IMPACT OF PRIOR AEROBIC EXERCISE ON ARTERIAL STIFFNESS DURING PROLONGED SITTING IN HEALTHY, ACTIVE MALES AND FEMALES

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A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Exercise and Sport Science (Exercise Physiology) in the College of Arts & Sciences.

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ABSTRACT

Sasha L. Riley: Impact of Prior Aerobic Exercise on Arterial Stiffness during Prolonged Sitting in Healthy, Active Males and Females (Under the direction of Erik D. Hanson)

Prolonged sitting is associated with arterial stiffening, however acute aerobic exercise can transiently decrease arterial stiffness. To examine the impact of aerobic exercise on brachial-femoral (bfPWV) and femoral-ankle pulse wave velocity (faPWV) during prolonged sitting, measurements were taken at baseline, after exercise but before sitting (pre), and post-sitting (post). Thirteen participants (22 [3] y, 40% F) performed 30-minutes of walking at moderate-intensity (EX) or 30-minutes of standing (STAND) followed by 2.5-hours of uninterrupted sitting. No effects were present for bfPWV (p = 0.667). A condition x time interaction was present for faPWV (p < 0.001). From baseline to pre, EX decreased by 6.5% while STAND increased by 6.1%, leading to a difference between conditions. However, during sitting, faPWV rebounded in EX by 14.1% that resulted in no difference between conditions at post. Therefore, prior aerobic exercise does not appear to attenuate increases in arterial stiffness caused by prolonged sitting.
ACKNOWLEDGEMENTS

I would like to dedicate this thesis to my best friend, Elizabeth Ottusch. I am indebted to you for all the memories, advice, and random daily thoughts you both listen to and provide me with. You inspire me to take more chances, to love with my whole heart, and to by myself no matter what anyone thinks. You are my sister in every sense of the word, and even in the face of cancer you are crushing life (and your ACL). Love, love, love. Lizz, Lizz, Lizz.

I would also like to thank my advisor, Dr. Erik Hanson, my committee Dr. Ondrak and Dr. Stoner, my cohort, my lab group, and my family and friends. Dr. Hanson you have endlessly supported me throughout these past two years while teaching me and challenging me to be a better researcher, writer, and person. To my committee, you’ve given me feedback, and a lab to collect data in, at every step of the way and helped me learn about an area I came in with no knowledge of. To my cohort and my lab group, I owe you all for the countless hours of prolonged sitting you’ve done for me and for all the memories you’ve provided. I couldn’t have asked for a better team! Lastly, to my family and friends you’re the ones who have gotten me to this point and have supported me through many life crises. I couldn’t have done it without each and every one of you.
<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES .............................. viii</td>
</tr>
<tr>
<td>LIST OF FIGURES ............................ ix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS ................... x</td>
</tr>
<tr>
<td>CHAPTER I: INTRODUCTION .................. 1</td>
</tr>
<tr>
<td>Research Questions ........................ 3</td>
</tr>
<tr>
<td>Research Hypothesis ....................... 4</td>
</tr>
<tr>
<td>Assumptions ................................ 4</td>
</tr>
<tr>
<td>Delimitations ............................... 4</td>
</tr>
<tr>
<td>Limitations .................................. 4</td>
</tr>
<tr>
<td>Significance of Study ..................... 4</td>
</tr>
<tr>
<td>CHAPTER II: LITERATURE REVIEW ........... 6</td>
</tr>
<tr>
<td>Section 1: Sedentary Behavior and Cardiovascular Disease ..................... 6</td>
</tr>
<tr>
<td>Section 2: Acute Sedentary Behavior and Arterial Stiffness ...................... 7</td>
</tr>
<tr>
<td>Section 3: Mechanisms of Acute Sedentary Behavior and Arterial Stiffening ........... 8</td>
</tr>
<tr>
<td>Section 4: Physical Activity and Arterial Stiffness ................................. 12</td>
</tr>
<tr>
<td>4.1 Sitting Interruption Strategies ................................................ 12</td>
</tr>
<tr>
<td>4.2 Acute Aerobic Exercise ...................... 13</td>
</tr>
<tr>
<td>Section</td>
</tr>
<tr>
<td>----------------------------------------------</td>
</tr>
<tr>
<td>Pulse Wave Velocity</td>
</tr>
<tr>
<td>Power Calculation</td>
</tr>
<tr>
<td>Statistical Analyses</td>
</tr>
<tr>
<td>CHAPTER IV: RESULTS</td>
</tr>
<tr>
<td>Participants</td>
</tr>
<tr>
<td>Central Arterial Stiffness</td>
</tr>
<tr>
<td>Peripheral Arterial Stiffness</td>
</tr>
<tr>
<td>Accelerometry</td>
</tr>
<tr>
<td>CHAPTER V: DISCUSSION</td>
</tr>
<tr>
<td>Strengths and Limitations</td>
</tr>
<tr>
<td>Comparison to Literature</td>
</tr>
<tr>
<td>Implications</td>
</tr>
<tr>
<td>Conclusion</td>
</tr>
<tr>
<td>REFERENCES</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1: A summary of prolonged sitting studies examining central and peripheral arterial stiffness and endothelial dysfunction in adults......................................................... 8

