Consequences and countermeasures of sedentary behaviour on arterial wave reflection

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Cardiac load is directly influenced by central wave reflections, with increases in reflected wave magnitude and reductions in return time contributing to increased systolic pressure, afterload, and impaired coronary blood flow. Prolonged sitting has known vascular consequences such as increased arterial stiffness and endothelial dysfunction, yet the impact on wave reflections and efficacy of interruption methods to mitigate these effects is unknown. This study was designed to investigate whether seated elliptical exercise or standing breaks alleviated the deleterious effects of prolonged sitting on central wave reflections. Eighteen healthy adults (9 females, 25±3 years) completed three hours of uninterrupted sitting, sitting with periodic standing, and sitting with seated elliptical breaks, on separate days. Central and lower limb pulse wave velocity were measured before and after the intervention as well as central wave reflection which was calculated via pressure-flow analysis of aortic blood flow and carotid arterial pressure. A main effect of time was observed for forward wave amplitude (40.0±8.2 to 35.2±6.0 mmHg; $p = 0.02$) and reflected wave amplitude (11.9±1.6 to 11.3±2.0 mmHg; $p = 0.03$). Central relative wave reflection magnitude increased during sitting control (0.31±0.05 to 0.35±0.05; $p < 0.01$) but did not change with seated elliptical (0.30±0.05 to 0.30±0.04; $p > 0.99$) or standing breaks (0.30±0.05 to 0.32±0.04; $p = 0.19$). Augmentation index increased with sitting control (-9.3±9.2 to -4.6±12.2 %; $p < 0.01$) and standing (-12.4±9.8 to -5.6±9.4 %; $p < 0.01$), but not with seated elliptical (-9.9±9.4 to -10.2±11.1 %; $p = 0.82$. Lower limb pulse wave velocity did not change with sitting control (9.5±1.7 to 9.6±1.0 $m \cdot s^{-1}$; $p = 0.73$) or standing (9.1±1.5 to 9.7±1.7 $m \cdot s^{-1}$; $p = 0.21$) but decreased with seated elliptical (9.8±1.4 to 9.1±1.5 $m \cdot s^{-1}$; $p = 0.03$) Central pulse wave velocity increased during sitting control

(5.3±0.8 to 5.7±0.9 $m \cdot s^{-1}$; $p < 0.01$) and standing (5.3±0.7 to 5.7±0.7 $m \cdot s^{-1}$; $p < 0.01$) but not with seated elliptical (5.5±0.5 to 5.6±0.6 $m \cdot s^{-1}$; $p = 0.43$). Prolonged sitting without interruptions increased central wave reflection, central pulse wave velocity, augmentation index, and decreased femoral blood flow while seated elliptical but not standing breaks were able to ameliorate these sitting-induced vascular consequences. Future work should examine the long-term effectiveness of interruption methods, as well as the optimal type, frequency, and duration for reducing CVD risk associated with sedentary behaviours.

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List of Abbreviations

Pf: forward pressure wave

Pb: reflected pressure wave

CWR: central wave reflection

FMD: flow-mediated dilation

RM: central relative wave reflection magnitude

SFA: superficial femoral artery

RWTT: reflected wave transit time

SSN: suprasternal notch

cfPWV: carotid-femoral pulse wave velocity

faPWV: femoral-ankle pulse wave velocity

SV: stroke volume

$\dot{Q}$: cardiac output

TPR: total peripheral resistance

HR: heart rate

MAP: mean arterial pressure

SBP: systolic blood pressure

DBP: diastolic blood pressure

1.0 LITERATURE REVIEW

1.1 Defining central wave reflection

1.1.1 Decomposition of a pressure wave

Pressure waves can be separated into the forward (Pf) and the reflected pressure wave (Pb), occurring as a result of left ventricular contraction and the arrival of Pf at a discontinuity in vessel structure, respectively (Nichols *et al.*, 2011). During systole, blood is ejected from the left ventricle, creating a forward wave of pressure traveling from central to peripheral locations. At points of discontinuity such as artery-arteriole conversion zones, bifurcations, and areas of arterial tapering, a portion of the forward wave is reflected back in the opposing direction of blood flow. Reflected pressure waves from numerous peripheral sites will unite, arriving back at central arteries where they are referred to as central wave reflections (CWR). Unlike flow, pressure is additive meaning the reflected wave will summate with the forward wave to form the measured wave (See Figure 1). Contributions from forward and reflected pressure waves can be viewed as the central relative wave reflection magnitude (RM), defined as Pb/Pf. RM provides a holistic view of the reflections in a given system, accounting for the changes in both Pb and Pf respective to one another. In healthy individuals at rest, the forward wave travels during systole and the reflected wave returns during diastole. The delayed return of the reflected wave relative to the incident wave helps enhance coronary blood flow without excessively augmenting systolic blood pressure (London & Guérin, 1999). The coronary vessels are unique in that during systole, contraction of the heart musculature restricts blood flow while diastole allows for relaxation of the heart and associated vessels, allowing for coronary flow to resume (Ramanathan & Skinner, 2005). Arrival of the wave reflection

during diastole allows for enhanced flow during this point of the cardiac cycle where the coronary arteries are not restricted. In pathology, the reflected wave may return during systole and augment Pf, contributing to systolic hypertension (Mitchell *et al.*, 2003). Due to the above two points, the timing of the reflective wave is critical to describe central cardiovascular dynamics and is measured using reflected wave transit time (RWTT). RWTT varies across individuals as a function of height and with cardiovascular stimuli such as exercise (Stock *et al.*, 2021) which will be discussed in depth below. Smulyan et al. (Smulyan *et al.*, 1998) investigated the impact of height on RWTT, concluding that reflected waves arrive earlier in shorter individuals because of their shorter arterial trees, which therefore have shorter distances from reflecting sites to the aorta. Cardiovascular stimuli can also alter wave reflection magnitude, resulting in increased pressure during systole when combined with reduced RWTT, and a negative impact on cardiac afterload (Murgo *et al.*, 1980; Westerhof & O'Rourke, 1995; Chirinos & Segers, 2010; Nichols *et al.*, 2011). Increased afterload resulting from arterial wave reflection puts strain on cardiac tissue, impacting ejection fraction, stroke volume, and overall left ventricular systolic function (Cebrowska *et al.*, 2021). While CWR has beneficial contributions to cardiovascular function, deviation from optimal conditions can prove detrimental with chronic exposure.

The relationship between Pf and Pb and the implications of independent changes from one another are unknown. Pf is associated with left ventricular ejection, caused by a blood pressure wave travelling away from the heart (Safar & O'Rourke, 1995). This association suggests that changes in Pf are associated with changes in cardiac contractility and/or end-systolic volume. In contrast, Pb is a result of Pf reaching

discontinuity points and impedance mismatches, resulting in a portion of the forward wave being reflected back as a summated wave (Westerhof *et al.*, 2010). This relationship indicates that changes in Pb may imply changes to either peripheral reflection points or how Pf behaves at these reflection sites. From this, one might assume that Pf and Pb are linked such that changes in one would result in changes in the other. However, research has shown that this is not the case, showing that Pf and Pb can change independently of one another. One example is lower limb exercise which increases Pf while decreasing Pb (Stock *et al.*, 2021). With increasing exercise intensity, heart rate and contractility increase to meet oxygen demands which increases Pf. Coincident with Pf increasing, local vasodilation at the active muscles reduces vascular resistance and Pb (Stock *et al.*, 2021). Although more research is required to understand all factors impacting the relationship between Pb and Pf, understanding where and why they originate can provide insight into their function.

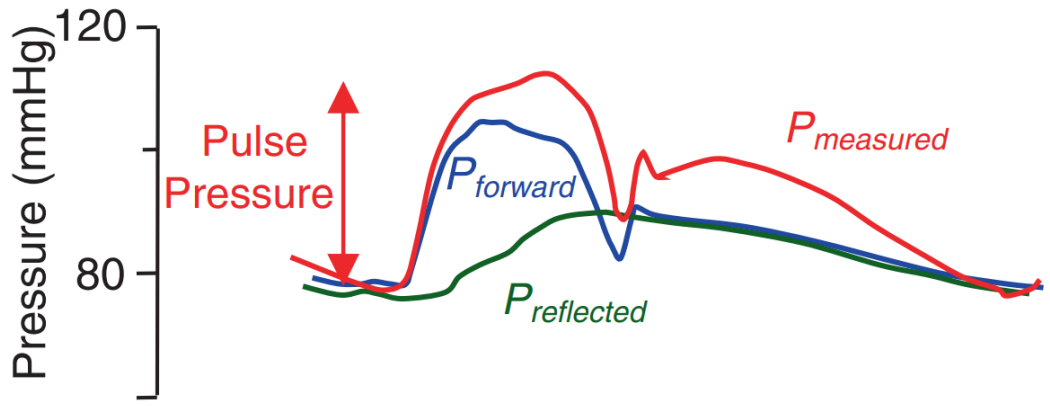

Figure 1: Decomposition of aortic pressure into its forward and reflected components. Adapted from Westerhof et al. (Westerhof *et al.*, 2010).

CWR is linked with various aspects of cardiovascular health including arterial stiffness, cardiac afterload, and hypertension. Greater arterial stiffness increases pulse

wave velocity, causing RWTT to decrease. Reductions in RWTT leads to the return of reflected waves during systole, augmentation of systolic blood pressure and increased afterload. Chronically increased left ventricular load as caused by the arrival of Pb during systole negatively impacts left ventricular structure and function, with animal models finding reduced hypertrophy and slower rates of left ventricular pressure fall (Gillebert & Lew, 1991; Kobayashi *et al.*, 1996; Nichols *et al.*, 2011). The relationship between CWR outcomes and cardiovascular health led research to examine their predictive capabilities, finding strong links with CWR and cardiovascular disease risk. Chirinos et al. (Chirinos *et al.*, 2012) measured CWR in 5960 individuals, finding that a 10% increase in RM was associated with greater risk of cardiovascular events when controlling for confounders (e.g., height, weight, diabetes, cholesterol, smoking, heart rate, and glomerular filtration rate). Additionally, Pb can predict cardiovascular mortality with greater magnitude of reflected waves associated with increased risk of cardiovascular events (Wang *et al.*, 2010). The predictive capabilities of CWR for cardiovascular disease risk provide evidence for their potential applications as a novel marker of vascular health.

1.1.2 Proposed origin of central wave reflection

Current research suggests that the lower limbs act as the driver of CWR due to the larger number of artery-arteriole conversion zones and greater muscle mass relative to the upper limbs (Avolio *et al.*, 1984, 1985; O'Rourke & Yaginuma, 1984; Nichols *et al.*, 2008; Stock *et al.*, 2020, 2021). The idea that the legs are the primary source of wave reflection has been acknowledged for years, with studies in comparative physiology providing rationale for this theory. Specifically, work examining the kangaroo found the pyramid-like arterial distribution with exaggerated lower body mass and minimal upper body mass to

have a substantial impact on how wave reflections behave. Avolio et al. (Avolio *et al.*, 1984) used a conceptual tubular model representing systemic arteries and compared the pyramid model seen in the kangaroo with a single tube model (McDonald & Taylor, 1959) and an asymmetrical T tube model (O'Rourke, 1967) (See Figure 2). In the simplified tube model, the behaviour of fluid injected into rubber tubing of varying lengths was examined. In tubing of greater lengths, the incident wave would propagate and, as it does so, pressure at a given point would fall to zero until the reflected wave returned from the termination point. In contrast, tubing of shorter lengths would have reflections arriving before the completion of the forward wave, resulting in summation and amplification of pressure (McDonald & Taylor, 1959). While the single tube model provides insight into how waves behave in artery-like structures, it does not consider the summation of reflected waves from various peripheral sources, which more realistically describes what occurs in human circulation. The asymmetrical T tube model was based on work done in dogs (O'Rourke, 1967) which, similar to humans, have upper and lower limb contributions to CWR. Although admittedly an oversimplified model, it does provide insight into the central response when wave reflections arrive from multiple peripheral sites. Centrally, reflected wave contributions from upper and lower limbs were apparent, with the reflected wave seeming to return sooner than in the single tube model, likely due to the reduced distance of reflecting sites from the aorta. Despite some similarities with the T tube model, the lower and upper limbs of humans are disproportionate in terms of volume, muscle mass, and artery-arteriole conversion zones. Therefore, the use of this model to explain wave reflection in humans is restricted. The kangaroo model resembles the T tube model apart from its larger lower limb portion, creating a pyramid shape. With manipulation of

the lower limb size, the reflected wave contributions from the upper limbs were no longer apparent as they were overpowered by the strong reflections from the lower limbs. Avolio et al. (Avolio *et al.*, 1984) concluded that the reflected pressure waves and impedance mismatches were more distinct in the kangaroo model compared to the single tube model, suggesting the introduction of greater lower limb mass enhanced CWR. However, a lack of *in vivo* evidence leaves these claims unsupported in human physiology.

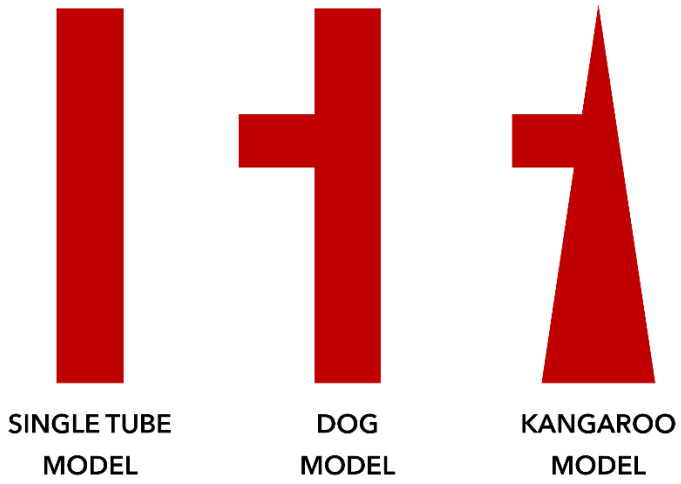

Figure 2:Model of systemic arterial system of a dog (left), kangaroo (middle), and single tube model (right). Adapted from Avolio et al. (Avolio *et al.*, 1984).

Prior to this MSc thesis, I conducted a study to investigate the origins of central wave reflection in humans, using peripheral limb heating as a stimulus (Athaide *et al.*, 2023). Fifteen healthy adults (8 females, 24±3.6 years) completed a within-subjects experimental crossover protocol with a washout period. The right arm and leg were warmed in a randomized order using 38°C water perfused tubing with a 30-minute break between protocols. There was a main effect of time for Pb (12.8 to 12.2 mmHg) and augmentation index (AIx) (-7.49 to -4.45%) (both $p < 0.05$) but no significant differences for condition or interaction. There were no significant main effects or interactions for Pf, RWTT, or RM (all $p > 0.05$). Peripheral limb heating reduced CWR,

however, the lack of difference between arm and leg conditions does not support the hypothesis that the lower limbs are the primary source of wave reflection. Future work should consider the potential importance of other aspects of human circulation like the splanchnic vasculature.

