduced lower-limb shear may affect brachial artery vascular function over days, weeks, months, and years. Indeed, bed rest studies assessing vascular function over multiple weeks have demonstrated sedentary behavior to be a strong stimulus for rapid structural remodeling of resistance and conduit arteries. Future studies that measure vascular function more frequently, across weeks and months, are needed to provide greater insight into the time course of vascular change and adaptation in response to prolonged sitting in both the upper and the lower limbs.

Subgroup analyses. For time points 60–180 min (Fig. 2), a reduction of 1% or greater was observed in FMD. A 1% chronic reduction in FMD has been associated with a 13% increase in future risk of cardiovascular events (12,62). Although the data in this study are reflective of acute changes, repeated exposures to this is additionally concerning for high-risk and clinical populations, where reduced vascular function can further compound preexisting cardiovascular risk factors such as older age, obesity, and T2D (63). Further, older individuals and those with metabolic disturbances typically spend more time in sedentary behavior relative to young and healthy individuals (15,64–67), further contributing to their cardiovascular disease risk. Despite this, most participants within this meta-analysis were young and healthy. Given that preserving or improving vascular function in these populations are fundamental to reducing atherosclerotic development, understanding the effect of prolonged uninterrupted sitting is imperative to quantify prolonged sitting thresholds and inform public health recommendations.

We observed that prolonged sitting had less apparent effect on vascular function in older adults and those with metabolic disturbances than in young healthy subjects. This may reflect the law of initial values (68). Given that older and diseased populations possess impaired vascular function *a priori*, the magnitude of response to prolonged sitting appears reduced. However, the small sample of older adults and those with metabolic disturbances makes it difficult to draw conclusions as to how prolonged uninterrupted sitting may influence individuals across the full spectrum of age and metabolic function. There also continues to be a primary focus on male participants in this research space. Eleven of 31 studies recruited only male participants (10,17,21–26,38,49,53), and an additional 13 studies recruited majority male participants (7,9,18,20,37,41, 42,44,46–48,51,54). Given many studies have reported excessive sitting time in female populations from young, healthy females (69) to older females experiencing cardiovascular complications (70), it is necessary to increase female participant recruitment in the area of vascular function and sedentary behavior. It is conceivable that sex differences may be present in the effect of prolonged sitting, given the well-described hormonal effects on endothelial function (71). Continued failure to recruit females, older adults, and individuals with preexisting cardiovascular risk factors not only widens the existing gap in the literature but also makes it difficult to provide public health recommendations for a large portion of the population.

Prolonged uninterrupted sitting and vascular assessment. An area of contention in previous studies of prolonged inactivity is the posture adopted during FMD assessment (8). Although many studies assumed the supine position during assessment, eight of the studies measured vascular function while sitting. Subanalysis of seated versus supine assessment in the lower-limb demonstrated that regardless of posture, vascular function significantly decreased when prolonged uninterrupted sitting was 180 min. Although there was considerable heterogeneity in the seated position, there were also fewer studies ($n = 8$) and a greater variability in participant health and age. When designing protocols that utilize FMD for vascular assessment, posture should be taken into consideration. If movement between seated and supine is active, increases in muscular activity may influence FMD results (25,54). Future research is needed to assess the validity and reliability of seated FMD measures.

Interrupting sitting with activity. Despite growing evidence indicating that reducing and interrupting prolonged sitting time positively influences glucose metabolism and shear stress profiles across the metabolic risk spectrum (72,73), limited research exists investigating the effects of regularly interrupting sitting on vascular function (8). Our meta-analysis included seven trials and demonstrated a nonsignificant increased FMD response (0.13, 95% CI = −0.20 to 0.45) when sitting was regularly interrupted with activity compared with prolonged, uninterrupted sitting over a few hours. Responses were similar between upper- and lower-limb vascular function relative to upper limb for interrupting sitting, which may be attributable to protocol design. Protocols assessing lower-limb vascular function were typically longer in duration, and thus participants were exposed to more frequent sedentary bouts. It is possible that a greater level of activity (increased frequency and duration) is needed to correspond to increased time spent in sedentary behavior.

Recent meta-analyses have demonstrated similar improvements in both short- and long-term sedentary behavior interventions (8,74). However, the varying interruption strategies, including mode, duration, and frequency, make it difficult to identify optimum activity interruptions. Nevertheless, this literature indicates that those with metabolic

disturbances benefit from an increase in FMD after activity interruptions.

Limitations. This study was the first meta-analysis to examine the time course between prolonged sitting and vascular function in both upper and lower limbs. Moreover, we provide additional recommendations for future research based on existing gaps in literature, including health status, age, and sex. However, there are limitations to address. It is important to acknowledge there are a small number of studies at the 30- and 60-min time points. Therefore, it is possible that with additional data, vascular impairment may be revealed earlier than 120 min. Given decreased vascular function has been reported as early as 30 min (7), future research should consider measuring vascular function at earlier time points. Second, our search criteria did not include terms surrounding "activity interruptions." However, given that to be eligible for study selection the studies needed to compare to prolonged sitting, we feel that we captured these articles in our search. Finally, as previously addressed, there are several gaps in the research with regard to female participants and older and clinical populations.

CONCLUSIONS

Prolonged uninterrupted sitting progressively impairs lower-limb vascular function up to 180 min. In healthy populations, vascular function may be more susceptible to the effects of prolonged sitting given those with metabolic disturbances report reduced vascular function *a priori*. Regularly interrupting sitting may improve vascular function for with metabolic disturbances; however, more data are needed to clarify. Future studies should also aim to include a wider range of participants, including females, older adults, and adults further along the metabolic spectrum to address to large gap in the literature. The findings of our analysis strongly suggest that prolonged uninterrupted sitting is detrimental for arterial function and health and that recommendations should focus on interrupting sitting with regular activity breaks spaced at most 120 min apart.

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Supplementary Material
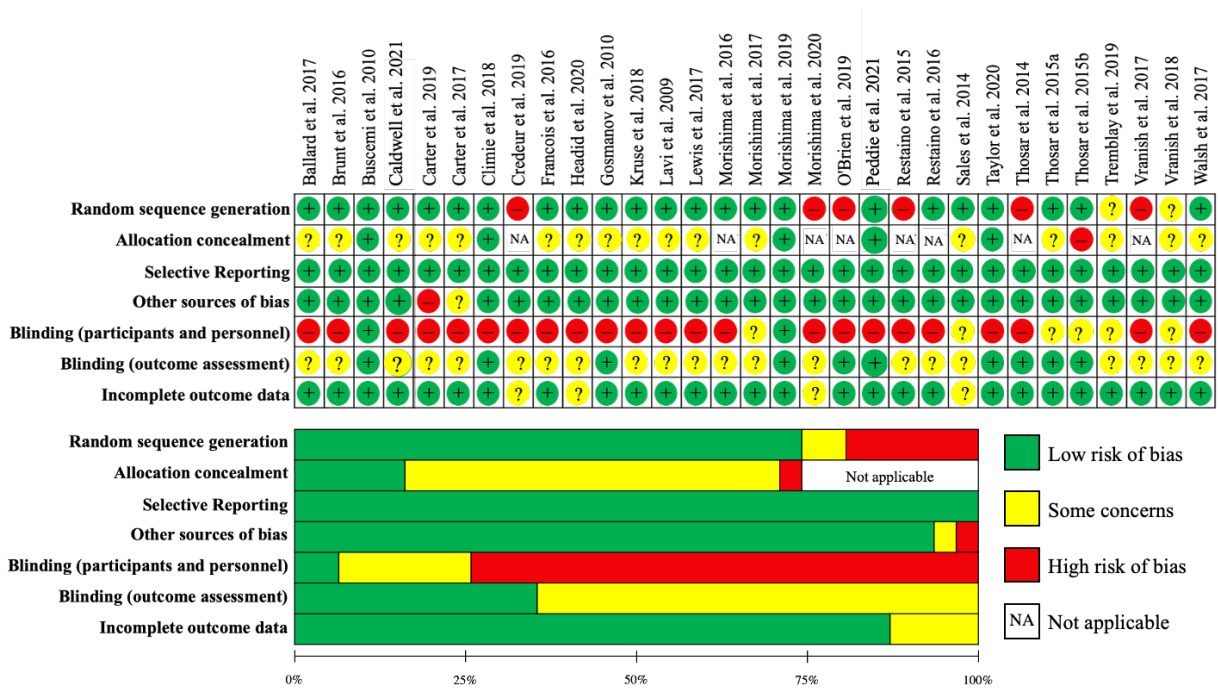
Figure 1. Cochrane Collaboration's risk of bias (RoB) for the included studies. Green, low risk of bias; Red, high risk of bias; Yellow, unclear risk of bias.
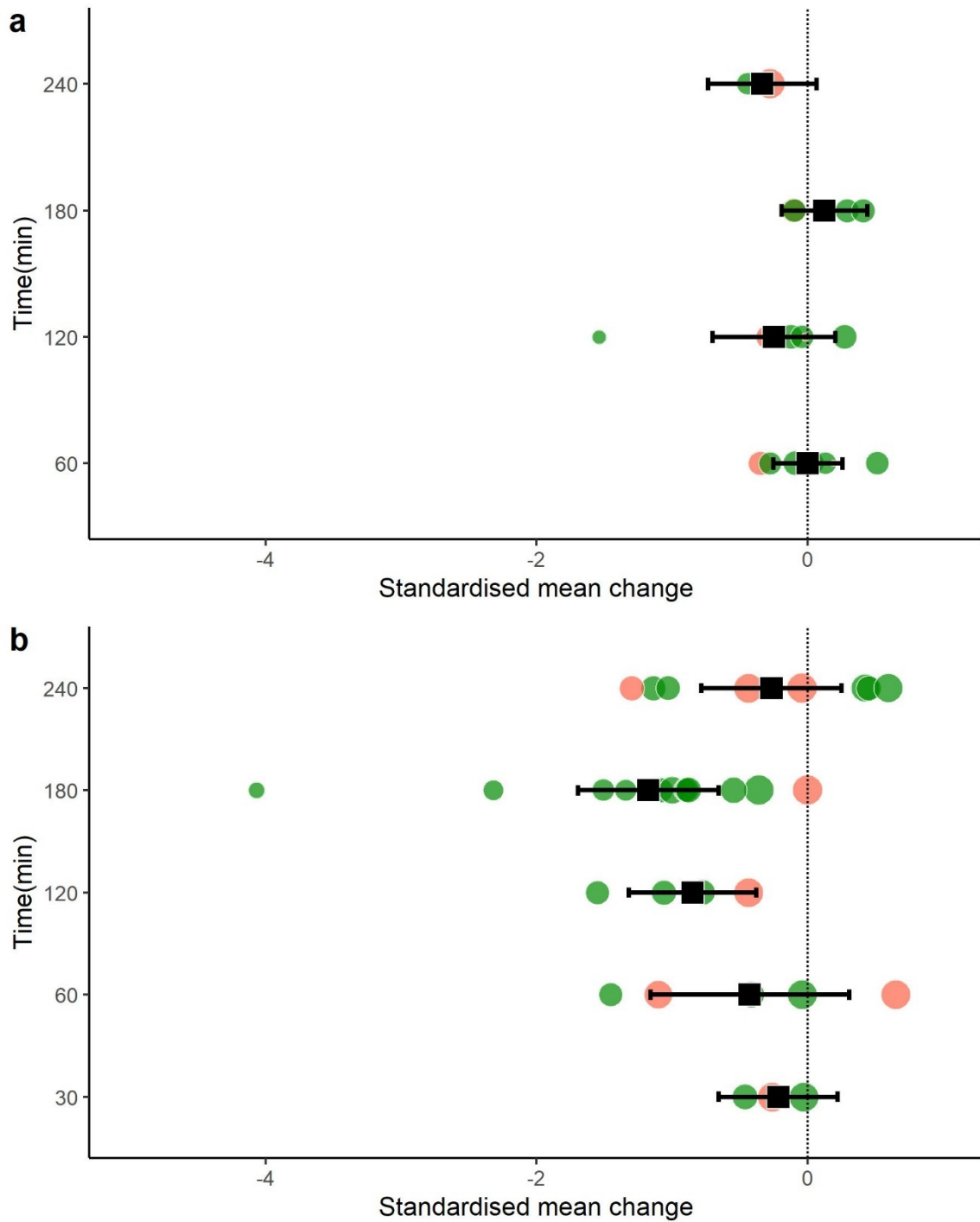
Figure 2. Time-course for standardised mean change in FMD after 30 min, 60 min, 120 min, 180 min, and ≥ 240 min of prolonged uninterrupted sitting. A) changes in SMC in the upper limb, B) depicts changes in SMC in the lower limb. Black square and error bars are presented as standardised mean change (95% CI). Dots represent the standardised mean change for each individual study. Green dots, healthy adults; red dots, adults with metabolic disturbances. Dots sizes are proportional to the weight of each study in the analysis. There was no upper-limb FMD data reported at the 30 min.
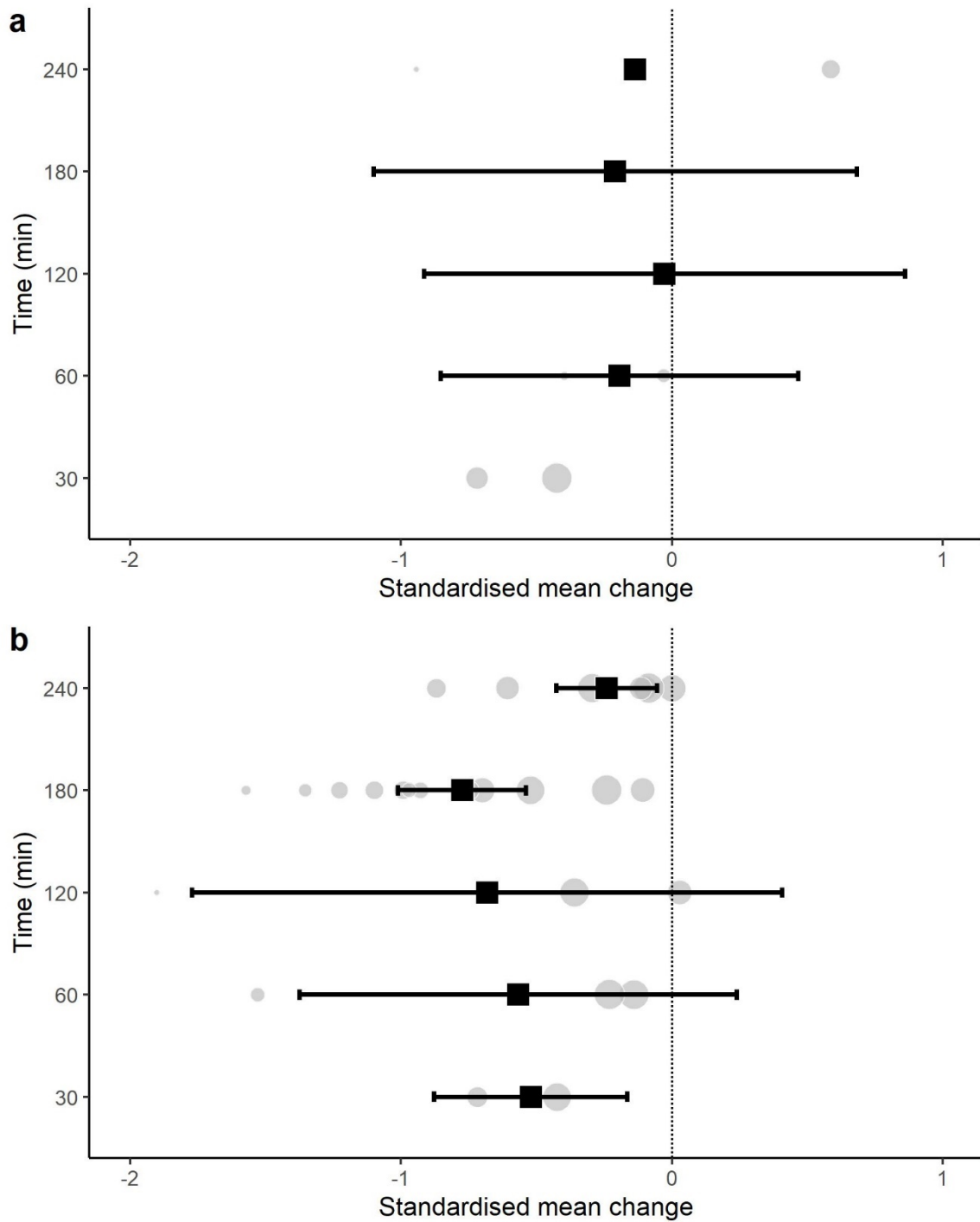
Figure 3. Time-course for standardised mean change in SRAUC after 30 min, 60 min, 120 min, 180 min, and ≥ 240 min of prolonged uninterrupted sitting. A) changes in SMC in the upper limb, B) changes in SMC in the lower limb. Data are presented as standardised mean change (95% CI). Dots sizes are proportional to the weight of each study in the analysis. There was no upper-limb SRAUC data reported at 30 min.
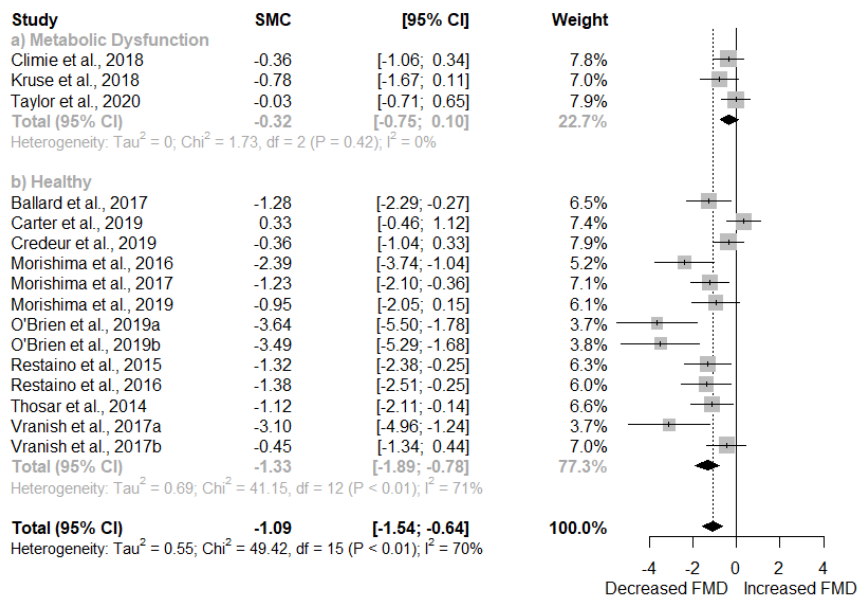
Figure 4. Effect of ≥180 min prolonged sitting on lower-limb vascular function (FMD) meta-analysis using a random-effects model grouped by health status. *SMC* standardised mean change, *CI* confidence intervals.
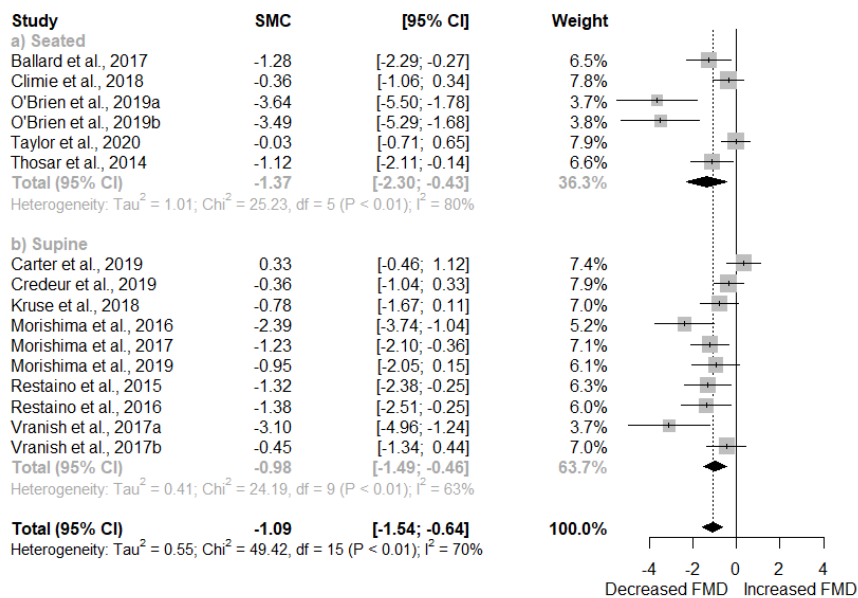
Figure 5. Effect of ≥ 180 min prolonged sitting on lower-limb vascular function (FMD) meta-analysis using a random-effects model grouped by posture during FMD assessment. *SMC* standardised mean change, *CI* confidence intervals.
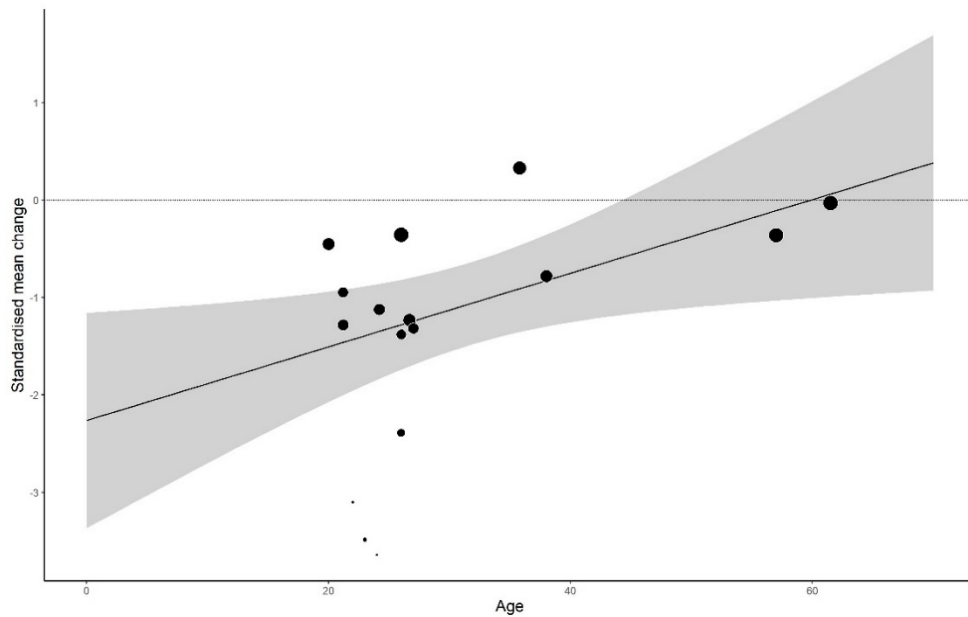
Figure 6. Meta-analytic bubble plot of age of participants against FMD. Dots sizes are proportional to the weight of each study in the analysis.
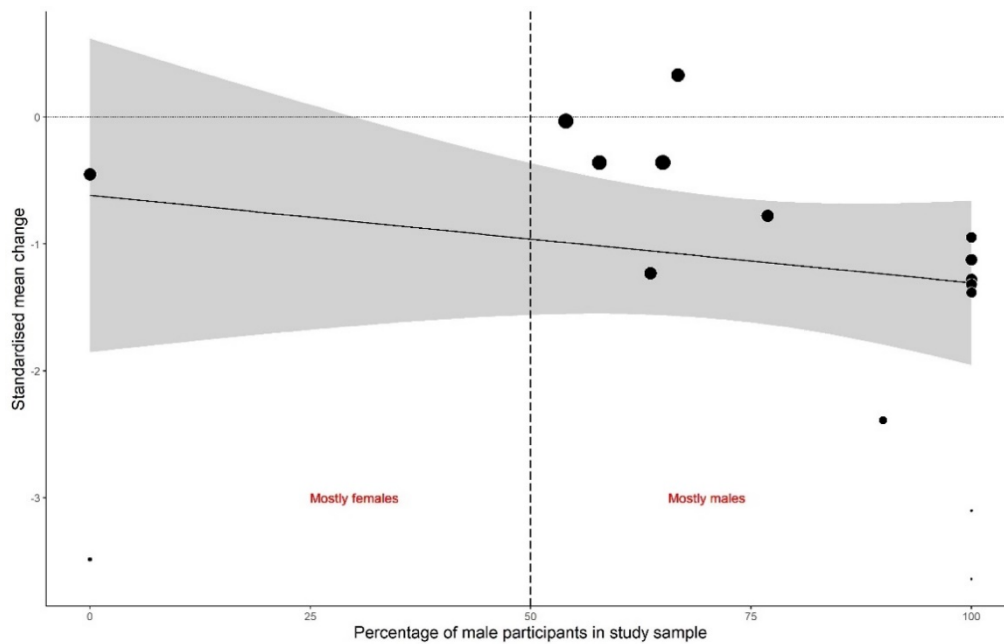
Figure 7. Meta-analytic bubble plot of percentage of male participants against FMD. Dots sizes are proportional to the weight of each study in the analysis. Mostly females, studies with more than 50% female participants; mostly males, studies with more than 50% male participants.
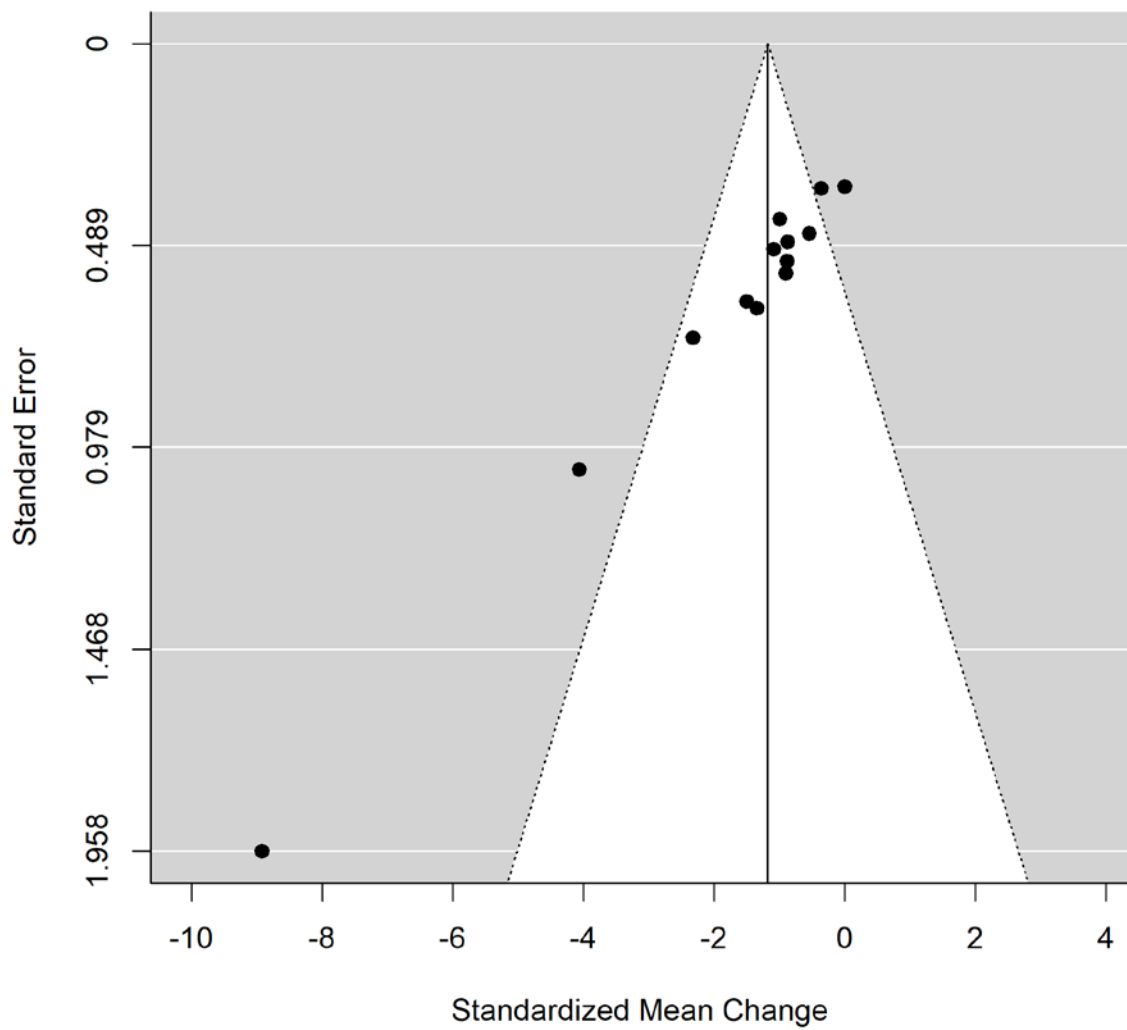
Figure 8. Funnel plot for effect of 180 min prolonged uninterrupted sitting on lower-limb vascular function meta-analysis.
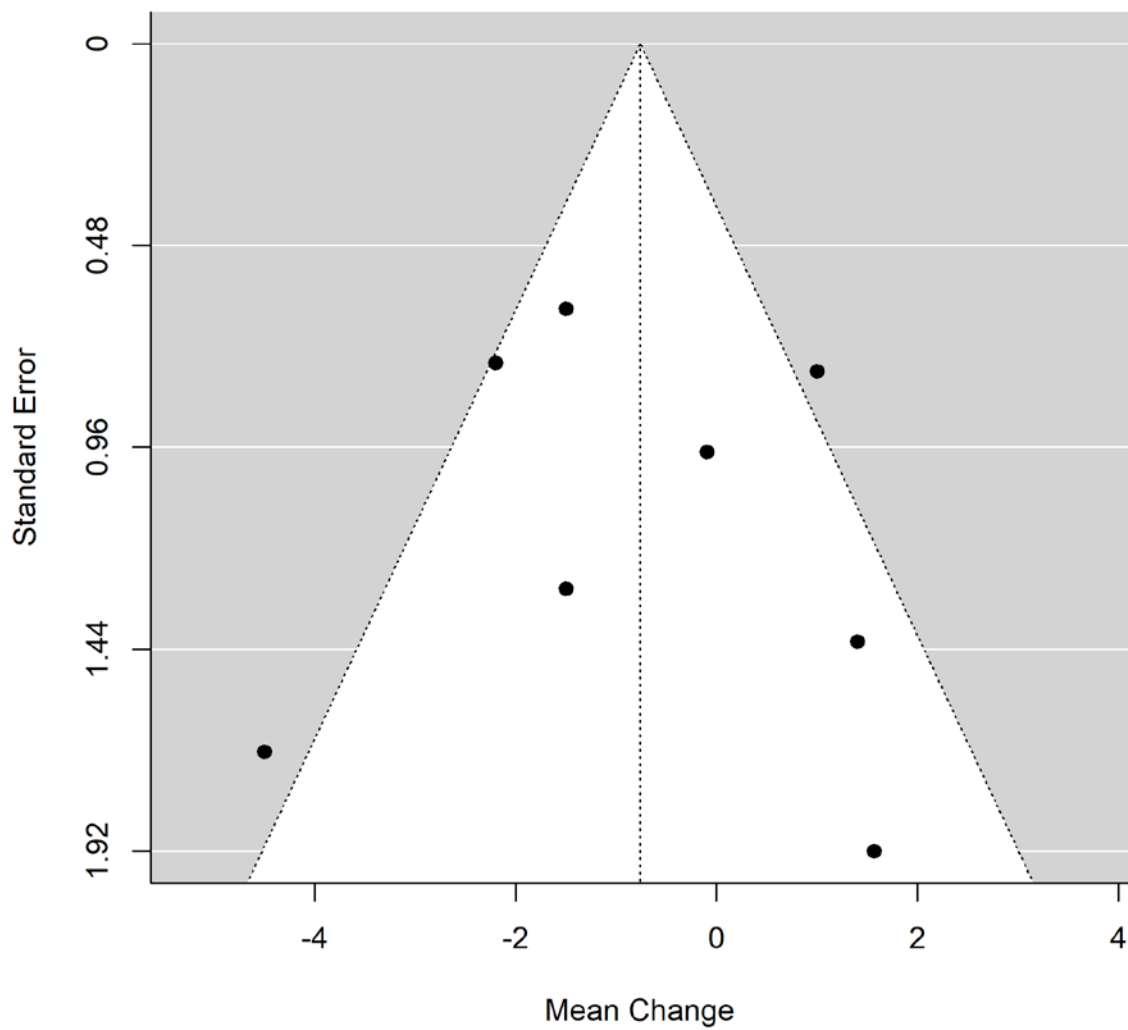
Figure 9. Funnel plot for effect of ≥240 min prolonged uninterrupted sitting on lower-limb vascular function meta-analysis.

Table 1. Characteristics of the included trials.