Table 2: Key methodological considerations................................................................. 17

Table 3: Procedures to control internal validity in participant recruitment and testing........ 22

Table 4: Eligibility and exclusion criteria........................................................................ 23

Table 5. Participant Characteristics (n = 13).................................................................. 33

Table 6. Baseline differences between hemodynamic measures in young healthy men and women. ................................................................. 34
LIST OF FIGURES

Figure 1: Proposed conceptual model theorizing how prolonged sitting leads to venous pooling, hemodynamic changes, and ultimately increases in arterial stiffness. .................................................................................................................................................. 9

Figure 2: Acute effect of exercise on pulse wave velocity by time period after exercise. .................. 14

Figure 3: Exercise intensities and arterial stiffness. Relationship between exercise intensities and the change of pulse wave velocity. .......................................................................................................................... 16

Figure 4: Response of central (bf-PWV) and peripheral (fa-PWV) arterial stiffness to 30-minutes of moderate-intensity treadmill walking across a 90-minute bout of lying supine. .......................................................................................................................... 21

Figure 5: Study flow using a randomized crossover design. ................................................................ 27

Figure 6: Representative timeline of one visit. .......................................................................................... 30

Figure 7. Acute changes in bfPWV (m/s) at baseline and with prolonged sitting with and without prior exercise. .................................................................................................................................. 34

Figure 8. Acute changes in faPWV (m/s) at baseline and with prolonged sitting with and without prior exercise. .................................................................................................................................. 35

Figure 9. A) Step count comparisons between condition over prolonged sitting bout and B) between condition. .................................................................................................................................. 36
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
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<tr>
<td>APL</td>
<td>Applied Physiology Laboratory</td>
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<tr>
<td>bfPWV</td>
<td>Brachial-femoral pulse wave velocity</td>
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<td>cfPWV</td>
<td>Carotid-femoral pulse wave velocity</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>D</td>
<td>Arterial path length</td>
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<td>EORL</td>
<td>Exercise Oncology Research Laboratory</td>
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<tr>
<td>faPWV</td>
<td>Femoral-ankle pulse wave velocity</td>
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<tr>
<td>FMD</td>
<td>Flow-mediated dilation</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
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<td>HR$_{\text{max}}$</td>
<td>Maximum heart rate</td>
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<td>HRR</td>
<td>Heart rate reserve</td>
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<tr>
<td>IPAQ-S</td>
<td>International physical activity questionnaire – short form</td>
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<td>MAP</td>
<td>Mean arterial pressure</td>
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<tr>
<td>METs</td>
<td>Metabolic equivalents</td>
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<td>mph</td>
<td>Miles per hour</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>PAR-Q</td>
<td>Physical activity readiness questionnaire</td>
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<tr>
<td>PTT</td>
<td>Pulse transit time</td>
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<tr>
<td>PWV</td>
<td>Pulse wave velocity</td>
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<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
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<tr>
<td>SB</td>
<td>Sedentary behavior</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
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</tr>
<tr>
<td>SV</td>
<td>Stroke volume</td>
</tr>
<tr>
<td>VO_{2\text{max}}</td>
<td>Maximal volume of oxygen consumption</td>
</tr>
<tr>
<td>VR</td>
<td>Venous return</td>
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CHAPTER I: INTRODUCTION