1.1.3 Methodology for central wave reflection acquisition

To acquire a measure of CWR, echocardiographic images in the parasternal (B-mode) and 5-chamber (pulsed-wave) views are taken, followed by some form of pressure acquisition. There are two primary methods in which pressure information is acquired: using applanation tonometry from a peripheral artery with use of a transfer function to predict aortic pressure, or directly from the carotid artery. Numerous studies exist to validate the use of radial artery transfer functions (Chen *et al.*, 1998; Pauca *et al.*, 2001; Sharman *et al.*, 2006; O'Rourke & Adji, 2014). Chen et al. (Chen *et al.*, 1998) conducted a study comparing direct invasive measures of central aortic pressure with transfer functions from radial tonometry. They confirmed the accuracy and reliability of central aortic pressure estimation from radial tonometry by use of a generalized transfer function, with central pressure estimated to ≤0.2±3.8 mmHg error, noting that augmentation index may be underestimated (Chen *et al.*, 1998). Transfer functions can also be used on the brachial artery as validated by Bultin et al. (Butlin *et al.*, 2012). Instead of using tonometry, they tested using the volume displacement waveform of the brachial artery with a cuff inflated to 10 mmHg below diastolic pressure. Comparing estimated central aortic pressure waveforms from radial tonometry and brachial waveforms revealed a high correlation for aortic diastolic ($r^2 = 0.98$) and systolic pressure ($r^2 = 0.99$), with an aortic SBP difference of 0.5±1.8 mmHg between the two methods (Butlin *et al.*, 2012). It was

concluded that volume displacement waveforms from the brachial artery were comparable to other tonometry methods for estimating aortic pressure waveforms (See Figure 3).

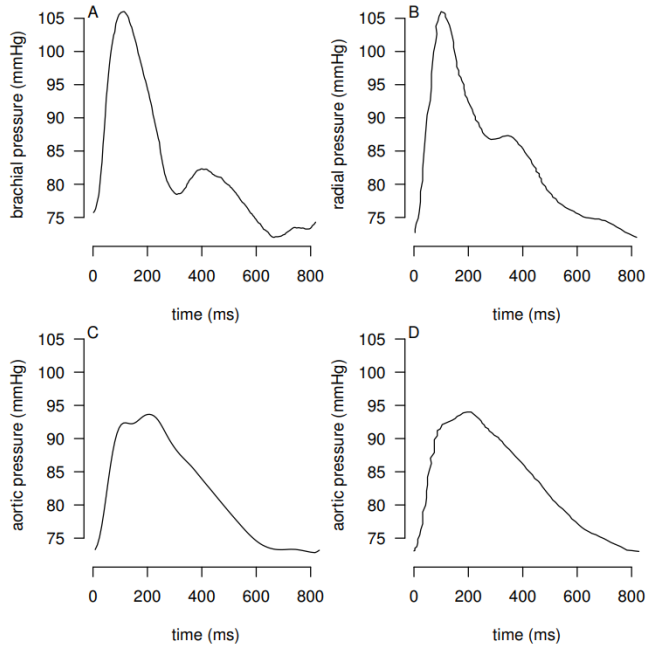

Figure 3:Wave forms acquired from brachial artery (A) and radial artery (B) along with the derived aortic waveforms from the brachial (C) and radial arteries (D) for one individual. Adapted from Butlin et al. (Butlin *et al.*, 2012).

Generalized transfer functions are commonly used in wave reflection research due to the simplistic and non-invasive methodology and, in most cases, are perfectly sufficient at rest, with high correlations between transfer functions and direct invasive methods (r = 0.88) (O'Rourke *et al.*, 2003). However, it is possible that this method of acquiring aortic pressure is not always adequate. Lower limb exercise drives a large pressure differential from the central arteries to the legs, including a large central pressure response that is not experienced to the same extent in the upper limbs. At the same time, whole body sympathetic responses will constrict the inactive upper vasculature (Remensnyder *et al.*, 1962; Paterson *et al.*, 2020). In this case, estimation of CWR from the upper limbs may

be inaccurate, resulting in an underestimation of the true central response. As an alternative, collecting tonometry information from the carotid artery is done without the use of a transfer function and instead assumes the carotid as a surrogate for aortic pressure. One of the earlier studies to support the relationship between carotid and aortic pressure was Kelly and Fitchett (Kelly & Fitchett, 1992). They concluded that carotid tonometry could be used as an accurate and reproducible surrogate for aortic pressure, as direct invasive methods were highly correlated with noninvasive tonometry (r = 0.98) (Kelly & Fitchett, 1992), even more so than previously found with transfer functions (r = 0.88) (O'Rourke *et al.*, 2003). Some favor the radial method over the carotid method because of the increased reliability from easier palpation. The radial artery pulse is superficial, easy to find, and anchored in place by adjacent bones and tissues, limiting its movement (O'Rourke & Adji, 2012). In contrast, the carotid artery is relatively deep in the neck, not always easy to find, and does not have strong supporting structures anchoring its location. However, the reliability of the carotid method can be improved with proper training, practice, and maintaining consistent researchers within a project. While this method has its own limitations, in a highly skilled environment, it may allow for a more accurate depiction of the central response for local vascular changes because of the location of data acquisition.

Once measures of central pressure and flow are acquired, they can be analyzed in either the time or frequency domains to estimate CWR. The time-domain considers pressure waveforms as a continuous waveform measured across seconds. It is a simplistic method that makes assumptions regarding the "upstroke" of the signal. Here, it is assumed that the upstroke is absent of all wave reflections and can be used as an

estimate for Pf; however, this assumption is difficult to confirm which results in reduced validity (Qureshi *et al.*, 2018). In contrast, the frequency domain considers pressure waveforms as the composition of various frequency waves. This method is more complex, requiring sufficient repeated cycles and a fast-Fourier transform to convert from the time domain to the frequency domain. The fast Fourier transform takes the pressure and flow waveforms in the time domain and decomposes them into their component frequency waves. The 4^{th} to 10^{th} harmonics of the pressure/flow frequency amplitudes are considered to be representative of the propagation properties of the arterial wall (Swillens & Segers, 2008). The mean amplitude of this range is also known as characteristic impedance and is used to calculate wave reflections in an arterial system. The frequency domain is often preferred as the more precise method due to its ability to filter out high frequency signals, making it less susceptible to noise when compared to the time domain (Swillens & Segers, 2008).

CWR measurements should occur while participants are fasted, without caffeine (Karatzis *et al.*, 2005; Grant *et al.*, 2018), in the supine position, and rested for a minimum of 20 minutes. Wave reflection is a variable measure requiring a high level of control for factors that may impact reliability. Stoner et al. (Stoner *et al.*, 2017) conducted a study to test the impact of common external factors on wave reflection reliability. CWR was measured in 20 healthy adults while fasted and nonfasted, supine and seated upright, all after 20 minutes of uninterrupted rest. The upright seated posture negatively affected reliability and precision while nonfasted measures impacted the amplitude and precision of reflected wave measures. Caffeine restriction is important for cardiovascular research, with studies confirming its ability to interfere with central blood pressure (Karatzis *et al.*,

2005; Grant *et al.*, 2018). Karatzis et al. (Karatzis *et al.*, 2005) performed a repeated measures study examining the prolonged effects of caffeine consumption from baseline to 120 minutes post-consumption. Consumption of caffeine acutely increased central systolic and diastolic blood pressure, and augmentation index, indicating a considerable effect on central hemodynamics (Karatzis *et al.*, 2005). The effect of increased blood pressure from caffeine is driven by increases in vascular resistance (Pincomb *et al.*, 1985). Thus, it is recommended to measure CWR in a fasted state, without caffeine, and in the supine position following 20 minutes of rest for optimal reliability. These recommendations are particularly important to avoid unwanted error and allow for more precise inferences on the vascular mechanisms behind lifestyle-induced changes in function.

1.2 Vascular response to prolonged sitting

1.2.1 Impact of prolonged sitting on vascular function and central wave reflection

A bout of prolonged sitting can be defined as anything longer than 3 hours (Thosar *et al.*, 2015; Morishima *et al.*, 2016; Climie *et al.*, 2018; Kruse *et al.*, 2018; Carter *et al.*, 2019). Prolonged sitting triggers a cascade of physiological responses, ultimately resulting in vascular dysfunction. With sitting, blood flow is reduced in the arteries of the lower limb including the superficial femoral artery (SFA) (Carter *et al.*, 2019) and popliteal artery (Restaino *et al.*, 2015) (See Figure 4).

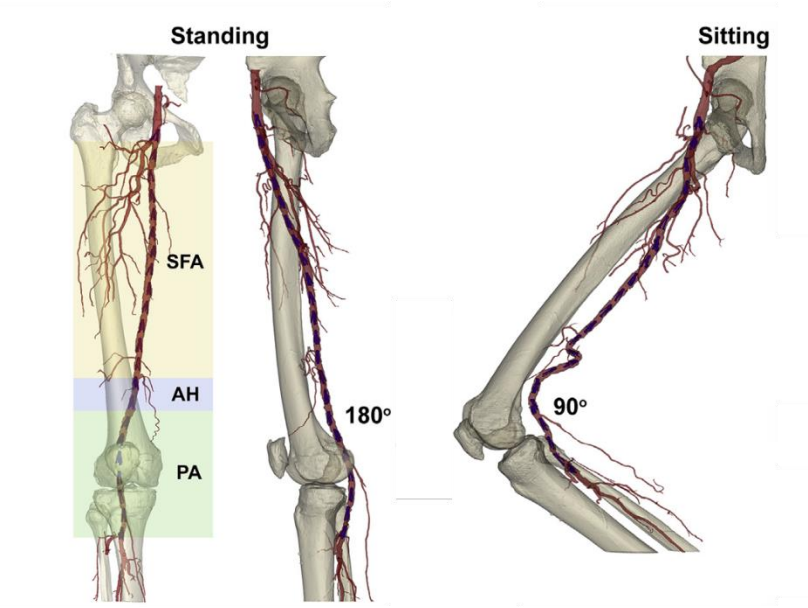

Figure 4:Position of lower limb arteries in standing and sitting positions. SFA, superficial femoral artery; PA, popliteal artery, AH, adductor hiatus. Adapted from Poulson et al. (Poulson *et al*., 2018).

Decreased blood flow leads to reductions in vascular shear stress which is described as the shearing force of blood along the endothelium (Davies *et al.*, 1984). Shear stress plays a crucial role in regulating the vascular milieu, most notably in the vasodilatory function of vessels through the release of vasoactive substances such as nitric oxide (Davies *et al.*, 1984). Reductions in shear stress leads to decreases in nitric oxide bioavailability and vasodilatory function. Carter et al. (Carter *et al.*, 2019) measured the effect of 4 hours of uninterrupted sitting on SFA function by measuring flow-mediated dilation (FMD), blood flow, and shear rate. The sedentary condition showed reductions in SFA blood flow. However, they did not find changes in endothelial function via FMD or shear rate, noting this discrepancy is likely due to methodological choices to not restrict participant leg movement (Carter *et al.*, 2019). Restaino et al. (Restaino *et al.*, 2015) investigated a similar question, examining 6 hours of uninterrupted sitting on popliteal artery blood flow, shear rate, and FMD among other measures. The popliteal artery

showed impairments in FMD, blood flow (See Figure 5), and shear rate with prolonged sitting (Restaino *et al.*, 2015).

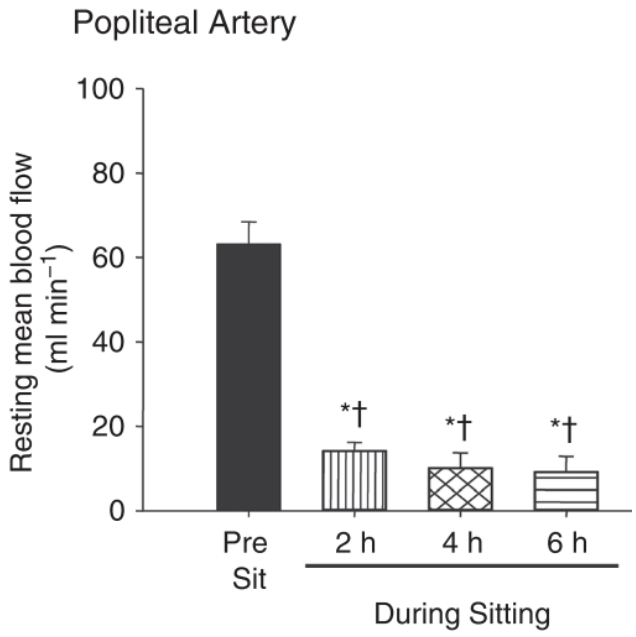

*Figure 5:Resting mean blood flow of the popliteal artery at baseline and after 2hrs, 4hrs, and 6hrs of uninterrupted sitting. * p<0.05 versus Pre Sit. †p,0.05 versus Post Walk (not shown). Adapted from* Restaino *et al.* (Restaino *et al.*, 2015)

In theory, the vasodilatory dysfunction seen with sedentary time creates favourable conditions for reflected waves. Pressure waves are reflected in response to transitions in vascular impedance caused by arterial taper, bifurcations, or artery-arteriole conversion zones (Nichols *et al.*, 2011). The relative vasoconstriction seen in prolonged sitting creates an impedance mismatch which may produces greater reflected waves (Westerhof *et al.*, 2010). Although logical, recent reports have not found increased wave reflections with prolonged sitting, likely due to methodological choices. Credeur et al. (Credeur *et al.*, 2019) examined participants before and after 3 hours of prolonged uninterrupted sitting and found a nonsignificant decrease in RM and Pb. Evans et al. (Evans *et al.*, 2019) also studied the effects of sedentary time on arterial waveforms, finding little to no change in forward or reflected wave amplitude with prolonged sitting. Both studies measured CWR

using a validated transfer function from upper limb pressure waveforms, which, as mentioned above, may not be adequate to detect central changes with lower limb interventions. However, without evidence for this theory, the root cause of this effect is unclear. Heffernan et al. (Heffernan *et al.*, 2013) took a different approach, examining self-reported sitting time and its relationship with wave reflection, finding that sedentary time was associated with greater forward and reflected pressure waves. This discrepancy in the literature raises questions as to the true response of wave reflections to prolonged sitting which may be accounted for through more direct methodology. Research examining the impact of prolonged sitting on CWR using alternative methods to transfer functions is warranted. Without evidence comparing brachial and radial to carotid pressure methods, the utility of transfer function estimation of central pressure with localized vascular stimuli is unclear.

1.2.2 Impact of prolonged sitting on pulse wave velocity

Pulse wave velocity is the gold standard measurement for arterial stiffness (Mikael *et al.*, 2017). Along with other cardiovascular changes to sedentary behaviours, pulse wave velocity (PWV) is negatively impacted with prolonged sitting. Credeur et al. (Credeur *et al.*, 2019) measured carotid-femoral PWV over 3 hours of uninterrupted sitting, finding increases in carotid-femoral PWV, indicating an increase in stiffness. Alansare et al. (Alansare *et al.*, 2020) measured PWV over 7.5 hours of prolonged sitting in a group of 25 adults classified as overweight/obese with elevated blood pressure. They found that prolonged sitting increased carotid-femoral PWV by 0.52 m/s and carotid-ankle PWV by 0.45 m/s. Studies focusing on long-term effects have found similar results regarding increased PWV with sedentary time. Ahmadi-Abhari et al. (Ahmadi-Abhari *et al.*, 2017)

conducted a longitudinal study examining the effect of physical activity and sedentary behaviour on PWV over a 5-year period. They found an increase of ~0.76m/s over 5 years which was reduced in those that engaged in more physical activity. Evidence of increased PWV with sedentary time is consistent and reproducible (Ahmadi-Abhari *et al.*, 2017; Credeur *et al.*, 2019; Evans *et al.*, 2019; Alansare *et al.*, 2020; Kelsch *et al.*, 2021). Because of this, PWV can be used in studies of prolonged sitting as a methodological "check" to ensure that the anticipated vascular changes occurred. Additionally, because PWV influences RWTT, changes in PWV should be related to changes in CWR. The use of PWV as a positive control allows for researchers to correctly discern whether the proposed intervention was ineffective or if the prolonged sitting bout was inadequate and therefore, unable to alter vascular physiology.