Author	Population	Study design	Protocol	Time – points (minutes)	Outcomes
Ballard et al. 2017 [38]	12 M; age: 21.2 ± 1.9 yr; BMI: 24.6 ± 1.1 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 135 + 180 min Meal: 58/14/6 g, 382 kcal	-135, 0, 60, 120, and 180	FMD - seated femoral artery, base diameter, SRAUC, and MAP
Brunt 2016 [39]	5 M 5 F; age: 23 ± 6 yr BMI: 22.8 ± 1.7 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 100 min Meal: none	0 and 100	FMD - supine brachial artery, base diameter, and SRAUC
Buscemi et al. 2010 [40][*]	10 M 10 F; age: 31 ± 2 yr BMI: 23.9 ± 0.7 kg/m² healthy	RCT double-blinded	Prolonged SB (placebo condition) Duration: 60 min Meal: decaffeinated coffee	0, 30, and 60	FMD - brachial artery

Caldwell et al. 2021 [49]	10 M; age: age: 24 ± 4 yr BMI: 24 ± 2 kg/m² healthy	RCT	Prolonged sitting Duration: 540 min	-30 and 510	FMD – femoral artery and SRAUC
Carter et al. 2017 [37]	6 M 4 W; age: 27.3 ± 8.3 yr healthy, active	RCT	Prolonged sitting Duration: 86 min Meal: none	0 and 86	FMD - supine brachial artery and MAP
Carter et al. 2019 [18]	10 M 5 F; age: 35.8 ± 10.2 yr BMI: 25.5 ± 3.2 kg/m² healthy, active	RCT	Prolonged sitting Duration: 240 min Meal: 61/8/5 g, 320 kcal	0 and 240	FMD - supine femoral artery, base diameter, blood flow, SRAUC, and MAP
Climie et al. 2018 [7]	11 M 8 F; age: 57 ± 12 BMI: 30.6 ± 3.4 overweight and obese, sedentary and inactive	RCT	Prolonged sitting Duration: 300 min Meal: 53-55%/30-33%/12-15%	Brachial: 0, 30, 60, 120, and 300 Femoral: 0 and 300	FMD - seated femoral and brachial artery, base diameter, blood flow, and SRAUC

Credeur et al. 2019 [41]	13 M 7 F; age: 26 ± 7 yr BMI: 30 ± 7 kg/m² healthy, overweight, and obese 6 M 6 F; age: 57.5 ± 5.0 yr BMI: 35 ± 7 kg/m² type 2 diabetes	Quasi-experimental	Prolonged sitting Duration: 180 min Meal: none	0 and 80	FMD - supine posterior tibial artery, base diameter, SRAUC, and MAP
Francois et al. 2016 [42]	6 M 6 F; age: 55.3 ± 9.1 yr BMI: 26 ± 5 kg/m² healthy, inactive 7 M 4 F; age: 55.1 ± 7.0 yr BMI: 23 ± 3 healthy, active	RCT	Prolonged sitting Duration 140 min Meal: none	0, 60, 120 and 180	FMD - supine brachial artery, base diameter, blood flow, and MAP
Gosmanov et al. 2010 [51]	4 M 9 F; age: 32.2 ± 9.8 yr	RCT	Prolonged lying down (placebo condition)	0, 240 and 480	FMD - supine brachial artery

			BMI: 36.7 ± 5.1 kg/m²	Duration: 480 min		
			obese	Meal: none		
	6 M 6 F; age: 22.3 ± 2.0 yr			Prolonged sitting		
Headid et al 2020 [43]	BMI: 23.9 ± 3.0 kg/m²	RCT		Duration: 150 min	0 and 150	FMD - brachial and popliteal artery and MAP
	healthy, recreationally active			Meal: none		
	10 M 3 F; age: 38 ± 3 yr			Prolonged sitting		FMD - supine popliteal artery, base diameter, blood flow, and SRAUC
Kruse et al. 2018 [52]	BMI: 29.7 ± 2.0 kg/m²	RCT		Duration: 240 min	0 and 240	
	overweight and obese, inactive			Meal: 46/9/16 g, 310 kcal		
	56 participants; age: 47.9 ± 5.8 yr			Prolonged SB (placebo condition)		
Lavi et al. 2009 [53]	BMI: 32.1 ± 4.3 kg/m²	RCT		Duration: 120 min	0 and 120	FMD - brachial artery
	overweight and obese			Meal: none		

Lewis et al. 2017 [44]	9 M 1 F; age: 27 ± 2 yr BMI: 23 ± 2 kg/m² healthy	RCT	Prolonged lying down Duration: 30 min Meal: none	0 and 30	FMD - supine brachial artery, base diameter, blood flow, and SRAUC
Morishima et al. 2016 [9]	7 M 4 F; age: 26 ± 1 yr BMI: 25.0 ± 1.1 kg/m² healthy, recreationally active	Unilateral model	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP
Morishima et al. 2017 [20]*	10 M 6 F; age: 26.7 ± 0.5 yr BMI: 25.6 ± 0.5 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 45 + 180 min Meal: none	-45 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP
Morishima et al. 2019 [21]*	9 M; age, 21.2 ± 2.0 yr BMI, 22.0 ± 3.0 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP

Morishima et al. 2020 [22]	9 M; age, 21.1 ± 1.8 yr BMI 24.8 ± 1.5 kg/m² healthy, recreationally active	Quasi-experimental	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP
O'Brien et al. 2019 [45]	10 M; age, 24 ± 2 yr BMI, 26.6 ± 2.0 kg/m² 10 F; age, 23 ± 2 y BMI, 24.2 ± 3.2 kg/m² healthy, active	Quasi-experimental	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - seated popliteal artery, base diameter, blood flow, SRAUC, and MAP
Peddie et al. 2021 [48]	11 M 7 F; age, 23.5 ± 5 yr BMI: 23.7 ± 2.6 kg/m² healthy, sedentary (≥ 5 h/day of sitting)	RCT	Prolonged sitting Duration: 360 min Meal: 62%/28%/10%	-30 and 360 min	FMD – supine popliteal artery, base diameter, and blood flow
Restaino et al. 2015 [23]	11 M; age, 27 ± 1 yr BMI: 25 ± 0.4 kg/m²	Quasi-experimental	Prolonged sitting Duration: 180 min	0 and 180	FMD - supine brachial and popliteal artery, base

	healthy, recreationally active		Meal: none		diameter, blood flow, and SRAUC
Restaino et al. 2016 [10]*	10 M; age, 26 ± 1 yr BMI, 26.8 ± 1.3 kg/m² healthy, recreationally active	Unilateral model	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP
Sales et al. 2014 [54]*	5 M; age, 39 ± 3 yr BMI, 31.4 ± 0.8 kg/m² MetS, sedentary	RCT	Prolonged sitting Duration 60 min + 60 min Meal: none	-60, 30 and 60	FMD - seated brachial artery and base diameter
Taylor et al. 2020 [55]	13 M 11 F; age: 61.5 ± 7.8 yr; BMI: 32.6 ± 3.5 kg/m² T2D with overweight or obesity, sedentary	RCT	Prolonged sitting Duration: 420 min + 60 min; Meal: 53-55%/30-33%/12-15% breakfast and lunch	0, 60, 210, 270 and 420	FMD - seated femoral artery, base diameter, blood flow, and SRAUC
Thosar et al. 2014 [25]	12 M; age, 24.2 ± 4 yr BMI, 23.7 ± 3.3 kg/m²	Quasi-experimental	Prolonged sitting Duration: 180 min	0, 60, 120 and 180	FMD - seated femoral and brachial artery, base diameter, and SRAUC

	healthy, inactive		Meal: none		
Thosar et al. 2015a [17]	12 M; age, 24.2 ± 4 yr BMI, 23.7 ± 3.4 kg/m²	RCT	Prolonged sitting Duration: 180 min Meal: none	0, 60, 120 and 180	FMD - seated femoral artery
Thosar et al. 2015b [24]	healthy, inactive 11 M; age, 24.2 ± 4.4 yr BMI: 23.6 ± 3.4 kg/m²	RCT	Prolonged sitting Duration: 180 min Meal: none	0, 60, 120 and 180	FMD - seated femoral artery
Tremblay et al. 2019 [46]	healthy, inactive 9 M 2 F; age: 23 ± 2 yr BMI: 24 ± 3 kg/m² healthy	RCT	Prolonged sitting Duration: 30 min Meal: none	0 and 30	FMD - supine femoral artery, base diameter, SRAUC, and MAP
Vranish et al. 2017 [36]*	8 M; age: 22 ± 1 yr BMI: 25.7 ± 0.9 kg/m² 12 W; age: 20 ± 0 yr BMI: 24.0 ± 0.8 kg/m²*	Quasi-experimental	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - semi-recumbent popliteal artery, base diameter, blood flow, SRAUC, and MAP

	healthy, recreationally active				
Vranish et al. 2018 [26]	18 M; age, 24.2 ± 4.4 yr BMI: 23.6 ± 3.4 kg/m² healthy, inactive	Quasi-experimental	Prolonged sitting Duration: 60 min Meal: none	0, 30, and 60	FMD - supine popliteal artery, base diameter, and blood flow
Walsh et al. 2017 [47]	8 M 4 W; age, 26.1 ± 1.1 yr BMI: 24.6 ± 0.4 kg/m² healthy, recreationally active	Unilateral model	Prolonged lying down Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, and SRAUC

Data presented as mean ± SD unless stated otherwise: [*] data presented as mean ± SE. Meal: carbohydrate/fat/protein. Abbreviations: BMI, body mass index; FMD, flow mediated dilation; MAP, mean arterial pressure; MetS, metabolic syndrome; RCT, randomized crossover trial; SB, sedentary behaviour; SRAUC, shear rate area under the curve; T2D, type 2 diabetes.

Table 2. Pooled acute effects of prolonged sitting on primary and secondary lower-limb vascular outcomes and leave-one-out sensitivity analysis.

Outcome	Main findings					Leave-one-out sensitivity analysis	
						Most benefit	Least benefit
	number of studies	number of participants	Pooled effect (95% CI)	P value	I^2, p value	Pooled effect (95% CI)	Pooled effect (95% CI)
FMD	18	258					
30 min	3	48	-0.22 (-0.66 to 0.22)	0.34	0%, p=0.34	-0.33 (-0.89 to 0.23)[a]	-0.19 (-0.75 to 0.38)[b]
60 min	5	81	-0.43 (-1.16 to 0.31)	0.25	74.5%, p<0.01	-0.70 (-1.33 to -0.07)[b]	-0.44 (-1.38 to 0.58)[a]
120 min	4	55	-0.85 (-1.32 to -0.38)	<0.01	9.9%, p=0.36	-1.09 (-1.65 to -0.52)[c]	-0.92 (-1.58 to -0.26)[a]
180 min	11	159	-1.18 (-1.69 to -0.66)	<0.01	67.8%, p<0.01	-1.26 (-1.89 to -0.63)[a]	-0.93 (-1.32 to -0.55)[d]
+240 min	8	118	-0.27 (-0.78 to 0.25)	0.31	66.90%, p=0.01	-0.40 (-0.92 to -0.12)[e]	-0.13 (-0.62 to 0.0.37)[c]
Blood flow	13	195					

30 min	2	37	-0.49 (-1.01 to 2.0)	0.49	89.3%, p=0.01	NA	NA
60 min	4	64	-0.10 (-0.94 to 0.74)	0.82	82.2%, p=0.01	-0.12 (-1.36 to 1.10)[f]	0.24 (-0.36 to 0.84)[g]
120 min	2	30	-0.36 (-2.41 to 1.70)	0.73	91.4%, p<0.01	NA	NA
180 min	8	115	-1.00 (-1.61 to -0.39)	<0.01	76.0%, p<0.01	-1.00 (-1.70 to -0.31)[h]	-0.76 (-1.24 to –0.28)[d]
+240 min	6	96	-0.05 (-0.92 to 0.83)	0.92	87.6%, p<0.01	-0.31 (-1.17 to 0.0.55)[k]	0.25 (-0.53 to 1.03)[i]
SRAUC	16	223					
30 min	2	30	-0.52 (-0.87 to -0.16)	0.01	0%, p=0.45	NA	NA
60 min	3	47	-0.57 (-1.37 to 0.24)	0.17	86.7%, p=0.01	-0.79 (-2.15 to 0.57)[i]	-0.19 (-0.48 to 0.11)[b]
120 min	3	43	-0.68 (-1.77 to 0.41)	0.14	90.4%, p<0.01	-0.90 (-2.79 to 0.99)[c]	-0.19 (-0.57 to 0.18)[b]
180 min	11	159	-0.77 (-1.01 to -0.54)	<0.01	45.3%, p<0.01	-0.83 (-1.06 to -0.60)[i]	-0.78 (-1.04 to -0.53)[j]
+240 min	7	100	-0.24 (-0.43 to -0.05)	0.01	0%, p=0.28	-0.29 (-0.52 to -0.09)[i]	-0.18 (-0.38 to 0.01)[k]
MAP	10	136					

30 min	5	66	0.13 (-0.11 to 0.37)	0.30	0%, p=0.88	0.18 (-0.08 to 0.44)[l]	0.09 (-0.17 to 0.36)[m]
60 min	5	65	0.12 (-0.12 to 0.36)	0.29	0%, p=0.49	0.19 (-0.07 to 0.46)[n]	0.05 (-0.24 to 0.34)[o]
120 min	6	79	-0.01 (-0.21 to 0.23)	0.90	0%, p=0.50	0.08 (-0.15 to 0.32)[a]	-0.02 (-0.26 to 0.22)[p]
180 min	9	127	0.15 (-0.05 to 0.35)	0.14	21.3%, p=0.26	0.19 (-0.02 to 0.39)[q]	0.08 (-0.11 to 0.27)[d]
+240 min	2	27	-0.05 (-0.42 to 0.33)	0.80	0%, p=0.40	NA	NA

Data are presented as standardised mean change (95% CI). Abbreviations: FMD, flow-mediated dilation; MAP, mean arterial pressure; SRAUC, shear rate area under the curve. [a] omitted Ballard et al. (2017) [38]; [b] omitted Thosar et al. (2014) [25]; [c] omitted Climie et al. (2018) [7]; [d] omitted O'Brien et al. (2019 – women) [45]; [e] omitted Peddie al. (2021) [48]; [f] omitted Vranish et al. (2018) [26]]; [g] omitted Morishima et al. (2016) [9]; [h] omitted Vranish et al. (2017) [36]; [i] omitted Taylor et al. (2020) [55]; [j] omitted Restaino et al. (2016) [10]; [k] omitted Restaino et al. (2015) [23]; [l] omitted Francois et al. (2016 – healthy, active) [42]; [m] omitted Francois et al. (2017 – type 2 diabetes) [42]; [n] omitted Carter et al. (2017) [37]; [o] omitted Credeur et al. (2019) [41]; [p] omitted Francois et al. (2016 – healthy, inactive) [42]; [q] omitted Morishima et al. (2017) [20].

4.3 Chapter 4 Summary

The findings presented in Section 4.2 are the first to suggest that two hours of continuous prolonged sitting may represent a critical threshold for susceptibility to lower-limb vascular dysfunction. While in isolation, short periods of lower-limb vascular impairment may not be particularly concerning, it is necessary to recognise the high volumes and repetitive nature of sedentary behaviour [31]. Indeed, rapidly advancing technologies in workplaces, transportation and home entertainment provide fewer opportunities for incidental activity, creating many settings that are conducive to bouts of prolonged sitting [64, 88].

Section 4.2 also demonstrated that in healthy populations vascular function may be more susceptible to the impacts of prolonged sitting, given those with metabolic disturbances report reduced vascular function *a priori*. Additionally, for all timepoints ≥ 60 min of prolonged sitting, a reduction of 1% or greater was observed in FMD. Given a 1% chronic reduction in FMD has been associated with a 13% increase in future risk of cardiovascular events [14, 149], it is plausible to suggest that repeated acute exposures of this magnitude may be detrimental to an individual's vascular health in the long term. This may be particularly relevant for high-risk and clinical populations, where reduced vascular function can further compound pre-existing cardiovascular risk factors such as older age, obesity, and T2D [150].

In light of these findings, it could be postulated that reducing and interrupting prolonged sitting in both healthy populations and those with metabolic disturbances may be beneficial to vascular health. However, limited research exists in regard to investigating the effect of regularly interrupting sitting in either healthy adults or those with metabolic disturbances. The meta-analysis in Section 4.2 demonstrated that interrupting sitting with activity significantly increased vascular function, compared to prolonged, uninterrupted sitting over a few hours. However, due to the limited number of studies to date, further research investigating differences between population groups, mode, duration, and frequency are required.

CHAPTER 5 - ACUTE EFFECTS OF INTERRUPTING PROLONGED SITTING ON VASCULAR FUNCTION IN TYPE 2 DIABETES

Publication statement:

This chapter is comprised of the following paper published in the *American Journal of Physiology – Heart and Circulatory Physiology:*

5.1 Chapter 5 Introduction

As outlined in Chapter 4, the World Health Organization has recently highlighted the need to quantify thresholds of prolonged sitting at which risk is more pronounced, and to examine the potential optimal frequency and duration of interrupting sitting with activity [93]. Such information is particularly relevant for clinical populations, who typically present with impaired vascular function contributing to increased cardiovascular risk [15]. However, many studies currently focus on the effect of prolonged sitting and interrupting prolonged sitting in young and healthy populations, rather than older populations with risk factors such as abnormal glucose metabolism. Given that those with abnormal glucose metabolism spend a greater proportion of time throughout the day in sedentary behaviour [25, 26], a better understanding of the effects of prolonged sitting on vascular function in clinical populations is germane. Additionally, determining the frequency and duration of interrupting sitting that provides positive vascular benefits in those with metabolic dysfunction may inform future public health recommendations. Chapter 5 therefore compared the effects of interrupting sitting using different break frequencies and intensities, with equivalent activity duration, on vascular function in adults with T2D.

RESEARCH ARTICLE

Vascular Biology and Microcirculation

Acute effects of interrupting prolonged sitting on vascular function in type 2 diabetes

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Abstract

In healthy and overweight/obese adults, interrupting prolonged sitting with activity bouts mitigates impairment in vascular function. However, it is unknown whether these benefits extend to those with type 2 diabetes (T2D), nor whether an optimal frequency of activity interruptions exist. We examined the acute effects on vascular function in T2D of interrupting prolonged sitting with simple resistance activities (SRA) at different frequencies. In a randomized crossover trial, 24 adults with T2D (35−70 yr) completed three 7-h conditions: *1*) uninterrupted sitting (SIT), *2*) sitting with 3-min bouts of SRA every 30 min (SRA3), and *3*) sitting with 6 min bouts of SRA every 60 min (SRA6). Femoral artery flow-mediated dilation (FMD), resting shear rate, blood flow, and endothelin-1 were measured at 0, 1, 3.5, 4.5, and 6.5–7 h. Mean femoral artery FMD over 7 h was significantly higher in SRA3 (4.1±0.3%) compared with SIT (3.7±0.3%, $P = 0.04$) but not in SRA6. Mean resting femoral shear rate over 7 h was increased significantly for SRA3 (45.3±4.1/s, $P < 0.001$) and SRA6 (46.2±4.1/s, $P < 0.001$) relative to SIT (33.1±4.1/s). Endothelin-1 concentrations were not statistically different between conditions. Interrupting sitting with activity breaks every 30 min, but not 60 min, significantly increased mean femoral artery FMD over 7 h, relative to SIT. Our findings suggest that more frequent and shorter breaks may be more beneficial than longer, less frequent breaks for vascular health in those with T2D.

NEW & NOTEWORTHY This is the first trial to examine both the effects of interrupting prolonged sitting on vascular function in type 2 diabetes and the effects of the frequency and duration of interruptions. Brief, simple resistance activity bouts every 30 min, but not every 60 min, increased mean femoral artery flow-mediated dilation over 7 h, relative to uninterrupted sitting. With further supporting evidence, these initial findings can have important implications for cardiovascular health in type 2 diabetes.

arteries; blood flow; sedentary behavior

INTRODUCTION

Those who are living with type 2 diabetes (T2D) are disproportionately affected by cardiovascular disease (CVD), with twofold increased risk of CVD mortality compared with those without T2D (1, 2). This is largely attributable to

atherosclerotic complications, including increased risk of myocardial infarction, stroke, and microvascular diseases (3). The importance of the endothelium in maintaining healthy vascular function is now well accepted (4, 5). Vascular impairment is recognized as an important early event in the progression of CVD, preceding obesity and

diabetes (6, 7). Measurement of vascular function, via endothelium-dependent vasodilation, is a widely used prognostic marker for the progression of CVD risk (8). In T2D, vascular reactivity is usually, but not invariably, reduced (9). Consequently, interventions that improve endothelial vasodilator function can provide antiatherogenic benefit and may be considered an integral tool of diabetic management (10–12).

Lifestyle modification, including increasing physical activity (PA), is considered a cornerstone for the prevention and management of T2D. Despite the known cardiometabolic benefits of PA (13), meeting recommended levels (at least 150 min of moderate to vigorous activity weekly) continues to be challenging in T2D, with numerous barriers to exercise reported (14, 15). Furthermore, in the social and economic context of rapidly advancing technologies in workplaces, transportation, and home entertainment, fewer opportunities exist for incidental activity, creating many contexts of daily life that are conducive to prolonged sitting. Sedentary behaviors, defined as seated posture with low energy expenditure ≤ 1.5 METS (metabolic equivalent of task), are now recognized as being strongly associated with all-cause and CVD-related mortality (16, 17). In particular, the deleterious consequences of prolonged periods of time spent sitting have been highlighted, with acute experimental studies reporting that prolonged uninterrupted sitting exacerbates postprandial cardiometabolic risk biomarkers (18) and may decrease vasodilatory function (19–21) via reduced bioavailability of vasodilators (i.e., nitric oxide) and increased production of vasoconstrictors [i.e., endothelin-1 (ET-1)] (20). Elevated ET-1 may be a marker of microvascular complications in those with T2D (22), and evidence suggests that endothelin receptor blockade reduces blood pressure and protects against renal events in patients with T2D (23, 24). Given that those with T2D report high basal ET-1 levels, are at increased risk of microvascular and macrovascular complications (1, 2), and report high levels of time spent in sedentary and low levels of participation in PA (15, 25), further research is needed to find practical strategies that may contribute to reducing CVD risk.

Recent experimental evidence shows that reducing and interrupting prolonged sitting time with brief bouts of light-intensity activity can negate the adverse effects of prolonged sitting on lower limb vascular function in healthy and overweight/obese adults (19, 26, 27). However, it is unknown whether interrupting sitting time can positively influence vascular function in those with T2D. Furthermore, although several studies (28–31) have reported the effects of interrupting sitting using different break frequencies and intensities, none have directly compared two different activity protocols with equivalent activity duration in those with T2D. This type of evidence is required to inform larger trials and to produce more specific public health guidelines around the optimal timing and duration of interruptions in sitting.

We examined the effects of interrupting prolonged sitting for 1) 3 min every 30 min and 2) 6 min every 60 min with simple resistance activities (SRA), on vascular function in those with T2D. As an exploratory outcome, we also examined the effect of interrupting prolonged sitting on plasma ET-1 levels, as a marker of vasoconstriction, in the same population. We hypothesized that regular interruptions involving SRA would acutely improve vascular function relative to uninterrupted sitting and that breaking up sitting either every 30 or 60 min (with equivalent total activity duration) would be efficacious in improving vascular function compared with prolonged sitting per se. Moreover, we anticipated that regularly interrupting sitting every 30 or 60 min compared with prolonged sitting would decrease plasma ET-1.

METHODS

Participants

Twenty-four men and women (BMI, 25–40 kg/m^2) aged 35–70 yr with T2D (1–3 hypoglycemic medications, ≥ 3 mo duration [based on the American Diabetes Association diagnostic criteria (32)] were recruited from local community advertisements, social media, and the Baker Heart and Diabetes Institute (ACTRN12617000392369). To be eligible, participants were required to be inactive (currently sitting for ≥ 5 h/day and not meeting PA guidelines of ≥ 150 min/wk of moderate-intensity exercise or high-intensity exercise ≥ 75 min/wk for >3 mo). Exclusion criteria included HbA$_{1c}$ $<6.5\%$ or $>10\%$; current use, or use within the previous 3 mo of insulin medication/s, current smoker, pregnancy, or major acute or chronic illness that might limit their ability to perform SRA. Based on previously published work, a sample size of 24 individuals (allowing for 15% attrition) would provide $>90\%$ power, assuming two-tailed $\alpha = 0.05$ (G*Power v.3.1.2) and a standard deviation of 1% between individuals, to detect a change in FMD of 1% between the intervention (interrupting sitting with activity breaks) and control (prolonged sitting) conditions (33). For longitudinal observational studies across heterogeneous populations, a 1% difference in FMD is associated with a clinically meaningful ~ 7–13% difference in cardiovascular events (6, 34).

Study Overview and Randomization

This three-arm randomized crossover trial took place at the Baker Heart and Diabetes Institute between July 2017 and April 2019. The study was approved by the Alfred Human Research Ethics Committee (50-17). Volunteers were initially screened via telephone questionnaire to determine their eligibility, during which they were asked to verbally confirm medical diagnosis of T2D for ≥ 3 mo, physical activity time of <150 min/wk, and sitting time of >5 h/day. Eligible participants provided written informed consent and attended the laboratory on four separate occasions: medical screening/familiarization, and three trial condition visits in a randomized order: 1) prolonged, uninterrupted sitting (SIT), 2) 3-min simple resistance activities every 30 min (SRA3), and 3) 6-min simple resistance activities every 60 min (SRA6).

The medical screening occurred 3–6 days before the first visit and included HbA$_{1c}$ and anthropometric measurements, resting blood pressure, resting 12-lead electrocardiogram, and a physical examination performed by the study physician (N.D.C.). Participants also provided information about medical history and current medications and were familiarized with the SRA. Additionally, they were familiarized with the study procedure, including weighed food

diaries and activity records, activity monitors, cuff occlusion, and requirements for the restrictive lead-in phase and fasting before each trial condition.

Experimental condition was randomly assigned by an independent third-party using computer generated random numbers and sealed in envelopes (balanced block randomization). Participants were blinded to the condition order until the start of the second visit.

Study Protocol

Experimental conditions.

Figure 1 shows the overall experimental protocol. A 6-day washout period between conditions was used to address any potential carryover effects. Each participant completed the 8-h experimental conditions, including an initial 1-h steady-state period. To mimic a free-living setting, habitual, unstandardized upper and lower body movements were permitted (e.g., reading a book, using a phone, readjusting seated position if uncomfortable). To minimize walking distance, participants were transported in a wheelchair for bathroom breaks. Participants completed the SRA in time with the video demonstration, which can be found at this link: https://www.youtube.com/watch?v=Ieb3wqDD_7Y&t=1s. The video was run twice for the SRA6 condition.

The choice for the activities was informed by previously published studies (19, 35). Exercises were selected on the basis of engaging large muscles in the lower body to promote increased leg blood flow and reduce vascular impairment (20). Additionally, these exercises were selected on the basis that that they could be performed in a static position with no equipment and therefore could be a practical choice for most adults. Regarding the break frequency, interrupting sitting every 30 min has previously been shown to improve lower limb vascular function (19). However, the 60-min break was used to overcome issues relating to the feasibility/

practicalities of high-frequency interruptions. To ensure that the activity was matched for duration, the 60-min break was doubled so that both conditions undertook an identical total amount of activity.

Participants were asked to refrain from moderate to vigorous PA for 48 h, and caffeine and alcohol for 24 h before each experimental condition, and they completed questions at the start of each experimental visit to ensure compliance. Participants resumed their habitual PA and dietary patterns during the washout period between experimental conditions. To objectively monitor daily activity levels throughout the study, participants wore an activPAL triaxial PA monitor (PAL Technologies Ltd., Glasgow, Scotland).

To minimize any potential diet-induced variability, participants were provided with standardized meals from the night before each trial visit to the end of the trial visit day. Consistent with previous investigations in our laboratory (19, 35), all meals provided 33.3% of estimated energy requirements [Schofield equation (36); 1.5 activity factor] with a target macronutrient profile of 12–15% energy from protein, 30–33% energy from fat, and 53–55% energy from carbohydrate. Participants were instructed to eat their standardized evening meal between 1900 and 2100.

On each experimental day, participants arrived at the laboratory at 0730 in a fasted state (>10 h). After participants voided and were weighed, they were asked to remain seated in an upright chair and minimize movement for the duration of the visit. Each experimental visit started with a 1-h "steady-state" period. During this time, baseline blood samples, including glucose and insulin, were collected, blood pressure (BP) was measured and femoral artery flow-mediated dilation (FMD) recorded. Participants received a standardized breakfast and lunch at 0 h, and 3.5 h, respectively, and were given up to 20 min to consume them. Breakfast options included bran-based cereals, ham-and-cheese
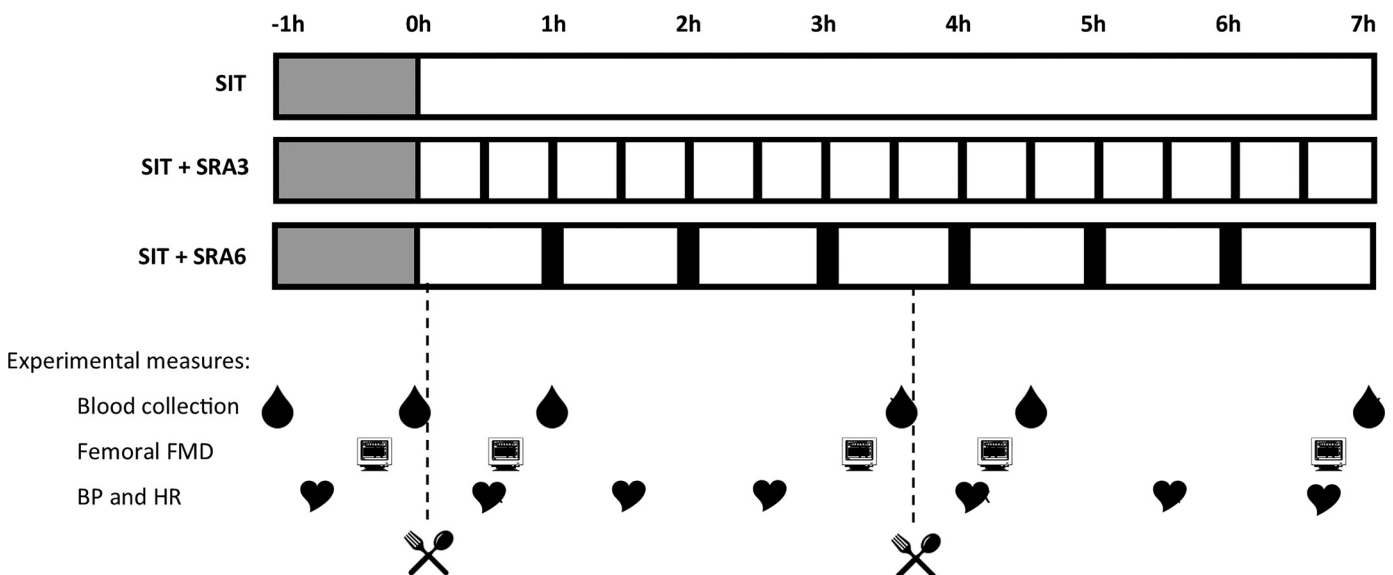
Figure 1. Study design and protocol. Participants were initially screened over the phone, followed by a medical screening and familiarization visit. Eligible participants then completed 3 experimental conditions in a random order. Gray bars represent steady-state hour when the following measures were taken: resting BP and HR, FMD, and fasted blood samples. Black bars in the SIT + SRA3 and SIT + SRA6 conditions represent SRAs. BP, blood pressure; HR, heart rate, FMD, flow-mediated dilation; SIT, uninterrupted sitting condition; SRA3, sitting interrupted by simple resistance activities every 30 min; SRA3, sitting interrupted by simple resistance activities every 60 min.

croissant, fruit salad, and juice. Lunch options included a salad and meat bread roll, sweet biscuits, and juice. A note was made regarding each individual's meal choice and replicated for subsequent visits. Participants were advised to take medications as normal. Blood samples were collected at 0 h (fasted) and 1, 3.5, 4.5, and 7 h for the analyses of ET-1. Due to the distance of the bathrooms from the clinic rooms, a wheelchair was used to take participants to the toilet. One participant's data were excluded from this analysis, as the blood draws were unable to be completed on the study days.

Measurements

Arterial function.

All vascular function assessments were performed in a quiet, dimly lit, temperature-controlled (22°C –25°C) room. Participants rested in the seated position for ~15 min before assessment and were instructed to place both feet flat on the floor. The superficial femoral artery was assessed in the right leg by using a 10-Mhz multifrequency linear array probe in conjunction with a high-resolution duplex ultrasound (Terason t3200; Teratech, Burlington, MA) machine at an isonation angle of 60°. A rapid inflatable cuff (SC-12-D, D.E.; Hokanson, Inc., Bellevue, WA) was placed around the thigh (distal femur). Once an optimal image of the artery was obtained, a 1-min recording of continuous resting vessel diameter and blood velocity was measured (live duplex mode). The cuff was then inflated (~220 mmHg) for 5 min. Following 5 min of inflation, the cuff was released to induce reactive hyperemia, and duplex ultrasound recording continued for a further 3 min to observe the postdeflation diameter and peak velocity response. All FMD measurements occurred before the SRA to avoid any transient effects of the SRA that might have influenced the measurement. Placement of the probe was marked and recorded on the first scan at the first visit and replicated for corresponding vascular measurements.

Data from four participants were excluded from this analysis, as complete valid datasets were not available due to poor image quality from patient movement or imaging artifact. Datasets were considered invalid if more than three of the five tests could not be assessed. Analysis of femoral artery diameter and blood velocity was performed using off-line, automated edge detection and wall tracking software by one scanner (33). Analysis of ultrasound recordings was performed (LabVIEW 6.02, National Instruments). This software has previously been demonstrated to overcome methodological issues, reproducing diameter measurements that significantly reduce observer error with an intraobserver coefficient of variation of 6.7% (33). FMD was calculated as the percentage rise in peak diameter from the preceding baseline diameter. Blood flow was defined as the product of cross-sectional area and velocity. Shear rate (s^{-1}), derived from blood velocity and diameter, was used as an estimate of shear stress on the artery wall. The shear stimulus was calculated as the shear rate area under the curve (AUC) from time of cuff release to peak dilation, using the sum of trapezoids method (37). Our sonographer has a between-visit reproducibility of 4.5%.

Resting BP.

Seated, resting brachial BP was measured at hourly intervals from −30 min with an additional measurement at 3 h. Measurements were taken in triplicate at 1-min intervals using an automated oscillometric BP monitor (HEM-907; Omron, Kyoto, Japan) and an appropriately sized cuff, per recommended guidelines (29). All measurements were repeated on the same arm for both conditions. An average of the three measurements each hour was used in analysis. Blood pressure measurements were taken immediately after the SRA (Fig. 1).

Biochemical analysis.

Whole blood samples were drawn into EDTA tubes and centrifuged within 5 min of collection, and the plasma fraction was separated and stored at −80°C. ET-1 samples were analyzed using sandwich immunoassay technique with DET-100 kits from R&D Systems (Minneapolis, MN) according to the manufacturer's instructions. The final product of the ELISA was quantified using a Benchmark Plus Microplate spectrophotometer and standard curve (Bio-Rad Laboratories, Hercules, CA) at 450 nm (38).

Statistical Analysis

All analyses were performed using the R statistical programming language (version 3.6.1, 2019, USA) (39). The total AUC across the 7-h protocol on each day was calculated for ET-1 using the trapezoidal method, where area is taken from a plasma concentration of zero. Generalized linear mixed models were used to examine FMD and hemodynamic measurements for 1) the average for each condition across all scans, excluding baseline, in the 7-h period and 2) between- and within-condition effects (i.e., condition × time interaction). Further analysis was undertaken to examine meal-specific responses on mean FMD. Generalized linear mixed models were used to examine mean femoral artery FMD for the pre- (1-h + 3.5-h) and post- (4.5-h and 7-h) lunch time periods. The generalized linear mixed models had the following fixed effects; age, sex, and BMI values and 0-h and condition order. We modeled participants as random effects. Additional fixed effects for resting diameter and shear stimulus were used on FMD models (8). To account for any residual effects of activity preceding the experimental conditions, primary outcomes were additionally adjusted for number of steps in the restrictive period. A condition × time interaction with postcomparisons was used to compare individual time points between and within conditions relative to 0 h. Post hoc comparisons between time points were adjusted for multiple comparisons using Sõidák corrections. Associations between variables were assessed using Spearman's rank correlation coefficients at each time point. Descriptive data are presented as means ± SD, and outputs from mixed-model analysis are presented as marginal means ± SE. $P \leq 0.05$ was considered statistically significant.