It is well established that sedentary behavior increases the risk for cardiovascular disease (CVD), which is the leading cause of death globally. Although there are numerous adverse effects of sedentary behavior, increasing arterial stiffness may be one key aspect contributing to increased CVD (Lattanzi et al., 2019; van Sloten, 2017). Sedentary behavior (e.g., prolonged sitting) may increase pulse wave velocity (PWV) (Credeur et al., 2019; Evans et al., 2019), a non-invasive, widely used marker of arterial stiffness. Interruption strategies such as walking breaks or standing desks used to prevent increases in PWV with prolonged inactivity yield conflicting findings (Barone Gibbs et al., 2017; Evans et al., 2019; Kowalsky et al., 2019). Moreover, disrupting sedentary behavior may not always be a feasible option, suggesting that alternative approaches may be required.

Technological developments in the workplace and at home have led to environments that encourage long bouts of uninterrupted sitting, with adults in the United States spending ~7.7 hours per day in sedentary behavior (Matthews et al., 2008). Laboratory investigations indicate that 3 hours of sitting without interruption induce a 0.3-0.4 m/s increase in PWV (Alansare et al., 2020; Credeur et al., 2019; Evans et al., 2019; Kelsch et al., 2021). Interruption strategies to prevent increases in PWV with prolonged sitting have shown mixed success. Alternating between standing and sitting at 30-minute intervals decreased peripheral PWV compared to staying seated (Barone Gibbs et al., 2017). In contrast, performing calf-raises every 10 minutes (Evans et al., 2019) or performing simple resistance training exercises once per hour
(Kowalsky et al., 2019) did not attenuate increases in PWV due to prolonged sitting. These discrepancies could be due to the duration differences of the interruption strategies, with 30 minutes of standing being a longer, potentially more sustained effort than a brief bout of calf-raises or simple resistance training exercises. These findings indicate that interruption strategies of longer durations may be necessary to offset the negative effects of sedentary behavior. However, there are numerous settings where a 30-minute standing break is not practical or feasible. Therefore, additional interruption strategies to attenuate changes in PWV with prolonged sitting need to be explored.

For situations where interruptions are not feasible, one strategy is an acute bout of aerobic exercise. Acute aerobic exercise may transiently reduce PWV in young, healthy adults (Heffernan et al., 2007; Kingwell et al., 1997; Kobayashi et al., 2017; Milatz et al., 2015; Naka et al., 2003). Arterial stiffness is believed to be improved by acute aerobic exercise due to an increase in shear stress, which then improves endothelium-dependent vasodilation via increased nitric oxide production (Goto et al., 2003; Hasegawa et al., 2018; Padilla & Fadel, 2017). While two meta-analyses indicated that acute aerobic exercise has a null overall effect on central arterial stiffness (Pierce et al., 2018; Sardeli et al., 2018), they failed to consider three important factors. These factors include the timing of post-intervention PWV measurement, arterial segment being measured, and the exercise mode, duration, and intensity. Large differences in exercise duration and intensity, along with small sample sizes may be factors contributing to the null findings. Arterial segment and measurement time also impact arterial stiffness (Mutter et al., 2017; Saz-Lara et al., 2021), with PWV increasing in upper body and decreasing in lower limb arterial segments measured 0-5 minutes post-exercise. Measurements obtained 5 to 60 minutes post-exercise demonstrated a decrease in PWV back to baseline or even below resting values for
all arterial segments. To account for different arterial segments, both a central (brachial-femoral PWV) and peripheral (femoral-ankle PWV) PWV measurement will be taken.

In light of the current evidence on sitting and vascular health, there remains a need to further explore strategies to mitigate the deleterious effects of prolonged sitting and how these strategies may impact different arterial segments. To address situations where sitting interruption may not be feasible an acute bout of aerobic exercise prior to prolonged sitting will be used. Arterial stiffness is altered in an exercise-intensity dependent manner, with increased intensities showing greater acute reductions in PWV (Kobayashi et al., 2018; Niebauer et al., 2020; Sapp et al., 2020). Therefore, the bout will be 30-minutes of moderate intensity (55-65% heart rate reserve) brisk treadmill walking/jogging at a grade. The treadmill was chosen for the exercise mode as it utilizes large amounts of muscle mass throughout the body, potentially allowing for increased shear stress in all arterial segments. Increased shear stress is hypothesized to lead to decreases in arterial stiffness (Goto et al., 2003; Hasegawa et al., 2018; Padilla & Fadel, 2017). If acute aerobic exercise is an efficacious strategy to mitigate increases in arterial stiffness, it could provide an alternative option to sitting interruption strategies. Therefore, the purpose of this project is to determine the impact of acute aerobic exercise, performed prior to prolonged sitting, on central and peripheral PWV in healthy, active adults, aged 18-to-35 years.