1.2.3 Relationship between sedentary behaviour and mortality/morbidity

Many meta-analyses have been conducted to examine the epidemiological studies investigating the relationship between prolonged sitting and cardiovascular health, finding negative long-term consequences of sedentary behaviours (Grontved & Hu, 2011; Ford & Caspersen, 2012; Wilmot *et al.*, 2012; Biswas *et al.*, 2015; Zhao *et al.*, 2020). Zhao et al. (Zhao *et al.*, 2020) conducted a meta-analysis and systematic review evaluating the relationships between sedentary behaviours and cardiovascular disease. They concluded that sitting time and television viewing were associated with cardiovascular disease in a dose-dependent manner, meaning increases in sedentary behaviours were met with a proportional increase in cardiovascular disease risk (Zhao *et al.*, 2020). In the meta-analysis conducted by Wilmot et al. (Wilmot *et al.*, 2012), sedentary behaviours were associated with increased cardiovascular disease risk, concluding that focus should be

put on reducing sitting time. Ford and Caspersen (Ford & Caspersen, 2012) conducted a similar review, investigating associations between common sedentary behaviours, sitting time, and cardiovascular disease risk. They found increased risk of cardiovascular disease with greater sedentary behaviours when accounting for confounders such as physical activity. The idea of adjusting for physical activity raises an important point; sedentary behaviour and physical inactivity are not synonymous. One can be both sedentary and physically active which would impact cardiovascular disease risk through the cardioprotective effects of exercise (Hamilton *et al.*, 2012). Although, the benefits of meeting the physical activity guidelines may not be enough to alleviate the consequences of sedentary behaviour (Hamilton *et al.*, 2012). Healy et al. (Healy *et al.*, 2008) studied a group of healthy adults classified as physically active and still found a dose-dependent response between sedentary behaviours and systolic blood pressure. Ekelund et al. (Ekelund *et al.*, 2016) conducted a meta-analysis investigating the ability of physical activity to eliminate all-cause mortality risk with sitting time. They concluded that 60 to 75 minutes per day of moderate intensity exercise eliminated mortality risk associated with sitting while only reducing mortality risk associated with television time. Notably, 60 to 75 minutes per day equates to 420 to 525 minutes per week which is well above the defined physically active value of 150 minutes per week (Ross *et al.*, 2020). Therefore, the question remains whether physically active individuals closer to the defined 150 minutes per week would still experience these effects. Thus, it is likely more important to reduce sitting time while increasing activity instead of treating them independently.

1.3 Alleviating the consequences of sedentary behaviour

1.3.1 Interruption methods for vascular function

Prolonged sitting negatively impacts cardiovascular health and vascular functioning. Paterson et al. (Paterson *et al.*, 2020) conducted a meta-analysis to examine the impact of prolonged sedentary time on vascular function and investigate suggested interruptive strategies and their effectiveness in protecting vascular function. Vascular function was impaired with prolonged sitting as shown through reductions in FMD in both the SFA and popliteal artery (Paterson *et al.*, 2020). With the understanding that sedentary time does negatively affect cardiovascular health, it is logical that interventions aimed at reducing sedentary time would alleviate these effects. The most common interruption methods suggested are resistance exercises (Climie *et al.*, 2018), under-desk cycling (Kruse *et al.*, 2018), walking breaks (Thosar *et al.*, 2015; Carter *et al.*, 2019), prolonged standing (Kruse *et al.*, 2018), and lower limb fidgeting (Morishima *et al.*, 2016) (Table 1). Climie et al. (Climie *et al.*, 2018) looked at the impact of sedentary time on cardiovascular disease risk in an overweight/obese population. Measures of FMD, blood flow, and shear rate were acquired before and after 5 hours of either uninterrupted sitting or interrupted sitting with light intensity resistance exercises every 30 minutes. They found a reduction in femoral artery vasodilatory function with prolonged sitting condition relative to the resistance exercise condition, indicating that short resistance exercise breaks were sufficient to reduce vascular dysfunction. Kruse et al. (Kruse *et al.*, 2018) studied the effects of prolonged sitting *vs* standing and under-desk cycling on popliteal endothelial function in a sedentary and overweight/obese population. The three conditions consisted of 4 hours of uninterrupted sitting, 4 hours of sitting with four 10-min periods of standing,

and 4 hours of sitting with four 10-min periods of under-desk cycling at a low intensity. They found that uninterrupted sitting caused an impairment in popliteal artery FMD while neither intervention conditions prevented popliteal endothelial dysfunction. It is likely that the interruption bouts were too brief to provide enough of a stimulus to maintain vascular function as well as the final hour of sitting before post measures acquisition may have been sufficient to impair endothelial function. Thosar et al. (Thosar *et al.*, 2015) and Carter et al. (Carter *et al.*, 2019) both investigated the benefits of walking as an interruption strategy on SFA endothelial function, each looking at different variations in timing of interruption. Thosar et al. (Thosar *et al.*, 2015) found SFA FMD declined with prolonged sitting while walking for 5 minutes at a light intensity every 30 minutes was able to maintain FMD. Carter et al. (Carter *et al.*, 2019) took a different approach with two experimental conditions, one with 2-minute walking breaks every 30 minutes and one with 8-minute walking breaks every 2 hours. SFA blood flow was lower after 4 hours of uninterrupted sitting which was improved in each of the walking conditions. Interestingly, they found that longer duration and less frequent walks were more beneficial on SFA blood flow *vs* shorter, more frequent breaks. Lastly, Morishima et al. (Morishima *et al.*, 2016) explored the idea of leg fidgeting as a sedentary interruption method, suggesting that periodic muscle contractions would effectively ameliorate the reductions in blood flow. They measured FMD in the popliteal artery before and after 3 hours of sitting with one leg sedentary and one fidgeting intermittently for 1 of every 5 minutes. They found that popliteal artery FMD was impaired in the sedentary limb while the fidgeting limb had improved FMD. Similar research has examined fidgeting-like movements and their effectiveness at eliminating the changes to PWV seen with prolonged sitting. Evans et al.

(Evans *et al.*, 2019) measured PWV in response to sedentary time as well as intermittent calf raises to determine their effectiveness in reducing changes to PWV. The control group saw increases in PWV which were not prevented in the experimental condition, indicating that intermittent calf raises were not adequate to prevent changes to stiffness with prolonged sitting. To date, an optimal strategy for the interruption of prolonged sitting has not been identified but appears to involve some form of lower limb activity. Time also seems to play a role in that less frequent longer duration bouts create a greater stimulus, allowing them to be more effective. However, the duration required for intervention effectiveness may be dependent on the activity itself.

Table 1:Summary of vascular findings in response to prolonged sitting and interruption methods. (Thosar *et al.*, 2015; Morishima *et al.*, 2016; Climie *et al.*, 2018; Kruse *et al.*, 2018; Carter *et al.*, 2019)

Author	Participant Characteristics	Sedentary Time	Interruption Method	Conclusion
Climie et al., 2018	n = 19, 8F Sedentary overweight/obese adults (57 ±12 yr)	5 hours	Resistance exercise	Sitting reduced femoral FMD which was alleviated with short resistance exercise
Kruse et al., 2018	n = 13, 3F Overweight/obese adults (38 ± 3 yr)	4 hours	Under-desk cycling (10 min / hour) Standing (10 min / hour)	Sitting reduced popliteal artery FMD. Both interventions were too brief to prevent dysfunction.
Carter et al., 2019	n = 15, 5F Healthy adults (35.8 ± 10.2 yr)	4 hours	Walking 1. 2 min / 30 min 2. 8 min / 2 hrs	Both conditions prevented decline in SFA blood flow seen with sitting but shorter, more frequent

				walks were more beneficial.
Thosar et al., 2015	n = 12, 0F Non-obese men (24.2 ± 4.2 yr)	3 hours	Walking 5 min / 30 min	SFA FMD declined with sitting which was prevented with walking breaks
Morishima et al., 2016	n = 11, 4F Young, healthy adults (26 ± 1 yr)	3 hours	Lower limb fidgeting	Popliteal artery FMD was impaired in sedentary limb but fidgeting improved FMD.

1.3.2 Impact of interruption methods on central wave reflection

Table 2 shows the current available research on the response of CWR to prolonged sitting, exercise and postural interventions, though not necessarily in combination. Most research in this area focuses on the acute response of CWR to different interventions without considering their use as an interruption method for sedentary behaviour. Stock et al. (Stock *et al.*, 2021) provided evidence for lower body aerobic exercise to reduce RM. Wave reflections were measured before and after 5-minute bouts of low intensity aerobic exercise at 40%, 50%, and 60% of age-predicted heart rate max. With each increase in exercise intensity, Pf increased, Pb decreased, and RM decreased. The reductions in reflected waves were attributed to local reductions in vascular resistance caused by the vasodilatory effect of exercise. Notably, wave reflection was measured before and immediately following 5 minutes of recumbent cycling, meaning the impact of the intervention on CWR is confounded by the exercise itself. Van Den Bogaard et al. (Van Den Bogaard *et al.*, 2011) examined the effects of posture change on CWR, acquiring data at supine, 30° and 70° head-up tilt, and 90° active standing. With increasing angle of head-up tilt, Pf decreased, Pb decreased, and consequently, RM decreased,

suggesting that a change in posture can positively impact wave reflection. Contradictory to the previously mentioned positive relationship between vascular resistance and wave reflection, total peripheral resistance was found to increase during active standing while wave reflections were at their lowest. They concluded that increases in TPR were likely due to blood pooling in the lower limbs which reduced venous return, causing a sympathetic response to increase vasomotor tone and total peripheral resistance. The concomitant decrease in RM with increases in total peripheral resistance raises questions as to whether there are other unknown factors at play that influence the wave reflection response. Similar to Stock et al. (Stock *et al.*, 2021), the standing intervention was acute (30 minutes), meaning the effect of standing over the course of hours was not investigated. There are currently no studies that examine the effectiveness of interruption methods to counteract the consequences of prolonged sitting on central wave reflections, although studies such as those above suggest a potential beneficial effect. Low intensity aerobic exercise and intermittent standing are both commonly suggested sedentary interruption methods because of their simple nature and accessibility with commercial products. Therefore, research investigating the effects on vascular conditioning with low intensity aerobic exercise and intermittent standing on CWR is warranted to determine their effectiveness as sedentary interruption methods.

Table 2:Summary of wave reflection findings in response to different experimental conditions. SD was not reported in Van Den Bogaard et al. (Van Den Bogaard *et al.*, 2011; Credeur *et al.*, 2019; Evans *et al.*, 2019; Stock *et al.*, 2021)

				Central Wave Reflection (Mean ± SD)	
Author	**Participant Characteristics**	**Method**	**Experimental Condition**	**Baseline**	**Post**

Stock et al., 2021	n = 25, 12F Healthy adults (24 ± 4 yr)	Radial tonometry with transfer function	Lower body dynamic exercise (light condition – 40%HRmax)	Pf = 26±7 mmHg Pb = 11±3 mmHg RM = 43±5%	Pf = 30±7 mmHg Pb = 10±2 mmHg RM = 33±6%
Van Den Bogaard et al., 2011	n = 10, 1F Healthy adults (22 – 39 yr)	Finger arterial pressure with transfer function	Posture (supine vs upright)	Pf = 34.9 mmHg Pb = 20.0 mmHg RM = 57.2%	Pf = 30.2 mmHg Pb = 13.7 mmHg RM = 45.6%
Credeur et al., 2019	n = 20, 7F Healthy adults (26 ± 7 yr)	Oscillometric pressure waveforms using brachial cuff with transfer function	Prolonged Sitting	Pf = 30±1 mmHg Pb = 15±1 mmHg RM = 50±2%	Pf = 28±1 mmHg Pb = 13±1 mmHg RM = 49±2%
Evans et al., 2019	n = 20, 14F Healthy adults (21.7 ± 2.5 yr)	Oscillometric pressure waveforms using brachial cuff with transfer function	Prolonged Sitting Intermittent Calf Raises	Pf = 26±2 mmHg Pb = 12±3 mmHg Pf = 27±5 mmHg Pb = 13±3 mmHg	Pf = 26±3 mmHg Pb = 11±2 mmHg Pf = 26±4 mmHg Pb = 11±2 mmHg

2.0 STUDY RATIONALE

With the understanding that augmented wave reflections and reduced RWTT directly impact cardiovascular risk, it is logical that interventions targeting CWR may positively impact long-term cardiovascular morbidity. One current area of interest is prolonged uninterrupted sitting and the risk body postures and sedentary behaviours may pose on chronic cardiovascular health; this has been highlighted previously as increased arterial stiffness and endothelial dysfunction following even one hour of sitting (Paterson *et al.*, 2020). Small changes in wave reflection will also impact central blood pressure, leading to non-trivial increases in systolic blood pressure and pulse pressure. However, there is potential for sedentary interruption methods to alleviate these cardiovascular consequences. Studies examining wave reflections while cycling at light intensities show reductions in wave reflection amplitude (Stock *et al.*, 2021). Additionally, standing when compared to supine causes reductions in wave reflection amplitude (Van Den Bogaard *et al.*, 2011). Therefore, we propose a randomized cross-over trial investigating the ability of seated elliptical exercise and standing interruptions to alleviate the deleterious effects of prolonged sitting on central wave reflections.

3.0 RESEARCH QUESTIONS AND HYPOTHESES

Research questions

1. Do periodic interruptions prevent sitting-induced impairments in central wave reflection?
2. Does the elliptical intervention attenuate increases in central wave reflections more than the prolonged standing intervention?

Hypotheses

1. The elliptical and periodic standing conditions will attenuate the increases in central wave reflections seen with prolonged sitting.
2. The elliptical intervention will attenuate increases in central wave reflections more than periodic standing.

4.0 METHODS

4.1 Ethics

This study received ethics clearance through a University of Waterloo Research Ethics Committee (ORE #43301). The research methods and protocols adhere to the recommendations outlines by the Declaration of Helsinki concerned with the use of human participants.

4.2 Participants

We recruited eighteen healthy men and women (18-35 years of age) into this study. Exclusion criteria included daily cigarette or marijuana smokers; current or past diagnosis of cardiovascular disease, respiratory disease, kidney disease, cerebrovascular disease, diabetes, or musculoskeletal disorders; current medical prescriptions that impact the cardiovascular system or receipt of vaccine within 14 days prior to study start date; active pregnancy or pregnancy in the last 12 months, amenorrhea, or menopause. We recruited an equal number of males and females to improve the generalizability of our findings; given the lack of research in this area, we did not have adequate information to power a sex-differences analysis.