RESULTS

Participant Characteristics

Of the 25 participants included, 24 randomized participants completed all three study arms (Fig. 2). Participant
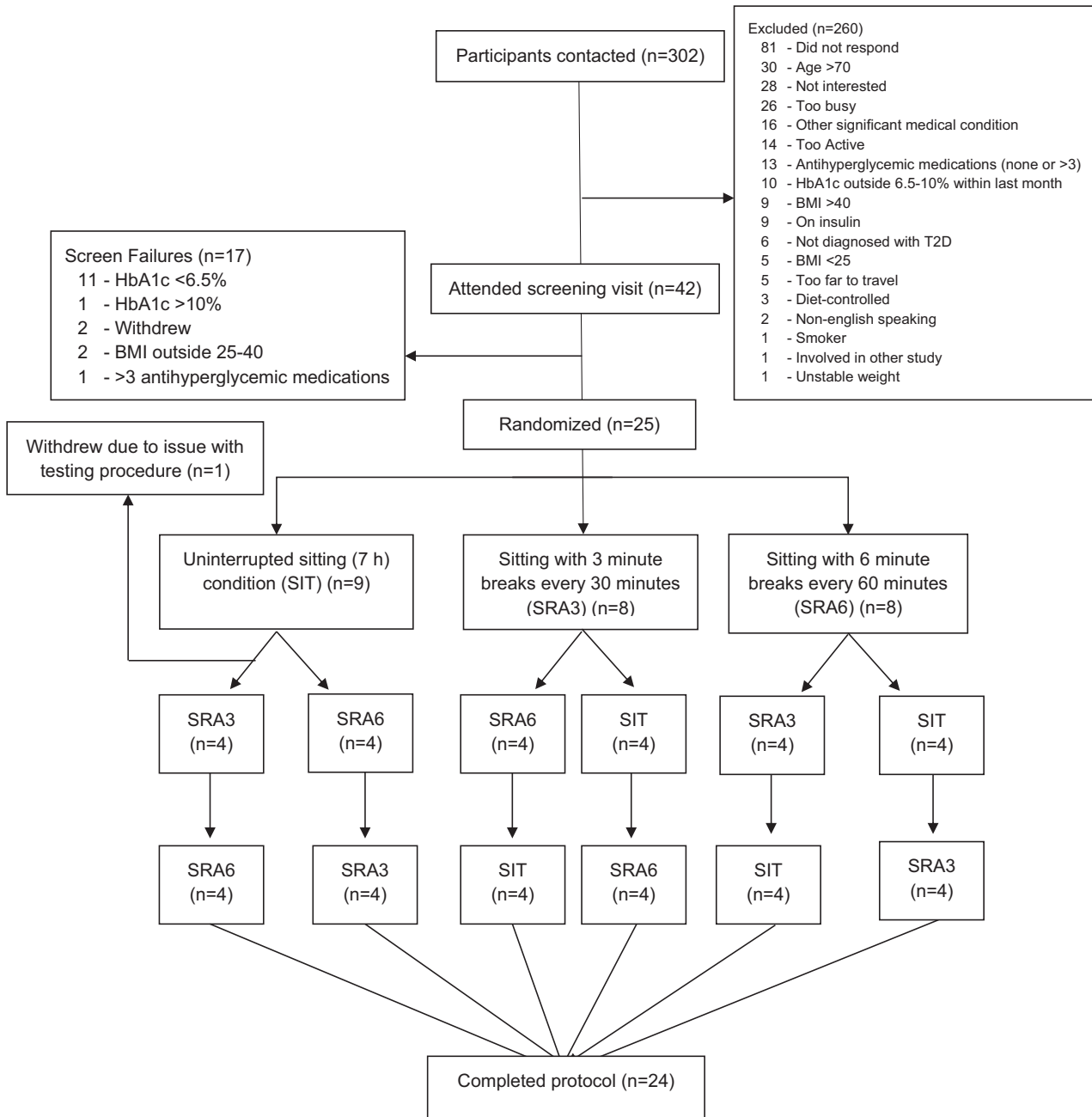
Figure 2. Consort standards of reporting trials (CONSORT) diagram. SIT, uninterrupted sitting condition; SRA3, sitting interrupted by simple resistance activities every 30 min; SRA3, sitting interrupted by simple resistance activities every 60 min.

characteristics are presented in Table 1. All female participants within the study were postmenopausal. Preexperimental period data on time spent sitting, standing, and stepping (inferred using activPAL data from a stepping cadence of >100 steps per minute for >1 min) and diet are presented in Supplemental Table S2 of the supplemental material (supplemental material is available at https://doi.org/10.6084/m9.figshare.12431696 and https://figshare.com/s/13f3805314af80e22474). Prior to the SRA3 condition, the total number of steps and total stepping time was significantly higher in the restricted period, but not the habitual period. No other significant differences were observed in activity level, sitting time and dietary indices between pre-experimental periods.

There were 14 participants on antihypertensive medication. All participants maintained their baseline antihypertensive treatment and other medications (Table 1) on the experimental day and throughout the course of the trial.

FMD and Hemodynamics

The hemodynamic and absolute (i.e., unadjusted) FMD data are presented in Supplemental Table S1, and Table 2 displays adjusted data with statistical comparisons. Femoral artery FMD averaged across the 7 h was significantly different between SIT: $3.7 \pm 0.3\%$ and SRA3: $4.1 \pm 0.3\%$, $P = 0.04$; however, there were no significant differences between SIT

Table 1. *Participant characteristics*

Characteristics	
Sex (male/female)	13/11
Age, yr	61.5 ± 7.8
BMI, kg/m^2	32.6 ± 3.5
Weight, kg	94.0 ± 13.4
Waist circumference, cm	111.0 ± 8.8
Waist-to-hip ratio	1.0 ± 0.1
SBP, mmHg	129 ± 10
DBP, mmHg	74 ± 10
T2D duration, yr	10.1 ± 7.0
Metabolic parameters	
Glycated hemoglobin, %	7.6 ± 0.8
Glycated hemoglobin, mmol/mol	59 ± 9
Fasting glucose, mmol/L	8.1 ± 1.5
Fasting insulin, mmol/L	76.2 ± 43.5
Fasting triglycerides, mmol/L	1.7 ± 0.6
HOMA2-IR	1.9 ± 1.0
Medication, *n* (%)	
Metformin	22 (92)
DPP4	8 (33)
Sulfonylureas	6 (25)
SGLT2$^+$	8 (33)
GLP agonists	4 (17)
ACE inhibitor or ARB	13 (54)
Calcium channel blocker	3 (13)
Beta blocker	3 (13)
Diuretic and other	2 (8)
Statin	14 (58)
Antidepressants	6 (25)

Values are means ± SD; n = 24 participants. ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; BMI, body mass index; DBP, diastolic blood pressure; DPP4, dipeptidyl peptidase-4 inhibitors; GLP, glucagon-like peptide-1 receptor agonists; HOMA-IR; homeostatic model assessment of insulin resistance, SBP, systolic blood pressure; SGLT2$^+$, sodium-glucose cotransporter 2 inhibitors; T2D, type 2 diabetes.

and SRA6, or SRA3 and SRA6 (Fig. 3B). When the day was split into pre- and postlunch periods, there was a statistically significant difference in the average of the two prelunch measures between SRA6: 3.7 ± 0.3% and SRA3: 4.4 ± 0.3%, $P <$ 0.001, and SRA6 and SIT: 4.3 ± 0.3%, P = 0.005. The average of postlunch measures revealed a statistically significant difference between SIT: 3.1 ± 0.5% and SRA6: 3.7 ± 0.5%, P = 0.02 suggesting that the impact of SIT was greater as the day progressed (Fig. 3A). Additional adjustments for resting diameter

and shear stimulus did not change the interpretation of the results. There were no statistically significant differences for between- or within-condition (i.e., condition × time interaction) effects at any of the time points for FMD (Table 2). However, within the SRA3 condition there was a trend for femoral artery FMD to increase following a meal at the 3.5- and 7-h time points (increase of 1.5% from 0 h to 3.5 h, and an increase of 1.3% from 4.5 h to- 7 h). A similar trend was not observed in the SRA6 or SIT condition, with FMD levels remaining relatively constant across the day (Table 2).

Mean resting femoral shear rate averaged across the 7 h was significantly lower in the SIT condition (33.1 ± 4.1/s) relative to SRA3 (45.3 ± 4.1/s, $P <$ 0.001) and SRA6 (46.2 ± 4.1/s, $P <$ 0.001). Mean resting femoral blood flow averaged across 7 h was significantly lower in the SIT condition (71.1 ± 9.2 mL/min) relative to SRA3 (96.4 ± 9.2 mL/min, $P <$ 0.001) and SRA6 (92.5 ± 9.1 mL/min, $P <$ 0.001). No differences in resting systolic or diastolic BP averaged across 7 h between the SIT and SRA3 (121 ± 3 vs. 121 ± 3 mmHg, P = 0.95; 68 ± 1 vs. 69 ± 1 mmHg, P = 0.81, respectively) or SIT and SRA6 (121 ± 3 vs. 120 ± 3 mmHg, P = 0.93; 68 ± 1 vs. 67 ± 1 mmHg, P = 0.57, respectively) conditions were observed. Additionally, there were no significant differences in resting mean HR over 7 h (SIT: 74 ± 2 beats/min, SRA3: 75 ± 2 beats/min, SRA6: 75 ± 2 beats/min, $P >$ 0.26 for all).

Blood Biomarkers

Plasma ET-1 total AUCs were 4% lower in the SIT (10.1 ± 0.9 pg·h·mL^{-1}) condition relative to SRA3 (10.5 ± 0.9 pg·h·mL^{-1}), and 1% lower relative to SRA6 (10.2 ± 0.9 pg·h·mL^{-1}); however, neither was statistically different (Fig. 4B). ET-1 concentrations were not significantly different between conditions ($P >$ 0.17 for all). ET-1 at 7 h was significantly lower relative to respective baselines for all conditions ($P <$ 0.007). Change relative to baseline was not statistically different between conditions ($P >$ 0.93). A weak positive relationship was observed across the day between resting femoral blood flow and ET-1 (r_s = 0.062, P = 0.02) and resting femoral shear rate and ET-1 (r_s = 0.163, $P <$ 0.001).

DISCUSSION

To our knowledge, this is the first study to examine the effect of interrupting prolonged sitting with different SRA

Table 2. *Hemodynamic and adjusted FMD data during 7 h of SIT, SRA3, and SRA6*

	0 h	1 h	3.5 h	4.5 h	7 h
SIT FMD, %	3.3 (2.1, 4.5)	5.0 (3.8, 6.2)	3.4 (2.2, 4.6)	3.4 (2.2, 4.6)	3.1 (1.8, 4.3)
SIT resting diameter, mm	6.5 (6.3, 6.8)	6.5 (6.2, 6.7)	6.8 (6.6, 7.0)	6.6 (6.4, 6.9)	6.7 (6.5, 7.0)
SIT resting blood flow, mL/min	50.2 (25.2, 75.3)	46.5 (20.9, 72.1)	58.6 (33.6, 83.7)	93.2 (67.6, 118.8)	94.6 (69.0, 120.2)
SIT resting shear rate/s	27.3 (18.3, 36.2)	26.1 (16.9, 35.2)	30.8 (21.9, 39.8)	38.4 (29.3, 47.5)	43.6 (34.5, 52.7)
SRA3 FMD, %	3.2 (2.0, 4.4)	4.0 (2.8, 5.2)	4.7 (3.4, 5.9)	3.4 (2.2, 4.6)	4.7 (3.5, 5.9)
SRA3 resting diameter, mm	6.6 (6.4, 6.9)	6.3 (6.3, 6.7)	6.5 (6.3, 6.7)	6.6 (6.3, 6.8)	6.7 (6.5, 6.9)
SRA3 resting blood flow, mL/min	61.1 (36.0, 86.1)	63.1 (38.1, 88.2)	89.2 (63.6, 114.8)	89.9 (64.8, 114.9)	137.7 (112.1, 163.2) †
SRA3 resting shear rate/s	29.5 (20.6, 38.4)	28.6 (19.7, 37.6)	47.6 (38.5, 56.6)	50.8 (41.9, 59.7)†	55.3 (46.2, 64.3)†
SRA6 FMD, %	3.4 (2.3, 4.6)	4.0 (2.9, 5.2)	3.3 (2.2, 4.5)	3.6 (2.5, 4.8)	3.7 (2.6, 4.9)
SRA6 resting diameter, mm	6.6 (6.4, 6.8)	6.7 (6.5, 6.9)	6.7 (6.5, 6.9)	6.6 (6.4, 6.8)	6.7 (6.5, 7.0)
SRA6 resting blood flow, mL/min	57.5 (33.0, 82.0)	43.8 (19.3, 68.4)	97.7 (73.2, 122.2)	102.1 (77.6, 126.7)	123.1 (98.6, 147.6)†
SRA6 resting shear rate/s	30.3 (21.5, 39.0)	21.0 (12.2, 29.7)	48.9 (40.2, 57.7)†*	53.2 (44.5, 62.0)†	59.9 (51.1, 68.6)†

Values are marginal means ± 95% confidence intervals for each condition. All models adjusted for age, sex, body mass index, treatment order, and multiple comparisons. Time points 1 h, 3.5 h, 4.5 h, and 7 h additionally adjusted for value at 0 h. FMD, flow-mediated dilation; SIT, uninterrupted sitting; SRA3, sitting interrupted with simple resistance activities every 30 min; SRA6, sitting interrupted with simple resistance activities every 60 min. *$P <$ 0.05 relative to SIT condition; †$P <$ 0.05 within condition vs. 0 h; n = 20.
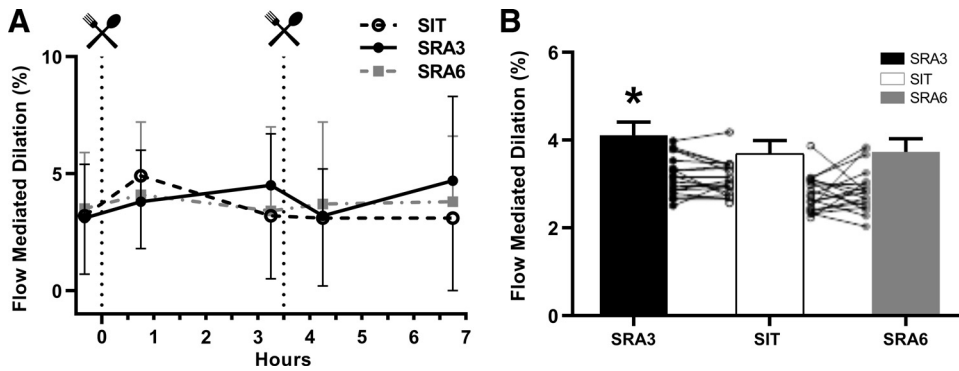
Figure 3. *A*: time course of unadjusted femoral artery flow-mediated dilation (FMD) in the 3 conditions. Data are means ± SD. *B*: unadjusted mean femoral artery FMD over 7 h in uninterrupted sitting (SIT), sitting interrupted by 3-min simple resistance activities every 30 min (SRA3), and sitting interrupted by 6-min simple resistance activities every 60 min (SRA6) conditions, adjusted for values at 0 h, age, body mass index, sex, and treatment order. Data are marginal means ± SE with paired individual values. *P = 0.04 vs. SIT.

break frequencies on vascular function in those with T2D. When averaged across 7 h, femoral artery function (measured via FMD) significantly increased in the SRA3 condition, but not the SRA6 condition, relative to SIT. Although no between- or within-condition differences in femoral artery function were observed at specific time points (Table 2), this average difference reflected increases in FMD in the SRA3 condition that occurred ~3 h after meal ingestion. Blood flow and resting shear rate were also significantly higher across the day in the SRA3 and SRA6 conditions relative to SIT. These findings provide insights into the effects of interrupting prolonged sitting on lower limb vasculature in adults with T2D.

Whereas FMD tended to increase across the day (baseline to 7 h) in the SRA3 condition, only small to negligible changes were observed for SRA6 and SIT. Others have reported similar findings in healthy desk workers and young, healthy males, observing an increase in FMD when prolonged sitting was broken up with PA, despite not reaching statistical significance relative to baseline (27, 28). Nevertheless, a 1.5% increase across the day (baseline to 7 h) was observed in the SRA3 condition (Table 2). Although this was not statistically significant, a FMD increase of 1% is considered clinically meaningful, given that it decreases the risk of cardiovascular events by 13% (34). Certainly, previous studies have reported that breaking up prolonged sitting prevents superficial femoral artery endothelial dysfunction (19, 27, 40).

The increase in superficial femoral artery FMD across the day in the SRA3 condition, relative to SRA6 condition, suggests that the frequency of SRA may be more important than the duration of SRA. This contradicts previous work, which found that infrequent walking breaks maintained superficial

femoral artery endothelial function at 30, 90, and 150 min (27) and 120 and 240 min (28). However, it should be noted that those two studies were examining endothelial function in healthy males and females, whereas participants in this study had T2D with overweight and/or obesity. Given that there is a progressive impairment in vascular function throughout the pathogenesis of T2D (7), it is possible that more frequent interruptions to sitting are needed to preserve leg blood flow (20) and therefore mitigate sitting-induced vascular impairments in this population. Further research that investigates interrupting prolonged sitting with activity breaks across the spectrum of dysmetabolism (from healthy individuals to those with T2D and complications) is needed to confirm this hypothesis.

There are several possible reasons why we did not observe statistically significant differences in condition × time interactions in FMD between conditions. Previous studies employed tighter control over restricted leg movements (27, 41) during sitting compared with this current study; thus, it is plausible that the habitual, unstandardized lower leg motion allowed in our study contributed to the lack of statistically significant difference in time × condition interaction (28). Indeed, low-level muscular contractions while sitting for 3 h versus sitting interrupted with leg fidgeting have been shown to prevent popliteal endothelial dysfunction that would have otherwise occurred in young, healthy participants (30). Our approach emulates a "real world" circumstance, and on average we observed improvements with interruptions to sitting across the day. Additionally, all of our FMD measurements were collected while the unbroken seated position was reatained. Although we acknowledge that current FMD guidelines stipulate that assessments be performed in the supine position, movement between
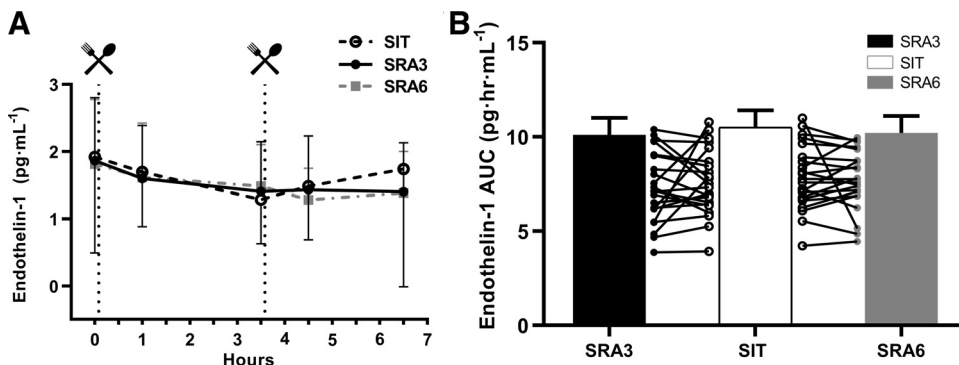
Figure 4. *A*: time course of plasma endothelin-1 (ET-1)in the 3 conditions (*n* = 23). Data are means ± SD. *B*: effect of uninterrupted sitting (SIT), sitting interrupted with 3-min simple resistance activities every 30 min (SRA3), and sitting interrupted with 6 min simple resistance activities every 60 min (SRA6) on plasma ET-1 total area under the curve (AUC) over 7 h, adjusted for age, sex, body mass index, and treatment order (*n* = 23). Data are marginal means ± SE with paired individual values.

seating and supine would necessitate muscular activity that might impact FMD measures (27). It should also be noted that offline analysis was not performed by a blinded observer. However, we used automated edge detection and wall tracking software, in keeping with published guidelines (8, 42), which is designed to minimize the potential for investigator influence and bias (33) compared with manual methods of analysis. Nonetheless, our findings are in line with previous studies investigating FMD (%) in overweight adults (28), and we still observed a change in average FMD, shear stress, and blood flow across the day. It is also possible that our sample size may have been marginal for detecting between-condition differences at individual time points. Finally, we cannot exclude the possibility of some variability in responsiveness to meals between participants, although our meals were standardized and the same meals were consumed for all thhree conditions.

The significant differences in vascular function we observed between conditions when data were averaged across time periods reflects a trend (Fig. 3A) in the repetitive response in FMD to the meals, particularly in the SRA3 condition. In line with previous findings, this suggests that the effect of the relative insulin resistance [induced by prolonged sitting after a meal (29, 35, 43)] on vascular function could be ameliorated by frequently interrupting sitting with activity breaks. This trend was in line with previous studies that have observed femoral artery FMD to increase when sitting was frequently interrupted after a meal or a snack (28, 29). However, given that relatively few studies explore the effect of interrupting sitting on vascular function in a non-fasted state over a 7-h time frame (19, 28, 44), and that our study is the first to observe the effects in adults with T2D, future research should determine whether frequently interrupting sitting following a meal is beneficial for adults with T2D.

Resting femoral shear rate and resting blood flow significantly increased from baseline across 7 h in both the SRA3 and SRA6 conditions (Table 2). Shear stress is recognized as an important physiological factor in maintaining endothelial health (20, 45), and sitting-induced decreases in blood flow and shear stress may contribute to vascular dysfunction (20, 45, 46). Episodic increases in blood flow and shear stress that accompany activity breaks may provide an antiatherogenic stimulus over the long term (47), particularly given that the lower limb is susceptible to atherosclerosis (20). The magnitude of increase in blood flow and, consequently, shear stress needed to induce a clinically meaningful improvement in vascular function is unknown. However, in adults with overweight and obesity, resting blood flow and shear rate increased from baseline nearly four- and threefold, respectively, corresponding to a FMD increase of 3.1% across 5 h in the SRA condition (19). Given that we did not observe an increase of this magnitude for blood flow and shear rate in our participants (Table 2), it is possible, that a larger increase is needed to stimulate a significant improvement in femoral artery FMD in those with T2D. Previous research has indicated that shear and function relationships decline with long-term exposure to CVD risk factors (48), where individuals with T2D are more resistant to the beneficial vascular adaptations of PA relative to healthy controls (12). This may partly explain the relatively small increase in blood flow and shear in our T2D participants relative to other studies assessing those with overweight and obesity who are otherwise healthy (19). Future research should explore the long-term impacts of interrupting prolonged sitting on lower limb blood flow and vascular function in populations across the spectrum of diabetes risk.

We observed high basal levels of plasma vasoconstrictor ET-1 in our participants with T2D (49, 50), and the observation of no significant changes in ET-1 levels is consistent with what others have reported following PA (51). The utility of plasma ET-1 as a marker of cellular concentrations has been questioned, since ~80% of ET-1 is secreted on the abluminal side (49). Thus, it is plausible that different results might have been observed had we directly measured ET-1 in the vessel wall. Furthermore, we observed a weak but positive correlation between ET-1 and blood flow and shear, which may partly explain why ET-1 remained relatively sustained across the day. Previous studies demonstrated that an ET-1 blockade significantly increases blood flow in those with T2D to a greater extent relative to individuals without T2D, suggesting that those with T2D have greater ET-1-mediated basal vasoconstrictor tone (52–54). In addition, studies have demonstrated that although ET-1 antagonists improve nitric oxide bioavailability in obese individuals, these effects are not observed at the same magnitude in those with T2D (52, 53), suggesting that ET-1 blockade alone may not be enough to significantly increase nitric oxide bioavailability. These studies indirectly demonstrate the importance of insulin resistance in altering ET-1-mediated vasoconstriction. Additionally, more than half of our participants were taking antihypertensive medications, which may play a role in interacting with mediators (ET-1) that influence endothelial homeostasis (55). Indeed, it is possible that the elevated basal ET-1 levels found in this study may partly explain the blunted blood flow and shear response to SRA in our participants relative to overweight and obese adults (19). However, given that these are complex pathways that involve multiple integrative mechanisms and very tightly controlled experimental conditions, more comprehensive data are needed to explain the vascular mechanisms relating to interrupting sitting with activity breaks in those with T2D.

No statistically significant changes in the mean systolic BP, diastolic BP, or heart rate measurements were observed between conditions over the trial period. These results contrast those of previous studies that reported a systolic BP-lowering effect with short-bouts of activity (56, 57). Since BP of our study population was well controlled (Supplemental Table S2), this may have precluded any BP changes due to the interruptions.

Interventions that may be used to inform larger trials and more specific public health advice surrounding optimal timing and duration of breaks must be feasible, so that cardiovascular health benefits from interrupting sitting are translated to high-risk populations such as office workers with T2D (28). Therefore, this study directly compared two different activity protocols with equivalent total activity duration and energy expenditure over a day. As previously noted, interrupting sitting more frequently with shorter duration SRA (SRA3) was more effective in increasing superficial femoral artery FMD relative to less frequent, longer-duration SRA (SRA6). Given the pragmatic approach to

interruptions in prolonged sitting employed in this study, which does not require people to move from their desk, or the use of equipment, a high-frequency break strategy may be suitable in sedentary workplaces. Certainly, previous studies that have examined interrupting sitting every 20 or 30 min have reported benefits for reducing glucose and insulin concentrations (18, 29) and blood pressure (56) and maintaining cerebral blood flow (58, 59). Future research should aim to examine break frequencies that mitigate sitting-induced impairments in both vascular and metabolic function (28). This may help to inform larger trials and to produce more specific public health guidelines around the optimal timing and duration of breaks in sitting necessary to improve cardiometabolic health outcomes.

This study was performed in a laboratory setting and utilized a well-controlled, randomized crossover design, affording smaller sample sizes as it provided control for person-specific factors. Trial conditions were standardized and restrictive periods implemented before testing days (minimal variance in PA levels and diet;Supplemental Table S2). Future research should establish the effect of interrupting prolonged sitting in home- and/or work-based settings that better reflect real life settings. Since we did not assess changes in nitroglycerine responses, our study could not determine impacts on vascular smooth muscle function per se. Moreover, this was an acute study, and we examined responses to interrupting sitting over only a 7-h period. Participants in this study were also taking a diverse range of medications that may have influenced our results. Of note, 54% of participants were taking angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and 58% were under statin therapy. It is possible that these medications might have modified vascular function, but we experimentally controlled for this according to established guidelines (8, 42, 60) by standardizing the timing and dose in our repeated-measures experiment. Importantly, the proof-of-concept nature of the study highlights the need for studies that examine vascular response to interrupting sitting and meals in those with T2D. Longer-term exposures to interrupting sitting may assist in gaining a better understanding for the long-term cardiovascular health adaptations in those with T2D.

In conclusion, breaking up prolonged sitting with SRA every 30 min significantly increased mean superficial femoral artery FMD% over 7 h relative to prolonged sitting, and clinically meaningful effect sizes (>1%) were evident (6). Vascular shear rate and blood flow across the intervention period were also enhanced by interrupting prolonged sitting. Our findings suggest that more frequent and shorter breaks may be more beneficial than longer, less frequent breaks for improvement in vascular function in those with T2D. Taken together, these results provide new insights into the frequency and duration of activity breaks needed to stimulate blood flow or improve vascular function during prolonged sitting. Future research should aim to examine potential mechanisms and the longer-term impacts of interrupting prolonged sitting in free-living settings on vascular function in adults with T2D.

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GRANTS

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.G., M.J.W., D.J.G., D.W.D., P.C.D., B.A.K., N.O., and R.N.L. conceived and designed research; F.C.T., M.J.W., M.K.T., and A.R.H. performed experiments; F.C.T., N.E. and N.M. analyzed data; F.C.T., D.J.G., D.W.D., and P.C.D. interpreted results of experiments; F.C.T. prepared figures; F.C.T. drafted manuscript; F.C.T., M.G., M.J.W., N.M., D.J.G., D.W.D., A.R.H., P.C.D., B.A.K., R.E.C., N.O., N.D.C., and R.N.L. edited and revised manuscript; F.C.T., M.G., N.E., M.J.W., M.K.T., N.M., D.J.G., D.W.D., A.R.H., P.C.D., B.A.K., R.E.C., N.O., N.D.C., and R.N.L. approved final version of manuscript.

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Table 1. *Hemodynamic and absolute (i.e., unadjusted) flow-mediated dilation data during 7h of uninterrupted sitting (SIT), sitting interrupted with 3-min of simple resistance activities every 30 minutes (SRA3), and 6-min of simple resistance activities every 60 minutes (SRA6).*

	0h	1hr	3.5h	4.5h	7h
SIT resting diameter, mm	6.4 (0.8)	6.3 (0.7)	6.7 (0.9)	6.6 (0.8)	6.7 (0.9)
SIT peak diameter, mm	6.7 (0.7)	6.7 (0.8)	6.9 (0.8)	6.8 (0.8)	6.9 (0.9)
SIT shear stimulus, SR_{AUC} (au)	11032 (6318)	9517 (4837)	9453 (4540)	10475 (5524)	11369 (5307)
SIT resting blood flow, ml/min	45 (31)	44 (37)	53 (39)	87 (58)	88 (38)
SIT resting shear rate, /s	25.5 (22.4)	25.6 (21.9)	29.1 (21.2)	35.7 (21.3)	40.9 (24.7)
SRA3 resting diameter, mm	6.6 (0.8)	6.5 (0.8)	6.5 (0.8)	6.5 (0.8)	6.7 (0.8)
SRA3 peak diameter, mm	6.8 (0.8)	6.8 (0.8)	6.8 (0.8)	6.7 (0.8)	7.0 (0.8)
SRA3 shear stimulus, SR_{AUC} (au)	9602 (7226)	9967 (4714)	12441 (5831)	14134 (5278)	10108 (5780)
SRA3 resting blood flow, ml/min	63 (68)	63 (44)	92 (66)	89 (50)	133 (119)
SRA3 resting shear rate, /s	29.9 (22.5)	29.4 (20.6)	50.7 (27.6)	50.9 (23.4)	56.3 (30.0)
SRA6 resting diameter, mm	6.6 (0.8)	6.7 (0.8)	6.7 (0.7)	6.5 (0.9)	6.7 (0.8)
SRA6 peak diameter, mm	6.8 (0.9)	6.9 (0.9)	6.9 (0.8)	6.8 (0.9)	6.9 (0.8)
SRA6 shear stimulus, SR_{AUC} (au)	10685 (6496)	7799 (4564)	14625 (14870)	13199 (5958)	13805 (6495)
SRA6 resting blood flow, ml/min	58 (30)	44 (36)	98 (65)	102 (70)	123 (63)
SRA6 resting shear rate, /s	32.3 (17.5)	22.9 (17.6)	50.9 (36.0)	55.2 (29.1)	61.9 (23.1)

Data are mean ± SD. au, arbitrary units; AUC, total area under the curve; FMD, flow-mediated dilation; SIT, uninterrupted sitting; SRA, sitting interrupted with 3-min simple resistance activities every 30-min; SR, shear rate.

Table 2. *Pre-experimental period participant demographic information.*

	SIT	SRA3	SRA6
Weight (kg)	93.2 ± 2.8	93.4 ± 2.9	93.4 ± 2.8
Systolic blood pressure (mmHg)	123 ± 3	122 ± 2	121 ± 2
Diastolic blood pressure (mmHg)	74 ± 2	72 ± 2	71 ± 2
Heart rate (bpm)	69 ± 2	69 ± 2	66 ± 2
activPAL data^			
Sitting time (min/day)			
Habitual	575 ± 29	597 ± 24	575 ± 41
Restricted	635 ± 36	623 ± 35	578 ± 50
Standing time (min/day)			
Habitual	232 ± 22	213 ± 26	282 ± 38
Restricted	211 ± 25	207 ± 22	223 ± 28
Total Stepping time (min/day)			
Habitual	90 ± 9	94 ± 10	99 ± 9
Restricted	75 ± 9	94 ± 7*	82 ± 7
Total number steps			
Habitual	7190 ± 680	7411 ± 839	7495 ± 636
Restricted	5774 ± 791	7695 ± 578*	6350 ± 556
Diet°			
Total daily energy (kJ/day)	9134 ± 533	8712 ± 438	8919 ± 412
Total CHO (g/day)	223 ± 13	216 ± 10	219 ± 10
Total fat (g/day)	91 ± 7	83 ± 7	88 ± 6
Total protein (g/day)	99 ± 9	101 ± 8	97 ± 5

Data are means ± SEM. ^activPAL data collected during habitual (free-living) days and the 48 h period preceding the trial condition. °Dietary intakes were assessed from 3-day diet records for three days prior to each trial condition, and analyzed using FoodWorks dietary analysis software (FoodWorks; Xyris Software, Highgate Hill, Queensland, Australia). *$p < 0.05$ relative to SIT condition. N=20. During the restricted period participants were instructed to avoid moderate-vigorous physical activity for 48 h, and caffeine and alcohol for 24 h prior to each experimental condition.

5.3 Chapter 5 Summary

The findings presented in Section 5.2 are the first to examine the impact of interrupting prolonged sitting with different SRA break frequencies on vascular function in subjects with T2D. Across the 7h intervention period, femoral artery function significantly increased in the SRA3 condition, but not the SRA6 condition, relative to SIT. Additionally, blood flow and resting shear rate were significantly higher across the day in the SRA3 and SRA6 conditions relative to SIT.

In the SRA3 condition, an FMD increase of 1.5% was observed across the day. While not statistically significant, an FMD increase of 1% is considered clinically meaningful given it decreases the risk of cardiovascular events by 13% [149]. Further, an increase in shear was reported for both SRA conditions. Given that shear stress is an important physiological factor in maintaining endothelial health, episodic increases following SRAs may provide a long term anti-atherogenic stimulus.

The findings in 5.2 provide initial 'proof-of-concept' evidence, in a laboratory-based setting, that interrupting sitting may be beneficial for vascular function in adults with T2D. Considering those with T2D are at increased cardiovascular risk, partly owing to reduced vascular function [151, 152], these findings have potential implications for the management of T2D. Indeed, interrupting sitting at regular intervals is likely to be a practical strategy for maintaining vascular health and can be implemented across the day. Future research should include free-living and longer-term interventions that allow for habitual patterns of movement. Additionally, such studies may be expanded to include more diverse population groups, such as women with PCOS.

CHAPTER 6 – INTERRUPTING PROLONGED SITTING AND ENDOTHELIAL FUNCTION IN POLYCYSTIC OVARY SYNDROME

Publication statement:

This chapter is comprised of the following paper published in *Medicine & Science in Sports & Exercise.*

6.1 Chapter 6 Introduction

Chapter 4 highlighted the difficulty in drawing conclusions regarding the effects of sitting on vascular function in females, due to the limited number of studies that have been conducted to date. Indeed, there continues to be a primary focus on male participants in vascular research. In my recent meta-analysis, 11 out of 31 studies recruited only male participants, and an additional 13 studies recruited majority male participants (> 50% males) [31]. Given that the studies reporting excessive sitting time in female populations have focussed on young [153] or older healthy females [154], it is necessary to include more female participants with cardiometabolic abnormalities in research investigating the effects of sedentary behaviour on vascular function. Further, it is conceivable that sex differences may be present in the impact of prolonged sitting, given the well-described hormonal impacts on endothelial function [155]. This is particularly relevant for women with PCOS who not only possess reduced vascular function, but also report increased sedentary time and decreased physical activity levels. Accordingly, the following study explored whether interrupting prolonged sitting with activity breaks was beneficial for improving vascular function in women with PCOS.

Interrupting Prolonged Sitting and Endothelial Function in Polycystic Ovary Syndrome

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ABSTRACT

TAYLOR, F. C., D. W. DUNSTAN, E. FLETCHER, M. K. TOWNSEND, R. N. LARSEN, K. RICKARDS, N. MANIAR, M. BUMAN, P. C. DEMPSEY, A. E. JOHAM, N. COHEN, N. OWEN, L. J. MORAN, and D. J. GREEN. Interrupting Prolonged Sitting and Endothelial Function in Polycystic Ovary Syndrome. *Med. Sci. Sports Exerc.*, Vol. 53, No. 3, pp. 479–486, 2020. **Purpose:** In healthy adults, the impairment of vascular function associated with prolonged sitting can be mitigated with intermittent brief bouts of activity. It is unknown whether these benefits extend to women with polycystic ovary syndrome (PCOS), in whom vascular function is typically impaired and sitting time is high. We examined the acute effect of regularly interrupting sitting time with brief simple resistance activities (SRA) on vascular function in PCOS. **Methods:** In a randomized crossover trial, 13 physically inactive women with PCOS (18–45 yr) completed two 3.5-h conditions: 1) uninterrupted sitting (SIT) and 2) sitting interrupted by 3-min bouts of SRA every 30 min. Femoral artery flow-mediated dilation (FMD), resting shear rate, and resting blood flow were measured at 0, 1, and 3.5 h. **Results:** Mean resting femoral shear rate, averaged across the 3.5 h, significantly increased in the SRA condition relative to the SIT condition (40.1 ± 6.1 vs 62.8 ± 6.1 s^{-1}, $P < 0.0001$). In addition, mean resting blood flow also significantly increased across the 3.5 h for SRA relative to SIT (45.0 ± 9.8 vs 72.8 ± 9.9 mL·min^{-1}, $P < 0.0001$). There were no differences between conditions in the temporal change in femoral artery FMD across 3.5 h ($P_{time–condition} > 0.05$ for all). **Conclusion:** Frequently interrupting sitting with SRA acutely increased resting shear rate and blood flow in women with PCOS but did not alter FMD. With sedentary behavior increasing in prevalence, longer-term studies of similar interventions to reduce and break up sitting time are warranted. **Key Words:** ARTERIES, BLOOD FLOW, SEDENTARY BEHAVIOR

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in females of reproductive age, with a prevalence of 10% when using the broader Rotterdam criteria (1).