**Research Questions**

1. Compared to standing, does 30-minutes of moderate intensity aerobic exercise prior to prolonged sitting attenuate increases in brachial-femoral PWV?

2. Compared to standing, does 30-minutes of moderate intensity aerobic exercise prior to prolonged sitting attenuate increases in femoral-ankle PWV?
Research Hypothesis

1. 30-minutes of moderate intensity aerobic exercise prior to prolonged sitting will attenuate increases in brachial-femoral PWV.

2. 30-minutes of moderate intensity aerobic exercise prior to prolonged sitting will attenuate increases in femoral-ankle PWV.

Assumptions

1. Participants will follow pre-assessment guidelines.

2. Pre-assessment guidelines will adequately control for baseline changes in outcomes.

3. All participants will answer the medical history questionnaire, physical activity readiness questionnaire, and physical activity questionnaire truthfully.

Delimitations

1. Women’s menstrual cycles will be recorded and controlled.

2. Repeated measures design will be used to control for condition specific variability.

3. All subjects will have similar dietary intake prior to and during testing.

4. All subjects will be between the ages of 18-35.

Limitations

1. Physical activity will be measured via self-report questionnaire, not objectively.

2. Maximal heart rate will be predicted, not measured.

Significance of Study

Arterial stiffness has been implicated in the pathogenesis of CVD, with a 1.0 m/s increase in central PWV corresponding to a 14% and 15% increase in total cardiovascular events and cardiovascular mortality respectively (van Sloten, 2017; Vlachopoulos et al., 2010). A bout of prolonged sitting has been shown to increase central and peripheral PWV by 0.3-0.5 m/s.
(Alansare et al., 2020; Credeur et al., 2019; Evans et al., 2019; Kelsch et al., 2021) but standard interruption strategies have only been successful in attenuating increases in peripheral measures of PWV (Barone Gibbs et al., 2017). Acute bouts of aerobic exercise have been shown to decrease central and peripheral PWV measures by 0.2-0.5 m/s (Heffernan et al., 2007; Kingwell et al., 1997; Milatz et al., 2015; Sugawara et al., 2005). Therefore, acute aerobic exercise prior to prolonged sitting may provide a strategy to mitigate increases in central and peripheral PWV. This could result in clinically meaningful decreases in arterial stiffness, improving cardiovascular health and decreasing risk of cardiovascular events and mortality.
CHAPTER II: LITERATURE REVIEW

This review is divided into the following sections: 1) Sedentary Behavior and Cardiovascular Disease 2) Acute Sedentary Behavior and Arterial Stiffness 3) Mechanisms of Acute Sedentary Behavior and Arterial Stiffening 4) Physical Activity and Arterial Stiffness 5) Gap in Knowledge 6) Methodological Considerations and 7) Statistical Considerations

Section 1: Sedentary Behavior and Cardiovascular Disease

It has been well established that more time spent in sedentary behavior (SB) increases the risk for developing cardiovascular disease (Biswas et al., 2015; Wilmot et al., 2012). SB is defined as any waking behavior characterized by an energy expenditure of 1.5 metabolic equivalents (METs) or less while in a sitting, reclining, or lying posture (Tremblay et al., 2017). It is important to note that SB is distinct from physical inactivity, which is defined as an individual failing to meet the recommended 150 minutes of moderate to vigorous physical activity per week (World Health Organization, 2010). This distinction is important as SB is a risk factor for CVD, independent of physical activity status. In fact, CVD risk significantly increases when adults sit for more than 6 hours per day, even after adjustments for physical activity (R. Patterson et al., 2018). As the average American currently spends ~7.7 hours per day in sedentary behaviors, these individuals are already at an elevated risk for CVD (Matthews et al., 2008). Yet, time spent in SB only seems to be increasing over time due to increased reliance on automobiles, increased work and leisure computer usage, and increased TV-viewing time
(Brownson et al., 2005; Yang et al., 2019). As CVD is already the number one cause of death worldwide, further increases in SB are highly concerning for vascular health across the world.