4.3 Sample size calculation

The primary outcome was RM, measured as the ratio of the reflected pressure wave to the forward pressure wave (Pb / Pf). The initial sample size estimate of n = 12 was based on simulation-based power calculations (2000 simulations) for an anticipated absolute change in RM of +0.06 for prolonged sitting control, +0.04 for standing, and -0.02 for seated elliptical with a common standard deviation of 0.05 (Cohen's f = 0.61) (Figure 6), yielding >80% power to detect differences between seated elliptical vs. prolonged sitting

control and seated elliptical vs. standing in a one-way repeated-measures ANOVA design with Bonferroni-Holm corrected dependent t-tests for significant main effects (Lakens & Caldwell, 2019). Reasonable effect sizes of detectable changes in RM were estimated from previous work with low intensity (40% heart rate max) cycling (RM Δ -0.10 from rest) as well as supine to stand (RM Δ -0.12 from supine) (Van Den Bogaard *et al.*, 2011; Stock *et al.*, 2021). The standard deviation range of 0.05 is what we anticipate seeing based on reported RM standard deviation values at baseline and post-intervention (e.g., lower body dynamic exercise, isometric and dynamic handgrip exercises) of 0.05 (Stock *et al.*, 2020, 2021). We recruited additional participants (n = 18) to account for data drop out as well as order and carry-over effects to use a balanced Latin square randomization design.

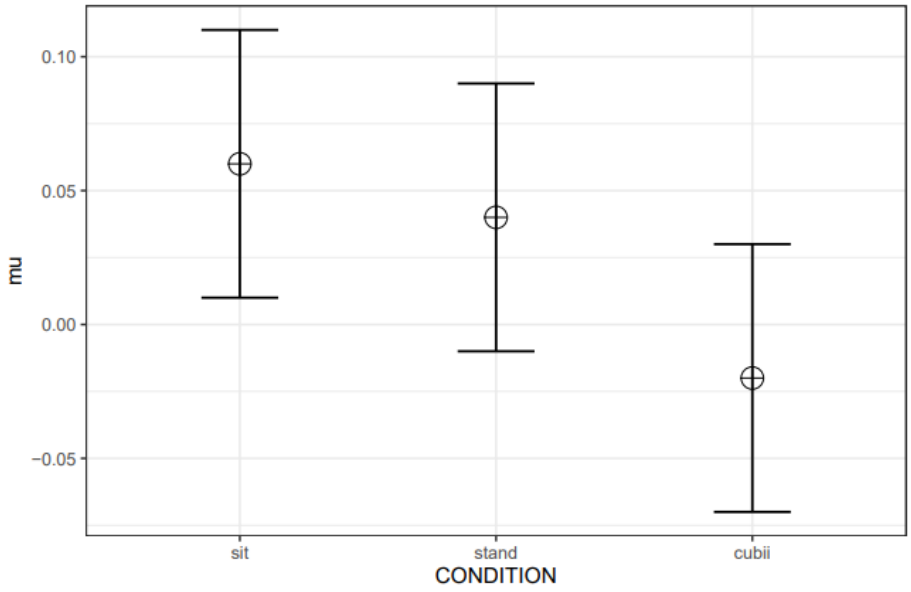

Figure 6: Anticipated change in central wave reflection during the prolonged sitting control, standing, and seated elliptical (cubii) conditions. Error bars reflect SD.

4.4 Study design

This study had a within-subject experimental crossover design. All participants completed the prolonged sitting control, standing, and seated elliptical conditions. The order of

conditions each participant experiences was block randomized, determined using a random number generator to allocate conditions until order and carry-over effects were controlled in a balanced Latin square design. All participants were required to arrive for their study visit having refrained from caffeine for the past 6 hours, refrained from alcohol and vigorous physical activity for the past 12 hours, and fasted for 4 hours prior to the start time.

Figure 7 provides an overview of the experimental protocol. After 20 minutes of supine rest, baseline measures were taken, including central wave reflection, pulse wave velocity, femoral blood flow, blood pressure and heart rate. The randomized intervention then took place for 3 hours with central wave reflection, pulse wave velocity, and femoral blood flow measured at baseline and 3 hours while blood pressure and heart rate were measured at baseline, 1 hour, 2 hours, and 3 hours. During each condition, participants were allowed to complete desk-based activities that did not generate emotional stress such as reading and working on a computer (Thosar *et al.*, 2014, 2015; Restaino *et al.*, 2015; Carter & Gladwell, 2017; Kruse *et al.*, 2018; Carter *et al.*, 2019). During the sitting control intervention, participants were seated in a typical desk chair of the appropriate height such that hip and knee angles were ~90°, and feet were flat on the floor. They were asked to minimize any fidgeting or crossing of the legs because of the potential for these habits to reduce the vascular dysfunction seen with sedentary time (Van Velthoven *et al.*, 2014; Morishima *et al.*, 2016). Adherence to the protocol was confirmed with auditory cues with lower limb movement. For the standing intervention, participants began the 3-hour period with 30 minutes of standing at a desk of the appropriate height. Previous research has used a 50:50 ratio of sit-to-stand, suggesting that standing for 50% of the

time would be well tolerated (Healy *et al.*, 2013; Thorp *et al.*, 2014*a*, 2014*b*). Once 30 minutes had elapsed, they returned to the quiet seated position, identical to the conditions of the sitting control intervention. This “on and off” pattern was repeated every hour for 3 hours. In the seated elliptical condition, a similar “on and off” pattern as seen in the standing intervention was used. At the start of the intervention, participants used the under-desk elliptical for 30 minutes at 60 rpm with the resistance set to “5” or ~8 watts (Cho, 2017). After 30 minutes, a 30-minute sitting period started, identical to the conditions of the sitting control intervention. A minimum of 24 hours was kept between study visits to ensure there is no contamination of one intervention on another.

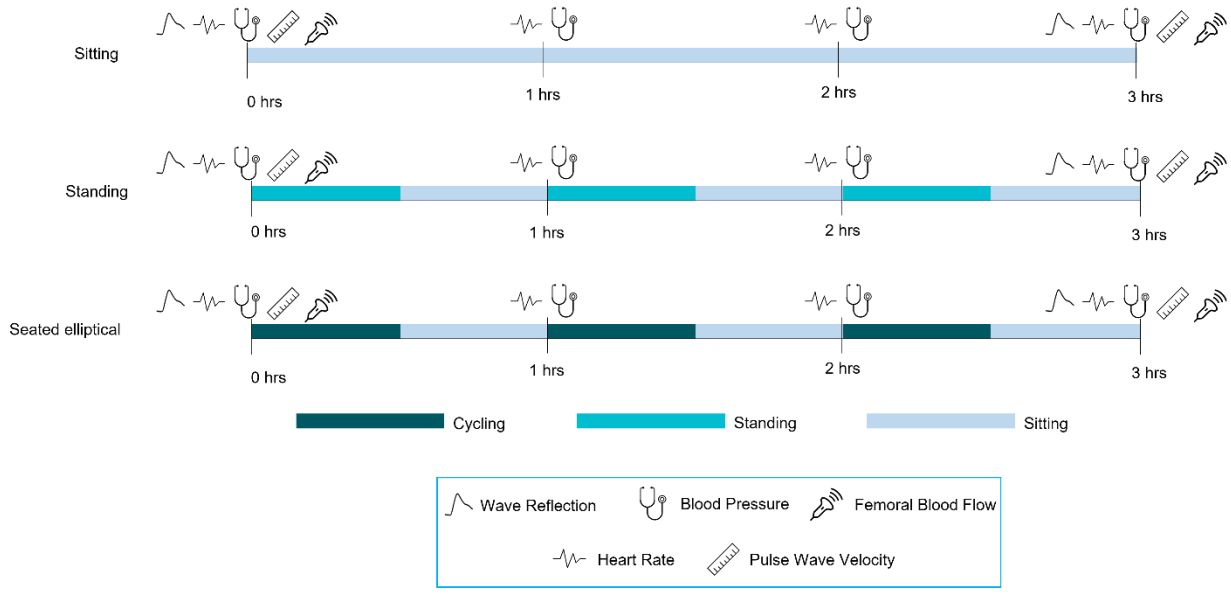

Figure 7:Outline of proposed experimental protocol. Central wave reflection, pulse wave velocity, and femoral blood flow was measured at baseline and 3 hours while blood pressure and heart rate are measured at baseline, 1 hour, 2 hours, and 3 hours.

4.5 Experimental measures

Central hemodynamics

Brachial artery blood pressure and lead II electrocardiogram were measured using the NIHem USB-II workstation (Cardiovascular Engineering Inc., Norwood, MA, USA). ECG and heart rate data were collected during each wave reflection and blood pressure

measurement. Brachial blood pressure was measured in triplicate or until two consecutive measures within 5 mmHg were acquired. Stroke volume (SV), cardiac output (Q̇), and total peripheral resistance (TPR) were calculated as:

$$SV = \frac{VTI\ x\ CSA}{1000}$$

Equation 1: Stroke Volume

$$\dot{Q} = HR\ x\ SV$$

Equation 2: Cardiac Output

$$TPR = \frac{MAP}{\dot{Q}}$$

Equation 3: Total Peripheral Resistance

where VTI is the velocity time integral of the left ventricular outflow tract in cm from echocardiography (below), CSA is the cross-sectional area of the aortic root in cm^2, HR is heart rate in bpm, and MAP is mean arterial pressure in mmHg.

Central wave reflection

RM, calculated as ratio of the reflected wave and incident wave, was estimated from the frequency domain pressure-flow relationship. The right common carotid artery pressure wave was measured using applanation tonometry to approximate aortic pressure (Kelly & Fitchett, 1992). Aortic blood flow was acquired through cardiac ultrasound as the product of the time-velocity integral of the Doppler velocity within the left ventricular outflow tract (LVOT) in the apical 5-chamber view and the cross-sectional area of the aortic root using the parasternal long axis view (Vivid S70N; GE Medical Systems, Milwaukee, WI). Characteristic impedance (Z_c) was calculated as the average of the 4^{th} to 10^{th} harmonics of the pressure-flow relationship in the frequency domain by the NIHem USB-II workstation. Zc allows for the calculation of the Pf and Pb which was used to

quantify RM (Pb/Pf) (Westerhof *et al.*, 1972). AIx was calculated as the ratio of augmented pressure to central pulse pressure. All calculations were completed by the NIHem USB-II workstation and exported as outcomes values for further analysis.

$$RM = \frac{Pb}{Pf}$$

Equation 4: Central relative wave reflection magnitude

Femoral blood flow

Vascular B-mode and pulsed wave Doppler ultrasound was acquired at the right common femoral artery to measure arterial diameters and blood flow velocities, respectively. Blood flow and conductance was calculated as:

$$Blood\ flow = \pi\left(\frac{d}{2}\right)^2 \times MBV \times 60$$

Equation 5: Blood flow

$$Conductance = \frac{Blood\ Flow}{Mean\ Arterial\ Pressure}$$

Equation 6: Conductance

where d is the end-diastolic diameter and MBV is the mean blood velocity taken as the intensity-weighted time-averaged mean velocity of the Doppler waveform (Totosy de Zepetnek *et al.*, 2015). Doppler waveforms were analyzed offline with an open-access MATLAB program based on time-varying pixel intensity (FloWave; Vanderbilt University Institute of Imaging Science, Nashville, TN, USA) (Coolbaugh *et al.*, 2016). Mean arterial pressure was calculated as diastolic pressure + 1/3 (systolic pressure – diastolic pressure).

Pulse wave velocity

Applanation tonometry was used to measure common carotid, common femoral, and posterior tibial pressure waveforms (NIHem USB-II). The time delay between the arrival of the pulse waves at the carotid and femoral was calculated using the foot-to-foot method, comparing the time difference between the foot of one wave to the foot of the other, relative to the R spike in the ECG. The distances of both the carotid and femoral artery tonometry sites to the suprasternal notch (SSN) and the distance from the femoral tonometry site to the posterior tibial site were measured. The distance between the carotid and femoral tonometry sites was calculated as the SSN-femoral distance minus the SSN-carotid distance. Carotid-femoral pulse wave velocity (cfPWV) was calculated as the distance between carotid and femoral sites divided by the pulse transit time (Van Bortel *et al.*, 2012). Lower-limb pulse wave velocity (faPWV) was calculated as the distance between the femoral and posterior tibial sites divided by the pulse transit time.

Prolonged sitting control intervention

The seated workstation included a standard desk chair, desk, and the participants' personal laptops. Participants were monitored and asked to refrain from behaviours such as fidgeting, excessive leg movements, and crossing of the legs to avoid interference with the anticipated effects on vascular dysfunction.

Low intensity aerobic exercise intervention

An under-desk elliptical device (Cubii, FitnessCubed, Inc., Chicago, IL, USA) was used to provide low intensity seated aerobic exercise. Cadence was maintained at 60 rpm which is found to be the most efficient for reducing attention loss (Schuna *et al.*, 2016; Cho *et al.*, 2017) and was monitored continuously by the participant through a camera to visualize the LCD monitor displaying current rpm. To maintain consistency in all

conditions, the same desk and personal laptop were used. Participants used a stationary chair during the exercise condition to increase comfort and ease of exercise. During the sedentary portions of this intervention, the same guidelines applied regarding fidgeting, leg movements, and crossing of the legs.

Standing intervention

A standing desk was used for this condition. Participants were instructed while standing to maintain an even weight distribution as much as possible. Desk height was determined for each individual based on comfort (typically around chest height). The sedentary portions of this condition used the same laptop, desk, and chair previously described with the same guidelines regarding fidgeting, leg movements, and crossing of the legs.

4.6 Statistical analysis

All statistical analysis and plotting was performed using R (R Core Team, 2021). Our primary outcome (RM) as well as our secondary outcomes (Pf, Pb, RWTT, cfPWV, faPWV, femoral blood flow, conductance, $\dot{Q}$, and TPR) were assessed by linear mixed models with time (baseline, post) and condition (sitting control, standing, seated elliptical) as fixed effects and participant as a random effect. In the case of significant effects, pairwise comparisons of the estimated marginal means were made with the Bonferroni-Holm correction applied for multiple comparisons. The change in RM (ΔRM) was assessed with a one-way repeated measures linear mixed model across conditions with significant effects followed with Bonferroni-Holm corrected contrast tests. Repeated measures correlation coefficients were used to assess associations between central wave reflection (Pf, Pb, RM), $\dot{Q}$, RWTT, and TPR. An *a priori* type I error rate was established at $\alpha = 0.05$, and the appropriate magnitude of effect (Cohen's d for t-tests,

partial eta-squared for linear mixed models) was calculated to supplement statistical inferences.

5.0 RESULTS

5.1 Participant characteristics

Participant demographic information is reported in Table 3.

Table 3:Participant characteristics . Values are mean ± standard deviation.