Traditional and novel cardiovascular risk factors associated with PCOS (endothelial dysfunction, dyslipidemia, oxidative stress, and inflammation) place women with PCOS at increased cardiovascular risk (2).

Growing evidence indicates that endothelial dysfunction is inherent in this population, irrespective of obesity and visceral adiposity (3,4). Endothelial dysfunction is an important early event in the progression of atherosclerotic cardiovascular disease, preceding obesity and diabetes, even in those considered otherwise healthy (5). Flow-mediated dilation (FMD) is a noninvasive approach to the assessment of endothelial function *in vivo* and has been widely used as a prognostic marker for the progression of cardiovascular disease risk (6). Despite this, there are limited data available on the effect of lifestyle modifications on endothelial function in women with PCOS. To date, only a small number of studies have investigated the effects of diet and exercise on endothelial function in PCOS, with mixed results (7–9).

Observational studies have shown that women with PCOS have increased sedentary time (10) and reduced physical activity (11) compared with women without PCOS. Further research

is needed to examine whether prolonged sitting is associated with increased cardiovascular risk for women with PCOS (10,12). Recently, several experimental studies have reported that regular interruptions in prolonged sitting that involve simple resistance activities (SRA) can improve lower-limb endothelial function and arterial compliance, relative to prolonged sitting in healthy and overweight and obese populations (13–15). However, the effects of interrupting prolonged sitting time with brief activity bouts in women with PCOS are currently unknown. Interrupting prolonged sitting may provide an additional therapeutic option for lifestyle intervention in women with PCOS. This may also be a useful intervention for women with PCOS who are not overweight and obese but who still seek advice on lifestyle interventions. In light of this, the aim of the present study was to explore whether interrupting prolonged sitting every 30 min with brief activity interruptions is an effective strategy for improving endothelial function in women with PCOS. Based on our previous findings in overweight and obese populations (14), we hypothesized that, when compared with prolonged, uninterrupted sitting, regular active interruptions would improve endothelial function in women with PCOS.

METHODS

Participants

We recruited 14 women with PCOS (18–45 yr) via local community advertisement, social media, and doctor's clinics. PCOS was defined using the Rotterdam criteria (16), requiring the presence of two of the following three criteria: (i) oligoanovulation, (ii) hyperandrogenism (hirsutism or male pattern alopecia or high levels of testosterone), or (iii) polycystic ovaries on ultrasound (follicle number per ovary of ≥25 and/or an ovarian volume >10 mL). Exclusion criteria included the following: nonsedentary occupation (e.g., nurse), body mass index (BMI) ≥ 45 kg·m^{-2}, pregnancy, self-reported regular engagement in moderate- to vigorous-intensity activity (≥150 min·wk^{-1}), major acute or chronic illness that limited the ability to perform SRA, use of medications interacting with glucose or insulin metabolism (e.g., metformin), or reproductive (contraceptive pill) hormone production. No women reported being menopausal or perimenopausal.

Study Overview and Randomization

This study was a randomized crossover trial (ACTRN12618000239268) and took place at the Baker Heart and Diabetes Institute between August 2018 and February 2019 and was approved by the Alfred Human Research Ethics Committee (91-18). Potential participants were initially screened using an online eligibility survey, which asked about their general health and medical history. Eligible participants underwent further screening that included nonfasted blood tests for testosterone, sex hormone binding globulin, prolactin and thyroid-stimulating hormone at a local pathology clinic (Melbourne Pathology; Sonic Healthcare Ltd., Sydney, Australia), and/or polycystic ovary ultrasound (MIA, Victoria).

The order of experimental conditions (described below) was randomly assigned by computer generated random numbers (balanced block randomization). Participants were not aware of the condition order until the day of the first experimental visit.

Study Protocol

Familiarization visit. Participants provided written informed consent and attended a familiarization visit 4 to 6 d before their first experimental visit (Fig. 1), at which they were familiarized with the study procedures and measurements. Height, weight, neck, waist, and hip circumference measurements were taken by standard methods, in duplicate, to minimize error. Resting blood pressure (BP) was taken and participants also provided information about medical history and current medications. To minimize diet-induced variability, participants were provided with standardized meal packs for consumption the evening before testing. Consistent with previous investigations (14,17), using FoodWorks Software (FoodWorks Xryris, 2012), all meals were matched for 33.3% of estimated energy requirements (Schofield equation [18], 1.5 activity factor) with a target macronutrient profile of 12%–15% energy from protein, 30%–33% energy from fat, and 53%–55% energy from carbohydrate. Participants were instructed to eat their standardized evening meal between 1900 and 2100 h and to fast until the next morning. Participants were also instructed to avoid moderate to vigorous physical activity (exercise) for 48 h, and caffeine and alcohol for 24 h before each experimental condition. To objectively monitor daily activity levels in the 48 h before, participants wore an activPAL^3 triaxial physical activity monitor (PAL Technologies Ltd., Glasgow, Scotland).

Experimental conditions. On the experimental days, participants arrived at the laboratory between 0730 and 0800 h in a fasted state (>10 h). They were asked to record the day their most recent menstrual cycle commenced, and weight was remeasured. A peripheral intravenous catheter was inserted into the antecubital vein for blood sampling. Each experimental visit started with a 1-h "steady-state" period where blood samples were collected, BP measured, and femoral artery FMD recorded. At 0 h, participants were given a 75-g glucose drink to consume. A 75-g glucose drink was chosen to eliminate potential intra- and intersubject variability associated with chewing solid meals and meal consumption. Blood samples were then collected at half-hourly intervals up until the 3 h mark.

Participants were asked to remain seated in an upright chair, and minimize movement, for the duration of the visit. In the uninterrupted sitting (SIT) condition, participants were asked to remain seated for 3.5 h, only rising from the chair to visit the bathroom. This was replicated during the SRA condition, but sitting was interrupted every 30 min for 3 min of light-intensity body weight exercises (half squats, calf raises, and single knee raises with gluteal contractions). Each exercise was performed for 20 s and repeated three times in a sequential order, while mimicking a standardized, prepared video recording (17). The participant was then asked to return to the seated position. A 10-d washout period was observed between conditions.
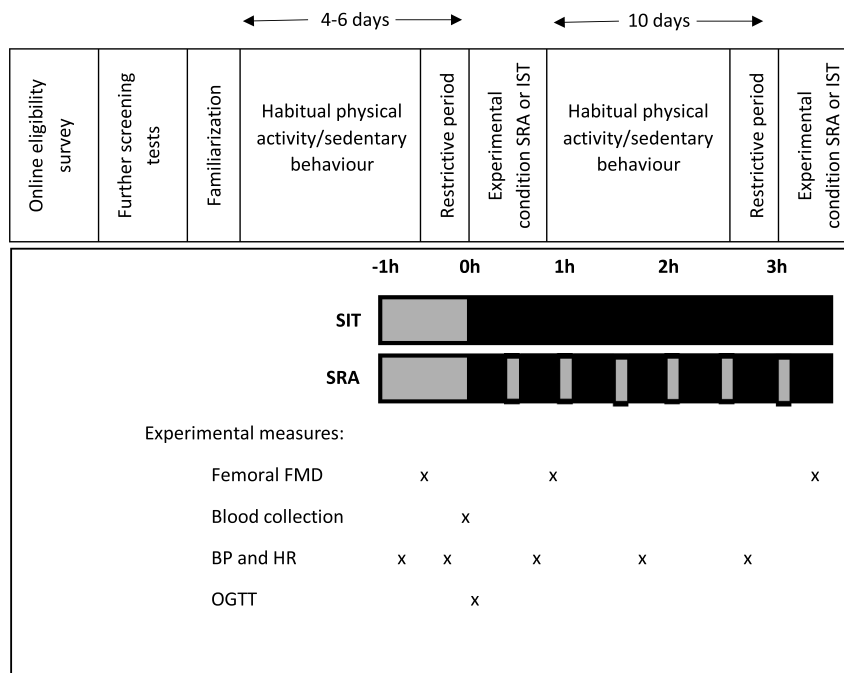
FIGURE 1—Study design and protocol. Participants were initially screened online, followed by further screening tests if eligible. Eligible participants then attended a familiarization visit followed by two experimental conditions in a random order. HR, heart rate; OGTT, oral glucose tolerance test; SIT, uninterrupted sitting condition; SRA, sitting interrupted by the simple resistance activities condition.

Measurements Arterial Function

All FMD measurements were completed in accordance with current evidence-based guidelines (6). Vascular function assessments were performed in a quiet, darkened, temperature-controlled (22°C–25°C) room in a seated position. Participants were left to equilibrate to the darkened room for ~15 min before assessment, and they were instructed to place both feet flat on the floor. The superficial femoral artery was measured in the right leg using a 10-MHz multifrequency linear array probe in conjunction with a high-resolution duplex ultrasound (Terason t3200; Teratech, Burlington, MA) machine at an insonation angle of 60°. A rapid inflation cuff (SC-12-D; D.E. Hokanson Inc., Bellevue, WA) was placed around the thigh, distal to the ultrasound probe. A 1-min recording of blood velocity and continuous resting vessel diameter was measured (live duplex mode) once an optimal image of the artery was obtained. The cuff was then inflated (~220 mm Hg) for 5 min. After 5 min of inflation, the cuff was released to induce reactive hyperemia, and continuous duplex ultrasound recording continued for a further 3 min to observe the postdeflation diameter and peak response. To avoid any transient effects of SRA that may have influenced the measurement, FMD measures occurred right before the SRA and 20 min after the previous activity bout. Placement of the probe was marked and recorded on the first scan at the first visit and replicated for corresponding vascular measurements.

One participant's FMD data were excluded from this analysis because we did not have a complete valid data set. One scanner performed analysis of femoral artery diameter and blood velocity using automated edge detection and wall tracking software (19). Analysis of ultrasound recordings was performed using LabVIEW (version 6.02; National Instruments, Austin, TX). This software has previously been demonstrated to significantly reduce observer error with an intraobserver CV of 6.7% (19). FMD was calculated as the percentage increase in peak diameter from the resting baseline diameter and was measured during the steady-state period (0 h), at 1 h, and at 3.5 h. Shear rate (s^{-1}), derived from blood velocity and diameter, was used as an assessment of shear stress on the artery wall. Shear rate area under the curve from time of cuff release to peak dilation was used to define shear stimulus (20). For this study, our between-visit reproducibility was 4.5%.

Resting BP

Seated resting brachial BP was measured at 5 points across the day, taken at hourly intervals in accordance with recommended guidelines. BP was measured 5 min after activity bouts and in triplicate, at 1-min intervals using an automated BP monitor (Dinamap Vital Signs Monitor 184465X, HEM-907; Omron, Kyoto, Japan) using an appropriately sized cuff (8). All measurements were repeated on the same arm between conditions. For analysis, an average of the three measurements was used.

Biochemical Analysis

To characterize baseline insulin resistance, fasting blood samples were collected during the steady-state period. Whole blood glucose levels were completed in duplicate via a point-of-care HemoCue glucose analyzer (HemoCue Glucose 201+ System, Canada AB) within 5 min of collection, using a modified dehydrogenase method and photometric detection.

For plasma equivalent results, the plasma conversion was made according to the International Federation of Clinical Chemistry using the factor of 1.11. Whole blood was also drawn into serum separator tubes and rested for 30 min before being centrifuged (2000 rpm for 15 min at 4°C). The serum fraction was then separated and stored at −80°C. Testosterone, sex hormone binding globulin, and serum insulin were determined using chemiluminescent microparticle immunoassay (Abbot Alinity) by an independent laboratory accredited by the National Association of Testing Authorities/The Royal College of Pathologists of Australasia (Alfred Pathology, Melbourne) according to manufacturer's instructions.

Statistical Analysis

All analyses were performed using R statistical programming language (version 3.6.1, 2019) (21). Based on previously published work (14), we anticipated an effect size of 3.28, and assuming >90% power and an alpha level = 0.05, we would require a sample size of 13. The primary outcome was FMD. We examined the within- and between-condition effects using generalized linear mixed models. Outcome variables were adjusted for age, BMI, day since commencement of last menstrual period, values at 0 h, and condition order. Additional adjustment for resting diameter and shear stimulus were used on FMD models (6). A condition–time interaction with *post hoc* comparisons was used to compare individual time points between conditions and within condition relative to 0 h. *Post hoc* comparisons between time points were adjusted for multiple comparisons using Šidák corrections. Descriptive data are presented as means ± SD, and output from mixed model analyses is presented as marginal mean ± SEM, where $P < 0.05$ was considered statistically significant.

RESULTS

Participant characteristics. Of the 14 participants randomized, 1 participant withdrew after the first visit because of personal reasons unrelated to the study, with 13 participants completing both experimental conditions (Fig. 2). The participant characteristics are presented in Table 1, and preexperimental
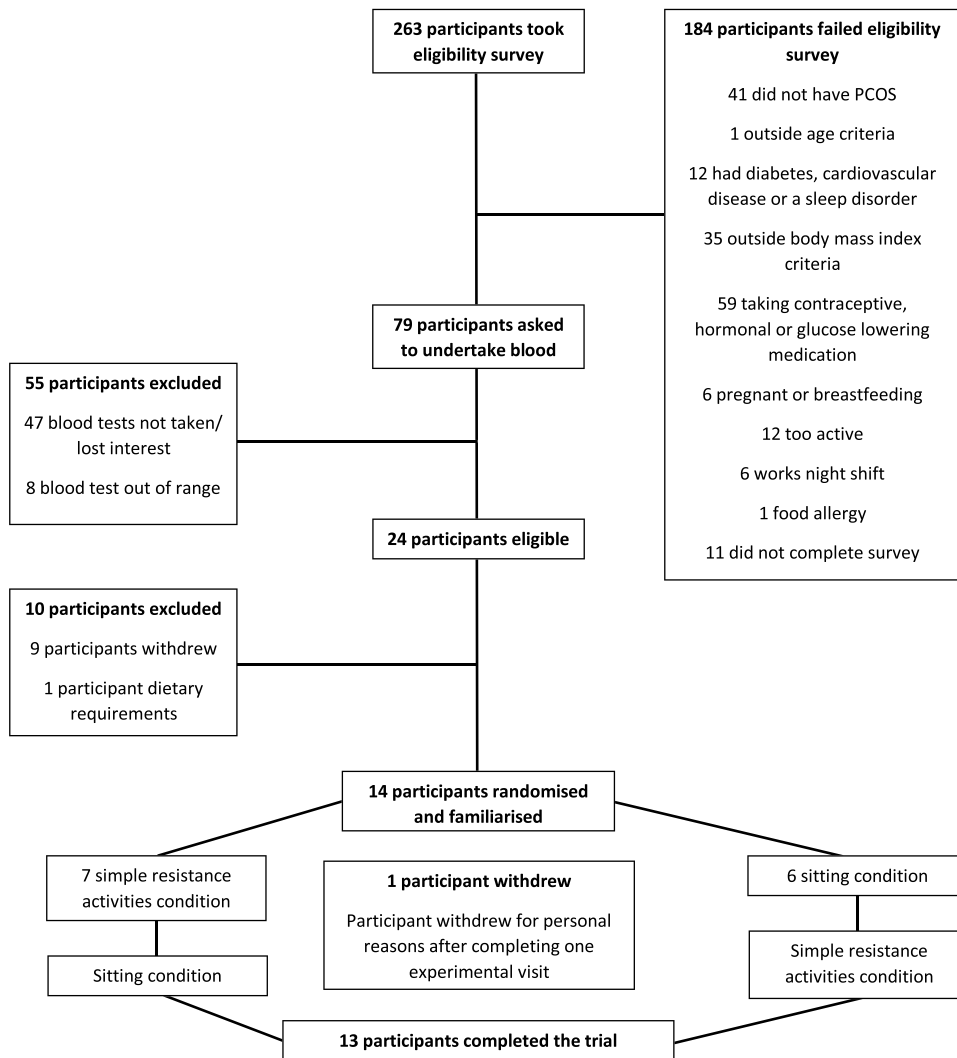
FIGURE 2—Consort standards of reporting trials (CONSORT) diagram.

TABLE 1. Participant characteristics.

	Participants ($n = 13$)
Clinical characteristics	
Age (yr)	32.2 ± 6.3
BMI ($kg \cdot m^{-2}$)	30.2 ± 5.3
Weight (kg)	81.5 ± 13.4
Waist circumference (cm)	98.5 ± 14.3
Waist-to-hip ratio	0.9 ± 0.1
SBP (mm Hg)	111 ± 10
DBP (mm Hg)	74 ± 10
Ferriman–Gallwey score	12.9 ± 8.1
Biochemical and metabolic parameters	
Testosterone ($nmol \cdot L^{-1}$)	1.5 ± 0.6
SHBG ($nmol \cdot L^{-1}$)	44.1 ± 29.6
FAI	4.0 ± 1.7
Fasting glucose ($mmol \cdot L^{-1}$)	4.7 ± 0.3
Fasting insulin (pmol)	51.6 ± 15.9
HOMA-IR	1.8 ± 0.5
HOMA2-IR	1.1 ± 0.3

Data are presented as mean ± SD. The Ferriman–Gallwey score is for hirsutism.
DBP, diastolic BP; FAI, free androgen index; HOMA-IR; homeostatic model assessment of insulin resistance; SBP, systolic BP; SHBG, sex hormone binding globulin.

period data are presented in the supplemental content (see Table, Supplemental Digital Content 1, Pre-experimental period, http://links.lww.com/MSS/C139). No differences in resting brachial systolic or diastolic BP, averaged across 3.5 h, were observed between the SIT and the SRA conditions, respectively (systolic BP: 105 ± 3 vs 106 ± 3 mm Hg, $P = 0.668$; diastolic BP: 68 ± 2 vs 67 ± 2 mm Hg, $P = 0.701$). There were also no differences in mean heart rate averaged over 3.5 h between SIT and SRA conditions, respectively (66 ± 3 vs 68 ± 4 bpm; $P = 0.685$).

FMD and hemodynamics. The data of 12 participants were analyzed for FMD. The hemodynamic and absolute (i.e., unadjusted) FMD data are presented in supplemental content [see Table, Supplemental Digital Content 2, Hemodynamic and absolute (i.e., unadjusted) flow-mediated dilation data during 3.5 h of uninterrupted sitting and sitting interrupted with simple resistance activities, http://links.lww.com/MSS/C140]. Supplemental Digital Content 3 shows the adjusted data with statistical comparisons (see Table, Supplemental Digital Content 3,

Hemodynamic and adjusted flow-mediated dilation data during 3.5 h of uninterrupted sitting and sitting interrupted with simple resistance activities, http://links.lww.com/MSS/C141). No significant between-condition ($7.31\% \pm 0.61\%$ vs $7.40\% \pm 0.63\%$, $P = 0.883$) or within-condition ($P > 0.438$ for all; see Table, Supplemental Digital Content 3, Hemodynamic and adjusted flow-mediated dilation data during 3.5 h of uninterrupted sitting and sitting interrupted with simple resistance activities, http://links.lww.com/MSS/C141) differences were observed for femoral artery FMD in SIT, relative to SRA condition. Femoral artery FMD averaged across 3.5 h was not significantly different between SIT and SRA conditions ($7.66\% \pm 1.27\%$ vs $7.50\% \pm 1.27\%$, $P = 0.447$; Fig. 3A). Femoral artery FMD change from baseline was not significant between conditions ($P = 0.923$), nor within conditions ($P > 0.543$ for all; Fig. 3B). Additional adjustment for resting diameter and shear stimulus did not change the interpretation of the results for SIT vs SRA.

Mean resting femoral shear rate, averaged across 3.5 h, was significantly higher in the SRA condition compared with SIT (62.8 ± 6.1 vs 40.1 ± 6.1 s^{-1}, $P < 0.001$). Mean resting femoral blood flow, averaged across 3.5 h, was significantly higher in the SRA condition compared with SIT (72.8 ± 9.9 vs 45.0 ± 9.8 $mL \cdot min^{-1}$, $P < 0.001$). No significant differences were observed between conditions for baseline diameter ($P = 0.597$). Further analysis on common phenotypic differences did not yield any common factors in our participants.

DISCUSSION

To our knowledge, this is the first study to examine the acute effects of interrupting prolonged sitting time on endothelial function in women with PCOS. Femoral artery function (measured via FMD) remained relatively constant between the SIT and the SRA conditions across the 3.5-h trial duration, with minimal evidence of a consistent improvement in FMD
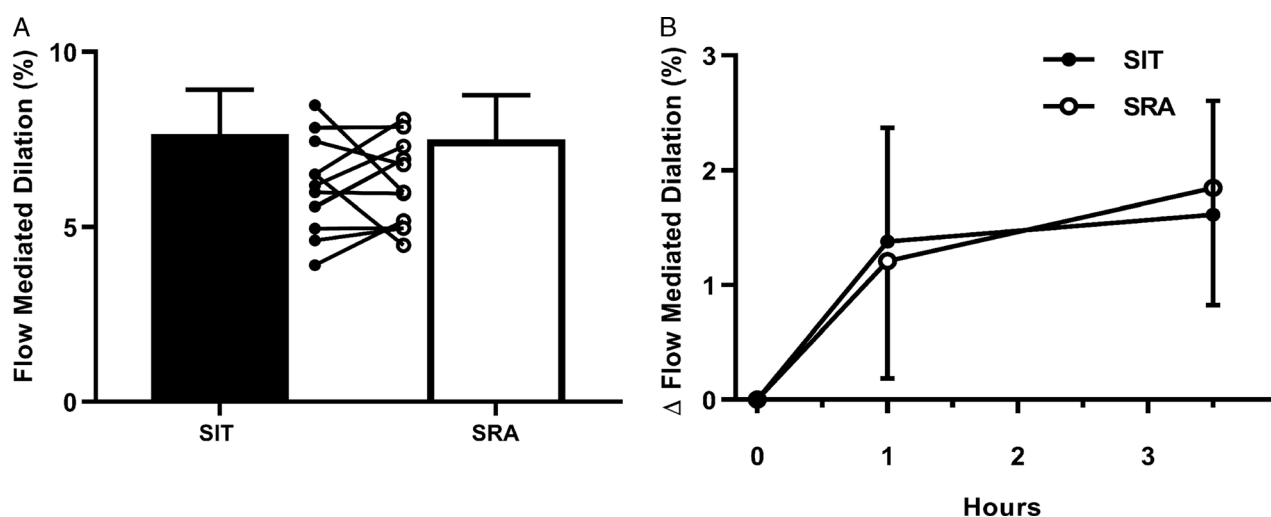
FIGURE 3—A, Mean femoral artery FMD over 3.5 h in the uninterrupted sitting (SIT) and sitting interrupted with SRA conditions. B, A time course of change from baseline of femoral artery flow-mediated (FMD) in the two conditions. Data are adjusted for values at 0 h, age, BMI, days since last period, and treatment order. Mean femoral artery FMD was additionally adjusted for resting diameter and shear stimulus. Data are marginal mean ± SE.

with SRA relative to SIT overall or at specific time points. However, statistically significant increases in both resting shear rate and blood flow were observed at 1 and 3.5 h in the SRA condition relative to the SIT.

Contrary to previous studies (13,14,22), vascular function did not decrease in our sample of women with PCOS after a bout of prolonged sitting. It is plausible that the effects of sitting on endothelial function were not as pronounced in our sample on the basis that we recruited women with PCOS who did not yet have clinically impaired vascular function. Women were recruited based on the Rotterdam criteria, which have been commonly used in observational studies examining endothelial function in PCOS (3). However, in many of these studies (23–26), women with PCOS had more severe insulin resistance and biochemical hyperandrogenism compared with our participants. For example, El-Kannishy et al. (2010) and Kravariti et al. (2005) observed testosterone measurements ranging from 2.50 to 2.95 nmol·L^{-1}. By comparison, participants in our study reported testosterone levels of 1.5 ± 0.6 nmol·L^{-1} despite being of similar age and BMI. Moreover, the homeostatic model assessment of insulin resistance index for our participants more closely resembled that of the control (i.e., healthy) women in the aforementioned studies. El-Kannishy et al. (2010) and Kravariti et al. (2005) also reported lower baseline FMD, ranging from 3.50% to 4.13%, relative to our participants 6.6%. Indeed, current literature indicates that the effect of diet and exercise on endothelial function in PCOS has mixed results (7–9). It is possible this is due to a variance in the severity of PCOS. The reduced biochemical hyperandrogenism and insulin resistance severity may partly explain why our study did not demonstrate impairments in the SIT condition, or improvements in the SRA condition.

There was a small, albeit nonsignificant, increase in femoral FMD from baseline in both the SIT and the SRA conditions (see Table, Supplemental Digital Content 3, Hemodynamic and adjusted flow-mediated dilation data during 3.5 h of uninterrupted sitting and sitting interrupted with simple resistance activities, http://links.lww.com/MSS/C141). This is surprising, given that previous work has reported prolonged sitting to impair macrovascular function in both young, healthy (13,27), and older overweight/obese populations (14). However, these studies have been performed primarily in young, healthy men, with recent investigations into the effects of prolonged sitting in women reporting mixed results (22,27). Of relevance, and in line with our work, both these studies observed a subset of women who reported small or no changes in FMD after the SIT protocol. This supports previous suggestions that some young women may be more susceptible to sitting-induced endothelial dysfunction than others (22,27). Given the relationship between habitual activity and vascular function (28), sedentary behavior may, in part, explain the variance among women. Nevertheless, the lack of objectively monitored activity data and small sample sizes in studies to date makes it difficult to develop definitive inferences (22,27).

Although we did not observe a marked increase in FMD for the SRA condition, we did observe a significant increase in resting blood flow and shear rate at 1 and 3.5 h for SRA, compared with SIT. This is noteworthy because reduced leg vascular shear stress is likely the primary mediator of impaired endothelial function in lower extremity conduit arteries (29–31). Although the magnitude of increase in blood flow and, consequently, shear stress needed to induce a clinically meaningful improvement in vascular function is currently unknown, similar increases in shear stress have been reported in women with PCOS after a 12-wk exercise intervention (8). The increase in resting blood flow and shear rate occurred in the SRA condition despite no observed increase in FMD, which has also been reported in previous work (32). This could be partly due to the relatively "normal" baseline FMD in our subset of women with PCOS, making it harder to improve FMD, on average. However, given that in some women FMD improved, a more appropriately powered study may find different results. Nevertheless, shear stress has been recognized as an important physiological factor in maintaining endothelial health (29,31). At the same time, reduced blood flow and shear rate have been implicated in endothelial impairment, with the development of atherosclerotic lesions noted in arterial regions characterized by low shear stress (30). Given that the lower-limb vasculature is susceptible to atherosclerosis, and reduced shear is the primary mediator of impaired vascular function, SRA that promotes increased blood flow and shear stress may benefit leg endothelial function for women with PCOS over the long term.

No statistically significant changes in the mean systolic BP, diastolic BP, or heart rate measures were observed between conditions over the trial period. These results contrast those of previous studies that reported a systolic BP-lowering effect with short bouts of activity (33,34). This discrepancy may be related to the relatively low resting BP and/or methodological differences, including the shorter times between active breaks and BP measurements compared with previous studies (35,36).

The well-controlled randomized crossover design is a strength of this study because it provides control for person-specific factors and affords smaller sample sizes. Trial conditions were also strictly supervised and standardized, with restrictive periods before testing days (minimal variance in physical activity levels and diet) monitored through the use of weighed food records and objectively monitored activity data. We also adopted a seated posture for FMD measurements, rather than supine, for "steady state" and throughout the experiment, to reduce the influence of postural changes throughout the study.

The present trial also has limitations future studies could address. This study was performed in a laboratory-based setting. Although beneficial for establishing initial proof of concept, home- and/or work-based sitting reduction studies may more accurately reflect the effect of prolonged sitting on vascular function in a real-life setting. Similarly, given that food has the potential to influence postprandial responses, the use of standardized mixed meals in place of an oral glucose tolerance test may have changed the blood flow response and confounded the results. Further, this was an acute exposure study, and we only examined responses to sitting and breaking up sitting over a 3.5-h period. It is possible that longer-term exposures

may produce different results and assist in gaining a better understanding for the long-term cardiovascular health implications (14). Future research may also establish the efficacy and dose–response relationships associated with SRA breaks, and how different frequencies, intensities, and durations may be applied in free-living settings. The study is also limited by the small sample size, although our findings are in line with previous studies investigating FMD (%) in women (27), and we still observed a change in resting shear stress. Finally, PCOS in this study was defined by the more variable Rotterdam criteria. Given that the Rotterdam PCOS phenotype is associated with a less severe metabolic profile, compared with the classic National Institutes of Health PCOS subtype (oligomenorrhea and elevated testosterone), it is possible that the study may be underpowered to find a difference in non-NIH phenotypes (37). Unfortunately, it was not within the scope of this study to measure estrogen levels, which may have indicated the severity of PCOS in our participants.

In this sample of women with PCOS, we demonstrated that breaking up sitting increased resting blood flow and shear rate but did not alter FMD across 3.5 h when participants undertook brief periods of SRA. Given that women with PCOS report increased sedentary time compared with controls, and that high volumes of sitting contribute to increased risk for all-cause and CVD-related mortality, frequent brief bouts of SRA may provide an additional therapeutic target to maintain healthy vascular function via improved blood flow and shear rate. Future research should aim to examine the longer-term effects of sedentary behavior in women with differing presentations and severities of PCOS and the effectiveness of interventions on arterial function that aim to reduce and break up sitting.

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Supplemental Digital Content - Table 1. *Pre-experimental period*

	SIT	SRA
Weight (kg)	81.1 ± 13.3	82.1 ± 14.2
Days since first day of last menstrual period	29 ± 41	31 ± 38
ActivPAL data[†]		
Sitting time (min/day)	509 ± 124	520 ± 155
Standing time (min/day)	251 ± 110	250 ± 117
Total stepping time (min/day)	101 ± 28	100 ± 33
Total number steps	4048 ± 1220	3808 ± 1331
Diet[‡]		
Total energy intake (kJ/day)	8244 ± 1297	7941 ± 1436
Total carbohydrate (energy %)	50 ± 11	49 ± 11
Total fat (energy %)	33 ± 9	34 ± 10
Total protein (energy %)	15 ± 5	15 ± 5

Data are mean ± SD. [†]ActivPAL data collected during the 48-h restricted period before the trial condition. Due to incomplete valid data sets 8 participants ActivPAL data were analyzed for the SIT condition, and 9 participants ActivPAL data were analyzed for the SRA condition. [‡]Dietary intakes were assessed from weighed/measured food records during the 24-h preceding the trial condition, using dietary analysis software (FoodWorks; Xyris Software, Highgate Hill, Queensland, Australia). No significant differences were found between conditions.

Supplemental Digital Content - Table 2. *Hemodynamic and absolute (i.e., unadjusted) flow-mediated dilation data during 3.5h of uninterrupted sitting and sitting interrupted with simple resistance activities.*

	0h	1h	3.5h
SIT FMD, %	6.9 (3.5)	7.4 (4.2)	8.0 (2.6)
SRA FMD, %	6.6 (2.1)	7.5 (3.8)	8.4 (3.5)
SIT resting diameter, mm	5.6 (0.6)	5.5 (0.5)	5.5 (0.6)
SRA resting diameter, mm	5.6 (0.4)	5.5 (0.4)	5.5 (0.5)
SIT peak diameter, mm	6.0 (0.6)	6.0 (0.6)	5.9 (0.5)
SRA peak diameter, mm	5.9 (0.5)	5.9 (0.5)	6.0 (0.4)
SIT shear stimulus, SR_{AUC} (au)	14548 (6585)	11176 (5700)	14945 (5582)
SRA shear stimulus, SR_{AUC} (au)	18420 (5310)	16389 (6419)	20785 (8866)
SIT antegrade, SR_{AUC} (au)	14904 (6718)	11653 (5801)	15933 (5129)
SRA antegrade, SR_{AUC} (au)	18747 (5396)	16882 (6850)	21496 (9181)
SIT resting blood flow, ml/min	44 (17)	44 (24)	47 (20)
SRA resting blood flow, ml/min	66 (42)	77 (31)	82 (39)
SIT time to peak, s	55 (21)	44 (27)	66 (36)
SRA time to peak, s	52 (13)	49 (33)	74 (26)
SIT resting shear rate, s^{-1}	40.6 (13.1)	40.1 (15.8)	42.4 (16.4)
SRA resting shear rate, s^{-1}	47.2 (25.8)	57.9 (20.9)	75.4 (25.3)

Data are mean ± SD. au, arbitrary units; AUC, total area under the curve; FMD, flow-mediated dilation; SIT, uninterrupted sitting; SRA, sitting interrupted with 3-min simple resistance activities every 30-min; SR, shear rate.

Table 3. *Hemodynamic and adjusted flow-mediated dilation data during 3.5h of uninterrupted sitting and sitting interrupted with simple resistance activities.*

	0h	1h	3.5h	Group	Time	Group x time interaction
SIT FMD, %	6.7 (5.1, 8.4)	8.6 (6.9, 10.3)	8.1 (6.6, 9.7)	P = 0.453	Baseline: 1h	Group: 1h
					P = 0.052	P = 0.829
					Baseline: 3.5h	Group: 3.5h
SRA FMD, %	6.0 (4.3, 7.7)	7.6 (5.9, 9.2)	7.6 (5.9, 9.3)		P = 0.127	P = 0.889
SIT resting diameter, mm	5.7 (5.2, 6.1)	5.6 (5.1, 6.0)	5.5 (5.1, 6.0)	P = 0.597	Baseline: 1h	Group: 1h
					P = 0.049	P = 0.342
					Baseline: 3.5h	Group: 3.5h
SRA resting diameter, mm	5.6 (5.2, 6.1)	5.6 (5.2, 6.1)	5.6 (5.2, 6.1)		P = 0.012	P = 0.112
SIT resting blood flow, ml/min	41.7 (21.4, 62.0)	45.6 (25.5, 65.8)	46.5 (26.3, 66.7)	P = 0.001	Baseline: 1h	Group: 1h
					P = 0.568	P = 0.365
SRA resting blood flow, ml/min	65.4 (45.0, 85.8)*	78.4 (58.0, 98.7)**	70.8 (50.4, 90.1)*		Baseline: 3.5h	Group: 3.5h

					P = 0.488	P = 0.955
SIT resting shear rate, s^{-1}	37.6 (24.4, 50.8)	39.6 (26.4, 52.8)	41.4 (28.2, 54.6)	P = 0.030	Baseline: 1h	Group: 1h
					P = 0.627	P = 0.161
					Baseline: 3.5h	Group: 3.5h
SRA resting shear rate, s^{-1}	47.1 (33.8, 60.4)	57.6 (44.4, 70.9)**	70.3 (57.0, 83.6)** †		P = 0.364	P = 0.002

Data are marginal means ± 95 CI's. Data are adjusted for age, body mass index, days since last period and treatment order. Additional adjustment for resting diameter and shear stimulus were used for FMD. Time points 1h and 3.5h additionally adjusted for value at 0h. *$P < 0.05$ relative to SIT condition, **$P < 0.001$ relative to SIT condition, †$P < 0.05$ within condition vs. 0 h. FMD, flow-mediated dilation; SIT, uninterrupted sitting; SRA, sitting interrupted with 3-min simple resistance activities every 30-min.