Section 2: Acute Sedentary Behavior and Arterial Stiffness

The relationship between SB and CVD is thought to be mediated by an array of factors including traditional CVD risk factors (e.g. blood pressure, BMI, high density lipoproteins, waist circumference) and measures of artery health (Carter et al., 2017). Specifically, one measure of artery health that may mediate the relationship between SB and CVD is arterial stiffness. Arterial stiffness is defined as a reduction in arterial compliance and distensibility as a result of changes in structure (i.e., vessel wall extracellular matrix) and function (i.e., central autonomic and local endothelial function) (Kucharska-Newton et al., 2019; Lattanzi et al., 2019; Stewart et al., 2003; Stoner et al., 2020). Of these, only vessel function changes acutely with prolonged sitting, as remodeling the physical structures takes additional time. Arterial stiffness is most commonly measured via pulse wave velocity, with carotid-femoral PWV being the gold standard measurement for central arterial stiffness (Van Bortel et al., 2012). Increases in arterial stiffness have been associated with acute and chronic SB. Given the nature of the study, this review will focus on acute SB.

One subset of acute SB is prolonged sitting, for which there is growing evidence to indicate it results in transient increases in arterial stiffness (Alansare et al., 2020; Credeur et al., 2019; Evans et al., 2019; Kelsch et al., 2021). Central and peripheral PWV have both been directly shown to increase during 3-hour prolonged sitting bouts in young, healthy adults as well as overweight and obese adults with hypertension (Table 1). Central PWV, commonly measured via carotid-femoral or brachial-femoral PWV, has been shown to increase by 0.3-0.5 m/s (Alansare et al., 2020; Credeur et al., 2019; Evans et al., 2019; Kelsch et al., 2021). Peripheral
PWV, often measured via carotid-ankle or femoral-ankle PWV, increases by increments of 0.38 – 0.55 m/s (Alansare et al., 2020; Kelsch et al., 2021). It is important to note that these increases do not reach the clinically significant value of 1.0 m/s increase in PWV, which corresponds to an age-, risk factor-, and sex-adjusted increase in CV event and CV mortality risk by 14% and 15%, respectively (Vlachopoulos et al., 2010). However, it must be recognized that these acute changes are being seen in relatively short windows and even acute sitting has the potential to contribute to development of CVD via arterial stiffness.

Table 1: A summary of prolonged sitting studies examining central and peripheral arterial stiffness and endothelial dysfunction in adults.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Details</th>
<th>Relevant Measures</th>
<th>Findings of Prolonged Sitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Credeur et al., 2019</td>
<td>3 hours uninterrupted sitting: measured aortic PWV and tibial artery FMD in healthy men and women (18 to 55)</td>
<td>aPWV increased by 0.4 m/s, while FMD decreased by 5%</td>
<td>1. Central PWV increased 2. Peripheral endothelial function decreased</td>
</tr>
<tr>
<td>Evans et al., 2019</td>
<td>3 hours uninterrupted sitting: measured aortic PWV in young healthy men and women</td>
<td>aPWV increased by 0.3 m/s</td>
<td>1. Central PWV increased</td>
</tr>
<tr>
<td>Alansare et al., 2020</td>
<td>7.5 hours prolonged sitting with supine break: measured cfPWV and caPWV in overweight/obesity adults with elevated BP</td>
<td>cfPWV increased by 0.52 m/s and caPWV 0.38 m/s</td>
<td>1. Central PWV increased 2. Peripheral PWV increased</td>
</tr>
<tr>
<td>Kelsch et al., 2021</td>
<td>3 hours uninterrupted sitting: measured G-PWV (bfPWV, cfPWV, and faPWV) in young healthy men and women</td>
<td>bfPWV increased by 0.36 m/s, faPWV increased by 0.55 m/s, and cfPWV decreased by 0.03 m/s (non-significant finding)</td>
<td>1. Central PWV increased (bfPWV) 2. Peripheral PWV increased (faPWV)</td>
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Section 3: Mechanisms of Acute Sedentary Behavior and Arterial Stiffening

Given that the structure of an artery is unlikely to be altered with a single bout of acute sedentary behavior, any functional changes to the artery via acute SB are likely to be driven by
alterations within the endothelium. Figure 1 illustrates the conceptual model by which acute SB is thought to cause arterial stiffening.