Variable	Female	Male	Total
n	9	9	18
Age (yrs)	24±2	26±2	25±3
Height (cm)	167±8	175±5	171±7
Body mass (kg)	59.1±7.7	81.0±6.7	70.0±13.1

5.2 Central hemodynamics

Systolic blood pressure did not change (all $p > 0.07$; all partial $\eta^2 < 0.01$; Figure 8A). Diastolic blood pressure increased from baseline to post during sitting control (65±7 to 70±8 mmHg; $p < 0.01$; Cohen's d = 0.54) and standing (64±6 to 69±6 mmHg; $p < 0.01$; Cohen's d = 0.82) but did not change for seated elliptical (66±6 to 67±7 mmHg; p = 0.50; Cohen's d = 0.10). ΔDBP was reduced with seated elliptical compared to sitting control (0.7±2.6 vs. 4.2±5.2 mmHg; p = 0.03; Cohen's d = 0.86) and standing (0.7±2.6 vs 5.0±4.2 mmHg; $p < 0.01$; Cohen's d = -1.25) with no difference between sitting control and standing (4.2±5.2 vs. 5.0±4.2 mmHg; p = 0.50; Cohen's d = 0.18) (Figure 8B). MAP increased with standing (82±6 to 85±7 mmHg; $p < 0.01$; Cohen's d = 0.47) and sitting (84±7 to 86±7 mmHg; p = 0.03; Cohen's d = 0.32) but not seated elliptical (84±7 to 83±6 mmHg; p = 0.42; Cohen's d = 0.12). ΔMAP was smaller during seated elliptical vs. sitting control (-0.8±3.2 vs. 2.3±4.3 mmHg; p = 0.05; Cohen's d = 0.80) and standing (-0.8±3.2 vs. 3.0±4.6 mmHg; p = 0.02; Cohen's d = 0.96) (Figure 8C). $\dot{Q}$ decreased from baseline to post (main effect of time: 4.9±0.9 to 4.5±0.9 $L \cdot min^{-1}$; p = 0.03; partial $\eta^2 = 0.19$; Figure 9A), likely due to similar reductions in HR (main effect of time: 64±8 to 61±8 bpm; p <

0.01; partial $\eta^2 = 0.18$; Figure 9C) and no change in SV (all $p > 0.40$; all partial $\eta^2 < 0.06$; Figure 9B). TPR did not change from baseline to post (all $p > 0.06$; all partial $\eta^2 < 0.06$; Figure 9D).

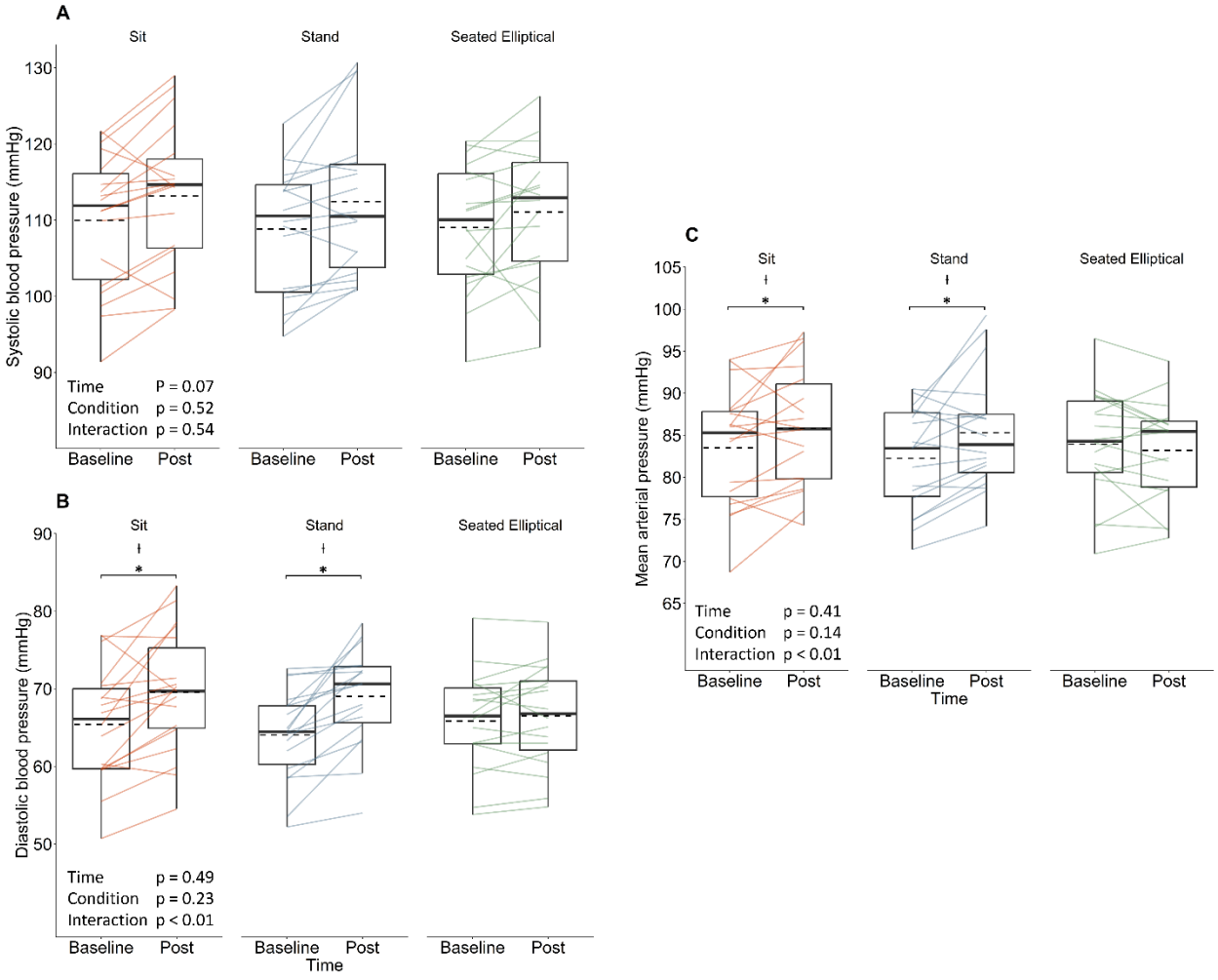

*Figure 8: Systolic (A), diastolic (B), and mean arterial pressure (C) before and after 3 hours of sitting (left), sitting with standing breaks (middle), and sitting with seated elliptical breaks (right). Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n = 18). *p < 0.05, † p < 0.05 vs seated elliptical (Δ). Overall effects were assessed by linear mixed models. Significant effects were examined using pairwise comparisons of the estimated marginal means with Bonferroni-Holm correction applied for multiple comparisons.*

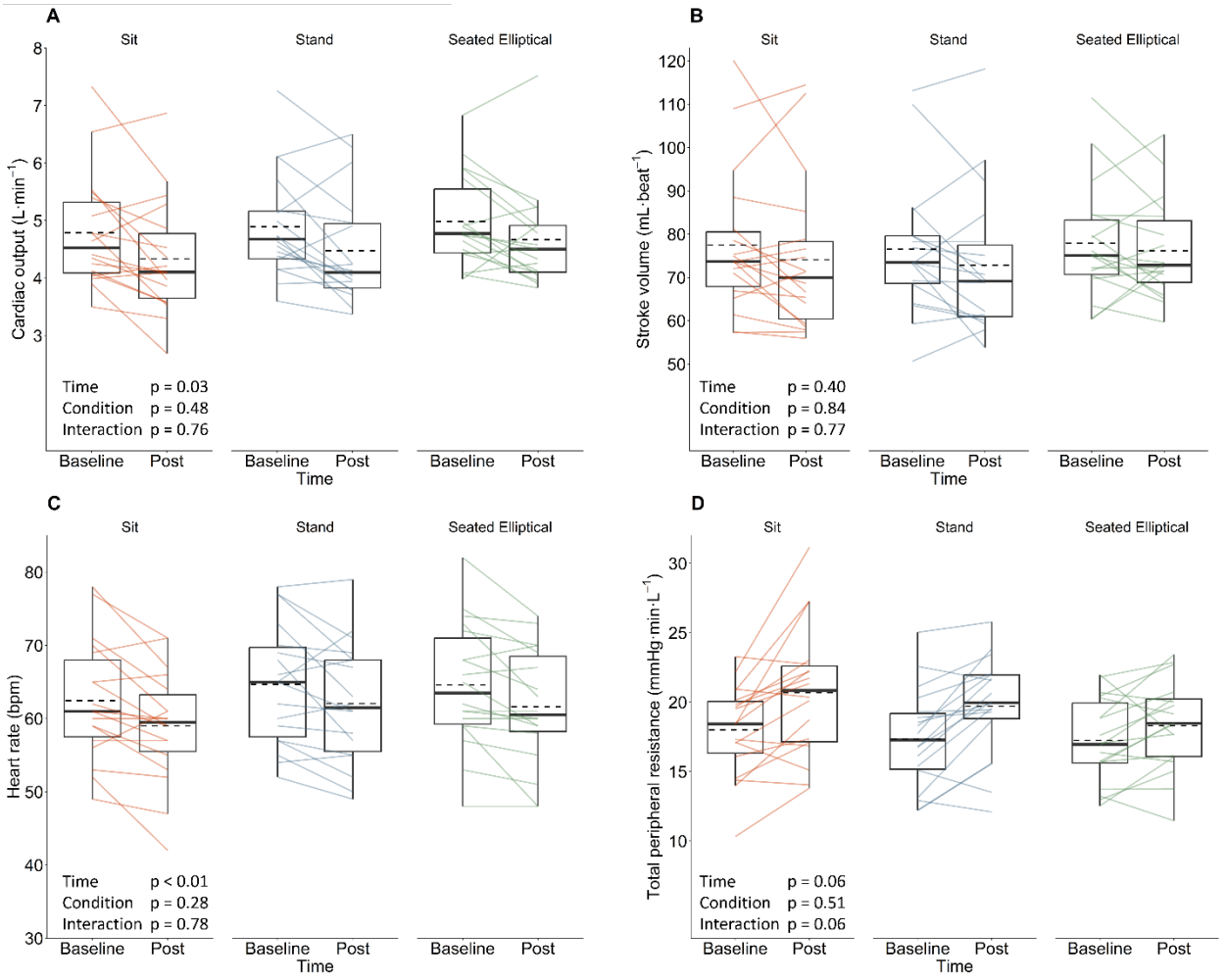

*Figure 9: Cardiac output(A), stroke volume (B), heart rate (C), and total peripheral resistance (D) before and after 3 hours of sitting (left), sitting with standing breaks (middle), and sitting with seated elliptical breaks (right). Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n = 18). Overall effects were assessed by linear mixed models.*

5.3 Central wave reflection

Pb decreased from baseline to post (main effect of time: 11.9±1.6 to 11.3±2.0 mmHg; p = 0.03; partial η^2 = 0.05; Figure 10A). Likewise, Pf decreased from baseline to post (main effect of time: 40.0±8.1 to 35.2±6.0 mmHg; p = 0.02; partial η^2 = 0.17; Figure 10B). RM increased from baseline to post with sitting control (0.31±0.05 to 0.35±0.05; p < 0.01; Cohen's d = 0.69) but did not change for standing (0.30±0.05 to 0.32±0.04; p = 0.19,

Cohen's d = 0.35) or seated elliptical (0.30±0.05 to 0.30±0.04; p > 0.99, Cohen's d = 0.00) (Figure 10C). Sitting control had a greater ΔRM compared to seated elliptical (0.04±0.05 vs. 0.00±0.03; p = 0.02; Cohen's d = 0.88) but there was no difference between seated elliptical and standing (0.00±0.03 vs. 0.02±0.05; p = 0.54; Cohen's d = 0.40) or sitting control and standing (0.04±0.04 vs. 0.02±0.05; p = 0.54; Cohen's d = 0.40). RWTT did not change over time (all p > 0.06; all partial η^2 < 0.04; Figure 11). AIx increased with sitting control (-9.3±9.2 to -4.6±12.2 %; p < 0.01, Cohen's d = 0.44) and standing (-12.4±9.8 to -5.6±9.4 %; p < 0.01, Cohen's d = 0.70) but not with seated elliptical (-9.9±9.4 to -10.2±11.1 %; p = 0.82; Cohen's d = 0.03). Additionally, ΔAIx was smaller during seated elliptical compared to standing (-4.1±14.5 vs. -15.7±30.8 %; p = 0.03; Cohen's d = 0.49) (Figure 12).

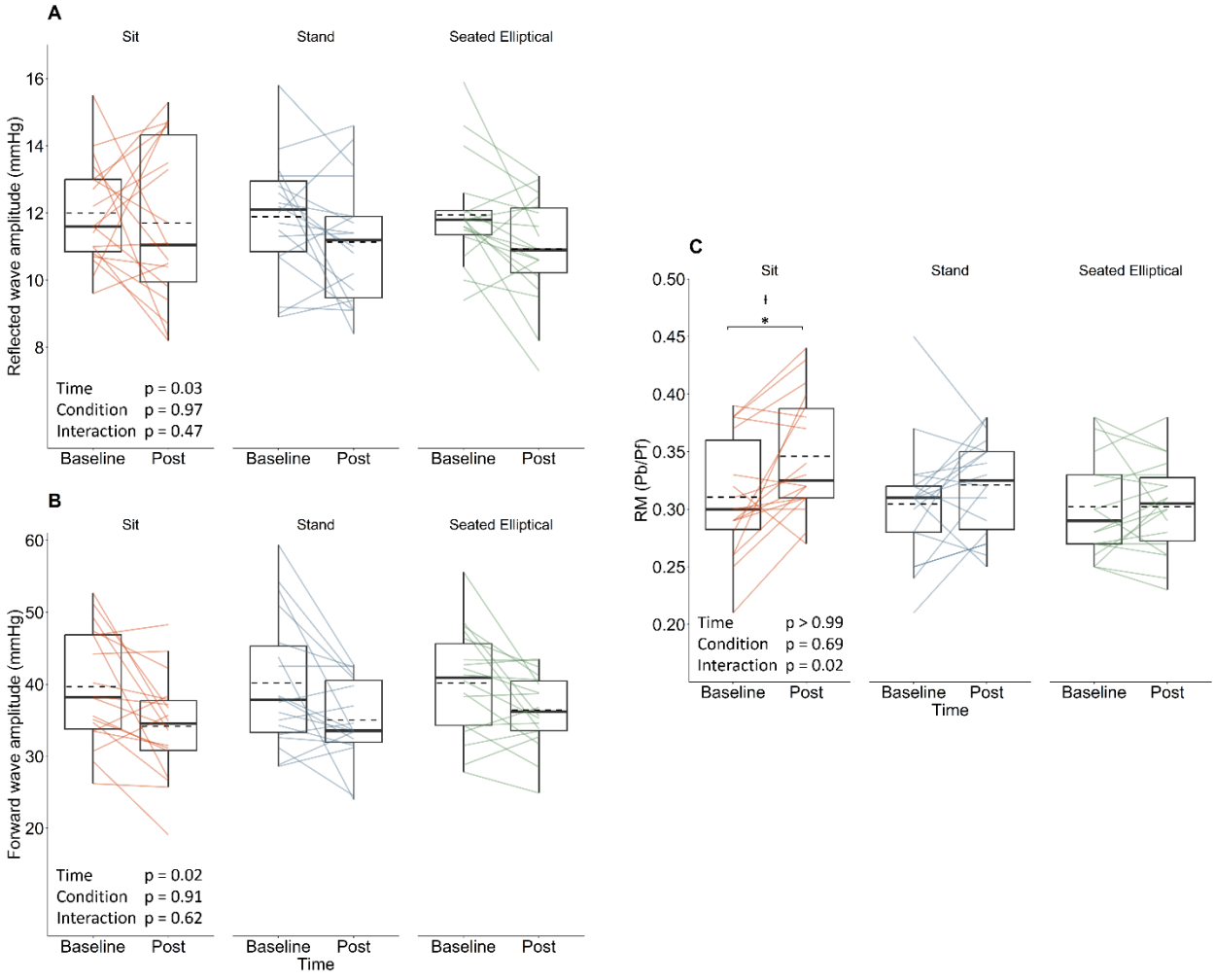

*Figure 10: Central wave reflection outcome variables (Pb(A), Pf(B), and RM(C)) before and after 3 hours of sitting (left), sitting with standing breaks (middle), and sitting with seated elliptical breaks (right). Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n = 18). *p < 0.05, † p < 0.05 vs seated elliptical (Δ). Overall effects were assessed by linear mixed models. Significant effects were examined using pairwise comparisons of the estimated marginal means with Bonferroni-Holm correction applied for multiple comparisons.*