6.3 Chapter 6 Summary

The findings presented in Section 6.2 are the first to examine the acute effects of interrupting prolonged sitting time on vascular function in women with PCOS. While femoral artery vascular function remained relatively consistent between the SRA and SIT conditions, statistically significant increases were observed in both resting shear rate and blood flow. Similar results for resting shear rate and blood flow were observed in Section 5.2. Given that reduced shear is a primary mechanism responsible for impaired vascular function, SRA's that promote increased blood flow and shear rate may benefit lower-limb vascular function for women with PCOS in the longer term.

The results observed in Section 6.2 were not in line with previous studies, in which prolonged sitting has been reported to impair vascular function in both young healthy [28, 126] and older/overweight [2] populations. However, as noted in Section 4.2, the majority of sedentary behaviour and vascular studies have been performed in men. Indeed, the lack of objectively monitored activity data, and studies involving small sample sizes, makes it difficult to develop definitive inferences for women, and specifically women with PCOS [126, 156].

This study was performed in a laboratory setting, and whilst beneficial for establishing initial proof-of-concept, future research should focus on assessing vascular function in a 'real-life' setting. Additionally, given two definitions for PCOS currently exist, future larger scale studies should take the severity of PCOS into consideration. Nevertheless, Section 6.2 has shown that interrupting sitting with activity may provide an additional therapeutic target to maintain health vascular function in women with PCOS.

CHAPTER 7 – GENERAL DISCUSSION, LIMITATIONS AND CONCLUSION

General Discussion

This thesis presents novel research which has investigated the impact of prolonged sitting, and interrupting prolonged sitting with intermittent activity, on vascular function across states of abnormal glucose metabolism. It includes one of the first studies to report progressive impairment in lower-limb vascular function as time spent sitting increases up to 180 minutes in adults. Additionally, this thesis contains studies which focus on underrepresented populations in the vascular function and sedentary behaviour research field, including women with PCOS and those with T2D, providing additional insight into the differential effects of prolonged sitting in people with metabolic disturbances. Importantly, this research highlights that interrupting sitting more frequently with shorter duration SRAs may be more effective to increase superficial femoral artery vascular health, relative to less frequent, longer SRAs for those with T2D. Finally, it provides recommendations for future research based on existing gaps in the literature such as in women with PCOS and older adults with T2D, which could assist with improving understanding of how vascular function changes across abnormal states of glucose metabolism in response to prolonged sitting.

Notably, meta-analyses of FMD data and acute prolonged sitting demonstrate that prolonged sitting leads to a significant decline in vascular function [30, 31, 103]. In Chapter 4 I demonstrate that 120-min of continuous prolonged sitting may represent a critical threshold at which lower-limb vascular susceptibility is exacerbated [31]. While in isolation, short periods of lower-limb vascular impairment may not be particularly concerning, repetitive exposure to sedentary behaviour exacerbates the impact of vascular dysfunction on longer term arterial health. With rapidly advancing technologies in workplaces, transportation and home entertainment, fewer opportunities exist for incidental activity, creating many settings in daily life that are conducive to multiple bouts of prolonged sitting. Indeed, accelerometer-based estimates indicate that adults spend approximately 8.2 h/day sedentary [157] which is concerning given mortality risk in adults increases for sitting times greater than 7 or 8/h day (Figure 7.1) [158, 159]. Accordingly, it is important to consider the cumulative effects of acute episodes of prolonged sitting on lower-limb vascular function over many days, weeks, months, and years. Indeed, there are reasonable grounds to suggest that episodic reductions in lower limb vascular shear stress and function, associated with prolonged sitting, may contribute to

the pathogenesis of arterial diseases [4]. This is supported by epidemiological evidence which suggests that sedentary time is associated with low levels of ankle-brachial index, a predictive measure of peripheral arterial disease [102].
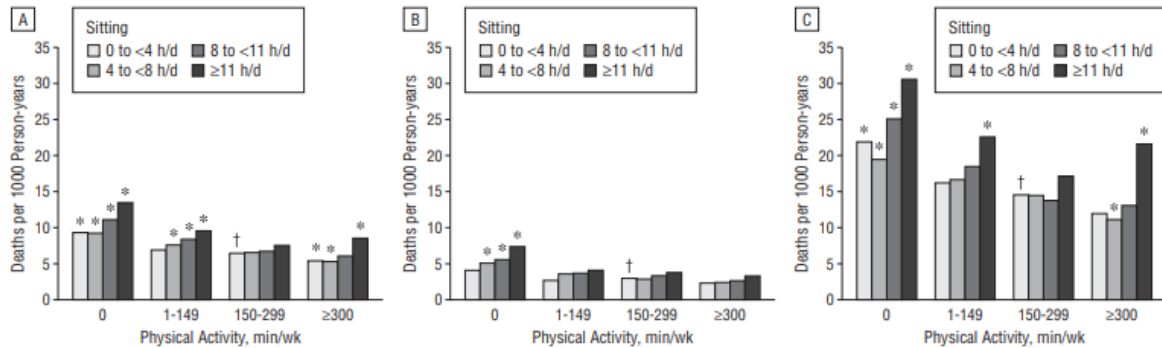
Figure 7.1 The combined relationships of sitting and physical activity with all-cause mortality. A, All participants (n = 222 497). B, Healthy participants who at baseline had no cardiovascular disease, diabetes mellitus, or cancer, with the exception of nonmelanoma skin cancer (n = 145 713). C, Participants with cardiovascular disease or diabetes at baseline (n = 52 229). Deaths per 1000 person-years were adjusted for sex, age, educational level, marital status, urban or rural residence, body mass index, smoking status, self-rated health, and receiving help with daily tasks for a long-term illness or disability. *P.05 compared with the reference group. †Reference group. Sourced from van der Ploeg (2011) [159].

Further, the observation in Chapter 4 that acute prolonged sitting has a more pronounced effect on lower limb vascular function, compared to upper limb function, supports the hypothesis that excessive sitting may predispose femoral and popliteal arteries to increased risk for atherosclerotic peripheral arterial disease [4, 31]. Evidence indicates that atherosclerotic peripheral arterial disease primarily manifests in the lower extremity vasculature, with upper limbs appearing more atheroresistant [4]. The reason for these differences in structural and functional changes between upper and lower limbs remains unclear [160]. However, research investigating cardiovascular disease risk in those with spinal cord injuries may provide insight. People with spinal cord injuries report greater arterial intima-media thickening [161], an important indicator for future cardiovascular disease [162]. Indeed, lack of physical activity in the lower limbs has been postulated as a mechanism for impaired lower-limb vascular function in those with spinal cord injuries. Consistent with these observations relating inactivity to arterial dysfunction, a cross-sectional analysis of 945 men reported an odds ratio of 1.19 (95% CI 1.07, 19 1.33) for a low ankle-brachial index with each additional 30-min increment in

sedentary time. Taken together, these studies suggest repetitive prolonged sitting may place the lower limb vasculature at increased risk for atherogenesis.

The observation in Chapter 4 that prolonged sitting has no significant effect on upper limb vascular function warrants further comment. Exercise studies have consistently reported improved vascular function in the untrained upper limbs following lower limb cycling training [138, 139]. It is therefore likely that, despite the modest effect of prolonged sitting on upper limb vascular function reported in Chapter 4, impacts may accumulate over many days, months, and years. Indeed, bed rest studies assessing vascular function over multiple weeks have induced rapid structural remodeling of resistance and conduit arteries [163]. Future studies that measure vascular function more frequently, across weeks and months, are needed to provide greater insight into the time-course of vascular change and adaptation in response to prolonged sitting in both the upper- and lower-limbs.

For adults who are older, or those with metabolic disturbances, the structural remodeling of resistance and conduit arteries following sedentary behaviour may have important implications. Indeed, prolonged sitting-induced vascular impairment may further compound the influence of pre-existing cardiovascular risk factors such as older age, obesity, and T2D [150]. The findings of the experimental studies in Chapter 5 and 6 expand on this area by including older adults with T2D, and women with PCOS, respectively. However, neither of these studies reported significantly reduced femoral vascular function following a bout of acute prolonged sitting [144, 164]. These results are consistent with the findings in Chapter 4, and may reflect the law of initial values [165]. That is, as these populations are predisposed to impaired vascular function, *a priori*, the magnitude of response to prolonged sitting is relatively diminished. Put simply, it is more difficult to demonstrate an impairment in vascular function if arterial health is already compromised. Nevertheless, it is important to note that following the first hour of sitting in the papers presented in both Chapters 5 and 6, shear rate, the key physiological stimulus responsible for maintaining vascular health, declined in both groups. Climie *et al*. (2019), reported similar findings, observing that shear stimulus declined over 5 hours of prolonged sitting in adults with overweight and obesity [2]. Given that older individuals and those with metabolic disturbances, spend more time in sedentary behaviour relative to young and healthy individuals [26, 166-169], the repetitive transient decline in shear following prolonged sitting induce to additional vascular dysfunction over time. Prospective

and longer duration intervention studies, in free-living settings, are needed to confirm this hypothesis.

Reducing and interrupting time spent in prolonged sedentary behaviours has been identified as a key area for the prevention and management of cardiovascular disease in adults with metabolic disturbances [93]. However, current guidelines on sedentary behaviour are broad and non-prescriptive, recommending adults 'sit less and move more.' It is currently unknown whether different frequencies and duration of breaks differentially influence vascular outcomes across the abnormal glucose metabolism spectrum. Chapter 5 provides new evidence by comparing different frequencies of interruptions to prolonged sitting over 7h, on vascular function in adults with T2D. This data demonstrated that more frequent, shorter activity interruptions may be more beneficial than longer, less frequent interruptions for improvements in vascular function in those with T2D. However, it should be noted that this sample of participants presented with more-advanced T2D (as evidenced by the high number of anti-hyperglycaemic medications used and duration of disease). Given that there is a progressive impairment in vascular function along the pathogenic spectrum of T2D [15], it is possible that more frequent interruptions to sitting (i.e. greater stimulus) are needed to preserve leg blood flow [4] and mitigate sitting-induced vascular impairments in this population.

The observation that interruptions to sitting may differentially affect the vascular function of distinct population groups was a hypothesis that was further explored in Chapter 6. As highlighted in Chapter 4, there remains a primary focus on young, healthy male subjects in the sedentary behaviour and vascular research field, making it difficult to extrapolate findings to the broader population. Indeed, it is conceivable that sex differences may be present in the physiological responses to sitting interruptions, given the well-described female hormonal impacts on vascular function [155]. Chapter 6 aimed to address this evidence gap by exploring whether interrupting prolonged sitting with activity breaks was beneficial for improving vascular function in women with PCOS. While femoral artery FMD did not increase for the activity breaks condition, relative to prolonged sitting, statistically significant increases were observed for resting shear and blood flow. Given the relatively 'normal' baseline FMD response in our subset of women with PCOS, it is likely that the potential for observing changes in FMD over the three-hour period was diminished. It should be noted that relatively 'normal' baseline FMD responses are not typically observed in women with PCOS, and this may suggest

that the women we recruited may not have exhibited clinically impaired vascular function. Still, the simple resistance activities in Chapter 6 promoted increased blood flow and shear stress in women with PCOS. Thus, this chapter provides preliminary evidence that interrupting sitting with activity breaks may represent a strategy to improve the underlying stimulus responsible for enhancing vascular function in women with PCOS.

Limitations of the Thesis

There are several inherent limitations to the research presented in this thesis that must be acknowledged. The paper in Chapter 4 was the first meta-analysis to examine the time course between prolonged sitting and vascular function in both upper and lower limbs. However, it is important to acknowledge that only a small number of studies included 30- and 60-minute time points. Therefore, it is possible that with additional studies including these time points, decreased vascular function may have been observed earlier than 120 minutes. Further, our search criteria did not include terms such as "activity interruptions." However, given that to be eligible for selection the studies needed to compare activity breaks to prolonged sitting, it is likely the articles were captured in the search. Finally, as previously highlighted in this thesis, due to small number of studies recruiting female participants and older and/or clinical populations, this meta-analysis included mostly young, male subjects.

Due to recruitment issues impacting the activities for the study reported in Chapter 5, the inclusion criteria were widened to T2D patients taking at least one, but no more than three anti-hyperglycaemic medications. While the inclusion of a wide range of T2D diagnosis was more reflective of the general T2D population, there was greater heterogeneity in the study sample, and this may have influenced vascular outcomes. However, we experimentally controlled for this according to established guidelines [52, 53]. Previous studies have also employed tighter control over leg movements during sitting [28, 170], compared with my study. It is possible that this habitual, unstandardised movement may have contributed to the lack of statistically significant difference in time-condition interaction [127]. Our approach attempted to emulate a "real world" circumstance as best as possible, and on average we observed improvements with interruptions to sitting across the day. All FMD measurements were collected in the seated position. Although it is acknowledged that current FMD guidelines stipulate that assessments be performed in the supine position, movement between seating and supine would necessitate muscular activity that may have impacted FMD measures [28]. Finally, offline analysis was not performed by a blinded observer. However, automated edge detection and wall tracking software was used,

consistent with published guidelines [52, 53]. It should also be noted that the sample size was calculated based on a previous study which included a more homogenous sample of diet- or metformin-controlled T2D patients [171].

Chapter 6 had similar limitations to Chapter 5 in regard to the following: FMD measurements were taken in a seated position and non-blinded observer FMD analysis was performed. However, there were additional limitations that future studies could address. This study was performed in a laboratory-based setting. Although beneficial for establishing initial proof of concept, home- and/or work-based sitting reduction studies may more accurately reflect the effect of prolonged sitting on vascular function in a real-life setting. Similarly, given that food has the potential to influence postprandial responses, the use of standardised mixed meals in place of an oral glucose tolerance test may have changed the blood flow response and confounded the results. An oral-glucose tolerance test was chosen for this study because it also assessed the impact of interrupting prolonged sitting on glucose metabolism. For this reason, we were only able to examine responses to sitting and breaking up sitting over a 3.5-h period, as beyond this, the participant would require a meal for a longer period of time. It is possible that longer-term exposures may produce different results and assist in gaining a better understanding for the long-term cardiovascular health implications (14). The study is also limited by the small sample size, although our findings are in line with previous studies investigating FMD (%) in women [126], and we still observed a change in resting shear stress. Finally, PCOS in this study was defined by the more variable Rotterdam criteria. Given that the Rotterdam PCOS phenotype is associated with a less severe metabolic profile, compared with the classic National Institutes of Health PCOS subtype (oligomenorrhea and elevated testosterone), it is possible that the study may be underpowered to find a difference in non-NIH phenotypes [172].

Opportunities for Future Research

As discussed within this thesis, there is opportunity to include a wider range of participants in future studies. Specifically, females, older adults, and adults across all stages of the abnormal glucose metabolism spectrum (IFG, IGT, T2D and T2D with complications). Indeed, this would aid in providing public health recommendations for these populations. Research focusing on FMD assessment during prolonged sitting is also needed. Currently, there is a mixture of studies measuring lower-limb FMD in varied postures. Indeed, it is possible that the

active movement between seated and supine may influence FMD results [164, 170]. Future research is needed to assess the validity and reliability of seated FMD measure.

Acute laboratory-controlled studies, like those in Chapters 5 and 6, are necessary to establish proof-of-concept, and contribute valuable knowledge which can lead to future studies. The next logical step is to focus on free-living and longer-term interventions. In free-living settings, it is rare for people to sit uninterrupted and natural interruptions to sitting are likely to occur. Additionally, it has recently been reported that free-living interruptions are less active than those that have been applied in a laboratory-controlled studies [173]. Future research should include longer-term, free-living studies which employ the use of activity monitors to report sitting accumulation patterns and breaks in sitting. When employed with regular measurements of vascular function (i.e., FMD measured over the space of several weeks or months), free-living studies that utilise body-worn activity monitors may help in understanding the long-term relationships between vascular function and sedentary behaviour. In particular, it may provide insight into the long-term impacts on the upper limbs.

Specific thresholds with respect to the frequency and intensity of activity interruptions beneficial for health are still unknown [93]. To address this and move towards more specific and prescriptive sedentary behaviour guidelines, future research may consider a 'dosing approach.' This was completed in Chapter 5 where the impact of interruptions at varying frequencies was compared. However, future studies may also employ a minimum stepping count, an intensity or activity [173]. This may help in determining whether a specific dosage of interruptions confers vascular health benefits. Additionally, given the co-existence of exercise and sedentary behaviour in the daily lives of many, future research may consider including structured exercise, in addition to breaks in sitting. Indeed, a morning bout of exercise combined with sitting interruptions have been shown to have an additional blood pressure lowering effect, relative to morning exercise alone [174].

Public Health and Clinical Implications of the Thesis Work

The outcomes of this program of research may have implications for sedentary behaviour guideline development in the future. Current evidence indicates that sedentary behaviour increases all-cause and cardiovascular mortality [24, 175]. However, in 2020 the WHO Guideline Development Group identified important gaps in the evidence which included; 1) lack of consensus on thresholds for sedentary time and disease risk, and 2) the absence of specific recommendations on how and how often to break up sitting with activity [140]. The

findings in this thesis provide additional evidence for these gaps. Chapter 4 reported that 2 hours of prolonged sitting may represent a critical threshold for adverse impacts on lower-limb vascular function. Additionally, it outlined future recommendations to recruit participants that are more representative of the general population, so that guidelines may be extrapolated across all population groups. Chapters 5 and 6 demonstrated that frequent half-hourly interruptions during sitting may benefit vascular health in both those with T2D and PCOS, respectively. However, while both studies observed increases in shear following activity breaks, that a greater exercise stimulus may be needed to observe FMD increases in populations further along the abnormal glucose metabolism spectrum.

Conclusion

The findings in this thesis provide further evidence that could assist in helping to inform the development and implementation of sedentary behaviour guidelines that improve vascular function across states of abnormal glucose metabolism. By seeking to further understand how the vascular system responds to sitting and interruptions in sitting, in high-risk populations, this thesis provides initial evidence on how sedentary behaviour reduction guidance may be modified to be more specific and relevant for varied population groups. The findings presented in this thesis have provided new perspectives for those living with abnormal glucose metabolism, and concurrently addressed the fundamental research questions posed in this thesis:

1. *What is the dose-response relationship between acute uninterrupted sitting and vascular function?*

Progressive impairment is observed in lower limb vascular function following acute prolonged sitting. Specifically, two hours of prolonged sitting was reported to significantly decrease femoral artery function. This study is a first step in helping to quantify thresholds for acute prolonged sitting and provides a foundation in determining the frequency and duration of activity interruptions.

2. *Does prolonged sitting affect the vascular function of individuals across the abnormal glucose metabolism spectrum differently?*

While prolonged sitting is detrimental to the vascular health of young, healthy adults, this adverse effect is less pronounced in adults with metabolic disturbances. Given there is a

progressive impairment in vascular function as the severity of metabolic disturbance increases, it is possible that the magnitude of response to prolonged sitting appears reduced in adults with metabolic disturbances. Future studies that focus on clinical populations with metabolic disturbances are needed determine whether the acute prolonged sitting threshold varies relative to healthy adults.

3. *What is the optimal frequency and duration at which sitting should be interrupted to improve vascular function in those with metabolic disturbance?*

For older adults with T2D, shorter and more frequent breaks may be more beneficial than longer, less frequent breaks for improvement in vascular function. However, resting shear rate and blood flow significantly increased relative to prolonged sitting for both frequencies. This evidence supports current evidence that reducing sedentary behaviour with activity of *any* duration and/or frequency is beneficial, but also provides new opportunity for more targeted public health recommendations regarding the patterns of break and bout accumulation.

CHAPTER 8 – APPENDICES

Appendix I: Research Portfolio

Publications related to thesis

1. **Taylor FC**, Pinto AJ, Maniar N, Dunstan DW, and Green DJ (2022). **The Acute Effects of Prolonged Uninterrupted Sitting on Vascular Function: A Systematic Review and Meta-analysis.** *Medicine and Science in Sport and Exercise.*

Contribution statement:

FCT was primarily responsible for development and execution of search strategy, preparation of figures, statistical analysis, writing and submission of manuscript, responding to reviewer's feedback and approval of final proofs. AJP assisted in the search and screening, preparation of tables, writing manuscript and response to reviewer's feedback. NM assisted with statistical analysis or interpretation. DWD and DJG contributed to the conception of the research question and study design and development of search strategy. All authors participated in critical revision of the manuscript for intellectual content and approved the final version of the manuscript. FCT and DWD are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Approximate percentage contributions: Taylor FC 50%; Pinto AJ 20%; Maniar N 10%; Dunstan DW 10%; and Green DJ 10%.

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2. **Taylor FC**, Dunstan DW, Fletcher E, Townsend MK, Larsen RN, Rickards K, Maniar N, Buman M, Dempsey PC, Joham AE, Cohen N, Owen N, Moran LJ, and Green DJ. (2020) **Interrupting Prolonged Sitting and Endothelial Function in Polycystic Ovary Syndrome**. *Medicine & Science in Sports & Exercise*.

Contribution statement:

FCT was primarily responsible for recruitment of participants, collection of data, analysis of data, statistical analysis, preparation of tables and figures, writing and submission of manuscript, responding to reviewer's feedback and approval of final proofs. EF, KR and MKT assisted with collection of data. DWD, EF, RNL, MB, PCD, AEJ, NC, NO, LJM and DJG assisted in the concept and design of the study and participated in critical revision of the manuscript for intellectual content. NM, PCD, DWD and DJG assisted with data cleaning or management and statistical analyses or interpretation. NC provided clinical support during data collection. All authors approved the final version of the manuscript. FCT and DWD are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Approximate percentage contributions: Taylor FC 52.5%; Dunstan DW 5%; Fletcher E 5%; Townsend MK 2.5%; Larsen RN 2.5%; Rickards K 2.5%; Maniar N 5%; Buman M 2.5%; Dempsey PC 5%; Joham AE 2.5%; Cohen N 2.5%, Owen N 5%, Moran LJ 2.5%, and Green DJ 5%.

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3. **Taylor FC**, Dunstan DW, Homer AR, Dempsey PC, Kingwell BA, Climie RE, Owen N, Cohen N, Larsen RN, Grace M, Eikelis N, Wheeler MJ, Townsend MK, Maniar N, and Green DJ. (2020) **Acute Effects of Interrupting Prolonged Sitting on Vascular Function in Type 2 Diabetes**. *American Journal of Physiology – Heart and Circulatory*.

Contribution statement:

FCT was primarily responsible for recruitment of participants, collection of data, analysis of data, statistical analysis, preparation of tables and figures, writing and submission of manuscript, responding to reviewer's feedback and approval of final proofs. ARH, MJW and MKT assisted with the recruitment of participants and collection of data. PCD, RNL, MJW, MG, DJG, NC, NO, BAK and DWD assisted in the concept and design of the study and participated in critical revision of the manuscript for intellectual content. NM, DWD and DJG assisted with statistical analysis or interpretation. NC provided clinical support during data collection. All authors approved the final version of the manuscript. FCT and DWD are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Approximate percentage contributions: Taylor FC 50%; Dunstan DW 5%; Homer AR 5%; Dempsey PC 5%; Kingwell BA 2.5%; Climie RE 2.5%; Owen N 5%; Cohen N 2.5%; Larsen RN 2.5%; Grace M 2.5%; Eikelis N 2.5%; Wheeler MJ 2.5%; Townsend MK 2.5%; Maniar N 5%; and Green DJ 5%.

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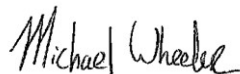
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Conference Presentations Related to Thesis

1. **Taylor FC,** Pinto AJ, Maniar N, Dunstan DW, Green DJ. (2021) The Acute Effects of Prolonged Uninterrupted Sitting on Vascular Function: A Systematic Review and Meta-Analysis, International Society for Physical Activity and Health; October 2021

    Contribution statement: This presentation was based on the work from publication one (see above for author contributions). The presentation was designed and delivered by FT.

2. **Taylor FC,** Dunstan DW, Homer AR, Kingwell BA, Dempsey PC, Climie RE, Owen N, Larsen RN, Wheeler MJ, Townsend MK, Maniar N, Green DJ. (2020) Acute Effects of Interrupting Prolonged Sitting on Vascular Function in Type 2 Diabetes. Thematic Poster Presentation at the Annual Meeting of the American College of Sports Medicine, June 2020 (Abstract published in conference proceedings).

    Contribution statement: This abstract and poster were based on the work from publication two (see above for author contributions). Due to COVID restrictions at the time, the abstract was published in conference proceedings and the poster was not displayed.

3. **Taylor FC.** The Sitting Spectrum. 3-minute thesis given at The Australian Society for Medical Research, Victorian Student Symposium; May 2019.

    Contribution statement: This presentation was based on the work from publication two (see above for author contributions). The presentation was designed and delivered by FT. AH and DD reviewed the presentation and provided feedback.

theAlfred

ETHICS COMMITTEE CERTIFICATE OF APPROVAL

This is to certify that

Project No: 50/17

Project Title: Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial

Principal Researcher: Professor David Dunstan

Protocol Version 2 **dated:** 9-Feb-2017

Participant Information and Consent Form Version 4 **dated:** 10-Mar-2017

*was considered by the Ethics Committee on **23-Feb-2017**, meets the requirements of the National Statement on Ethical Conduct in Human Research (2007) and was **APPROVED** on **28-Mar-2017***

It is the Principal Researcher's responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or progress report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- Any expiry of the insurance coverage provided with respect to sponsored clinical trials and proof of re-insurance;
- A delay of more than 12 months in the commencement of the project; and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit

- A Progress Report on the anniversary of approval and on completion of the project *(forms to be provided)*;

The Ethics Committee may conduct an audit at any time.

All research subject to the Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Hospital Ethics Committee is a properly constituted Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research (2007).

SPECIAL CONDITIONS

None   **SIGNED:**

Professor John J. McNeil
Chair, Ethics Committee

Please quote project number and title in all correspondence

theAlfred

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:
Change to inclusion criteria and study visit structure and procedures; Addition of retinal imaging; Use of different glucose monitor and addition of measure of habitual blood glucose control; Change to electronic data storage

Attachments:
Project Description version **3** dated **5-May-2017**
PICF version **5** dated **3-May-2017**
Participant Handbook version **3** dated **15-May-2017**

have been approved in accordance with your amendment application dated **18-May-2017** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **31-May-2017**
Chair, Ethics Committee

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:
Changes to inclusion criteria

Attachments:
Project Description version **4** dated **27-Sep-2017**
PICF version **6** dated **27-Sep-2017**
Phone Screening Questionnaire version **2** dated **10-Oct-2017**
Recruitment Poster version **2** dated **10-Oct-2017**
Facebook ad version **3** dated **10-Oct-2017**

have been approved in accordance with your amendment application dated **2-Oct-2017** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **13-Oct-2017**
Chair, Ethics Committee

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

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Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:

Change to research personnel
(departure of Dr Megan Grace, change of role for Ms Ashleigh Homer to trial coordinator)

Particiapnt Information & Consent Form **Version 7** dated: **30-Nov-2017**

have been approved in accordance with your amendment application dated **30-Nov-2017** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **5-Dec-2017**
Chair, Ethics Committee

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:

Addition of wrist worn ActiGraph Gt3x, questionnaire and radio advertisement

Change to research personnel (addition of research assistant Mr Hamza Ali and student Ms Emily Wordie-Thompson)

Participant Information & Consent Form **Version 8** dated: **19-Jan-2018**
Project Description **Version 5** dated: **19-Jan-2018**
CRF Sleep Ap & Insomnia **Version 1** dated: **19-Jan-2018**
Radio Script **Version 1** dated: **19-Jan-2018**

have been approved in accordance with your amendment application dated **30-Jan-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **7-Feb-2018**
Chair, Ethics Committee

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:

Addition of sub-study: "Reliability of a trained operator for femoral flow mediated dilation in healthy participants "
Sub-study Protocol **Version 2** dated: **4-Apr-2018**
Sub-study Participant Information and Consent Form **Version 2** dated: **4-Apr-2018**
Change to research personnel (addition of student researcher Ms Frances Taylor)

have been approved in accordance with your amendment application dated **22-Mar-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **16-Apr-2018**
Chair, Ethics Committee

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:

Further extend eligibility criteria from current eligible HbA1c range of 7-10% to 6.5-10%; removal of retinal imaging from Protocol

Project Description **Version 6** dated: **18-Apr-2018**
Participant Information & Consent Form **Version 10** dated: **18-Apr-2018**

have been approved in accordance with your amendment application dated **18-Apr-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **1-May-2018**
Chair, Ethics Committee

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:
Revised radio advertising

Radio Script dated: **16-May-2018**

have been approved in accordance with your amendment application dated **16-May-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **16-May-2018**
Chair, Ethics Committee

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

theAlfred

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:

Newspaper advertisement **Version 1** dated: **18-5-18**

have been approved in accordance with your amendment application dated **18-May-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil
Chair, Ethics Committee

Date: **21-May-2018**

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **50/17 Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE trial**

Principal Researcher: **Professor David Dunstan**

Amendment:

Removal of Ambulatory Blood Pressure Monitoring (ABPM) from the protocol

Project Proposal **Version 7** dated: **6-Aug-2018**
Participant Information & Consent Form **Version 11** dated: **6-Aug-2018**

have been approved in accordance with your amendment application dated **6-Aug-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **14-Aug-2018**
Chair, Ethics Committee

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

Ever wondered if too much sitting is harming your health?

**M**itigating the adverse effects **O**n blood **VE**ssel health with frequent breaks in sitting.

The MOVE study

Baker Heart and Diabetes Institute is currently examining whether or not interrupting prolonged sitting can improve blood vessel health in individuals with type 2 diabetes.

Studies suggest that breaking up sitting time can improve blood glucose control and blood fat levels in patients with type 2 Diabetes. Researchers from the Baker Heart and Diabetes Institute seek to determine whether or not breaking up sitting time also improves vascular function.

Baker
HEART & DIABETES INSTITUTE

Study Summary:

Participants will be required to:

- Attend 4 visits (1 screening visit and 3 day-long visits) over a period of 4 weeks
- Provide blood samples at regular intervals (by means of intravenous catheter)
- Wear a continuous blood glucose monitor for a period of 4 weeks.
- Undergo tests that measure blood vessel health.
- Consume meals specifically prepared by a research kitchen.

Participants will be reimbursed for their time and all parking costs will be covered.

Who can participate:

Eligible participants must be aged between 35 and 70 years and have type 2 diabetes.

Further information:

Interested persons should contact MOVE@baker.edu.au,or Hamza Ali on 8532-1852, or Ashleigh Homer on 8532-1786.

Ever wondered if sitting down all day is harming your health?

Thank you for registering you interest in participating in research at the Baker Heart and Diabetes Institute. The Physical Activity Laboratory is excited to announce a new initiative – the **MOVE** study – that we think will be of interest to you.

For your participation you will receive:
- ✓ **FREE** *cutting-edge* and *individualised reports* and *advice* from medical research professionals about:
    - ✓ Your current biological health status (including retinal images)
    - ✓ How this changes over the course of a normal day
    - ✓ How you can improve your health & reduce the risk of chronic disease
- ✓ On top of all this, you will also be reimbursed **$375** for your time along with **FREE** meals during and around the trials.

Further details on the study and your eligibility to participate are provided in the INFORMATION LEAFLET.

Please make contact if you are interested. We look forward to hearing from you.

Ashleigh Homer – Physical Activity Laboratory

T: 8532-1786   **E**: ashleigh.homer@baker.edu.au

INFORMATION LEAFLET

Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes: the MOVE study.

Introduction

You are invited to take part in this research project, the Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes (MOVE) study. This is because you are **aged between 35 and 70 years** and have had **type 2 diabetes mellitus for over 3 months** for which you are **taking a maximum of three anti-hyperglycaemic medications**. You belong to an important target group for evaluating strategies for disease prevention and management. In a recent study, we found that reducing sedentary behaviour (prolonged sitting time) had beneficial effects on blood glucose control and blood fat levels in patients with type 2 diabetes – two important risk factors for diabetes complications and cardiovascular disease. The present study aims to investigate whether breaking up sitting time with light-intensity simple resistance activities also has beneficial effects on vascular function.

What does participation in this research project involve?

Participation in this project will involve **four visits** to the Baker Heart & Diabetes Institute, over a period of 3-4 weeks. At the **first visit** (medical screening & familiarisation) we will require you to undergo a medical check to assess your ability to participate, including a glycated haemoglobin (HbA1c) test. This will simply require a finger prick blood sample and we will be able to determine your eligibility to participate immediately. Based on the results of these tests, along with other criteria, we will either admit or exclude you from the study. If admitted to the study, you will then proceed to the familiarisation stage of the visit in which we will familiarise you with our testing procedures and measurement devices. During **visits 2, 3 and 4** (separated by at least 6 days), you will be required to complete each of the three experimental conditions described below in a random order. For visits 2, 3 and 4, you will be required to *fast* (not consume any food or drinks) from 10pm the night before your appointment. You may only drink water. For an overall diagram of the *study schedule* just described, please see page 7.

Part 1:   Consent/Screening/Familiarisation

<u>**Visit 1 (Day 1):**</u> Medical screening & Familiarisation – (120 min).

You will be asked to attend an initial visit at the **Physical Activity Laboratory, Baker Heart & Diabetes Institute.** During this visit you will be given an opportunity to ask questions before giving signed consent to participate. You will then complete:

i) *A medical and physical examination*

A quick, routine medical profile and physical examination will be conducted by both the study coordinator (Ashleigh) and one of our qualified physicians (Dr Neale Cohen) to ensure it is safe for you to take part in this study. This will involve a blood test, vital sign checks (including a resting 12 lead electrocardiogram (ECG) – which involves placing sticky electrodes on your skin to check the electrical activity of your heart), and an overall body inspection.

ii) *A glycated haemoglobin (HbA1c) test*

The glycated haemoglobin (HbA1c) test measures your body's ability to use and control your blood sugar, called glucose, which is the body's main source of energy. Hemoglobin is a protein in your red blood cells which is responsible for carrying oxygen. Sugar remains attached to your hemoglobin for approximately 120 days, which allows the HbA1c test to measure the percentage of hemoglobin which is glycated (or coated in sugar). This test is one of the tools often used to diagnose diabetes. Other aspects of your blood will also be assessed at this collection (e.g. liver/kidney function and full blood analysis).

Preparing for the blood test is quick and simple. Unlike fasting blood sugar tests (another type of laboratory test to check your blood sugar), HbA1c testing is unaffected by what you ate immediately prior. A lancet will be used to prick your finger, to enable the collection of a small sample of blood.

As with all visits, *wearing a loose fitted short sleeve shirt and short* will make access to your chest area (for the 12 lead ECG) and thigh area (FMD measurement) easier for the researchers.