Figure 1: Proposed conceptual model theorizing how prolonged sitting leads to venous pooling, hemodynamic changes, and ultimately increases in arterial stiffness.

The first step in the alterations leading to arterial stiffening, is lower limb venous pooling caused by prolonged sitting (Shvartz et al., 1983; Thosar et al., 2012; Vranish et al., 2017).
Venous pooling results in decreased venous return (VR) leading to decreased stroke volume (SV), which is essential in the maintenance of cardiac output (Magder, 2016; S. W. Patterson & Starling, 1914). In healthy adults, venous pooling led to an immediate drop in SV of 40% and an overall drop in cardiac output of 20% (Tansey et al., 2019). During prolonged sitting there is an increase in blood pooling, seen via non-invasive measures of calf circumference. Across a 3 hour bout of uninterrupted sitting a 1-2 cm increase in calf circumference has been observed (Credeur et al., 2019; Vranish et al., 2017). This increase could possibly be due to lack of muscle pump activation in the lower limb musculature (Restaino et al., 2015). The muscle pump is activated during skeletal muscle contraction and works to squeeze blood from the lower body back to the right side of the heart, helping to maintain venous return (Brooks et al., 1984). In the case of the lower limbs, the calf muscle pump plays a partial role in ensuring an adequate VR to the heart (Pollack & Wood, 1949). Without sufficient VR cardiac output will decrease, as over the course of a few heart beats cardiac output equals VR (Brooks et al., 1984). Furthermore, venous pooling is exaggerated in response to an increase in hydrostatic pressure in the lower limbs. Hydrostatic pressure is the pressure exerted by a fluid at rest due to the force of gravity. The hydrostatic pressure experienced by intravascular fluid can be dramatically increased depending on bodily position, increasing specifically in the seated and standing positions (Hinghofer-Szalkay, 2011; Malhotra et al., 2002).

The decrease in venous return due to venous pooling impacts the function of the endothelium through multiple processes. In the aorta, a decrease in cardiac output causes a resultant decline in aortic shear stress. In the peripheral arteries, the bending at the hip and knee during sitting can reduce lower limb blood flow by up to 45%, ultimately reducing shear forces (Morishima et al., 2017; Padilla & Fadel, 2017; Walsh et al., 2017). Shear stress is the frictional
force from blood flow as it moves across the endothelium (Davies, 2009) and is essential in maintaining proper endothelial function. The effects of shear stress on endothelial function were reviewed by Ando and Yamamoto (2011). In that review it was stated that endothelial cells, which line the lumen of the blood vessel, recognize shear stress as a mechanical stimuli. When stimulated by shear stress, the endothelial cells then release nitric oxide, a potent vasodilator, via activation of endothelial NO synthase. In contrast, when shear stress decreases vasoconstriction is upregulated due to increased production of endothelin-1 and angiotensin-converting enzyme (Ando & Yamamoto, 2011). Furthermore, the decrease in cardiac output will subsequently cause a decrease in mean arterial pressure (MAP). This will unload the carotid and aortic baroreceptors resulting in increased activation of the sympathetic nervous system, ultimately leading increased systemic vascular resistance and further decreasing vascular compliance (Cooke et al., 1999).

Laboratory results have shown that prolonged sitting causes endothelial dysfunction via reduced shear stress, as described above. A 3-6 h bout of prolonged sitting results in decreases in shear rate in the popliteal and femoral arteries, with a concomitant decrease in endothelial function measured via flow-mediated dilation (FMD) (Kruse et al., 2018; Morishima et al., 2016, 2017, 2020; O’Brien et al., 2019; Paterson et al., 2020; Restaino et al., 2015, 2016; Thosar, Bielko, Mather, et al., 2015; Thosar, Bielko, Wiggins, et al., 2015; Thosar et al., 2014; Vranish et al., 2017). These negative hemodynamic changes begin to occur with just 1-hour of uninterrupted sitting and continue to decline as sitting prolongs (Paterson et al., 2020). The decline in endothelial function has been implicated in the development of atherosclerotic lesions (Cheng et al., 2004, 2006). Furthermore, blood pooling induced by a 3-hour prolonged sitting bout was associated with a 14.8% decrease in stroke volume, a major determinant of cardiac output (Horiuchi & Stoner, 2021). Ultimately, these changes lead to endothelial dysfunction,
causing an inability of the blood vessels to dilate properly in response to volume changes, indicating arterial stiffening.