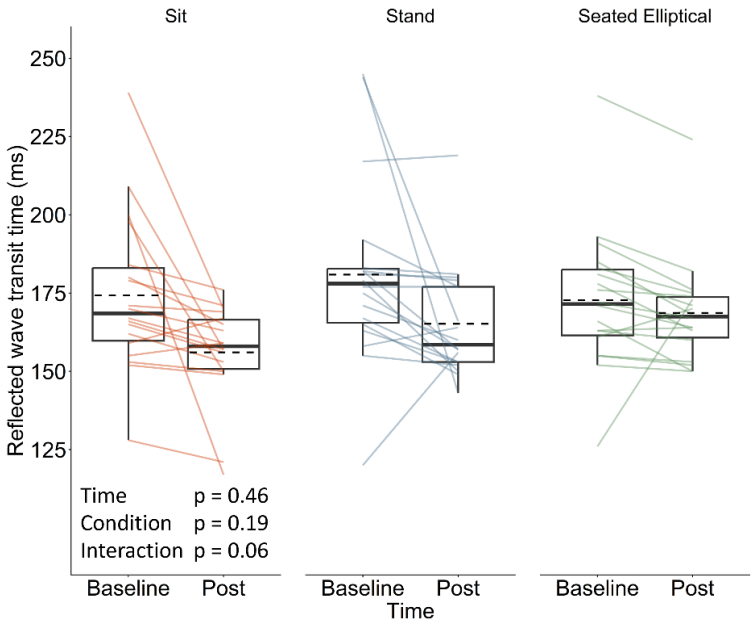

*Figure 11: Reflected wave transit time before and after 3 hours of sitting (left), sitting with standing breaks (middle), and sitting with seated elliptical breaks (right). Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n = 18). Overall effects were assessed by linear mixed models.*

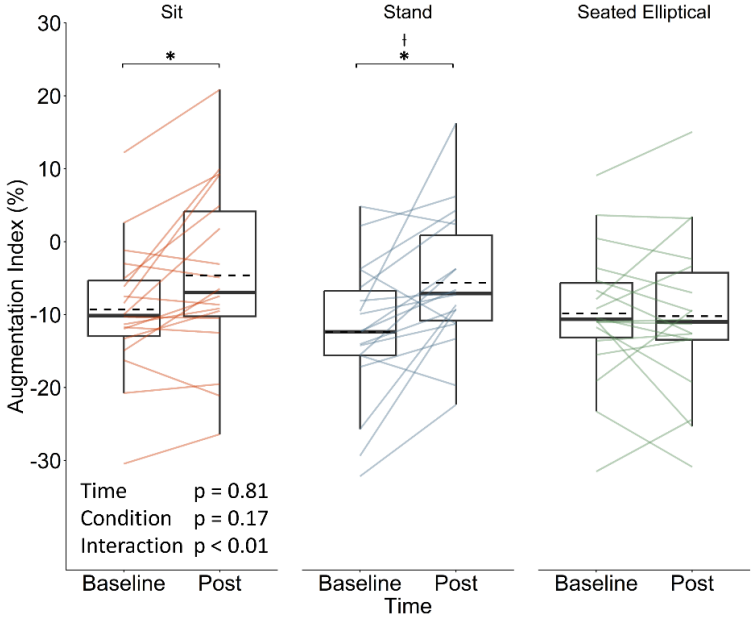

*Figure 12: Augmentation index before and after 3 hours of sitting (left), sitting with standing breaks (middle), and sitting with seated elliptical breaks (right). Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n = 18). *p < 0.05, † p < 0.05 vs seated elliptical (Δ). Overall effects were assessed by linear mixed models. Significant effects were examined using pairwise comparisons of the estimated marginal means with Bonferroni-Holm correction applied for multiple comparisons.*

5.4 Femoral blood flow

Femoral blood flow increased after seated elliptical (158±68 to 216±100 mL·min^{-1}; p = 0.04; Cohen's d = 0.68) but did not change during sitting control (183±71 to 193±110 mL·min^{-1}; p = 0.70; Cohen's d = 0.10) or periodic standing (188±88 to 171±104 mL·min^{-1}; p = 0.56; Cohen's d = 0.18) (Figure 13A). Additionally, Δ Femoral blood flow was larger with seated elliptical compared to sitting control (59±87 vs. 9±85 mL·min^{-1}; p = 0.02; Cohen's d = 0.57) and standing (59±87 vs. -17±85 mL·min^{-1}; p = 0.02; Cohen's d = 0.88). Similarly, conductance increased with seated elliptical breaks (1.9±0.8 to 2.6±1.2 mL·min^{-1}·mmHg^{-1}; p = 0.02; Cohen's d = 0.72) but did not change with sitting control (2.2±0.8 to 2.2±1.2 mL·min^{-1}·mmHg^{-1}; p = 0.84; Cohen's d = 0.07) or standing (2.3± 1.0 to 2.0±1.0 mL·min^{-1}·mmHg^{-1}; p = 0.19; Cohen's d = 0.33) (Figure 13B). Δ Conductance was greater during seated elliptical compared to sitting control (0.7±1.1 vs. 0.1±0.9 mL·min^{-1}·mmHg^{-1}; p < 0.01; Cohen's d = 0.67) and standing (0.7±1.1 vs -0.3±0.9 mL·min^{-1}·mmHg^{-1}; p < 0.01; Cohen's d = 1.08), and greater during sitting control compared to standing (0.1±0.9 vs. -0.3±0.9 mL·min^{-1}·mmHg^{-1}; p = 0.04; Cohen's d = 0.43).

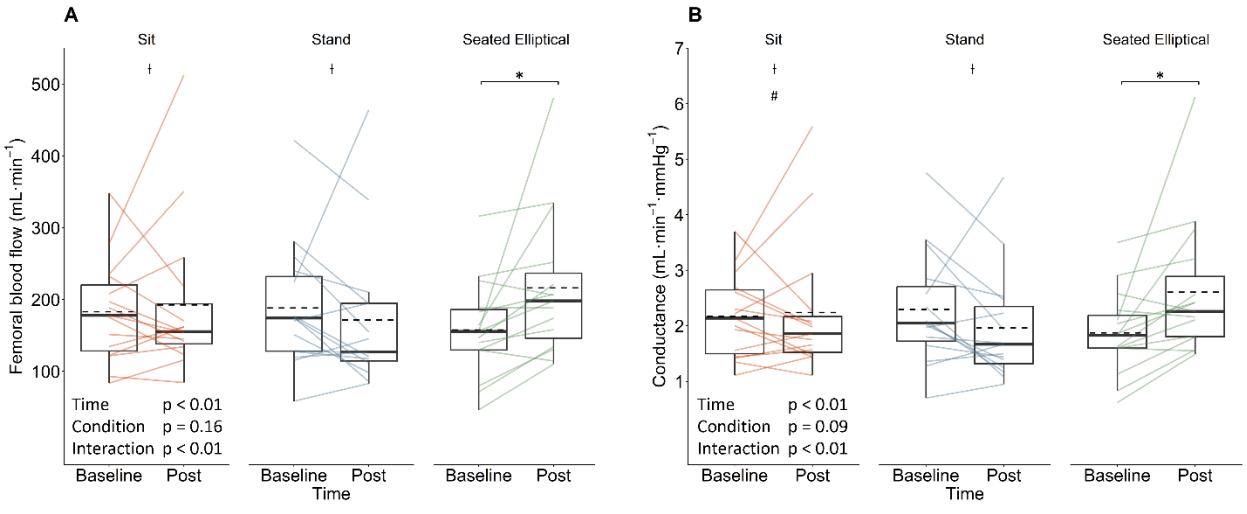

*Figure 13: Common femoral blood flow (A) and conductance (B) before and after 3 hours of sitting (left), sitting with standing breaks (middle), and sitting with seated elliptical breaks (right). Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n = 18). *p < 0.05, † p < 0.05 vs seated elliptical (Δ), # p < 0.05 vs. standing (Δ). Overall effects were assessed by linear mixed models. Significant effects were examined using pairwise comparisons of the estimated marginal means with Bonferroni-Holm correction applied for multiple comparisons.*

5.5 Pulse wave velocity

Central pulse wave velocity increased for sitting control (5.3±0.8 to 5.7±0.9 $m \cdot s^{-1}$; $p < 0.01$; Cohen's d = 0.55) and standing (5.3±0.7 to 5.7±0.7 $m \cdot s^{-1}$; $p < 0.01$; Cohen's d = 0.63), but not for seated elliptical (5.5±0.5 to 5.6±0.6 $m \cdot s^{-1}$; $p = 0.43$, Cohen's d = 0.13) (Figure 14A). Additionally, ΔcfPWV was reduced with seated elliptical compared to sitting control (0.1±0.4 $m \cdot s^{-1}$ vs. 0.5±0.4; $p < 0.01$; Cohen's d = 0.92) and standing (0.1±0.4 vs. 0.4±0.3 $m \cdot s^{-1}$; $p = 0.05$; Cohen's d = 0.90) but there was no difference between standing vs. sitting control (0.4±0.3 vs. 0.5±0.4 $m \cdot s^{-1}$; $p = 0.76$; Cohen's d = 0.09). Lower limb PWV decreased during seated elliptical (9.8±1.4 to 9.1±1.5 $m \cdot s^{-1}$; $p = 0.03$; Cohen's d = 0.51) but did not change for sitting control (9.5±1.7 to 9.6±1.0 $m \cdot s^{-1}$; $p = 0.73$; Cohen's d = 0.08) or standing (9.1±1.5 to 9.7±1.7 $m \cdot s^{-1}$; $p = 0.21$; Cohen's d = 0.38) (Figure 14B). ΔfaPWV was greater with standing compared to seated elliptical (0.6±1.1 vs. -0.7±1.3 $m \cdot s^{-1}$; $p = 0.02$; Cohen's d = 1.08) but there was no difference between sitting control and standing (0.1±1.2 vs. 0.6±1.1 $m \cdot s^{-1}$; $p = 0.25$; Cohen's d = 0.42) or seated elliptical (0.1±1.2 vs -0.7±1.3 $m \cdot s^{-1}$; $p = 0.06$; Cohen's d = 0.68).

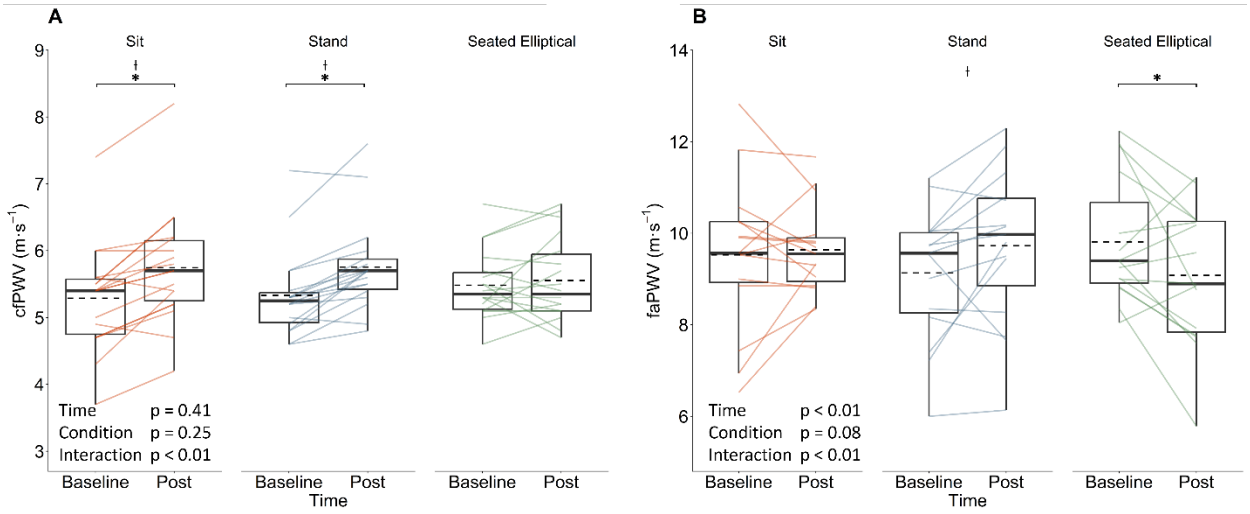

*Figure 14: Carotid-femoral pulse wave velocity (A) and femoral-ankle pulse wave velocity (B) before and after 3 hours of sitting (left), sitting with standing breaks (middle), and standing with seated elliptical breaks (right). Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n = 18). *p < 0.05, † p < 0.05 vs seated elliptical (Δ). Overall effects were assessed by linear mixed models. Significant effects were examined using pairwise comparisons of the estimated marginal means with Bonferroni-Holm correction applied for multiple comparisons.*

5.6 Correlations

Pf was significantly correlated with $\dot{Q}$ (r_{rm} = 0.34; p < 0.01; Figure 15A), and HR (r_{rm} = 0.37; p < 0.01; Figure 15C) but not with SV (r_{rm} = 0.16; p = 0.13; Figure 15B) while Pb showed no relationship with $\dot{Q}$, HR, or SV (r_{rm} < 0.09; p > 0.38). RM was correlated with $\dot{Q}$ (r_{rm} = -0.32; p < 0.01) but not SV (r_{rm} = -0.09; p = 0.39). There was a significant correlation between RM and TPR (r_{rm} = 0.34; p < 0.01; Figure 16) and between Pf and TPR (r_{rm} = -0.34; p < 0.01) but not between Pb and TPR (r_{rm} = -0.09; p = 0.40). RWTT was positively correlated with Pf (r_{rm} = 0.29; p < 0.01; Figure 17A) and negatively correlated with RM (r_{rm} = -0.26; p = 0.01; Figure 17B). DBP was significantly correlated with AIx (r_{rm} = 0.35; p < 0.01, Figure 18A), RWTT (r_{rm} = -0.32; p < 0.01; Figure 18B), and TPR (r_{rm} = 0.49; p < 0.01; Figure 18C).