We will be able to let you know your results and whether you are eligible or ineligible immediately after the medical screening is complete. If you are eligible, we will then proceed to the familiarisation part of visit 1.

During familiarisation you will get the opportunity to practice and become acquainted with the simple (body weight) resistance exercises – half squats, calf raised and knee raises with gluteal clenches. You will also be acquainted with the processes required for the measurement of Flow Mediated Dilation (FMD) and Retinal Imaging. The FMD measurement involves using ultrasound to identify and

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image the femoral artery (in your mid-upper thigh), inflating a cuff similar to that used in a standard blood pressure measurement for 5 minutes, then deflating the cuff and using the ultrasound to record how well your vessels react. Retinal imaging is a standard procedure involving sitting in a dark room (to dilate the pupils), placing your head in a brace and taking a close up picture of your eye with a bright flash to get a picture of the small vessels at the back of the eye.

At the completion of this visit, you will receive a meal pack, to be consumed on the evening prior to the first experimental condition (Visit 2). You will also be fitted with two small, lightweight devices which will measure and store information regarding the frequency and duration of times spent sitting, standing and walking. One instrument, known as an *accelerometer*, will be worn with a belt at hip height. The other instrument, known as an *ActivPal inclinometer*, will be fixed to the front of your upper thigh with hypoallergenic tape. Both instruments can be easily removed and refitted after sleeping or engaging in water activities (e.g. shower or swimming).

You will be required to wear both devices everyday (during waking hours) starting from your familiarisation visit (Visit 1) to the completion of the 3rd and final experimental condition (Visit 4). This will be a maximum of 21-22 days. We will ask you to keep a log of your physical activity to help us gather information about non-walking physical activities (e.g. weight lifting, yoga, cycling).

At the end of the familiarisation visit you will be fitted with a Freestyle Libre Flash Glucose Monitor. The Libre is a small sensor which is fixed to the back of your upper arm. We will also provide you with a small handheld device called the reader, with which you will scan the sensor at regular times of the day to upload your blood glucose information. The sensor is fully waterproof and non-invasive, and does not require finger-prick calibration. Before you leave the clinic, we will make sure you are comfortable with the scanning process. You will wear this sensor for 14 days, which should coincide with Visit 3. At the beginning of Visit 3, you will be fitted with a new sensor to wear until the end of the trial (a total of 21-22 days wearing a sensor).

Part 2: Experimental procedures

Once all the inclusion and exclusion criteria have been met and you have been deemed suitable to take part in the study, appointments will be made for the remaining three visits. The three experimental conditions will be assigned to you in a random order.

Preparation for each experimental condition: In the 72 hour period prior to each experimental condition, we request that you do not engage in any moderate (e.g.

gentle swimming, yoga) and/or vigorous (e.g. running, aerobics) physical activity. We will also ask that you keep a record of your food and drink intake in the provided diary during this 48 hour period and avoid alcohol and caffeine (e.g. tea, coffee, caffeinated soft drinks) for 24 hours beforehand. _On the night before and after each experimental condition, we ask that you consume only the foods provided to you in your 'meal pack' and no other foods or drinks, except water_.

Visits 2, 3 and 4: Experimental conditions

Total blood collected per visit: 150 mL per condition

Upon arrival, you will have your weight measured. At the beginning of visits 2 and 4 you should still be wearing a Libre sensor. We will fit a new sensor at the beginning of visit 3. At the end of each experimental condition an Ambulatory Blood Pressure Monitor (ABPM) will be fitted to your non-dominant arm. The cuff will inflate every 20 minutes during the day, and every 30 minutes overnight. Whenever the cuff inflates, you are requested to stop what you are doing, stay still, not talk, and relax your arm. We will ask you to keep this device on overnight after each trial to assess what happens to your blood glucose and blood pressure during this period. During this time you will need to scan your Libre with the reader provided by us on the occasions detailed in the handbook. The next day at 12:00 you can remove the ABPM device, and bring with you to your next clinic visit. If you have completed your clinic visits, you can remove both the ABPM and the Libre sensor, and place them in the packaging and envelope provided, and mail it back to Baker (unless collected by researchers).

A cannula (small plastic tube) will be inserted into a vein in your arm to enable the collection of blood samples. Cannulation involves inserting the small, soft indwelling plastic tube into a vein in your arm via a needle, where it will stay until all blood samples have been collected. This tube will be removed before you leave the laboratory. Each experimental day will involve small samples of blood being collected for the analysis of blood glucose, fats, insulin, catecholamines, and inflammatory markers. You will be asked to provide small blood samples (up to 20 mL, just over a tablespoon) on 16 occasions, totalling 150 mL per day. This volume is relatively small (less than the volume of a typical blood bank donation), and is quickly replenished naturally by the body. We will be monitoring your blood count at the beginning and the end of the study visit to ensure your safety. We advise you to stay well hydrated throughout the study visits and to avoid any moderate to vigorous physical activity after the visit to reduce any side effects that the small reduction in blood volume could have.

A test for blood vessel health, called flow mediated dilation (FMD), will be performed periodically throughout each experimental condition. This technique uses a small ultrasound device about the size of a mobile phone, which will rest

against your leg to visualise your main femoral artery). We use a special hypoallergenic water soluble gel with this device to be able to see an image of your artery. A cuff, like a blood pressure cuff, will be wrapped around the lower part of your thigh. The test involves looking at your artery at rest for 1 minute, after which the cuff will be inflated for 5 minutes, and then deflated. We will continue to look at your artery for a further 3 minutes after cuff deflation. Doing this allows us to view your artery under conditions of altered blood flow to assess artery health.

In addition to FMD, to assess the health of your smaller blood vessels, we will perform retinal imaging at the beginning and end of each experimental condition. This involves you sitting in a darkened room for 10 minutes, which allows your pupils to dilate. You will then place your head in a rest close to a camera and have close-up photos taken of the back of your eye. The camera emits a bright flash which is necessary to capture the small vessels, and any discomfort caused will wear off within a few minutes. We will take two photographs of each eye using the flash. You will be given regular breaks to minimise any eye discomfort, but please do not hesitate to inform the examiner if longer breaks are required. The whole procedure takes approximately 15 minutes to complete.

On each experimental day, you will sit quietly in a chair for an initial 1 hour "steady-state" period before consuming a standardised breakfast meal, commencing the experimental condition thereafter. Lunch will also be provided around midday, and you will be given dinner and breakfast meal packs, to be consumed at home following each condition.

Experimental condition A: Prolonged uninterrupted sitting

The experimental condition will commence following the baseline measurements and steady-state (quiet sitting) period. During the 7 hour period, you will be asked to sit quietly in a comfortable lounge chair. You will be able to watch television programs, DVD's, or read whilst seated.

Experimental condition B: Sitting + simple resistance activities every 30 minutes

Following the steady state period, you will be asked to complete a 3 minute bout of simple resistance activities every 30 minutes throughout the 7 hour condition. This will involve alternating between 20 second periods of body weight half-squats, calf raises, and brief gluteal clenches in between single leg knee raises. Alternating between these activities will allow your various muscle groups to recover. You will then return to the seated position. This procedure will be completed on 12 occasions, totalling 36 minutes of activity.

Experimental condition C: Sitting + simple resistance activities every 60 minutes

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As for condition B, except you will be asked to complete a 6 minute bout of simple resistance activities every 60 minutes throughout the 7 hour condition. This procedure will be completed on 6 occasions, totalling 36 minutes of activity.

Randomisation (i.e. "coin flipping") will decide in what order you complete conditions A, B and C. For example, if it is decided that you will follow experimental condition B at visit 2, you will then complete experimental conditions A or C at visits 3 and 4

Study Schedule

Visit	1		2		3		4
Day	1	2-6	7	8-13	14	15-20	21
Activity	Medical Screening & Familiarisation	✗ ➔ ✔	Experimental Condition A, B or C	Wash-out	Experimental Condition A, B or C	Wash-out	Experimental Condition A, B or C

Note: participant eligibility is dependent on study inclusion/exclusion criteria, medical screening and confirmation of Type 2 Diabetes.

Fires in our new suburbs cost CFA

ALEX WHITE

MORE than 1500 fires that caused an estimated $42 million damage statewide were preventable, CFA data reveals.

Cooking, smoking, heating, gas and electrical faults were the leading causes of fires, and the biggest damage bills were in Melbourne's booming suburban fringe — with Dandenong, Caroline Springs and Narre Warren topping the list.

CFA chief officer Steve Warrington said the figures showed the changing nature of call-outs as once rural areas were consumed by the city.

Most preventable fires the CFA dealt with were in Melbourne's suburban fringe.

It comes as the Andrews Government struggles to restructure the state's fire services.

It wants to create a new body for paid firefighters from the MFB and CFA called Fire Rescue Victoria.

"These figures show how the services of CFA are changing," Mr Warrington said.

"Yes, we fight bushfires but we do so much more.

"We are also an urban firefighting authority and we are adapting to the communities we serve.

"The best way to stop a fire is to teach a person how to be safe in the home and stop a fire before it even begins.

"Our message is clear — check your smoke alarm's battery and if your smoke alarm is aged 10 years or more the whole unit must be changed."

The Andrews Government's bid to break up the CFA was sensationally derailed on Good Friday when the Coalition broke a longstanding parliamentary pairing arrangement to kill the Bill.

The number of blazes and damage bills was down on previous years.

BITTER ATTACKS BURN US

STEPHEN DRILL

Shannon sets out how TV claims hurt not just him but his staff

MASTERCHEF star Shannon Bennett has hit back at claims he has underpaid staff and ripped off suppliers, saying he is speaking out now because the attacks are hurting his staff.

The acclaimed Melbourne chef says he has been the target of a vicious smear campaign.

This includes accusations of taking tips off staff, refusing toilet breaks and being chased over bills he claims had been paid.

The storm of criticism started soon after the regular *MasterChef* mentor paid $17 million for a house in Toorak late last year.

Staff say the false claims make them angry and tarnish all the hard work they have done to build and maintain a successful restaurant empire under Bennett's Vue Group brand.

"I must have walked under a ladder," Bennett said from his offices in the Rialto Tower in Collins St.

"I'm really proud of hospitality in this city and this takes the shine off all the good work that we do.

"I thought I would just keep my head down but now staff have been hurt and I had to address this."

The first hit came in April when a program on a rival channel took aim at him over claims he had not paid $6000 for a lobster tank delivered in 2011.

Bennett disputed the bill and claimed he had settled the matter at the time, and came to a confidential final settlement recently.

He was filmed while jogging as a TV reporter chased him down the street.

A supplier claimed he had not been paid for $25,000 of work on Bennett's inner eastern suburbs property, but Bennett claimed the supplier had made threats against his family and the family of a staff member, and said they had been reported to police.

The TV probe detailed a butcher claiming he had not been paid for some sausages, but that was in 2002, and newspaper reports at the time claimed Bennett had questioned the sausages' quality and wanted to show them to the health department.

Then the ABC last week claimed Bennett had underpaid staff and withheld tips from those who turned up late or failed to pass a "tip test".

But Bennett also disputed those claims — saying a former staff member who complained was sacked for making racist comments towards a co-worker.

Texts provided by Bennett show that a staff member had called a black colleague a "dumb n----r" and made another highly offensive comment.

"I could see that those messages broke (the co-worker) — he was in tears," he said.

"I will never ever forgive (the staff member) for that — I immediately fired him."

Bennett's restaurant manager Hugo Simoez-Santos debunked the "tip test" claims, saying a woman who complained to the ABC did not lose any money because he had reimbursed her from his own pocket.

"An email was sent out to everyone and since then only one person, who left early this year, suffered the consequences of this (lateness) rule.

"When this system was introduced, this one person managed to arrive late three times in two days.

"When the day of collecting tips came, I spoke with this person and I told her: 'You won't get your tip this week, but as your friend and because I know everything that you are going through, I will give you my own tips and I hope you learn your lesson'."

Hugh Allen, who now works at Noma in Denmark — voted world's best restaurant four times — said

MHSE01Z01MA - V2

Participant Information Sheet/Consent Form
Non-Interventional Study - *Adult providing own consent*

Baker Heart and Diabetes Institute

Title	Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes.
Short Title	MOVE
Project Number	50/17
Project Sponsor	NHF Vanguard Grant
Principal Investigator	Prof David Dunstan
Associate Investigator(s)	Dr Rachel Climie, Prof Neville Owen, Prof Bronwyn Kingwell, Prof Daniel Green, Ms Kym Rickards, Dr Neale Cohen, Ms Ashleigh Homer, Mr Michael Wheeler, Mr Hamza Ali, Ms Emily Wordie, Ms Frances Taylor.
Location	Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project, the Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes (MOVE) study. This is because you are **aged between 35 and 70 years** and have had **type 2 diabetes mellitus for over 3 months** for which you are **taking a maximum of three antihyperglycemic agents**. You belong to an important target group for evaluating strategies for disease prevention and management. In a recent study, we found that reducing sedentary behaviour (prolonged sitting time) had beneficial effects on blood glucose control and blood fat levels in patients with type 2 diabetes – two important risk factors for diabetes complications and cardiovascular disease. The present study aims to investigate whether breaking up sitting time with light-intensity simple resistance activities also has beneficial effects on vascular function.

This Participant Information Sheet & Consent Form tells you about the research project. It explains the procedures and research involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:
• Understand what you have read
• Consent to take part in the research project
• Consent to the tests and research that are described
• Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2   What is the purpose of this research?

It is well known that being physically active is important for maintaining good health. However, new evidence has emerged which shows that being sedentary (sitting for prolonged periods) is adversely associated with indicators of poor health, such as elevated blood sugars and blood fats. Interestingly, too much sitting may even be a unique risk factor, independent to lack of physical activity.

Recent experimental evidence suggests that breaking up sitting time throughout the day with light-intensity activity (i.e. walking or simple resistance activities) results in lower blood sugars and blood fat levels than sitting for prolonged periods without activity breaks, in adults with type 2 diabetes. In addition, in healthy adults, it has been shown that prolonged, uninterrupted periods of sitting impairs vascular function (the ability of the blood vessels to respond appropriately). While these results are interesting, such experimental findings are yet to be verified in those at high risk of vascular disease (and who are likely to derive the greatest benefits from breaking up sitting), such as those with type 2 diabetes. This study aims to test the effect of 8 hours of prolonged sitting (i.e. a normal working day) on vascular function, blood sugars and blood fats in adults with type 2 diabetes with and without intermittent breaks of brief activity. We also aim to determine how often these activity breaks need to occur in order to observe meaningful benefits.

We anticipate that the approach of breaking up prolonged sitting with practical and brief 'activity breaks' will result in improvements in vascular function, and lowering of blood sugars and fats levels – potentially offering realistic tools, alongside or in place of drug therapy, for those with type 2 diabetes.

This research has been initiated by Professor David Dunstan and has been funded by a Vanguard Project Grant from the National Heart Foundation, and a Centre of Research Excellence grant from the National Health and Medical Research Council.

3   What does participation in this research involve?

Approximately 24 volunteers will be required for this study. Participation in this project will involve **four visits** to the Baker Heart and Diabetes Institute, over a period of 3-4 weeks. At the **first visit** (medical screening & familiarisation) we will require you to undergo a medical check to assess your ability to participate, including a glycated haemoglobin (HbA1c) test. This will simply require a finger prick blood sample and we will be able to determine your eligibility to participate immediately. Based on the results of this test, along with other criteria, we will either admit or exclude you from the study. If admitted to the study, you will then proceed to the familiarisation stage of the visit in which we will familiarise you with our testing procedures and measurement devices. During **visits 2, 3 and 4** (separated by at least 6 days), you will be required to complete each of the three experimental conditions described below in a random order. For visits 2, 3 and 4, you will be required to *fast* (not consume any food or drinks) from 10pm the night before your appointment. You may only drink water. For an overall diagram of the *study schedule* just described, please see page 5.

Part 1: Consent/Screening/Familiarisation

Visit 1 (Day 1): Medical Screening & Familiarisation – 2 hours

You will be asked to attend an initial screening visit at the clinical research rooms at the Baker Heart and Diabetes Institute. During this visit, you will be given an opportunity to ask questions before giving signed consent to participate. Research staff will then measure your height, weight, blood pressure, hip and waist circumference, and assess your risk of sleep apnoea and insomnia. During this visit you will also complete:

   i)   *A medical and physical examination*

A quick, routine medical profile and physical examination will be conducted by one of the research staff, and our qualified physician (Dr Neale Cohen) to ensure that it is safe for you to take part in this study. This will involve a blood test, vital sign checks (including a resting 12 lead electrocardiogram (ECG), which involves placing sticky electrodes on your skin to check the electrical activity of your heart), and an overall body inspection.

ii) A glycated haemoglobin (HbA1c) test

The HbA1c test measures your body's ability to use and control your blood sugar, called glucose, which is the body's main source of energy. Haemoglobin is a protein in your red blood cells which is responsible for carrying oxygen. Sugar remains attached to your haemoglobin for approximately 120 days, which allows the HbA1c test to measure the percentage of haemoglobin which is glycated (or coated in sugar). This test is one of the tools often used to diagnose diabetes.

Preparing for the blood test is quick and simple. Unlike fasting blood sugar tests (another type of laboratory test to check your blood sugar) or finger sticks, HbA1c testing is unaffected by what you have previously eaten that day. A lancet will be used to prick your finger to enable the collection of a small sample of blood.

As with all visits, *wearing a loose fitted short sleeve shirt and shorts* will make access to your chest area (ECG test) and thigh area (FMD measurement) easier for the researchers.

We will be able to let you know your results and whether you are eligible or ineligible immediately after the medical screening is complete, after which we will proceed to the familiarisation part of visit 1.

During the familiarisation part of this visit you will get the opportunity to practice and become acquainted with the simple (body weight) resistance exercises – half squats, calf raises and knee raises with gluteal clenches. You will also be acquainted with the processes required for the measurement of Flow Mediated Dilation (FMD). The FMD measurement involves using ultrasound to identify and image the femoral artery (in your mid-upper thigh), inflating a cuff similar to that used in a standard blood pressure measurement for 5 minutes, then deflating the cuff and using the ultrasound to record how well your vessels react.

At the completion of this visit, you will receive a meal pack, to be consumed on the evening prior to the first experimental condition (Visit 2). You will be fitted with two small, lightweight devices which will measure and store information regarding the frequency and duration of times spent sitting, standing and walking. One instrument, known as an *accelerometer*, will be worn on the wrist, similar to a normal wristwatch. The other instrument, known as an *ActivPal inclinometer*, will be fixed to the front of your upper thigh with hypoallergenic tape. Both instruments can be easily removed and refitted after engaging in water activities (e.g. shower or swimming).You will be required to wear both devices everyday starting from your familiarisation visit (Visit 1) to the completion of the 3rd and final experimental condition (Visit 4). This will be a maximum of 21-22 days. We will ask you to keep a log of your physical activity to help us gather information about non-walking physical activities (e.g. weight lifting, yoga, cycling).

At the end of the familiarisation visit you will be fitted with a Freestyle Libre Flash Glucose Monitor. The Libre is a small sensor which is fixed to the back of your upper arm. We will also provide you with a small handheld device called the reader, with which you will scan the sensor at regular times of the day to upload your blood glucose information. The sensor is fully waterproof and non-invasive, and does not require finger-prick calibration. Before you leave the clinic, we will make sure you are comfortable with the scanning process. You will wear this sensor for 14 days, which should coincide with Visit 3. At the beginning of visit 3, you will be fitted with a new sensor to wear until the end of the trial (a total of 21-22 days wearing a sensor).

It is anticipated that the first visit (medical screening & familiarisation) will take approximately 2 hours.

Part 2: Experimental procedures

Preparation for each experimental condition:

In the 72 hour period prior to each experimental condition, we request that you do not engage in any moderate (e.g. gentle swimming, yoga) and/or vigorous (e.g. running, aerobics) physical activity. We will also ask that you keep a record of your food and drink intake in the provided diary during this 72 hour period and avoid alcohol and caffeine (e.g. tea, coffee, caffeinated soft drinks) for **24** hours beforehand. *On the night before and after each experimental condition, we ask that you consume only the foods provided to you in your 'meal pack' and no other foods or drinks, except water*.

Visits 2, 3 and 4: Experimental Conditions – 8-9 hours per visit

Total blood collected: 150 mL per condition

On each testing day, you will be asked to report to the clinical testing rooms at Baker at 7:30am after having fasted from 10pm. You will be asked to wear a loose fitting t-shirt and shorts, which will allow easy access for various measurements.

Upon arrival, you will have your weight measured. At the beginning of visits 2 and 4 you should still be wearing a Libre sensor. We will fit a new sensor at the beginning of visit 3. A cannula (small plastic tube) will be inserted into a vein in your arm to enable the collection of blood samples. Cannulation involves inserting the small, soft indwelling plastic tube into a vein in your arm via a needle, where it will stay until all blood samples have been collected. This tube will be removed before you leave the laboratory. Each experimental day will involve small samples of blood being collected for the analysis of blood glucose, fats, insulin, catecholamines, and inflammatory markers. You will be asked to provide small blood samples (up to 20 mL, just over a tablespoon) on 16 occasions, totalling 150 mL per day. This volume is relatively small (less than the volume of a typical blood bank donation), and is quickly replenished naturally by the body. We will be monitoring your blood count at the beginning and the end of the study visit to ensure your safety. We advise you to stay well hydrated throughout the study visits and to avoid any moderate to vigorous physical activity after the visit to reduce any side effects that the small reduction in blood volume could have.

A test for blood vessel health, called flow mediated dilation (FMD), will be performed periodically throughout each experimental condition. This technique uses a small ultrasound device about the size of a mobile phone, which will rest against your leg to visualise your main femoral artery. We use a special hypoallergenic water soluble gel with this device to be able to see an image of your artery. A cuff, like a blood pressure cuff, will be wrapped around the lower part of your thigh. The test involves looking at your artery at rest for 1 minute, after which the cuff will be inflated for 5 minutes, and then deflated. We will continue to look at your artery for a further 3 minutes after cuff deflation. Doing this allows us to view your artery under conditions of altered blood flow to assess artery health.

On each experimental day, you will sit quietly in a chair for an initial 1 hour "steady-state" period before consuming a standardised breakfast meal, commencing the experimental condition thereafter. Lunch will also be provided around midday, and you will be given a dinner pack, to be consumed at home following each condition.

Experimental condition A: Prolonged uninterrupted sitting

The experimental condition will commence following the baseline measurements and steady-state (quiet sitting) period. During the 7 hour period, you will be asked to sit quietly in a comfortable lounge chair. You will be able to watch television programs, DVD's, or read whilst seated.

Experimental condition B: Sitting + simple resistance activities every 30 minutes

Following the steady state period, you will be asked to complete a 3 minute bout of simple resistance activities every 30 minutes throughout the 7 hour condition. This will involve alternating between 20 second periods of body weight half-squats, calf raises, and brief gluteal clenches in between single leg knee raises. Alternating between these activities will allow your various muscle groups to recover. You will then return to the seated position. This procedure will be completed on 12 occasions, totalling 36 minutes of activity.

Experimental condition C: Sitting + simple resistance activities every 60 minutes

As for condition B, except you will be asked to complete a 6 minute bout of simple resistance activities every 60 minutes throughout the 7 hour condition. This procedure will be completed on 6 occasions, totalling 36 minutes of activity.

Randomisation (i.e. "coin flipping") will decide in what order you complete conditions A, B and C. For example, if it is decided that you will follow experimental condition B at visit 2, you will then complete experimental conditions A or C at visits 3 and 4.

Study Schedule

Visit	1		2		3		4
Day	1	2-6	7	8-13	14	15-20	21
Activity	Medical Screening & Familiarisation	✗ ➜ ✓	Experimental Condition A, B or C	Wash-out	Experimental Condition A, B or C	Wash-out	Experimental Condition A, B or C

Note: participant eligibility is dependent on study inclusion/exclusion criteria, medical screening and confirmation of type 2 diabetes.

Reimbursement

At the completion of the study, you will be reimbursed $375 ($125 per condition) for your time. Parking at Baker Heart and Diabetes Institute and meals during experimental conditions will also be provided at no cost to you. There are no associated costs with participating in this research project.

4   What do I have to do?

In order to participate in the study, you must be willing to attend all scheduled visits and have the relevant tests. If any medical issues are identified, you will be notified as soon as possible so that you can contact your treating physician and commence appropriate clinical management and education.

You will also be asked to wear the accelerometer and inclinometer, and Libre (glucose) devices outside of the laboratory, for the specified periods of time outlined in the previous section. The Libre sensor will require at least 5 calibration scans each day outside of the lab. Both the Libre and activPAL^{TM} devices will be fitted with a gentle hypoallergenic breathable adhesive, and both instruments should be worn at all times, including in bed and in the shower. The wrist-worn accelerometer should be removed if taking a shower, but is easily put back on. You will be shown how to do this before you leave the laboratory.

You will also be asked to follow specific dietary and activity instructions. In the 72 hour period prior to each experimental condition, we ask that avoid any moderate (eg. gentle swimming, yoga) and/or vigorous (e.g. running, aerobics) physical activity. You will be provided with a diary in which you will keep a record of your activity, food and drink intake in the 72 hour period and we ask that you avoid any caffeine (coffee, tea, caffeinated soft drinks, etc.) and alcohol for 24 hours prior. In addition, on the evening prior to each experimental condition, you will be asked to only consume food provided to you in your pre-experimental meal pack, and fast from 10pm onwards.

5   Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage. Your decision whether to take part or not take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you, or your relationship with the Baker Heart and Diabetes Institute and the Alfred Hospital.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

6   What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research, however, possible benefits include a full medical assessment and individualised health profile, knowledge and understanding of your baseline metabolic and vascular health and what happens to these indicators of health when you are sedentary and/or actively breaking up your sitting. We expect that the findings from this study will make an important contribution to the design, implementation and evaluation of intervention strategies in the community to reduce the risk of diabetes and cardiovascular disease through the adoption of healthier behaviours and lifestyles.

7   What are the possible risks and disadvantages of taking part?

This research involves a number of procedures in which you may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with the study doctor. Your study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms.

Many side effects go away following the completion of a procedure. However, sometimes side effects can be serious, long lasting or permanent. Your study doctor should discuss the best way of managing any side effects with you.

The risks associated with the individual procedures involved in the study are as follows:

Cannulation / blood test: Having a blood sample taken may cause some discomfort or bruising. Sometimes, the blood vessel may swell, or blood may clot in the blood vessel, or the spot from which tissue is taken could become inflamed. Some people may feel faint when having blood taken, and may occasionally faint. Rarely, there can be a minor infection or bleeding. If this happens, it can be easily treated.

Flash Blood Glucose Monitoring: The Abbott Freestyle Libre Flash Glucose Monitor is a Therapeutic Goods Administration-approved device (manufactured by Abbott Diabetes Care Australia) that records blood glucose levels throughout the day and night. It involves having a tiny sensor inserted into the skin of the back of your upper arm, (sensor is roughly the size of a

20 cent piece). Insertion of the sensor is virtually painless and is comfortable to wear. Irritation or inflammation may occur at the sensor site, but this is unlikely as the device is minimally invasive and only worn for a short amount of time. A team of dedicated and experienced diabetes nurse educators are also on hand to help treat any eventualities, if they occur.

Blood vessel function test: This common procedure, known as Flow Mediated Dilatation (FMD), is quick and painless. You may feel a strange 'pins and needles' sensation in your arm while blood flow is briefly restricted (5 minutes) with a cuff (similar to the feeling with blood pressure measurement). Following release of the cuff, blood flow is measured using an ultrasound device passed lightly over the skin above an artery (femoral) in the thigh.

Resistance activity breaks: As part of this study, you will be asked to perform simple body weight resistance activities involving your lower body. To help minimise your risk, all experimental sessions will be supervised by trained staff and will be tailored to your ability. There is a small possibility that you will experience minor and transient muscle soreness initially following the exercise sessions. There may be additional unforeseen or unknown risks and participation in this study can be suspended or terminated if a medical issue or distress occurs.

There may be additional unforeseen or unknown risks and participation in this study can be suspended or terminated if a medical issue or distress occurs.

8   What will happen to my test samples?

On the experimental days, blood samples will be collected on 16 occasions via a cannula. The blood collected will be analysed for blood glucose, insulin, catecholamines, and inflammatory markers. A total of 150 ml will be collected over the course of each experimental condition.

At each blood collection, some of the blood that is taken will be in addition to what is required. This spare sample may be needed to re-run the tests described above to ensure accurate results. We would also like your permission to store these extra samples for future research into diabetes and heart disease. These samples will be stored indefinitely at -80°C in a locked freezer at Baker Heart and Diabetes Institute. Your samples will not undergo genetic testing. Only study investigators will have access to your samples. By signing the Consent Form for tissue sample storage and use, you consent to the analysis of markers relevant to diabetes and heart disease in your blood samples. The storage of any excess sample is voluntary.

Once the study has been completed you will be provided with a summary of your individual results by email. The unidentified group data will also be published in a peer reviewed scientific journal.

9   What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, the study coordinator will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, the study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, the study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

If you provide consent, we will store some extra blood for future analysis. Although it is unlikely that this analysis will generate information that will be important to you, should this occur the study coordinator will inform you. If this happens, he/she will explain this information to you and (if consented) your local doctor and any subsequent care requirements will be arranged.

10   Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell the study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell the study coordinator about any changes to these during your participation in the research project. The study coordinator should also explain to you which treatments or medications need to be stopped for the time you are involved in the research project.

11   What if I withdraw from this research project?

If you decide to withdraw from this research project, please notify a member of the research team before you withdraw. A member of the research team will inform you if there are any special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study coordinator and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly. You should be aware that data collected up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them when you withdraw from the research project.

12   Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons including unacceptable side effects, but this is unlikely.

13   What happens when the research project ends?

At the completion of the study, you will be provided with an individual report of your results. If requested, you can also receive a report of the main findings of the study and copies of any subsequent publications at the conclusion of the research.

Part 2   How is the research project being conducted?

14   What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research study that can identify you will remain confidential and will only be used for the purpose of this research study. It will only be disclosed with your permission, or in compliance with the law.

Study data will be stored on password-protected computers, belonging to the researchers involved in the study. Hard copy data will be stored in a locked filing cabinet in the Study Coordinator's office at the Baker, Alfred Centre, 99 Commercial Rd, Melbourne. Your data will be stored in a re-identifiable (coded) format with your personal details stored in a separate file. Data will be stored indefinitely as per Alfred Hospital Study policy. At the completion of the study, electronic and hard copy data will be archived securely off site.

Your contact details, pathology (blood) results in your name and medical history will be kept in a locked filing cabinet in the Study Coordinator's office at the Physical Activity laboratory, at Baker Heart and Diabetes Institute. Identifying information will not be entered into the study research forms, the study database or appear in any data reports. Your information will only be used for the purpose of this research study, however if you give permission for any remaining samples to be used for related research (by signing the second consent form), your coded data will be used

for further analysis. Your information will only be disclosed with your permission, or in compliance with the law.

Information that has been de-identified (i.e. does not have any of your personal information attached) will be stored on a secure cloud based eNotebook called LabArchives. This allows us to ensure that all of the data we have gained from you remains intact and safe. There will be no personal identifying information entered into LabArchives, and the account will be password protected, with data available only to study investigators.

This research study involves the establishment of a databank. When you sign the attached consent form, you are consenting your information being used for this specific study and, if you give consent, to future related research.

If you have a regular local doctor, it is desirable that they be advised of your decision to participate in this research study. If you do have a local doctor, by signing this consent form, you are agreeing to inform him/her of your participation in the study. With your permission, we will write to your local doctor to inform them of your participation in this study.

It is anticipated that the results of this research study will be published and/or presented in a variety of forums, which may include publication in scientific journals, presentations at scientific conferences and clinical trial registries such at www.clinicaltrials.gov. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. Only group data will be published and presented.

In accordance with relevant Australian and Victorian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

15   Complaints and compensation

If you suffer any injuries or complications as a result of this research study, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital. If you are ineligible for Medicare, you may be able to receive medical treatment through your private health insurer. You should contact your insurer before enrolling in this trial to ensure any medical treatment you require in connection with participation falls within your policy's terms and conditions.

In the event of loss or injury, the institutes conducting the research project each have insurance policies for an amount sufficient to cover its own liabilities. Baker holds insurance to cover adverse events if Baker contributed in some way to that event and the type of loss suffered is covered under the policy. Each situation would be considered by Baker insurers on a case by case basis.

16   Who is organising and funding the research?

This research study is being conducted and funded by the Baker Heart and Diabetes Institute (with the aid of an NHMRC and Vanguard Grant).

You will not benefit financially from your involvement in this research study even if, for example, your samples (or knowledge acquired from analysis of your samples) prove to be of commercial value to the Baker Heart and Diabetes Institute. In addition, if knowledge acquired through this research leads to discoveries that are of commercial value to the study doctors or their institutions, there will be no financial benefit to you or your family from these discoveries.

The Baker Heart and Diabetes Institute will receive payment for the direct research costs for this study from the National Heart Foundation and National Health and Medical Research Council. No member of the research team will receive a personal financial benefit from your involvement in this research study (other than their ordinary wages).

17   Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research study have been approved by the HREC of The Alfred Hospital, Melbourne.

This study will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

18   Further information and who to contact

The person you may need to contact will depend on the nature of your query.
If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact any of the following people:

Study co-ordinator: Ashleigh Homer
Telephone: (03) 8532-1786

Research Nurse: Kym Rickards
Telephone: (03) 8532-1864

Principal investigator: Prof David Dunstan
Telephone: (03) 8532-1873

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Position: Complaints Officer, Office of Ethics & Research Governance, Alfred Hospital
Telephone: 03 9076 3619
Email: research@alfred.org.au

Please quote the following Alfred Health project number: 50/17

Consent Form - *Adult providing own consent*

Title	Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes
Short Title	MOVE
Project Number	50/17
Project Sponsor	NHF Vanguard Grant
Principal Investigator	Prof David Dunstan
Associate Investigator(s)	Dr Rachel Climie, Prof Neville Owen, Prof Bronwyn Kingwell, Prof Daniel Green, Ms Kym Rickards, Dr Neale Cohen, Ms Ashleigh Homer, Mr Michael Wheeler, Mr Hamza Ali, Ms Emily Wordie, Ms Frances Taylor.
Location	Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.

Declaration by Participant

I have read the Participant Information Sheet.

I understand the purposes, procedures and risks of the research described in the project.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the project without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Baker concerning my condition and treatment for the purposes of this project. I understand that such information will remain confidential.

Name of Participant (please print)

Signature _____ Date _____

Name of Witness* to
Participant's Signature (please print)

Signature _____ Date _____

Declaration by Study Doctor/Senior Researcher[†]

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher[†] (please print)

Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Consent Form for the Storage and Use of Additional Blood Samples

Title	Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes
Short Title	MOVE
Project Number	50/17
Project Sponsor	NHF Vanguard Grant
Principal Investigator	Prof David Dunstan
Associate Investigator(s)	Dr Rachel Climie, Prof Neville Owen, Prof Bronwyn Kingwell, Prof Daniel Green, Ms Kym Rickards, Dr Neale Cohen, Ms Ashleigh Homer, Mr Michael Wheeler, Mr Hamza Ali, Ms Emily Wordie, Ms Frances Taylor.
Location	Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.

Declaration by Participant

I have read Section 8 of this Participant Information Sheet and am aware that extra blood is being collected for verification of pathology tests if required.