Section 4: Physical Activity and Arterial Stiffness

4.1 Sitting Interruption Strategies

The current work that has been done on acutely reducing arterial stiffness has focused on two strategies, the first of which is sitting interruption. To date, only three studies focusing on interruption strategies have measured pulse-wave velocity as an outcome (Barone Gibbs et al., 2017; Evans et al., 2019; Kowalsky et al., 2019). Two of which were unable to attenuate any increases in pulse-wave velocity. These interventions utilized 2 sets of 10-15 reps of one simple resistance training exercise (e.g. chair stands with calf raises, desk-pushups, alternating lunges with knee raises, and standing bicep curls with upright row) once per hour in middle-aged individuals at risk of cardiometabolic disease (Kowalsky et al., 2019) and calf-raises every 10-minutes in young, healthy individuals (Evans et al., 2019). The other study successfully reduced peripheral PWV by alternating between standing and sitting at 30-minute intervals in overweight-to-obese individuals with hypertension (Barone Gibbs et al., 2017). These interruption strategies may have had limited success due to the lack of duration and intensity of the interventions decreasing their ability to restore proper hemodynamics to offset the effects of prolonged sitting. Kowalsky (2019) and Evans (2019) both implemented strategies that utilized simple exercises that would last between 30 seconds to 2-minutes per round. Due to the lack of duration, it could be expected that a higher intensity would be needed to elicit a sufficient heart rate or stroke volume response to increase shear stress. Especially, considering that arterial stiffness is changed in an intensity-dependent manner, with exercise performed at intensities higher than 50% of VO2max significantly reducing PWV (Kobayashi et al., 2017; Niebauer et
al., 2020; Peres et al., 2018; Sapp et al., 2020; Tordi et al., 2010). While Barone Gibbs (2017) utilized an intervention that was of sufficient duration (30-minute change of posture) to see a 0.27 m/s decrease in peripheral PWV, it may have lacked a sufficient intensity to see a concurrent decrease in central PWV.

4.2 Acute Aerobic Exercise

The second strategy aiming to acutely reduce arterial stiffness are acute bouts of aerobic exercise. The effects of acute aerobic exercise on PWV have been assessed in numerous studies and have been summarized by one systematic review and three meta-analyses (Mutter et al., 2017; Pierce et al., 2018; Sardeli et al., 2018; Saz-Lara et al., 2021). Despite a substantial quantity of literature (n > 50 studies), the results are still conflicting due to 1) discrepancies in timing of post-intervention measurement, 2) the arterial segment being measured, and 3) variability in the mode, intensity, and duration of the exercise intervention.

Timing of Post-Intervention Measurement

Although the meta-analyses by Sardeli (Sardeli et al., 2018) and Pierce (Pierce et al., 2018) indicated that acute aerobic exercise failed to impact central PWV on the whole, the timing of the post-intervention arterial stiffness measurements were not taken into account. When considering timing of PWV measurement, central PWV is shown to initially increase 0-5 minutes post-intervention and then decrease to resting levels or below >5 minutes post-intervention (Mutter et al., 2017). This finding was backed-up in another meta-analysis that showed central PWV initially increased and then dropped below resting values by 30-59 minutes post-intervention (Saz-Lara et al., 2021). This is particularly important as certain studies that have only measured PWV immediately upon completion of the exercise bout may have missed the true hemodynamic changes that are occurring due to exercise.
Arterial Segment of Interest

Furthermore, the arterial segment that is being measured must be considered as peripheral and central arterial segments have been shown to react differently to acute aerobic exercise (Figure 2). Central PWV reacts by initially increasing before dropping further post-intervention (effect size: -0.15). Peripheral PWV has been shown to initially decrease post-intervention and then continues to decrease but by smaller amounts before eventually returning to baseline around the 24 hour mark (effect size: -0.50). Therefore, the timing of measurement must be considered along with the arterial segment of interest to accurately identify how acute aerobic exercise may be changing vascular function.