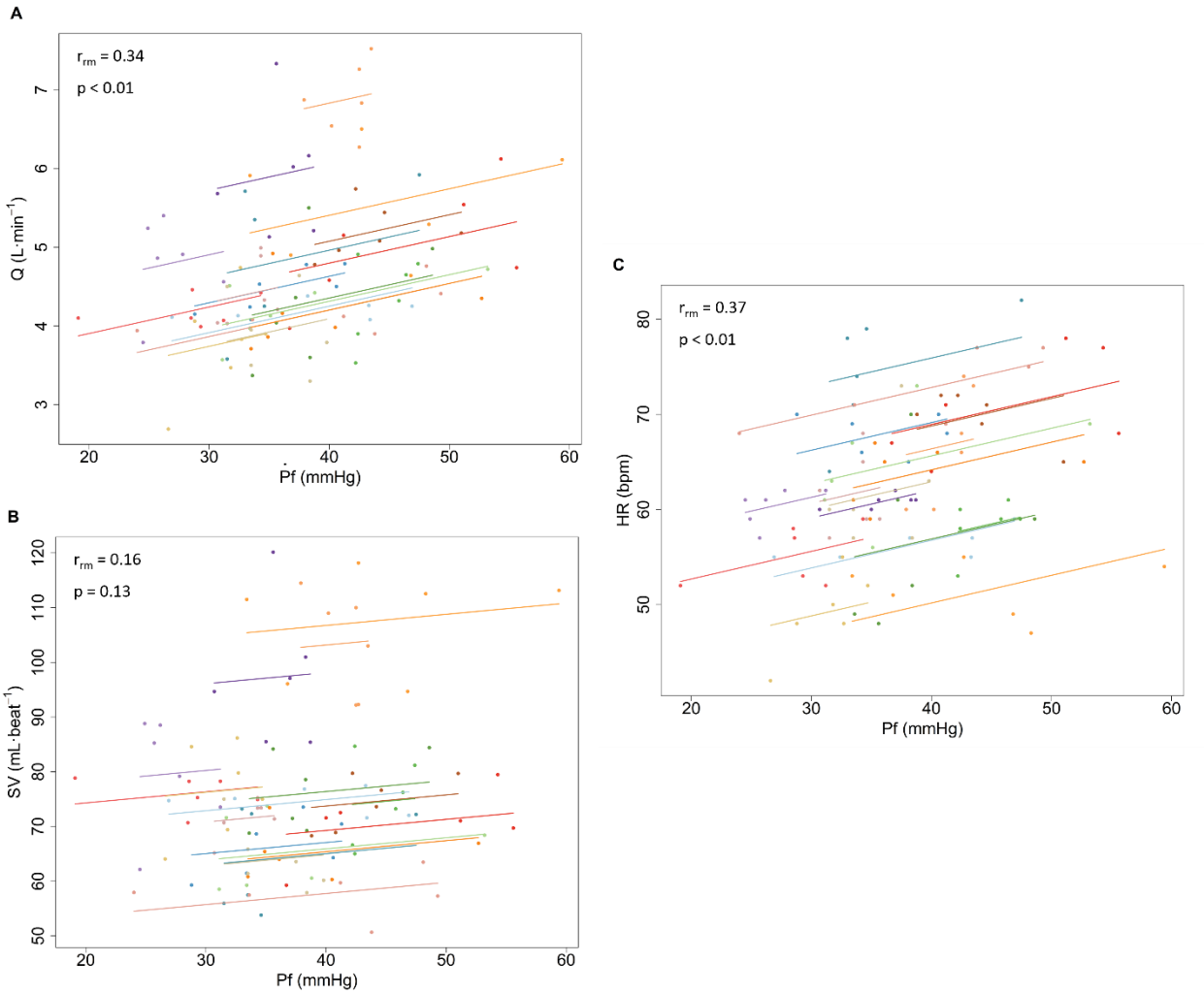

Figure 15: Repeated measures correlations between Pf and $\dot{Q}$(A), Pf and SV (B), and Pf and HR (C) across all conditions and timepoints.

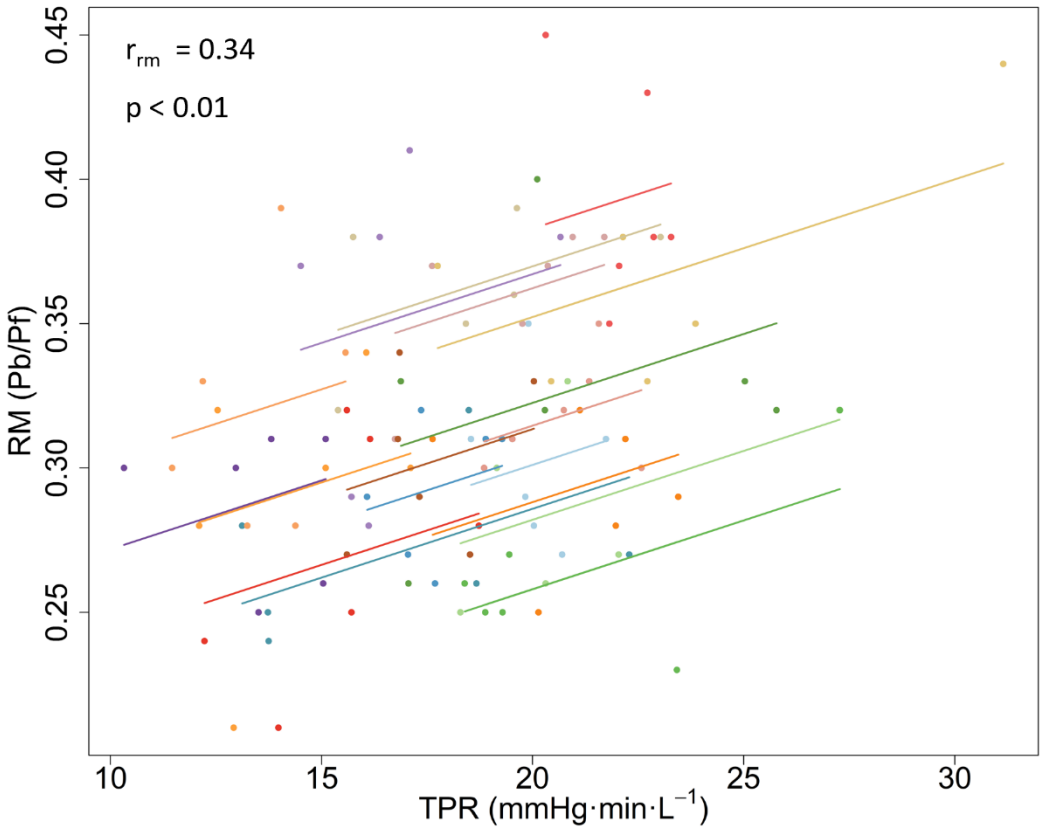

Figure 16: Repeated measures correlation between RM and TPR across all conditions and timepoints

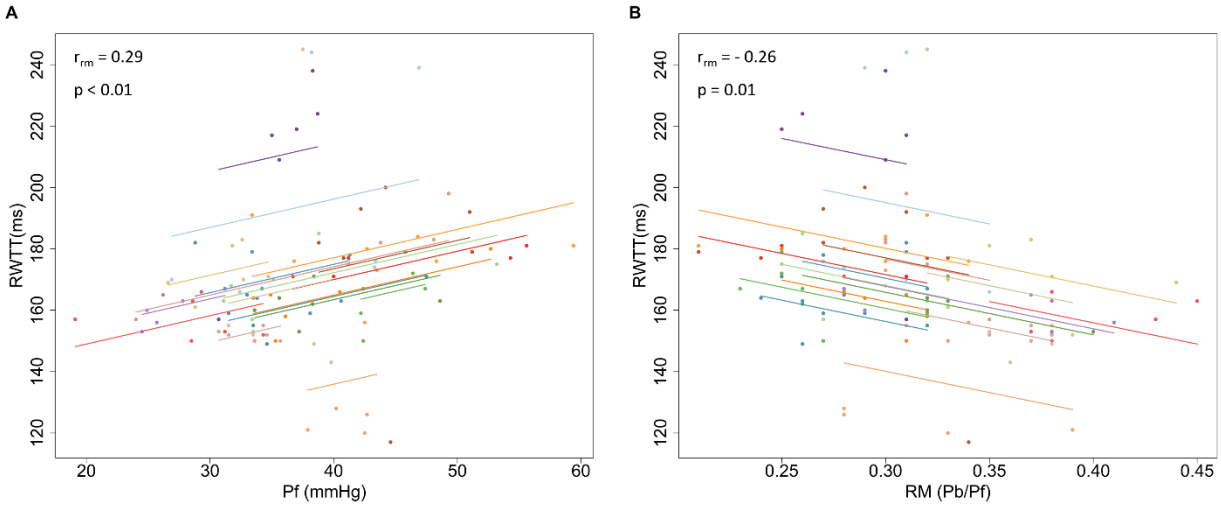

Figure 17: Repeated measures correlations between RWTT and Pf (A) and RWTT and RM (B) across all conditions and timepoints.

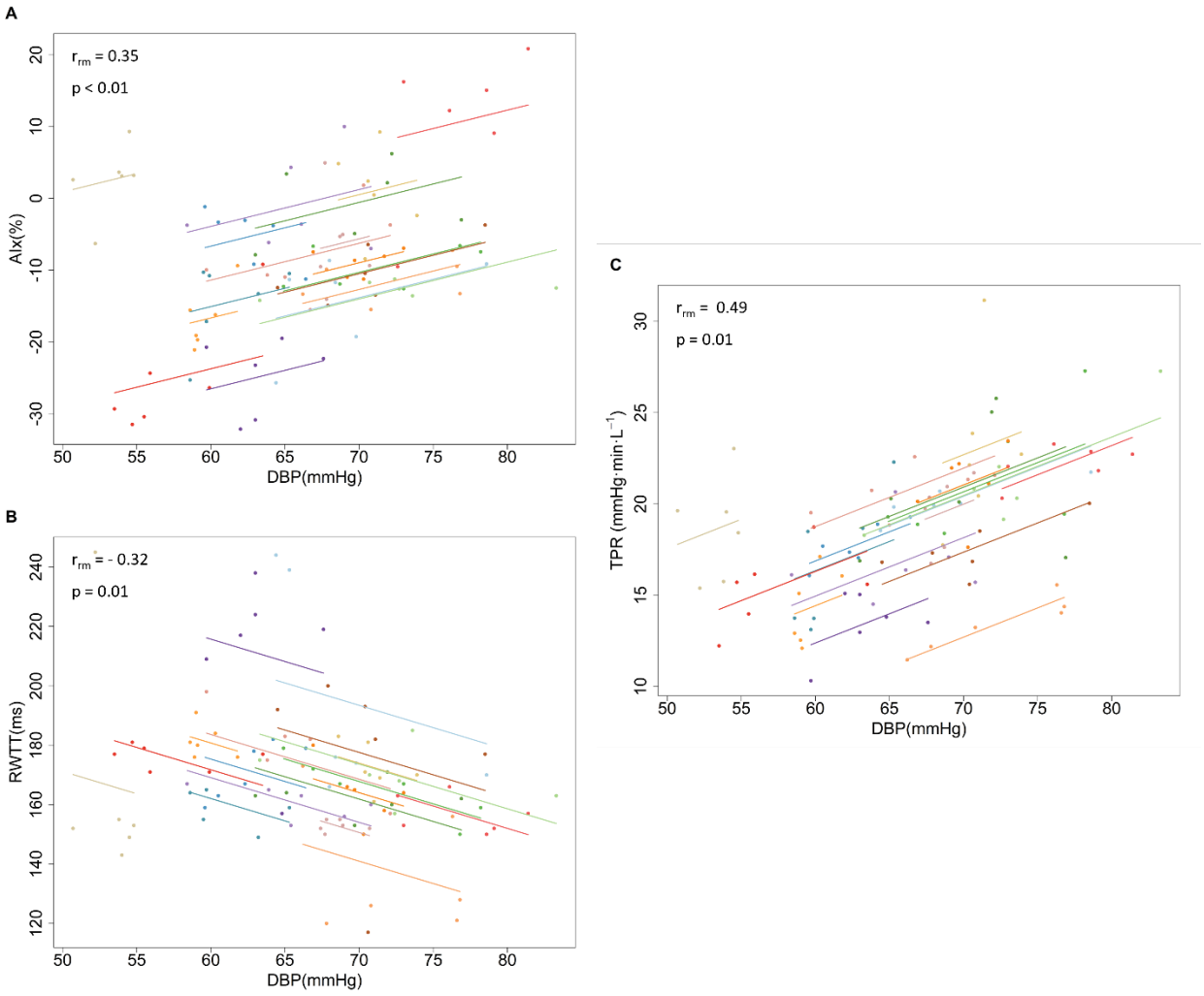

Figure 18: Repeated measures correlations for DBP vs. AIx (A), DBP vs. RWTT (B), and DBP vs. TPR (C) across all conditions and timepoints.

6.0 DISCUSSION

In this study, we investigated the impact of sedentary behaviour on central wave reflection and whether seated elliptical exercise or standing interruptions could alleviate the negative effects of prolonged sitting. Cardiac output decreased in all conditions, potentially indicative of reduced metabolic demand and blood pooling occurring with prolonged sitting. SBP did not change while DBP only increased during sitting control and standing. Sitting for 3 hours increased RM, but this effect was mitigated with the seated

elliptical interruption strategy. RM during standing was not different from sitting control, indicating no alleviation of negative effects. AIx was increased with sitting control and standing but not seated elliptical. Prolonged sitting induced increases in cfPWV, which was not prevented by intermittent standing, but the elevation was eliminated with elliptical cycling breaks. Our findings suggest intermittent seated elliptical interruptions attenuate increases in central wave reflection with prolonged sitting, while periodic standing is unable to provide the same benefit.

6.1 Central wave reflection

Prolonged sitting control

Pf and Pb decreased in all conditions. The decrease in Pf and consequently Pb, is likely driven by reduced $\dot{Q}$. With prolonged sitting, there is an increase in lower limb blood pooling (Shvartz *et al.*, 1983; Restaino *et al.*, 2015; Horiuchi & Stoner, 2021; Park *et al.*, 2022), resulting in decreased central venous pressure, cardiac filling, and cardiac output. Since Pf results from ventricular ejection, a decrease in $\dot{Q}$ would likely decrease Pf. We did find a weak positive correlation between $\dot{Q}$ and Pf but no correlation between SV and Pf, suggesting other factors may be contributing to this effect. RM increased with sitting control, indicating a greater portion of the forward wave, although diminished, was reflected back. Vascular resistance is believed to be the driving variable in the RM response, with increased TPR producing a greater magnitude and earlier return of the reflected wave (Wilkinson *et al.*, 2001; Casey *et al.*, 2008; Lydakis *et al.*, 2008). The root cause of increased TPR with prolonged sitting is still unknown but may be related to sympathetic activity from reduced venous return and baroreflex activation (Zoller *et al.*, 1972; Skagen, 1982; Cai *et al.*, 2000), venous distension-induced arterial constriction

(Kitano *et al.*, 2005), and arterial bending (Morishima *et al.*, 2017). Previous work found that increased vascular resistance results in reduced RWTT and increased AIx, suggesting this is indicative of increased reflected waves, although neither Pb nor RM were measured (Wilkinson *et al.*, 2001; Casey *et al.*, 2008; Lydakis *et al.*, 2008). The current study did not observe increases in TPR during sitting control however, the significant positive correlation between TPR and RM does suggest an effect for TPR in the RM response, though other mechanisms likely play a stronger role.