In the case that verification is not required, we ask for your consent to store and use these samples for future research into cardiovascular disease and diabetes (i.e. for future research to be approved by the Alfred Hospital Ethics Committee)

Note: Storage of extra samples is voluntary and will not affect your participation in the study. If you do not give consent, your samples will be destroyed at the conclusion of the study.

Name of Participant (please print) _____

Signature _____ Date _____

Name of Witness* to
Participant's Signature (please print) _____

Signature _____ Date _____

Declaration by Study Doctor/Senior Researcher[†]

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher[†] (please print) _____

Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes.
Short Title	MOVE
Project Number	50/17
Project Sponsor	NHF Vanguard Grant
Principal Investigator	Prof David Dunstan
Associate Investigator(s)	Rachel Climie, Prof Neville Owen, Prof Bronwyn Kingwell, Prof Daniel Green, Ms Kym Rickards, Dr Neale Cohen, Ms Ashleigh Homer, Mr Michael Wheeler, Mr Hamza Ali, Ms Emily Wordie, Ms Frances Taylor.
Location	Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Baker Heart and Diabetes Institute.

Name of Participant (please print)

Signature   Date

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher[†]

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher[†] (please print)

Signature   Date

[†] A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

Participant Registration/Contact Details

Screening Date: ____ ____ / ____ ____ / ____ ____ ____ ____

Name

First: Middle: Last:

Date of Birth

Day: ____ _____   Month: ____ ____   Year: ____ ____ ____ ____   Age: ____ ____

Gender

Male ◯   Female ◯

Contact Details

Street:

Suburb:

State:

Postcode:

Email Address:

Home Phone:

Mobile:

Preferred method of contact/reminders during the trial (please circle):

Email   Text message   Phone Call

Local/Family Doctor

Name:

Healthcare company/institute:

Emergency Contact/Next of Kin

Name: Address: _____

Relationship: _____

Phone: _____

Visit _____; Condition _____

Participants Initials: _____   Participant ID code: _____

Date of Visit: ____ ____ / ____ ____ / ____ ____ ____ ____

Time of Visit: ____ ____ : ____ ____ am/pm

Study condition: ◯ Uninterrupted Sitting   ◯ 30 min SRA Breaks   ◯ 60 min SRA Breaks

Researchers for specific tasks to sign:

Signature of Research Assistant/Study Coordinator: Printed:

_____   _____

Signature of Research Nurse: Printed:

_____   _____

Signature of FMD Operator: Printed:

_____   _____

Not listed tasks; sign, initial and specify task:

Signature: Printed: Task

_____   _____   _____

Signature: Printed: Task

_____   _____   _____

Signature: Printed: Task

_____   _____   _____

PRE-TRIAL CHECKLIST

Has the clinic room been booked and in the MOVE calendar? ◯ Y ◯ N

Participant booked into VIP? ◯ Y ◯ N

Ingredients for breakfast and lunch bought and prepared? ◯ Y ◯ N

All staff informed of their participation in today's testing? ◯ Y ◯ N

Pathology forms prepared? ◯ Y ◯ N

Enough vacutainers and aliquots? (EDTA, LH, EGTA-GSH) ◯ Y ◯ N

Consumables for blood taking ready? ◯ Y ◯ N

Scales, BP Cuffs? ◯ Y ◯ N

Enough transmission gel? ◯ Y ◯ N

Labels for tubes and aliquots prepared/printed? ◯ Y ◯ N

If last visit, envelope prepared with zip lock bags (posting of handbook, activity monitors, Libre and ABPM)? ◯ Y ◯ N

PRELIMINARY CHECKLIST

Has the Principal Investigator been informed of the study visit? ◯ Y ◯ N

Has the participant been informed of all procedures to take place at Visit ___? ◯ Y ◯ N

Has this participant consented to the storage and use of blood samples? ◯ Y ◯ N

Has the participant been fasting since 10pm? ◯ Y ◯ N

No caffeine, alcohol or MVPA in the previous 72 hours? ◯ Y ◯ N

Has the participant consumed the food pack and not anything else? ◯ Y ◯ N

Has the participant completed the 7-day sleep and activity diary? ◯ Y ◯ N

Has the participant been completed the 3-day food diary? ◯ Y ◯ N

Has the participant been wearing the activity monitors? ◯ Y ◯ N

Is the Libre sensor ready for the participant (Visit 3 only)? ◯ Y ◯ N

Is the 24 ABPM ready for the participant? ◯ Y ◯ N

Ultrasound equipment for FMD set up & turned on? ◯ Y ◯ N

RPE printed? ◯ Y ◯ N

If Pre-Menopausal Female; has the participant had their period since their last visit? ◯ Y ◯ N

Time of arrival: _____

Temperature in clinic room at 0h: _____

Weight: ____ ____ ____ . ____kg

Libre old sensor fitted & reader activated?   ◯ Y   ◯ N

How many days left on old sensor?   _____

New sensor fitted & reader activated?   ◯ Y   ◯ N

Time: ____ ____ : ____ ____ am

Cannula Inserted?   ◯ Y   Time: ____ ____ : ____ ____ am

PREPARATION FOR EXPERIMENTAL CONDITION – PARTICIPANT TO SIT

-60 min (-1 h)

Blood Collection: Time: ____ ____ : ____ ____am

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **3 mL EDTA**; Full Blood Examination   ◯ Y
1 x **4.5 mL LH**; whole blood to Alfred Path (Trigs)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (1 x ins, 3 x spare)   ◯ Y

(-45 min)

Seated Blood Pressure *(1 min between)*:   Time: ____ ____ : ____ ____am

Arm Used   ◯ L   ◯ R

Cuff Size   ◯ S   ◯ L

*Average of 3 measures taken automatically 1 min apart (Omron)***:**

Systolic (mmHg): ___ ___ ___   Diastolic (mmHg): ___ ___ ___   HR (bpm): ___ ___ ___

-20 min

Femoral Flow Mediated Dilation Complete and Saved?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

End Time: ____ ____ : ____ ____ am/pm

Comments:

_____

_____

_____

Prepare Breakfast   ◯ Y   ◯ N

(-5 min)

Libre scan completed? *(ensure 1 hour has passed since fitting Libre)*   ◯ Y   ◯ N

Time: ____ ____ : ____ ____ am

____ ____ . ____ mmol/L

0 min (0 h)

Blood Collection: Time: ____ ____ : ____ ____ am

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **10 mL EDTA**; plasma, 9 x 0.5 mL aliquots (Insulin, RPA, ET-1, ICAM, VCAM, lipidomics, 3 x spare)
     ◯ Y

1 x **5 mL EGTA+GSH**; plasma stored in 1 mL aliquots (Catecholamines)   ◯ Y
1 x **4.5 mL LH**; whole blood to Alfred Path (TAG)   ◯ Y

BREAKFAST

CONDITIONS BEGIN – START TIMERS Timer start (**real time**) ____ ____ : ____ ____ am

Serve Breakfast: Time: ____ ____ : ____ ____ am   Breakfast Finished:Time: ____ ____ : ____ ____ am

Participant comfortable and understands must remain seated for next 7 h (<u>toilet breaks</u>)   ◯ Y

30 min (0.5 h)

Blood Collection: Time: ____ ____ : ____ ____ am

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, spare)   ◯ Y
1 x **5 mL EGTA+GSH**; plasma stored in 1 mL aliquots (Catecholamines)   ◯ Y

(32 min)

Completed 3 min **SRA**?   ◯ Y   ◯ N

Time: ____ ____ : ____ ____ am

RPE Scale Complete?   After 1ˢᵗ round   ◯ Y; _____   ◯ N

                             After 2ⁿᵈ round   ◯ Y; _____   ◯ N

                             After 3ʳᵈ round   ◯ Y; _____   ◯ N

(35 min)

Seated Blood Pressure *(1 min between)*:   Time: ____ ____ : ____ ____am

                                            Arm Used   ◯ L   ◯ R

                                            Cuff Size   ◯ S   ◯ L

Average of 3 measures taken automatically 1 min apart (Omron):

Systolic (mmHg): ___ ___ ___   Diastolic (mmHg): ___ ___ ___   HR (bpm): ___ ___ ___

(45 min)

Femoral Flow Mediated Dilation Complete and Saved?   ◯ Y   ◯ N

                                            Start Time: ____ ____ : ____ ____ am/pm

                                            End Time: ____ ____ : ____ ____ am/pm

Comments:

_____

_____

_____

1:00

Blood Collection: Time: ____ ____ : ____ ____am

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **8 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, ET-1, ICAM, VCAM, 3 x spare)
                                                                            ◯ Y
1 x **4.5 mL LH**; whole blood to Alfred Path (TAG)   ◯ Y

(1:02)

Completed **6 min** SRA?   ◯ Y   ◯ N

                                            Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N

                                       After 3rd round   ◯ Y; _____   ◯ N

                                       After 6th round   ◯ Y; _____   ◯ N

Completed **3 min** SRA?   ◯ Y   ◯ N

                                       Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N

                                       After 2nd round   ◯ Y; _____   ◯ N

                                       After 3rd round   ◯ Y; _____   ◯ N

1:30

Blood Collection: Time: ____ ____ : ____ ____ am/pm
1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, 3 x spare)   ◯ Y

(1:32)

Completed **3 min** SRA?   ◯ Y   ◯ N

                                       Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N

                                       After 2nd round   ◯ Y; _____   ◯ N

                                       After 3rd round   ◯ Y; _____   ◯ N

(1:35)

Seated Blood Pressure *(1 min between)*:   Time: ____ ____ : ____ ____ am/pm

                                       Arm Used   ◯ L   ◯ R

                                       Cuff Size   ◯ S   ◯ L

*Average of 3 measures taken automatically 1 min apart (Omron)***:**

Systolic (mmHg): ___ ___ ___   Diastolic (mmHg): ___ ___ ___   HR (bpm): ___ ___ ___

2:00

Blood Collection: Time: ____ ____ : ____ ____ am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4.5 mL LH**; whole blood to Alfred Path (TAG)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, RPE, 3 x spare) ◯ Y
1 x **5 mL EGTA+GSH**; plasma stored in 1 mL aliquots (Catecholamines)   ◯ Y

(2:02)

Completed **6 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1ˢᵗ round   ◯ Y; _____   ◯ N

After 3ʳᵈ round   ◯ Y; _____   ◯ N

After 6ᵗʰ round   ◯ Y; _____   ◯ N

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1ˢᵗ round   ◯ Y; _____   ◯ N

After 2ⁿᵈ round   ◯ Y; _____   ◯ N

After 3ʳᵈ round   ◯ Y; _____   ◯ N

2:30

Blood Collection: Time: ____ ____ : ____ ____ am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, 3 x spare)   ◯ Y

2:32

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1ˢᵗ round   ◯ Y; _____   ◯ N

After 2ⁿᵈ round   ◯ Y; _____   ◯ N

After 3ʳᵈ round   ◯ Y; _____   ◯ N

(2:35)

Seated Blood Pressure *(1 min between)*:   Time: ____ ____ : ____ ____am/pm

Arm Used   ◯ L   ◯ R

Cuff Size   ◯ S   ◯ L

Prepare Lunch   ◯ Y   ◯ N

*Average of 3 measures taken automatically 1 min apart (Omron)***:**

Systolic (mmHg): ___ ___ ___   Diastolic (mmHg): ___ ___ ___   HR (bpm): ___ ___ ___

3:00

Blood Collection: Time: ____ ____ : ____ ____am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, 3 x spare)   ◯ Y

(3:02)

Completed **6 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N
                                 After 3rd round   ◯ Y; _____   ◯ N
                                 After 6th round   ◯ Y; _____   ◯ N

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N
                                 After 2nd round   ◯ Y; _____   ◯ N
                                 After 3rd round   ◯ Y; _____   ◯ N

(3:10)

Seated Blood Pressure *(1 min between)*:   Time: ____ ____ : ____ ____am/pm

Arm Used   ◯ L   ◯ R

Cuff Size   ◯ S   ◯ L

*Average of 3 measures taken automatically 1 min apart (Omron)***:**

Systolic (mmHg): ___ ___ ___   Diastolic (mmHg): ___ ___ ___   HR (bpm): ___ ___ ___

(3:15)

Femoral Flow Mediated Dilation Complete and Saved?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

End Time: ____ ____ : ____ ____ am/pm

Comments:

_____

_____

_____

_____

3:30

Blood Collection: Time: ____ ____ : ____ ____am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4.5 mL LH**; whole blood to Alfred Path (TAG)   ◯ Y
1 x **10 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, RPA, ET-1, ICAM, VCAM, lipidomics, 3 x spare)
    ◯ Y
1 x **5 mL EGTA+GSH**; plasma stored in 1 mL aliquots (Catecholamines)   ◯ Y

Libre Scan completed?   ◯ Y   ◯ N

Time: ____ ____ : ____ ____am/pm   ____ ____ : ____ ____mmol/L

LUNCH

Serve Lunch: Time ____ ____ : ____ ____am/pm   Lunch Finished:Time ____ ____ : ____ ____am/pm

4:00

Blood Collection: Time: ____ ____ : ____ ____ am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, 3 x spare)   ◯ Y
1 x **5 mL EGTA+GSH**; plasma stored in 1 mL aliquots (Catecholamines)   ◯ Y

(4:02)

Completed **6 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____ ◯ N
                                       After 3rd round   ◯ Y; _____ ◯ N
                                       After 6th round   ◯ Y; _____ ◯ N

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____ ◯ N
                                       After 2nd round   ◯ Y; _____ ◯ N
                                       After 3rd round   ◯ Y; _____ ◯ N

(4:10)

Seated Blood Pressure *(1 min between)*:   Time: ____ ____ : ____ ____ am/pm

Arm Used   ◯ L   ◯ R

Cuff Size   ◯ S   ◯ L

*Average of 3 measures taken automatically 1 min apart (Omron)***:**

Systolic (mmHg): ___ ___ ___   Diastolic (mmHg): ___ ___ ___   HR (bpm): ___ ___ ___

(4:15)

Femoral Flow Mediated Dilation Complete and Saved?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

End Time: ____ ____ : ____ ____ am/pm

Comments:

_____

_____

_____

_____

4:30

Blood Collection: Time: ____ ____ : ____ ____am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4.5 mL LH**; whole blood to Alfred Path (TAG)   ◯ Y
1 x **10 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, RPA, ET-1, ICAM, VCAM, lipidomics, 3 x spare)
                                                                            ◯ Y

(4:32)

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N

After 2nd round   ◯ Y; _____   ◯ N

After 3rd round   ◯ Y; _____   ◯ N

5:00

Blood Collection: Time: ____ ____ : ____ ____am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, 3 x spare)   ◯ Y

(5:02)

Completed **6 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N

After 3rd round   ◯ Y; _____   ◯ N

After 6th round   ◯ Y; _____   ◯ N

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N

After 2nd round   ◯ Y; _____   ◯ N

After 3rd round   ◯ Y; _____   ◯ N

5:30

Blood Collection: Time: ____ ____ : ____ ____ am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4.5 mL LH**; whole blood to Alfred Path (TAG)   ◯ Y
1 x **6 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, RPA, 3 x spare)   ◯ Y
1 x **5 mL EGTA+GSH**; plasma stored in 1 mL aliquots (Catecholamines)   ◯ Y

(5:32)

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N

After 2nd round   ◯ Y; _____   ◯ N

After 3rd round   ◯ Y; _____   ◯ N

(5:35)

Seated Blood Pressure *(1 min between)*:   Time: ____ ____ : ____ ____ am/pm

Arm Used   ◯ L   ◯ R

Cuff Size   ◯ S   ◯ L

Average of 3 measures taken automatically 1 min apart (Omron):

Systolic (mmHg): ___ ___ ___   Diastolic (mmHg): ___ ___ ___   HR (bpm): ___ ___ ___

6:00

Blood Collection: Time: ____ ____ : ____ ____am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, 3 x spare)   ◯ Y

(6:02)

Completed **6 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N
                                  After 3rd round   ◯ Y; _____   ◯ N
                                  After 6th round   ◯ Y; _____   ◯ N

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?   After 1st round   ◯ Y; _____   ◯ N
                                  After 2nd round   ◯ Y; _____   ◯ N
                                  After 3rd round   ◯ Y; _____   ◯ N

6:30

Blood Collection: Time: ____ ____ : ____ ____am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **4 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, 3 x spare)   ◯ Y

(6:32)

Completed **3 min** SRA?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

RPE Scale Complete?

	After 1st round	◯ Y; _____	◯ N
	After 2nd round	◯ Y; _____	◯ N
	After 3rd round	◯ Y; _____	◯ N

(6:35)

Seated Blood Pressure *(1 min between)*:   Time: ____ ____ : ____ ____ am/pm

Arm Used   ◯ L   ◯ R

Cuff Size   ◯ S   ◯ L

Average of 3 measures taken automatically 1 min apart (Omron):

Systolic (mmHg): ___ ___ ___   Diastolic (mmHg): ___ ___ ___   HR (bpm): ___ ___ ___

(6:45)

Femoral Flow Mediated Dilation Complete and Saved?   ◯ Y   ◯ N

Start Time: ____ ____ : ____ ____ am/pm

End Time: ____ ____ : ____ ____ am/pm

Comments:

_____

_____

_____

_____

7:00

Blood Collection: Time: ____ ____ : ____ ____ am/pm

1 x **0.1 mL Syringe**; whole blood (Haemocue)   ◯ Y
1 x **3 mL EDTA**; Full blood count for Alfred Path
1 x **4.5 mL LH**; whole blood to Alfred Path (TAG)   ◯ Y
1 x **10 mL EDTA**; plasma stored in 0.5 mL aliquots (Insulin, RPA, ET-1, ICAM, VCAM, lipidomics, 3 x spare)
        ◯ Y
1 x **5 mL EGTA+GSH**; plasma stored in 1 mL aliquots (Catecholamines)   ◯ Y

CONDITIONS END – STOP TIMERS Timer stop (**real time**)   ____ ____ : ____ ____pm

Complete final checklists BEFORE participants departs

PARTICIPANT WATER CONSUMPTION LOG

Tally of 1 L jugs consumed (mark off over trial)

_____

1 L x number = ____ ____ ____ ____mL ▬ amount in final 1 L jug (____ ____ ____ ____mL)

             Total volume of water participant consumed over trial period: ____ ____ ____ ____mL

PARTICIPANT TOILET BREAKS LOG

Time participant got out of chair	Time participant got back into chair	Wheelchair used	Comments
		◯ Y ◯ N	
		◯ Y ◯ N	
		◯ Y ◯ N	
		◯ Y ◯ N	
		◯ Y ◯ N	
		◯ Y ◯ N	

FINAL CHECKLIST

Participant understands they must scan Libre on data sheet before dinner, before bed and when they wake up (before breakfast)? Recording sheets provided?   ◯ Y   ◯ N

CGM and 24 h ABPM are fitted and comfortable – participant has no issues?   ◯ Y   ◯ N

Participant has been given new activity monitors and old ones collected?   ◯ Y   ◯ N

Participant has dinner pack and understands that they are only to eat what is provided?   ◯ Y   ◯ N

If final visit: Has participant completed Requisition for Payment (RFP) form?   ◯ Y   ◯ N

Participant has received postage envelope and protective wrapping for equipment?   ◯ Y   ◯ N

RESEARCH PERSONNEL

Final Comments:

_____

_____

_____

_____

_____

_____

_____

Role	**Name**	**Signature**
Principal Investigator /Trial Coordinator		
Clinical Nurse		
FMD Operator		
Other Signatures		

ETHICS COMMITTEE CERTIFICATE OF APPROVAL

This is to certify that

Project No: 91/18

Project Title: Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study

Principal Researcher: Professor David Dunstan

Protocol Version 1.1 **dated:** 12-Apr-2018

Participant Information and Consent Form Version 1.4 **dated:** 17-Apr-2018

*was considered by the Ethics Committee on **22-Mar-2018**, meets the requirements of the National Statement on Ethical Conduct in Human Research (2007) and was **APPROVED** on **30-Apr-2018***

It is the Principal Researcher's responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or progress report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- Any expiry of the insurance coverage provided with respect to sponsored clinical trials and proof of re-insurance;
- A delay of more than 12 months in the commencement of the project; and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit

- A Progress Report on the anniversary of approval and on completion of the project *(forms to be provided)*;

The Ethics Committee may conduct an audit at any time.

All research subject to the Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Hospital Ethics Committee is a properly constituted Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research (2007).

SPECIAL CONDITIONS

None   **SIGNED:**

Professor John J. McNeil
Chair, Ethics Committee

Please quote project number and title in all correspondence

theAlfred

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **91/18 Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study**

Principal Researcher: **Professor David Dunstan**

Amendment:
Protocol changes – inclusion/exclusion criteria, sample processing and transfer process, experimental procedure (3xFMD); PICF changes – consent for future contact, FMD risk wording; Addition of participant registration form and sleep screener

Attachments:
PICF version **2.0** dated **17-May-2018**
Protocol version **2.0** dated **17-May-2018**
Eligibility survey+screening version **2.0** dated **17-May-2018**
Participant registration form version **1.0** dated **17-May-2018**
Sleep disorders screener version **1.0** dated **14-May-2018**

have been approved in accordance with your amendment application dated **17-May-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil
Chair, Ethics Committee

Date: **4-Jun-2018**

theAlfred

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **91/18 Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study**

Principal Researcher: **Professor David Dunstan**

Amendment:
Changes to inclusion/exclusion criteria

Attachments:
Protocol version **3.0** dated **20-Jul-2018**
Advertisement version **2.0**

have been approved in accordance with your amendment application dated **9-Jul-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **31-Jul-2018**
Chair, Ethics Committee

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **91/18 Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study**

Principal Researcher: **Professor David Dunstan**

Amendment:
**Addition of smoking questionnaire and heart rate variability measurement;
Change to research personnel – Addition of Ms Melanie Townsend**

Attachments:
Protocol version **4.0** dated **24-Aug-2018**
PICF version **4.0** dated **24-Aug-2018**
Smoking Questionnaire version **2.0** dated **18-Sep-2018**

have been approved in accordance with your amendment application dated **4-Sep-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **27-Sep-2018**
Chair, Ethics Committee

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **91/18**
Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study

Principal Researcher:
Professor David Dunstan

Amendment:
Changes to protocol: increasing the BMI range to 18.5-45 kg/m2

Documents:
Protocol version 5 dated 17-Jan-2019
PICF version 5 dated 17-Jan-2019

have been approved in accordance with your amendment application dated **17-Jan-2019** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil   Date: **24-Jan-2019**
Chair, Ethics Committee

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

PCOS-BREAKS Study

Women with PCOS

Ever wondered if breaking up your sitting time could help control your blood glucose levels?

Researchers at the **Baker Heart and Diabetes Institute** are conducting a study looking at whether or not breaking up long periods of sitting time can help lower glucose and insulin levels in women with polycystic ovary syndrome (PCOS).

We are looking for women aged 18 to 45 years who have been medically diagnosed with PCOS to take part in this study.

To find out whether you are eligible to participate in this study, please visit https://baker.edu.au/pcos

For more information, please contact:
Dr Elly Fletcher
Study Coordinator
T: 03 8532 1834
E: PCOS-BREAKS@baker.edu.au

ABOUT THE STUDY

What is the aim of the study?

We aim to identify whether breaking up long periods of sitting time can help lower glucose and insulin levels in women with PCOS, to ultimately decrease the future risk of developing type 2 diabetes.

What does the study involve?

Participants will be required to:

- Attend 3 visits at the Baker Clinic over a three week period:
  - 1 familiarisation visit (2 hrs) and;
  - 2 testing study visits (4 hrs each)

- Provide blood samples at regular intervals (by means of intravenous catheter) during the testing visits

- Wear physical activity and continuous blood glucose monitors for a 5-day period on two occasions.

- Keep a record of your food and drink intake over a 24 hour period on two occasions.

Participants will be reimbursed for their time

CONTACT DETAILS

Dr Elly Fletcher
Level 4, 99 Commercial Road
Melbourne VIC 3004
E: PCOS-BREAKS@baker.edu.au
T: 03 8532 1834
www.baker.edu.au

Participant Information Sheet/Consent Form
Non-Interventional Study - *Adult providing own consent*
Baker Heart and Diabetes Institute

Title	Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study
Short Title	PCOS BREAKS Study
Protocol Number	V5.0
Project Sponsor	Baker Heart and Diabetes Institute
Principal Investigator	Prof David Dunstan
Associate Investigators (Australia)	A/Prof Lisa Moran, Prof Neville Owen, Dr Elly Fletcher, A/Prof Neale Cohen, Dr Paddy Dempsey, Dr Robyn Larsen, Dr Anju Joham, Mr Hamza Ali, Ms Kym Rickards, Miss Frances Taylor, Miss Melanie Townsend
Associate Investigator (International)	A/Prof Matthew Buman (USA)
Location	Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC

Part 1   What does my participation involve?

1   Introduction

You are invited to take part in this research project *"**Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study"**.* This is because you have polycystic ovary syndrome (PCOS) – a condition that affects a women's hormone levels, and as a result can increase their risk of developing future type 2 diabetes and cardiovascular disease (CVD). As 1-in-3 overweight women in Australia are diagnosed as having PCOS, you represent an important target group for evaluating strategies to decrease the future risk of developing type 2 diabetes and CVD.

The primary aim of this research project is to study the effect of frequent light physical activity breaks from sitting on blood glucose and insulin levels (well-known risk factors for type 2 diabetes and CVD) when compared with uninterrupted sitting. The secondary aims of this research project is to study the effects of frequent light activity breaks from sitting on: 1) common PCOS androgen markers (e.g. total testosterone and sex hormone binding globulin [SHBG]); 2) fatigue/alertness levels during the day; and 3) sleep quality, when compared to uninterrupted sitting.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and research involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:
• Understand what you have read
• Consent to take part in the research project
• Consent to the tests and research that are described
• Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

This research project has been registered with the "Australian New Zealand Clinical Trial Registry" (ANZCTR): ACTRN12618000239268p

2   What is the purpose of this research?

Currently, the first-line of treatment for women with PCOS is weight management through optimising diet and physical activity. However, dropout rates of up to 45% have been reported for both the diet and physical activity based lifestyle-interventions that involve women with PCOS. It is therefore important to identify alternative lifestyle strategies that will help reduce any future risk of developing type 2 diabetes and CVD.

Recent evidence suggests that reducing and breaking up sitting time may be a suitable self-care behaviour to improve risk factors relating to type 2 diabetes and CVD, such as high blood sugar and insulin resistance. Our laboratory has recently shown in overweight/obese individuals and those with type 2 diabetes that regular (every 20-30 minutes), brief (2-3 minutes) bouts of light-intensity activity (such as light walking and simple resistance activities) can lower blood glucose levels after a meal by 24-35% when compared to prolonged sitting. We have also shown that these types of breaks can help lower blood pressure and contribute to improvements in measurements of vascular health. However, it remains to be determined whether there is a potential benefit of reducing and breaking up sitting time in women with PCOS.

This study will evaluate whether breaking up sitting time throughout the day with repeated bouts of simple body-weight resistance activities can result in improve blood glucose and insulin levels when compared to sitting for prolonged periods without activity breaks. The study will also evaluate whether breaking up sitting time throughout the day will have improvements in common PCOS androgen markers, reduce fatigue throughout the day and improve sleep quality at night.

The results of this trial will help to better understand the potential importance of breaking up prolonged sitting in women with PCOS and how this may improve their cardiometabolic profile, PCOS-related symptoms and fatigue/sleep quality. The findings will also help to inform similar studies involving women with PCOS, as well as providing initial evidence to help inform the PCOS future guidelines update.

The results of this research will also be used by PhD candidate, Miss Frances Taylor, to obtain a Doctor of Philosophy degree. Specifically, Miss Taylor will use the flow mediation dilation (FMD) results to understand how breaking up prolonged sitting influences endothelial function.

This research has been initiated by Professor David Dunstan and has been funded by a NHMRC Centre of Research Excellence (CRE) in PCOS from the Monash Health Clinic and by a NHMRC CRE on sitting time and chronic disease prevention from the Baker.

3   What does participation in this research involve?

Approximately 22 volunteers will be required for this study. To determine eligibility for the study, all potential participants will undergo a screening process via an online survey, and if necessary, undergo a blood test and/or ultrasound (at no expense to the participant). If eligible to participate, consent will be obtained. Participation in this project will involve three visits to the Baker Heart & Diabetes Institute, over a period of three weeks. During the first week, you will visit the Baker on two occasions (visit one will be to fit the monitors and visit two will be to complete one of the two testing days). During the second week, you are not required to do anything except to go about your normal, everyday activities. This is because we need a 7-day wash out period in between completing the two testing days. During your third and final week, you will complete the second testing day.

Please see below for more details on what is involved during the screening process and at each visit to the Baker.

SCREENING PROCESS (PRIOR TO THE STUDY)

To determine eligibility, you will initially be asked to complete a short online survey. This involves answering a range of questions about your: age, height and weight, PCOS history, medical history (including if you are pregnant or breastfeeding), medication use (including insulin/glucose and hormonal medications), physical activity/sedentary behaviour levels, work, and sleep.

To confirm whether you have PCOS, you may need to have a blood test and/or ultrasound. The blood test is performed by your local Melbourne Pathology Centre and will measure whether you have hyperandrogenism (e.g. high levels of testosterone) and if you have any other endocrinology disorders other than PCOS. The ultrasound is performed by Women's Ultrasound Melbourne (three locations in Melbourne) and will determine whether you have polycystic ovaries (e.g. cysts on the ovaries). The blood test and ultrasound will be at no cost for potential participants.

Disclosure of your medication use is required to determine eligibility for the study. This is because participants must not be taking any insulin sensitising agents (e.g. metformin) or hormonal medication (e.g. oral contraceptive pill) at least three months prior to the study starting. If you are currently taking any insulin sensitising agents or hormonal medication we will ask you to speak to your local GP or endocrinologist about potentially ceasing this medication for three months prior to the study starting.

Once all the inclusion and exclusion criteria have been met and you have been deemed suitable to take part in the study, your three study visit appointments at the Baker will be scheduled. At your first appointment, you will be given the opportunity to ask questions before giving consent to participate.

WHAT IS INVOLVED IN THE STUDY

<u>VISIT 1 – FAMILIARISATION & FITTING MONITORS (60 MINUTES)</u>

Location: Clinical research rooms at Baker Heart and Diabetes Institute (Level 4, 99 Commercial Street, Melbourne 3004

During visit 1, you will be asked to attend the clinical research rooms at Baker Heart and Diabetes Institute. Here you will undergo the following:

❖ Sign the consent form in the presence of a research staff member and to complete study related forms/questionnaires.

❖ Research staff will measure your height, weight, blood pressure and neck, hip and waist circumference.

❖ You will be fitted with three monitors that measure and store information regarding your blood glucose and physical activity patterns. You will be required to wear all three monitors during all times (including sleeping, with the exception of removing the ActiGraph watch prior to bathing/showering) until the morning after you have completed testing day 1. You will then receive new monitors to wear for the 48-hours prior to testing day 2 and to wear them until the morning after you have completed testing day 2. Each day while wearing the monitors,

you will be asked to complete a short online activity/monitor log (takes 2-3 minutes). Please see below for details about the monitors:

Monitor 1 is a **Continuous Blood Glucose Monitor** (CGM) and is a small sensor that is inserted just beneath the skin of your upper arm and measures the amount of glucose in the fluid underneath your skin for the period that it is worn. Research staff will demonstrate how to insert the first CGM at the familiarisation visit as you will be required to insert the second CGM at your home at week 2. The CGM used in this study has been designed to be easily inserted by the individual.

Monitor 2 is known as an **activPAL™ inclinometer** (worn on your upper thigh) and records information regarding the frequency and duration of times spent sitting, standing and walking.

Monitor 3 is known as an **actiGraph GT3X+ accelerometer** (worn on your wrist) and records information regarding the frequency, intensity and duration of times spent sleeping and moving.

❖ Research staff will demonstrate the flow mediated dilation (FMD) test (see details below) and the simple (body weight) resistance exercises. You will have the opportunity to practice the simple resistance exercises so you become familiar with them.

❖ At the end of the visit, you will be given a food diary and meal pack. The food diary is for you to record all the food and beverages that you consume on the day prior to the first testing day. You will be then asked to consume this same diet on the day prior to the second testing day. The meal pack will contain 1 x standardised dinner. You will be asked to consume this dinner the evening prior to the first testing day.

It is anticipated that visit 1 will take approximately 90 minutes.

VISIT 2 & 3 – TESTING DAYS 1 AND 2 (APPROXIMATELY 5 HOURS, COMPLETED IN WEEK 1 AND WEEK 3)

Location: Clinical research rooms at Baker Heart and Diabetes Institute (Level 4, 99 Commercial Street, Melbourne 3004

Preparation for the testing days:
In the 48 hour period prior to each testing day, we ask that you avoid alcohol and caffeine (e.g. tea, coffee, caffeinated soft drinks) and do not engage in any moderate (e.g. gentle swimming, yoga) and/or vigorous (e.g. aerobics) physical activity. On the day prior to the first testing day, you will be asked to record all your food and drink intake in a food diary. You will then asked to consume this same diet on the day prior to the second testing day. On the night before each testing day, we ask that you consume only the foods provided to you in your 'meal pack' and no other foods or drinks, except water.

Testing days 1 and 2
On each testing day, you will be asked to report to the clinical testing rooms at Baker at 8:00am after having fasted from 10:00pm the prior evening. On arrival, a cannula will be inserted into a vein in your arm so the researchers can take the half hourly collection of venous blood samples (1.5 tablespoons/25 mLs per hour) on 8 occasions. A small, leadless ECG patch (***CardioWave™***) will be attached to your chest via two sticky electrodes during the study visit. This device is designed to allow longer duration electrocardiogram monitoring. A non-invasive measurement of your blood vessel health (the FMD) test) will also be taken in your upper leg (right thigh) three times throughout the experimental procedure. This procedure measures how your blood vessels respond to an increase in blood flow after occluding the main artery in your leg with a blood pressure cuff for a 5 minute period. The increase in blood flow will be measured with the use of ultrasound. Mild discomfort (e.g. pins and needles) may arise during the inflation of blood pressure cuffs on the right leg during testing measures. The longest period of cuff inflation will be five minutes. Cuffs will not be inflated to such an extent or duration that will cause permanent damage to your arms and leg. Throughout the testing days, blood pressure will also be measured every hour and you will be asked to complete a brief fatigue questionnaire on three different occasions.

The two testing days will be completed in random order:

Testing day 1 - Prolonged sitting: Testing day 1 will commence with an initial 60 minute 'steady state' period whereby you will be asked to sit quietly in a firm, but comfortable chair. Afterwards, baseline measurements of a blood test will be collected and you will be asked to consume a 75 g glucose drink (known as an oral glucose tolerance test). During the next 3 hour period, you will be asked to sit quietly in a comfortable lounge chair. You will be able to watch television programs or DVD's, use your computer/tablet and/or read. At the end of the 3 hour period, lunch will be provided (your choice from the Café's menu).

After leaving the laboratory, you will be asked to write down all food and beverages consumed for the rest of the day in a food diary provided. We will ask you to replicate this diet for testing day 2 after leaving the laboratory. We will also ask you to continue to wear the monitors until the following morning. This is because the monitors will provide an opportunity for us to examine any flow on effects of the testing days on blood glucose patterns, which persist beyond the laboratory period.