RWTT was correlated with Pf (r_{rm} = 0.29) and RM (r_{rm} = - 0.26) but did not see any change across time or condition. Based on the RWTT results (all $p > 0.06$; all partial $\eta^2 < 0.04$) it is possible that the study was underpowered to detect changes in RWTT, as the study was primarily designed to have sufficient power to detect changes in RM. Normally, reflected waves return during diastole, allowing for increased coronary blood flow and adequate blood supply to cardiac tissues (London & Guérin, 1999). When RWTT is reduced, the reflected wave may return during systole while the coronary vessels are closed, decreasing coronary flow and delivery to cardiac tissues. Additionally, earlier return of the reflected wave may cause Pb to merge with Pf, augmenting SBP, AIx and RM. Our findings demonstrate an inverse relationship between RWTT and RM, showing that as the reflected wave arrives earlier, RM increases. Prolonged sitting and the associated increases in RM, AIx, and decreased RWTT, have negative consequences for cardiovascular disease risk. AIx is a cardiovascular disease risk indicator and is often used as a surrogate for wave reflections (Nürnberger *et al.*, 2002; Chirinos *et al.*, 2005; Weber *et al.*, 2005). Multiple factors can influence AIx including RM, RWTT, HR and height (Hope *et al.*, 2005; McEniery *et al.*, 2005; Mitchell, 2006; Nichols *et al.*, 2011;

Chirinos *et al.*, 2012), making it sensitive to confounding variables that would impact its effectiveness as a independent CVD indicator. In contrast, RM is less sensitive to confounders (Chirinos *et al.*, 2012), enhancing its ability to quantify central wave reflections and potentially, making it a more robust CVD indicator. Supporting RM as a CVD predictor, Chirinos et al., (Chirinos *et al.*, 2012) found RM was associated with incident cardiovascular events and congestive heart failure while AIx was only associated with hard cardiovascular events (i.e., myocardial infarction, resuscitated cardiac arrest, CVD related death, stroke, or stroke related death). This relationship between wave reflections and cardiovascular disease risk may be linked to increased afterload occurring from abnormal wave reflection behaviours. With increased SBP, RM, and decreased RWTT, there is greater requirements from the heart to eject blood to overcome the augmented pressure gradient (i.e., increased afterload). Acutely, this may not impact CVD risk substantially, but chronic consequences of increased afterload may accumulate to cause negative structural adaptations (Weber *et al.*, 1993; Kahan & Bergfeldt, 2005) and contractile dysfunction (Saba *et al.*, 2014). Although the current study only touches on acute effects, it provides insight to the physiological response of prolonged sitting on CWR, which is crucial for examining potential interventions to eliminate these negative effects under chronic conditions.

Periodic standing

RM did not change over 3 hours of sitting with periodic standing interruptions. Notably, AIx increased with standing and was greater than seated elliptical. This provides further evidence for AIx to be affected by confounding variables like RWTT when compared to RM which did not see a difference over time. As a result, AIx cannot isolate vascular

mechanisms of dysfunction. Importantly, there was no difference in RM between sitting control and standing, suggesting that periodic standing is not an effective interruption method to eliminate sitting-induced increases in RM. While previous work examining wave reflections in active standing proposed a positive effect on wave reflections, it is possible that discrepancies in the RM response to standing is due to methodology. Van den Bogaard et al., (Van Den Bogaard *et al.*, 2011) found postural effects on RM, with the supine posture showing a diminished AIx, Pb, and RM but greater TPR compared to active standing, suggesting that TPR is not the only contributing factor to the RM response. Davis et al., (Davis *et al.*, 2011) also found reduced RM with increased TPR during standing, but it should be noted that their Pb values were much greater than average for young healthy individuals. Both studies used transfer functions to mathematically estimate central pressure from peripheral arteries which may have inaccurately estimated the central response to standing, as discussed above. The discrepancy in the RM response to standing may also be due to the timing of post-measurements. In the mentioned studies, wave reflections were measured either immediately after (Van Den Bogaard *et al.*, 2011) or 5 minutes after standing (Davis *et al.*, 2011) whereas the current study measured wave reflections 30 minutes after the last standing bout. It is possible that RM does initially decrease in response to standing but the effects of standing are not long lasting or protective against sitting. Further work is required to confirm the immediate RM response to standing and the duration of benefits, if any, from periodic standing interruptions.

Seated elliptical

Seated elliptical interruptions eliminated the change in RM seen with prolonged sitting control. We hypothesized that seated elliptical breaks would benefit RM through increased blood flow and shear stress, resulting in a shift towards a vasodilatory state and reduced wave reflections (Birk *et al.*, 2012). While we did not measure endothelial function, the observed prolonged increases in femoral blood flow and conductance with seated elliptical and lack thereof with sitting control suggest that the lower limb vasculature was likely shifted towards a more vasodilatory state from increased shear stress, resulting in the prevention of sitting-induced increases in RM. Other than local vasodilation, the RM response to seated elliptical breaks may also be related to lower limb pooling and associated cardiovascular changes. Park et al. (Park *et al.*, 2022) measured lower limb pooling via calf circumference during prolonged sitting with and without seated elliptical interruptions, with pooling occurring during sitting but was absent with active breaks likely due to muscle pump action. While we did not measure lower limb pooling, the longer duration of our seated elliptical interruption suggests that we likely had similar effects. As mentioned previously, lower limb pooling results in reduced venous return, $\dot{Q}$, and likely Pf (Shvartz *et al.*, 1983; Restaino *et al.*, 2015; Horiuchi & Stoner, 2021; Park *et al.*, 2022) as well as maintained or increased Pb from reduced shear stress and resulting vasoconstriction (Zoller *et al.*, 1972; Skagen, 1982; Cai *et al.*, 2000). If seated elliptical interruptions were able to reduce pooling, $\dot{Q}$ and Pf would not change and Pb would be protected by the shift to a more vasodilatory state, allowing for maintenance or reductions in RM. Even though there was still a net decrease in $\dot{Q}$ and Pf, this may be due to the time elapsed between end of the intervention period and post measurements (30 minutes). There is limited research on the wave reflection and cardiac

output response to prolonged sitting, with available work finding no change in cardiac output over time (Shvartz *et al.*, 1983; Credeur *et al.*, 2019; Evans *et al.*, 2019). It is possible that $\dot{Q}$ and Pf were maintained relative to baseline immediately after seated elliptical breaks but the sitting period before post measures reduced this effect. Assessment of $\dot{Q}$ and Pf immediately after intervention termination in addition to measurements after 30 minutes of sitting would aid investigation of this response but with measurements occurring supine, this was not done in the current study.

Regardless of the mechanisms, intermittent seated elliptical exercise successfully attenuated sitting-induced increases in RM. This suggests potential long term protective effects through successful mitigation of negative hemodynamic exposure from prolonged sitting. As mentioned above, RM has been suggested as a CVD indicator (Chirinos *et al.*, 2005) with greater RM resulting in increased CVD risk. Sedentary behaviour is known to increased CVD risk, but the role wave reflections play in risk determination is unclear. This study provides evidence of increased wave reflection with prolonged sitting while seated elliptical breaks are successful at eliminating these negative effects. Although the long-term consequences are not examined in this study, we can infer that sedentary behaviours would result in the chronic accumulation of greater wave reflection exposure periods relative to non-sedentary individuals and that seated elliptical exercise may eliminate this increased risk. However, studies examining the chronic effects of sedentary behaviour on wave reflection is warranted to support this claim. Given the lack of research in this area, the optimal type, dose, and frequency of sitting interruptions has yet to be determined. Our study used a 50:50 ratio of intervention to sitting and while improvements were seen in wave reflections. The chosen ratio is larger and may not be feasible in

practice; it is unclear if similar effects could have been achieved with reduced frequency or duration. Previous work found longer duration, less frequent interruptions is beneficial for superficial femoral artery blood flow (Carter *et al.*, 2019) although it is unclear if this varies based on type and intensity of interruption. Future work examining sitting interruption methods is required to determine the optimal dose, frequency, and type for CVD risk improvement and individual retention.

6.2 Femoral blood flow

Femoral blood flow and conductance increased during the seated elliptical condition and was significantly greater than sitting control and periodic standing, which did not show any changes over time. Previous work has found decreased blood flow with prolonged sitting (Climie *et al.*, 2018; Kruse *et al.*, 2018; Carter *et al.*, 2019) and increased blood flow following lower limb aerobic exercise (Jorfeldt & Wahren, 1971; Parker *et al.*, 2008). The presence of increased flow with seated elliptical suggests that there may have been a reduction in lower limb pooling and an increase in shear stress, shifting the vasculature to a vasodilatory state and allowing for RM to be maintained. While blood flow and conductance were reduced compared to seated elliptical, it is unclear why our sitting control condition did not align with previous work showing reductions in femoral blood flow. One possibility is related to the post measurement conditions and associated hemodynamic shifts. It is thought that 20 minutes of supine rest may mask the effects of sitting as blood pooling dissipates, which would alter sitting induced femoral blood flow changes (Paterson *et al.*, 2023). To overcome this, a 10-minute resting period is recommended to allow for enough rest to achieve accurate and reliable measures without eliminating the effects of the intervention. While the current study did adopt a 10-minute

rest, blood flow measures were done last to prioritize wave reflection assessments. It is possible that blood flow measures did not begin until closer to the 20-minute mark, potentially reducing the observed effects of the condition.

6.3 Pulse wave velocity

Lower limb PWV decreased with seated elliptical but saw no change with sitting control or standing. In contrast, cfPWV increased with sitting control and standing but did not change with seated elliptical. Increased cfPWV with prolonged sitting is a common finding (Germano-Soares *et al.*, 2018; Credeur *et al.*, 2019; Evans *et al.*, 2019; Alansare *et al.*, 2020, 2022), indicating that sedentary behaviours can increase central PWV and therefore, arterial stiffness. However, the effectiveness of sitting interruptions seems to be dependent on the type of intervention used. Standing breaks were unable to prevent sitting-induced increases in cfPWV, whereas seated elliptical breaks effectively maintained pre-sitting central stiffness and reduced lower limb arterial stiffness. While the effects of standing interruptions on CWR were unclear, previous work has shown standing to be ineffective at reducing sitting-induced increases in cfPWV. Barone Gibbs et al., (Barone Gibbs *et al.*, 2017) found no difference in cfPWV between prolonged sitting and period standing groups in overweight adults. Wright et al., (Wright *et al.*, 2022) had similar conclusions, with prolonged sitting and sitting with standing breaks both increasing cfPWV with no difference between conditions. In contrast, seated elliptical interruptions did not change cfPWV relative to baseline and reduced faPWV. This aligns with previous work showing reductions in leg PWV with acute low intensity single leg cycling (Sugawara *et al.*, 2003). With cfPWV indicative of arterial stiffness, the preservation of cfPWV through seated elliptical interruptions indicates a potential protective effect against adverse

vascular changes with sedentary behaviours, although longitudinal studies are required to confirm this claim. It is worthwhile investigating the optimal dose of sitting interruptions if they hold potential cardiovascular protective effects. Other studies investigating low intensity interruption methods such as calf raises were unable to attenuate increases in cfPWV with prolonged sitting (Evans *et al.*, 2019), suggesting a greater intensity, duration, or frequency of activity is required to elicit this effect. Research examining varying types, intensities, and dosages of sitting interruption methods is crucial for guideline development with individual adherence and cardiovascular disease risk in mind.

6.4 Blood pressure

The change in DBP was greater during sitting control compared to seated elliptical, suggesting that seated elliptical interruptions eliminated the sitting-induced increases in pressure. Our results suggest that prolonged sitting may have a greater impact on DBP compared to SBP, and thus DBP benefits more from interruptions to sitting. A recent meta-analysis concluded similar associations between blood pressure and sedentary behaviour, with each hour of self-reported sedentary time associated with greater increases in DBP compared to SBP (Lee & Wong, 2015). Similarly, Gopinath et al., (Gopinath *et al.*, 2012) found certain sedentary behaviours were associated with increased DBP in early adolescence. It is thought the impact of sedentary behaviours on DBP is related to vascular resistance because SBP is affected by resistance to a lesser extent (Rowell, 1993). Changes to DBP may also be related to other cardiovascular variables such as AIx and RWTT, which are correlated with DBP (Nürnberger *et al.*, 2003). In the current study, DBP was associated with AIx, RWTT, and TPR, providing further evidence for these relationships.

7.0 LIMITATIONS

Acquisition of measures in the seated posture would be ideal given a practical exposure stimuli, however, methodological standards limit the validity of seated assessments and require post measures to occur in the supine position (Thijssen *et al.*, 2019; Stoner *et al.*, 2021; Paterson *et al.*, 2023). To avoid hemodynamic effects due to moving from sitting to supine for measurements, some studies utilized a mechanical lift or manually carried subjects between positions (Restaino *et al.*, 2015; Morishima *et al.*, 2016; Credeur *et al.*, 2019). These strategies were not possible in the current study. Therefore, it is plausible that the effects of standing and walking a few steps to the measurement bed may have reduced the effects of prolonged sitting or the interventions. To reduce the interference of standing and walking on post measures, a 10-minute supine resting period was implemented post-transition. Ten minutes is recommended as the ideal time to ensure the participant is rested without being too long that the effects of the intervention would subside (Paterson *et al.*, 2023). To prioritize our main outcome, wave reflection assessments were done first, meaning blood flow measurements may have occurred closer to the 20-minute mark. Additionally, because the purpose of our study was to examine the impact of these interventions as sitting interruption methods, we increased the time between intervention and post measures to avoid direct assessment of the intervention on RM. As a result, the response magnitude may have been reduced due to the sitting period prior to post measures. We chose to have a 20-minute sitting period followed by 10 minutes of supine rest before post measures began because of available evidence showing cardiovascular changes to sitting occurring at 1 hour (Thosar *et al.*, 2014, 2015; Ballard *et al.*, 2017) and to eliminate the acute effects of exercise and

standing. However, the inclusion of a sitting period between interventions and post measures may have resulted in reduced effect sizes and provides possible explanation for the lack of differences with certain variables across condition. Also, it is possible that sex differences exist in the current study but due to the lack of existing data in this area, we did not power for a sex-differences analysis and are unable to evaluate this question with appropriate error control. To address this, data will be made open access for future groups to use raw data in power analyses. Lastly, while acute studies offer insight in understanding the physiological mechanisms behind certain phenomena, they are limited in their ability to inform recommendations regarding the long-term effects and their effect on cardiovascular disease risk. Future research should explore the consequences of chronic sedentary behaviour on central wave reflection and the effectiveness of interruptions in mitigating these effects.

8.0 CONCLUSION

Seated elliptical breaks effectively attenuate sitting-induced increases in central wave reflections while periodic standing breaks are unable to alleviate the cardiovascular consequences of prolonged sitting. Understanding the acute effects of prolonged sitting and interruption methods on central wave reflections is crucial for determining the consequences of sedentary behaviours and the ability of specific interventions to mitigate these effects and associated disease risk. Future work should examine the long-term effectiveness of seated elliptical interruptions in reducing CVD risk while continuing to investigate the optimal type, duration, and frequency of sitting interruptions for overall cardiovascular health.

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