Testing day 2: Breaking up sitting with simple resistance activities: Nine days later, you will again be required to report to the clinical research rooms at 8:00am and will undertake all of the procedures described in testing day 1. The exception will be that every 30 minutes you will be asked to perform a 3 minute bout of simple resistance activities such as body weight half-squats, calf raises, brief gluteal contractions and single leg knee raises. You will then return to your seated position. This procedure will be repeated every 30 minutes on another 5 occasions (total activity time: 18 minutes). Lunch will also be provided your choice from the Café's menu).

After leaving the laboratory, you will be asked to consume the same diet as you did on testing day 1 (a copy of your food diary on testing day 1 will be provided to help with this). Similar to testing day 1, you will also continue to wear the monitors until the following morning.

On the day of both your study visit, we ask that you go about normal activities, representative of your daily routine, and refrain from any moderate-vigorous physical activity.

Figure 1: Experimental protocol
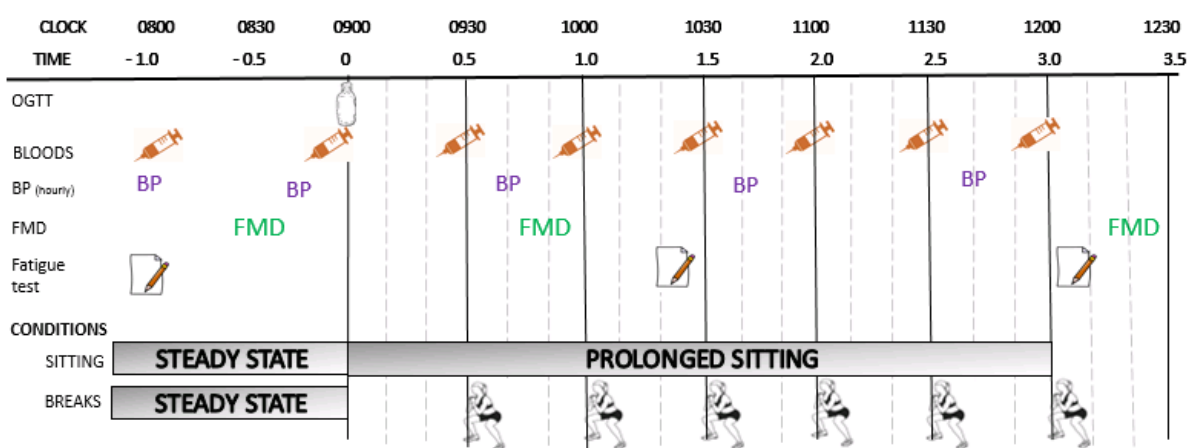
Reimbursement

At the completion of the study, you will be reimbursed $100 for your time and the parking/transport costs associated with your involvement in the study. You will also be provided with lunch prior to leaving. There are no other associated costs with participating in this research project.

4   What do I have to do?

In order to participate in the study, you must be willing to attend all three scheduled visits and be willing to undergo the necessary tests. During the study period, you will wear three monitors (a CGM, an activPAL™ and an actiGraph) that measures your blood glucose concentrations and physical activity levels. The CGM captures blood glucose patterns and you will be required to measure your blood glucose three times a day to ensure the data is transferred to the Abbott Libre glucose reader. The CGM and activPAL monitors will be fitted with tegaderm (a gentle, hypoallergenic, breathable tape) and all three monitors should be worn at all times, including in bed and in the shower.

You will also be asked to follow specific dietary instructions. In the 48-hour period prior to each testing day, we ask that you avoid any caffeine (coffee, tea, caffeinated soft drinks, etc.) and alcohol. We also ask that you complete a food diary the day prior to the testing days and the day of the testing day and to replicate this diet for testing day 2.

For the 3-week period that you will be participating in the study, we would also like to keep track of your menstrual cycle and any medications that you are taking. If you are feeling unwell on the day of a scheduled study visit, please contact a study co-ordinator to reschedule your study visit.

5   Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your relationship with Baker Heart and Diabetes Institute.

6   What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research, however, possible benefits may include: an individualised health profile; knowledge and understanding of your metabolic health; and what happens to your blood vessel health when you are sedentary and/or actively breaking up your sitting. We expect that the findings from this study will make an important contribution to the design, implementation and evaluation of intervention strategies to reduce the risk of developing future type 2 diabetes and/or CVD in women with PCOS.

If an abnormal test result comes to light during the study, the project coordinator or the study endocrinologists will inform you of your abnormal results. A copy of the results will be provided to you and you will be advised to discuss the results with your local GP. If further care is provided, the research coordinator can arrange an appointment with any member of our health care team, including endocrinologists, who can help you to manage your PCOS symptoms.

7   What are the possible risks and disadvantages of taking part?

This research involves a number of procedures in which you may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, please talk with the study nurse who is part of the research team. Your study nurse will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study nurse immediately about any new or unusual symptoms.

Many side effects go away following the completion of a procedure. However, sometimes side effects can be serious, long lasting or permanent. A member of the research team will discuss the best way of managing any side effects with you.

The risks associated with the individual procedures involved in the study are as follows:

Prolonged sitting – sitting uninterrupted for three hours may be uncomfortable for some participants. Where necessary, participants will be allowed to attend the bathroom.

Cannulation / blood test – Having a blood sample taken may cause some discomfort or bruising. Sometimes, the blood vessel may swell, or blood may clot in the blood vessel, or the spot from which tissue is taken could become inflamed. Some people may feel faint when having blood taken, and may occasionally faint. Rarely, there can be a minor infection or bleeding. If this happens, it can be easily treated.

Continuous Blood Glucose Monitoring – CGM is a TGA-approved device (manufactured by Abbott Australiasia) that records blood glucose levels throughout the day and night. The Abbott CGM system is commonly used for the management of type 1 and type 2 diabetes and is routinely used within Baker's specialist diabetes clinics. It involves having a tiny sensor inserted into the back of your upper arm (sensor is roughly the size of a 50 cent piece), which communicates with the Abbott Libre blood glucose reader. Insertion of the sensor is virtually painless and the sensor and data monitor are comfortable to wear. Irritation or inflammation may occur at the sensor site, but this is unlikely as the device is minimally invasive and only worn for a short amount of time. A team endocrinologist is also on hand to help treat any eventualities, if they occur.

FMD test – Mild discomfort (e.g. pins and needles) may arise during the inflation of blood pressure cuffs on the right leg during the FMD tests. The longest period of cuff inflation will be five minutes. Cuffs will not be inflated to such an extent or duration that will cause permanent damage to your arms and leg

Resistance activity breaks - As part of this study, you will be asked to perform simple body weight resistance activities involving your lower body. To help minimise your risk, all experimental sessions will be supervised by trained staff. There is a small possibility that you will experience minor and transient muscle soreness initially following the exercise sessions.

Skin irritations – In the unlikely event, participants may develop a skin irritation from the tape used to keep the activPal in position on the thigh.

Electrocardiogram (CardioWave) monitoring – you may find that the electrodes on your chest area become a little itchy. If this is the case, please consult a member of a research team and the device will be removed.

Disclosing personal information – This study involves disclosing personal information such as your menstrual cycle history. Although all information relating to menstrual history will remain confidential, some participants may experience some mild anxiety/distress while answering these questions.

There may be additional unforeseen or unknown risks. Participation in this study can be suspended or terminated if a medical issue or distress occurs.

8   What will happen to my test samples?

On each testing day, a fasting blood sample will be taken to measure glucose, insulin, total testosterone and sex hormone binding globulin (SHBG). These blood markers are used to assess cardiometabolic health and PCOS related symptoms, and are routinely assessed in conditions such as PCOS. Further blood samples (measuring glucose and insulin) will be collected half-hourly during the oral glucose tolerance test, with a final blood sample taken on completion of the experimental trial (measuring glucose, insulin, total testosterone and SHBG).

At each visit, 10mls of blood collected may be stored in case verification of any measurement is required at a later date. If no verification is required, these samples will be destroyed at the conclusion

of the study. However, we would like your permission to store these samples for future research into PCOS, CVD and diabetes. Your samples for the current study and for any future studies will not undergo genetic testing. Only study investigators will have access to your samples. By signing the Consent Form for blood sample storage and use, you consent to the analysis of substances relevant to diabetes and heart disease in your blood samples. The storage of any excess sample is voluntary.

Once the study has been completed you will be provided with a summary of both your individual results and group data by mail. If the study results are published in a scientific journal you will also be sent a copy of the publication.

9   What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, a member of the research team will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, a member of the research team will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

10   Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or treatments you have been taking for your PCOS or for other reasons. All participants who are currently taking any insulin sensitising agents (e.g. metformin) or hormonal medication (e.g. oral contraceptive pill), are asked to speak with their GP or endocrinologist about potentially ceasing this medication for 3 months prior to the study starting.

It is important to tell a member of the research team about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell the research team about any changes to these during your participation in the research project.

11   What if I withdraw from this research project?

If you decide to withdraw from this research project, please notify a member of the research team before you withdraw. A member of the research team will inform you if there are any special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly. You should be aware that data collected up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them when you withdraw from the research project.

12   Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons including unacceptable side effects, but this is unlikely.

13   What happens when the research project ends?

At the completion of the study, you will be provided with an individual report of your results. If requested, you can also receive a report of the main findings of the study and copies of any subsequent publications.

Part 2   How is the research project being conducted?

14   What will happen to information about me?

By signing the consent form you consent to the relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research study that can identify you will remain confidential and will only be used for the purpose of this research study. It will only be disclosed with your permission, or in compliance with the journal requirements and the law.

This research study involves the establishment of a databank. When you sign the attached consent form, you are consenting your information being used for this specific study.

All study data will be stored using a Baker-approved data management tool called *Research Electronic Data Capture* ('REDCap'). REDCap is a secure web application for building and managing online surveys and databases. The database will only be accessed by the researchers involved in the study. The data stored in REDCap is in a re-identifiable (coded) format.

Hard copy data, such as the consent form and medical history form, will be stored in a locked filing cabinet in the Study Coordinator's office at the Physical Activity laboratory, Baker Heart & Diabetes Institute, 99 Commercial Rd, Melbourne. The hard copy data will be stored in a re-identifiable (coded) format and will be stored indefinitely as per Alfred Hospital Study policy. At the completion of the study, hard copy data will be archived securely off site.

Identifying information will not appear in any data reports or publications. Your information will only be used for the purpose of this research study. Your information will only be disclosed with your permission, or in compliance with the law.

If you have a regular local doctor, it is desirable that they be advised of your decision to participate in this research study. If you do have a local doctor, by signing this consent form, you are agreeing to inform him/her of your participation in the study.

It is anticipated that the results of this research study will be published and/or presented in a variety of forums, which may include publication in scientific journals, presentations at scientific conferences and clinical trial registries such as the Australia New Zealand Clinical Trial Registry (https://www.anzctr.org.au). Only group data will be published and presented. As a requirement for some scientific journals to ensure integrity of the study's findings, the non-identifiable data underlying the findings described in the publication may need to be submitted to the journal for review. If this is the case, the data file provided will not contain information that can identify you (e.g. no names, contact details or addresses will be provided). Your identity as a participant in this study will remain confidential.

In accordance with relevant Australian and Victorian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

In accordance with the NHMRC National Statement, the Research Ethics Committee is required to conduct audits of research projects from time to time. It may therefore be possible that the Research Ethics Committee which has approved this research will seek to view a copy of your signed consent form, or to contact you, to ensure that the research is being conducted according to the ethical standards required by the National Statement.

15   Complaints and compensation

If you suffer any injuries or complications as a result of this research study, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital. If

you ineligible for Medicare, you may be able to receive medical treatment through your private health insurer. You should contact your insurer before enrolling in this trial to ensure any medical treatment you require in connection with participation falls within your policy's terms and conditions.

16   Who is organising and funding the research?

This research study is being conducted and funded through a Monash NHMRC Centre for Research Excellence in PCOS and NHMRC Centre for Research Excellence on sitting time and chronic disease prevention.

You will not benefit financially from your involvement in this research study even if, for example, your samples (or knowledge acquired from analysis of your samples) prove to be of commercial value to the Baker Heart and Diabetes Institute. In addition, if knowledge acquired through this research leads to discoveries that are of commercial value to the study team or their institutions, there will be no financial benefit to you or your family from these discoveries.

The Baker Heart and Diabetes Institute will receive payment for the direct research costs for this study from Monash. No member of the research team will receive a personal financial benefit from your involvement in this research study (other than their ordinary wages).

17   Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research study have been approved by the HREC of The Alfred Hospital, Melbourne.

This study will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

18   Further information and who to contact

The person you may need to contact will depend on the nature of your query. If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact any of the following people:

Trial Co-ordinator: Dr Elly Fletcher
Telephone: (03) 8532 1834
Email: PCOS-BREAKS@baker.edu.au

Research Assistant: Mr Hamza Ali
Telephone: (03) 8532 1852

Principal Investigator: Prof David Dunstan
Telephone: (03) 8532 1873

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Position: Complaints Officer, Office of Ethics & Research Governance, Alfred Hospital
Telephone: (03) 9076 3619

Please quote the following Alfred Health project number: 91/18

Consent Form - *Adult providing own consent*

Title	Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study
Short Title	PCOS BREAKS Study
Protocol Number	V5.0
Project Sponsor	Baker Heart and Diabetes Institute
Principal Investigator	Prof David Dunstan
Associate Investigators (Australia)	A/Prof Lisa Moran, Prof Neville Owen, Dr Elly Fletcher, A/Prof Neale Cohen, Dr Paddy Dempsey, Dr Robyn Larsen, Dr Anju Joham, Mr Hamza Ali, Ms Kym Rickards, Miss Frances Taylor, Miss Melanie Townsend
Associate Investigator (International)	A/Prof Matthew Buman (USA)
Location	Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.

Declaration by Participant

I have read the Participant Information Sheet.

I understand the purposes, procedures and risks of the research described in the project.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the project without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Baker Heart and Diabetes Institute concerning my condition and treatment for the purposes of this project. I understand that such information will remain confidential.

I agree that research data gathered for the study may be published provided my name or other identifying information is not used.

Name of Participant (please print) _____
Signature _____   Date _____

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher† (please print) _____
Signature _____   Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Consent Form For The Storage And Use Of Additional Blood Samples

Title	Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study
Short Title	PCOS BREAKS Study
Protocol Number	V5.0
Project Sponsor	Baker Heart and Diabetes Institute
Principal Investigator	Prof David Dunstan
Associate Investigator (Australia)	A/Prof Lisa Moran, Prof Neville Owen, Dr Elly Fletcher, A/Prof Neale Cohen, Dr Paddy Dempsey, Dr Robyn Larsen, Dr Anju Joham, Mr Hamza Ali, Ms Kym Rickards, Miss Frances Taylor, Miss Melanie Townsend
Associate Investigator (International)	A/Prof Matthew Buman (USA)
Location	Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.

Declaration by Participant

I have read Section 8 of this Participant Information Form and am aware that extra blood is being collected for verification of pathology tests if required.

In the case that verification is not required, we ask for your consent to store and use these samples for future research into cardiovascular disease and diabetes (i.e. for future research to be approved by the Alfred Human Ethics Committee)

Note: Storage of extra samples is voluntary and will not affect your participation in the study. If you do not give consent, your samples will be destroyed at the conclusion of the study.

Do you give consent for researchers to store and use these samples for future research into PCOS, CVD and/or diabetes?   *Please write "Yes" or "No"*

Name of Participant (please print) _____
Signature _____ Date _____

Declaration by Study Doctor/Senior Researcher[†]

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher[†] (please print) _____
Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	Do regular activity breaks from prolonged sitting improve the cardiometabolic profile of women with polycystic ovary syndrome? The PCOS BREAKS Study
Short Title	PCOS BREAKS Study
Protocol Number	V5.0
Project Sponsor	Baker Heart and Diabetes Institute
Principal Investigator	Prof David Dunstan
Associate Investigator (Australia)	A/Prof Lisa Moran, Prof Neville Owen, Dr Elly Fletcher, A/Prof Neale Cohen, Dr Paddy Dempsey, Dr Robyn Larsen, Dr Anju Joham, Mr Hamza Ali, Ms Kym Rickards, Miss Frances Taylor, Miss Melanie Townsend
Associate Investigator (International)	A/Prof Matthew Buman (USA)
Location	Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Baker IDI Heart and Diabetes Institute.

Name of Participant (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

PCOS-BREAKS STUDY REGISTRATION FORM (v1.0)

ABOUT YOU

Full legal name: _____

Home address: _____ Suburb: _____ Postcode: _____

Home phone: _____ Mobile: _____

Email: _____

Date of birth: _____

EMERGENCY CONTACT

Name: _____ Relationship: _____

Mobile: _____ Work: _____ Home: _____

INFORMATION FOR MEDICAL TREATMENT

Your doctor's name: _____

Doctor's Clinic: _____

Address: _____ Suburb: _____ Postcode: _____

Phone number: _____

Medicare card number: _____ Ref number: _____ Expiry: _____

Private Health Insurance? Y / N

If yes: Name of health fund: _____ Card # _____

MEDICATION USE

Please list any medications you take on a regular basis:

#1 Name: _____ Dose/Freq: _____

     Reason: _____

#2 Name: _____ Dose/Freq: _____

     Reason: _____

#3 Name: _____ Dose/Freq: _____

     Reason: _____

BANK DETAILS

We require your bank details so we can transfer your compensation directly into your bank account.

Name of bank: _____

BSB: _____ Account: _____

THANK YOU FOR COMPLETING THIS FORM. PLEASE HAND BACK TO A RESEARCH STAFF MEMBER

EXPERIMENTAL CONDITION: CRF FOR SV01 AND SV02

Participants Initials: ☐☐☐   Participant ID code: ☐☐☐☐

Date of visit: ☐☐ / ☐☐ / ☐☐   Time of Arrival: ☐☐ : ☐☐

Signature of study personnel: _____   Personnel ID: ☐☐

EXPERIMENTAL CONDITION:

Experimental Condition: ○ SIT Condition   ○ SRA Breaks Condition

PRELIMINARY CHECKLIST

1. Has the participant visited the bathroom?   ○ Y   ○ N

2. Has the participant fasted this morning?   ○ Y   ○ N

3. Did the participant consume last night's meal pack?   ○ Y   ○ N   Time of meal: ☐☐ : ☐☐

4. Did the participant complete a 1-day food record?   ○ Y   ○ N   ○ N/A

5. Has the participant avoided caffeine for 48hrs?   ○ Y   ○ N

6. Has the participant avoided alcohol for 48hrs?   ○ Y   ○ N

7. Has the participant avoided moderate-vigorous physical activity for 48hrs?   ○ Y   ○ N

8. Did the participant go to bed at their normal bedtime?   ○ Y   ○ N   Time of bed: ☐☐ ☐☐

MEASUREMENTS

9. Seated blood pressure: Arm used: ○ R   ○ L

Blood pressure to be measured on the non-dominant arm

(a) HR(60sec): ☐☐   (b) Systolic (mmHg): ☐☐☐   (c) Diastolic (mmHg): ☐☐☐

(d) HR(60sec): ☐☐   (e) Systolic (mmHg): ☐☐☐   (f) Diastolic (mmHg): ☐☐☐

(g) HR(60sec): ☐☐   (h) Systolic (mmHg): ☐☐☐   (i) Diastolic (mmHg): ☐☐☐

10. Weight (kg) (a): ☐☐☐ . ☐   (b): ☐☐☐ . ☐   *(use calibrated scales in rooms 1-3)*

11. Libre Flash blood glucose swipe: ☐☐ . ☐ mmol/L   Real time: ☐☐ : ☐☐

Participant ID:

Initials   *Code*

EXPERIMENTAL CONDITION START – STEADY STATE 1 HOUR

TIME: -1.00 – VENOUS BLOOD COLLECTION (STEADY STATE)
Cannulation of dominant arm is preferred

12. Actual time of blood collection: ☐☐ : ☐☐   (Steady state period begins)

13. Blood tubes collected: ◯ 3ml Glucose (Grey) ◯ 6ml Insulin (Gold) ◯ 3ml spare (Purple)

14. Is the participant comfortable and do they understand that they must remain seated for the next 4.5 hrs (participant can read, watch television, watch dvds etc)?   ◯ Y   ◯ N

TIME: -0.45 – FATIGUE TEST (STEADY STATE)

15. Actual time of fatigue test: ☐☐ : ☐☐
16. Test completed?   ◯ Y   ◯ N

TIME: -0.30 – FMD TEST (STEADY STATE)

17. Actual time of FMD test: ☐☐ ☐☐
18. Test completed?   ◯ Y   ◯ N

19. Comments: _____

_____

_____

TIME: -0.10 – BLOOD PRESSUE TEST (BASELINE MEASUREMENT)

20.   Seated blood pressure: Arm used: ◯ R   ◯ L

(a) HR(60sec): ☐☐   (b) Systolic (mmHg): ☐☐☐   (c) Diastolic (mmHg): ☐☐☐

(d) HR(60sec): ☐☐   (e) Systolic (mmHg): ☐☐☐   (f) Diastolic (mmHg): ☐☐☐

(g) HR(60sec): ☐☐   (h) Systolic (mmHg): ☐☐☐   (i) Diastolic (mmHg): ☐☐☐

TIME: -0.05 – VENOUS BLOOD COLLECTION (BASELINE MEASUREMENT)

21. Actual time of blood collection: ☐☐ : ☐☐

22. Blood tubes collected: : ◯ 3ml Glucose (Grey)   ◯ **8ml Insulin+SHBG+TES (Gold)**   ◯ 3ml spare (Purple)

-CONDITION BEGINS -

TIME: 0.00 – GLUCOSE DRINK *Glucaid to be consumed within <5mins*

23. Drinking start time: ☐☐ : ☐☐   **22.** Drinking finish time: ☐☐ : ☐☐

24. Has the participant received a 600mL water bottle?   ◯ Y   ◯ N

TIME: 0.30 - BLOOD COLLECTION

25. Actual time of blood collection: ☐☐ : ☐☐

Participant ID: ☐☐☐ ☐☐☐

Initials   *Code*

26. Blood tubes collected: ○ 3ml Glucose (Grey)   ○ 6ml Insulin (Gold)   ○ 3ml spare (Purple)

27. WALKING BREAK #1 COMPLETED POST 0.30?   ○ Y   ○ N   ○ N/A (sedentary condition)

TIME: 0.40 - BLOOD PRESSURE

28. Seated blood pressure: Arm used: ○ R   ○ L

(a) HR(60sec): ☐☐☐   (b) Systolic (mmHg): ☐☐☐   (c) Diastolic (mmHg): ☐☐☐

(d) HR(60sec): ☐☐☐   (e) Systolic (mmHg): ☐☐☐   (f) Diastolic (mmHg): ☐☐☐

(g) HR(60sec): ☐☐☐   (h) Systolic (mmHg): ☐☐☐   (i) Diastolic (mmHg): ☐☐☐

FMD TEST POST 0.40

29. Actual time of FMD test: ☐☐ : ☐☐

30. Test completed?   ○ Y   ○ N

31. Comments: _____

_____

_____

TIME: 1.00 - BLOOD COLLECTION

32. Actual time of blood collection: ☐☐ : ☐☐

33. Blood tubes collected: ○ 3ml Glucose (Grey)   ○ 6ml Insulin (Gold)   ○ 3ml spare (Purple)

34. WALKING BREAK #2 COMPLETED POST 1.00?   ○ Y   ○ N   ○ N/A (sedentary condition)

TIME: 1.25 – FATIGUE TEST

35. Actual time of fatigue test: ☐☐ : ☐☐

36. Test completed?   ○ Y   ○ N

TIME: 1.30 - BLOOD COLLECTION

37. Actual time of blood collection: ☐☐ : ☐☐

38. Blood tubes collected: ○ 3ml Glucose (Grey)   ○ 6ml Insulin (Gold)   ○ 3ml spare (Purple)

39. WALKING BREAK #3 COMPLETED POST 1.30?   ○ Y   ○ N   ○ N/A (sedentary condition)

TIME: 1.40 - BLOOD PRESSURE

40. Seated blood pressure: Arm used: ○ R   ○ L

(a) HR(60sec): ☐☐☐   (b) Systolic (mmHg): ☐☐☐   (c) Diastolic (mmHg): ☐☐☐

(d) HR(60sec): ☐☐☐   (e) Systolic (mmHg): ☐☐☐   (f) Diastolic (mmHg): ☐☐☐

(g) HR(60sec): ☐☐☐   (h) Systolic (mmHg): ☐☐☐   (i) Diastolic (mmHg): ☐☐☐

Participant ID: ☐☐☐ ☐☐☐

Initials   *Code*

FMD TEST POST 1.40

41. Actual time of FMD test: :

42. Test completed?   ◯ Y   ◯ N

43. Comments: _____

_____

_____

TIME: 2.00 - BLOOD COLLECTION

44. Actual time of blood collection: ☐☐ : ☐☐

45. Blood tubes collected: ◯ 3ml Glucose (Grey) ◯ 6ml Insulin (Gold) ◯ 3ml spare (Purple)

46. WALKING BREAK #4 COMPLETED POST 2.00?   ◯ Y   ◯ N   ◯ N/A (sedentary condition)

TIME: 2.30 - BLOOD COLLECTION

47. Actual time of blood collection: ☐☐ : ☐☐

48. Blood tubes collected: ◯ 3ml Glucose (Grey) ◯ 6ml Insulin (Gold) ◯ 3ml spare (Purple)

49. WALKING BREAK #5 COMPLETED POST 2.30?   ◯ Y   ◯ N   ◯ N/A (sedentary condition)

TIME: 2.40 - BLOOD PRESSURE

50. Seated blood pressure: Arm used: ◯ R   ◯ L

(a) HR(60sec): ☐☐☐   (b) Systolic (mmHg): ☐☐☐   (c) Diastolic (mmHg): ☐☐☐

(d) HR(60sec): ☐☐☐   (e) Systolic (mmHg): ☐☐☐   (f) Diastolic (mmHg): ☐☐☐

(g) HR(60sec): ☐☐☐   (h) Systolic (mmHg): ☐☐☐   (i) Diastolic (mmHg): ☐☐☐

TIME: 3.00 - BLOOD COLLECTION

51. Actual time of blood collection: ☐☐ : ☐☐

52. Blood tubes collected: ◯ 3ml Glucose (Grey)   ◯ **8ml Insulin+SHBG+TES (Gold)**   ◯ 3ml spare (Purple)

53. WALKING BREAK #6 COMPLETED POST 3.00?   ◯ Y   ◯ N   ◯ N/A (sedentary condition)

FATIGUE TEST (POST 3.00)

54. Actual time of fatigue test: ☐☐ : ☐☐

55. Test completed?   ◯ Y   ◯ N

FMD TEST (POST 3.00)

56. Actual time of FMD test: ☐☐ : ☐☐

57. Test completed?   ◯ Y   ◯ N

58. Comments: _____

_____

_____

TIME: 3.30 – LUNCH *To be consumed within 20 minutes*

59. Eating start time: ☐☐ ☐☐　　　　**22**. Eating finish time: ☐☐ ☐☐

60. Did participant consume all of the lunch? ◯ Y　　◯ N

61. If No, Has the left over lunch been recorded?

62. Libre Flash blood glucose swipe: ☐☐.☐ mmol/L　　Real time: ☐☐:☐☐

63. Volume of water consumed: ☐☐☐ g -15.5g (bottle weight) = ☐☐☐ ml　*Assume 1g=1ml*

CHECKLIST FOR COMPLETION OF STUDY VISIT

64. Has a copy been made of the participant's completed food diary?　◯ Y　◯ N

65. Has the participant been given a parking ticket?　◯ Y　◯ N　◯ taxi voucher

66. Has the participant received their afternoon meal pack post visit?　◯ Y　◯ N

67. Has the participant received their evening meal pack post visit?　◯ Y　◯ N

68. Has the participant received their breakfast meal pack post visit?　◯ Y　◯ N

69. Have CGM swipe reminders **been set** on pt's mobile and reader?　◯ Mobile　◯ Reader　◯ N

70. Has the participant filled out an ATO "Statement by a supplier" form?　◯ Y　◯ N

71. Has the participant received their compensation?　◯ Y　◯ N

72. Scheduled date + time of next visit: ☐☐/☐☐/☐☐　Time: ☐☐:☐☐　◯ N/A

Participant ID: | | | | | | | |

Initials　　　　　*Code*

COMMENT LOG:

This is log is to be used for clarification of CRF data or entry of significant comments by investigator or study co-ordinator. Please initial and date any comments made.

☐ **Check if NONE**

Item No.	Comment

Appendix III: Candidate Contribution and Media

Chapter 4

The candidate was involved extensively in conducting this study from initial design and concept to write-up. Along with co-author Ana Pinto (PhD student), the candidate was equally responsible for the search strategy, data extraction, quality assessment and figures relating to methods. The candidate handled statistical analysis and associated R code. Additionally, the candidate was responsible for the remaining figures and initial write-up.

Chapter 5

The candidate was involved extensively in conducting this study including data collection and to the analysis and presentation of results. The candidate and one of the co-authors from the paper presented in Chapter 5 (Ashleigh R. Homer, PhD student) were equally responsible for recruitment, screening, and enrolling participants in the study. During the experimental conditions, the candidate performed all the flow-mediated dilation and blood pressure measurements. Additionally, the candidate supervised the SRAs being performed, prepared standardised meals and activity monitors, and generally tended to the participants' needs while they attended the clinic. Subsequent to data collection, the candidate was responsible for cleaning, analysing and presenting the data pertaining to the research questions addressed in this thesis.

Chapter 6

The candidate was involved extensively in conducting this study from initial ethics application through to data collection and then the analysis and presentation of results. The candidate was responsible for recruitment, screening, and enrolling participants in the study. During the experimental conditions, the candidate was solely responsible for the flow-mediated dilation and blood pressure measurements. With assistance from the research nurse, the candidate handled the venous blood samples, including inserting the cannula, drawing, processing, analysing and storing the samples. Additionally, the candidate supervised the SRAs being performed, prepared standardised meals and activity monitors, and generally tended to the participants' needs while they attended the clinic. For data collection, the candidate was responsible for entering, cleaning, analysing and presenting the data pertaining to the research questions addressed in this thesis.

Additional Work Completed During Candidature

Taylor FC, Brakenridge CJ, Sethi P, Dempsey PC, Owen N, Head G, and Dunstan DW. **Associations of device measured sitting, standing and stepping time with 24-hr ambulatory blood pressure monitoring in Australian adults** *Manuscript under preparation*

This manuscript uses a compositional approach to characterise the relative contributions of sitting, standing, stepping and sleeping in a large sample of Australian adults with varied blood pressure profiles. In addition to coding the R code for the above publications, the end of my candidature involved teaching myself compositional analysis, and learning how to code it in R.

Taking Regular Breaks from Sitting to Improve Glycemic Control and Endothelial Function in Type 1 Diabetes (TARGET)

The candidate was also involved in a clinical trial that compared the acute effects of prolonged uninterrupted sitting versus sitting interrupted by bouts of light-intensity activities on endothelial function and associated mechanisms (inflammatory markers and oxidative stress) in adults with Type 1 Diabetes. The secondary aim of this study was to explore in adults with Type 1 Diabetes the effects of breaking up sitting on postprandial blood glucose, 24-hour glycaemic control and blood pressure. The candidate was involved extensively in recruitment, screening and data collection which included; FMD assessment, EndoPAT assessment, blood pressure and blood collection (via cannulation). Additionally, the candidate supervised the SRAs being performed, prepared standardised meals and activity monitors, liaised with diabetes educators and generally tended to the participants' needs while they attended the clinic. For data collection, the candidate was responsible for entering and cleaning the data. This data was then used for a University of Melbourne Honour's students thesis.

Your body loves exercise 'snacks' – even if they only last a minute

Back in the early '80s when fitness became a thing, the only way to exercise for a healthy heart was to do 20 to 30 minutes of non-stop aerobic activity, like running – or so exercise science thought.

"Now we know that fitting frequent short bursts of movement, even as brief as one or two minutes, into the day can benefit heart health and have positive effects on blood glucose levels and insulin sensitivity," says Professor David Dunstan, head of the Physical Activity Laboratory at Melbourne's Baker Heart and Diabetes Institute.

A 30-minute jog is still good – but clocking up multiple exercise "snacks" during the day helps too, and breaks up the prolonged sitting that ups the risk of rising levels of blood sugar and sluggish blood flow.

"When blood flow is reduced, blood pressure increases in an effort to pump blood around the body, and when muscles don't move there's less need for them to take up glucose from our blood to use for fuel," Dunstan explains.

There's also another way that prolonged sitting messes with blood vessels. When the forward flow of blood slows down, it reduces the force of blood pushing against the wall of the blood vessels.

"That continuous force is a benefit – it helps prevent plaque building up inside blood vessel walls," says Frances Taylor, a PhD student at the Institute's Physical Activity Laboratory.

"You want blood to keep flowing forward rather than slow down or stagnate – which can happen if you sit for long periods," says Taylor whose recent study of sedentary workers with type 2 diabetes shines new light on improving blood vessel health in people with the condition.

"We found that frequent breaks to interrupt sitting with leg raises, squats and calf raises significantly improved blood vessel function. Taking six-minute breaks every hour was good, but three minute breaks every 30 minutes was better. This suggests that shorter, more frequent breaks from prolonged sitting may be better for people with type 2 diabetes – a problem that already compromises blood vessel health."

Not everyone needs to leap out of their chair every 30 minutes – but we should get serious about interrupting long bouts of sitting, stresses Dunstan. He suggests identifying "danger zones" of continuous sitting like the first three hours of the working day, the post-dinner slump – or the summer holiday Netflix binge – and set a timer as a prompt to get up and move for a few minutes every hour.

But although "every move counts", as the catchcry of the World Health Organisation's new physical activity guidelines goes, it's the more vigorous bites of movements, like climbing stairs, walking uphill or playing with kids that deliver the most benefit says Emmanuel Stamatakis, Professor of Physical Activity, Lifestyle and Population Health at the University of Sydney's Charles Perkins Centre.

"Vigorous physical activity embedded in daily life is time efficient – as a rough guide, one minute of vigorous movement like walking fast uphill equals two minutes of moderate movement like a brisk walk on the flat. It also has a more profound effect on our cardiovascular

fitness compared to light or moderate exercise: by increasing the body's capacity to transport oxygen to muscles, it makes any physical activity easier so that we can do more for longer," he explains. "This is important for less sporty people, especially as we age, because it improves our ability to stay independent."

He believes that splicing enough vigorous 'exercise snacks' into our daily lives could also be a valid, more achievable alternative to structured exercise.

"The gym or sport are great but many people are time poor and not keen on structured exercise," says Stamatakis whose recent paper in the journal *Sports Medicine* calls for more research into what exercise scientists call 'vigorous intermittent lifestyle physical activity' – or VILPA.

Some small studies show a benefit. Just three minutes of brisk stair climbing a week improved fitness in young adults, according to one 2019 study. But we still need to know more – like how many minutes of VILPA each week are ideal, and how can we be persuaded to fit it into our daily lives?

"Making physical activity convenient helps," he says, pointing to Copenhagen where changes to the city centre mean it's now faster to get around by bike than by car.

At his own workplace, the stairs are a broad, attractive feature. "They invite you to climb them – but in many office buildings, stairs are hidden. If we make stairs appealing and visible more people will use them."

But while we wait for urban planners and architects to fix the environment, let's grab as many exercise snacks each day as we can – a walk around the block, some brisk vacuuming or a play date with the dog – and embrace climbing stairs and escalators.

Link: https://www.smh.com.au/lifestyle/health-and-wellness/your-body-loves-exercise-snacks-even-if-they-only-last-a-minute-20201230-p56qve.html

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