Figure 2: Acute effect of exercise on pulse wave velocity by time period after exercise. PWv: pulse wave velocity; SD standard deviation; * Values p<0.05 were considered significant. From “The Acute Effect of Exercise on Arterial Stiffness in Healthy Subjects: A Meta-Analysis,” by Saz-Lara et al., 2021, J Clin Med, 10(2), p 291.

Variability in Exercise Intervention

Lastly, the large variability in exercise protocols has made it difficult to know the ideal duration, intensity, and mode of exercise that may decrease central and peripheral PWV.
However, it does appear that PWV is altered in an exercise-intensity dependent manner (Figure 3), with higher intensities showing greater acute reductions in central and peripheral PWV (Kobayashi et al., 2018; Niebauer et al., 2020; Peres et al., 2018; Sapp et al., 2020; Tordi et al., 2010). Specifically, it was found that exercise performed at intensities higher than 50% of VO\textsubscript{2max} significantly reducing PWV compared to exercise intensities below 50% of VO\textsubscript{2max} (Niebauer et al., 2020). Furthermore, an intervention comparing high intensity interval exercise (4 min at 60-70% HR\textsubscript{max} followed by 1 min at 90-100% HR\textsubscript{max}, repeated 9 times) and continuous exercise (45 min of exercise at average HR achieved during high intensity interval exercise), found a 8.6% greater decrease in peripheral PWV during the high intensity exercise than continuous (Peres et al., 2018). These findings held true in varying studies utilizing a variety of modes (cycle ergometry, alpine skiing, cross-country skiing) and durations (30 min, 45 min, 60 min), possibly indicating that intensity may be the most important variable when considering the impact of acute aerobic exercise on PWV (Kobayashi et al., 2017; Sapp et al., 2020; Tordi et al., 2010).
Figure 3: Exercise intensities and arterial stiffness. Relationship between exercise intensities and the change of pulse wave velocity. Exercise intensities are defined by the mean relative oxygen uptake during an exercise session (VO2mean, in ml/min/kg) related to the VO2max of the all-out cycling ergometry [exercise intensity (%) = (100 & VO2mean)/VO2max]. PWV: pulse wave velocity, rxy: Pearson’s correlation coefficient, p: p-value. Acute Effects of Winter Sports and Indoor Cycling on Arterial Stiffness,” by Niebauer et al., 2020, J Sports Sci Med, 19(3), p 464.

Gap in Knowledge

Table 1 is a selection of studies that examine the impact of prolonged sitting on peripheral and central PWV in young, healthy adults. Furthermore, figures 2 and 3 also demonstrate the impact of acute aerobic exercise on central and peripheral PWV in young, healthy adults, while taking into consideration the time at which the post-intervention measurement was made, and the type of exercise being done. Through these tables and figures it can be seen that the effects of prolonged sitting and acute aerobic exercise on PWV have been elucidated separately. However, to our knowledge no study has identified if acute aerobic exercise completed prior to a prolonged sitting bout will be a sufficiently stimulus to mitigate increases in peripheral and central PWV.
Methodological Considerations

**Table 2: Key methodological considerations.**

<table>
<thead>
<tr>
<th>Methodological Area</th>
<th>Consideration</th>
<th>Chosen Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial Segment Measurements</td>
<td>Central and peripheral pulse wave velocity respond differently to acute aerobic exercise (Mutter et al., 2017; Saz-Lara et al., 2021).</td>
<td>Central (bfPWV) and peripheral (faPWV) measurements will be taken.</td>
</tr>
<tr>
<td>Timing of Pulse Wave Velocity Measures</td>
<td>The timing of post-exercise pulse wave velocity impacts the outcome. Central pulse wave velocity will increase if measurement is taken &lt;5 min post-exercise but will return to baseline or decrease when taken &gt;5 minutes post-exercise (Mutter et al., 2017).</td>
<td>All pulse wave velocity measurements were taken &gt;5 minutes post-exercise to capture the longer-term effects of prior exercise.</td>
</tr>
<tr>
<td>Exercise Modality</td>
<td>A variety of exercise modalities exist, all of which require different amounts of muscle mass.</td>
<td>Treadmill was chosen for the exercise mode as it utilizes large amounts of muscle mass throughout the body, potentially allowing for increased shear stress in all arterial segments.</td>
</tr>
<tr>
<td>Exercise Duration and Intensity</td>
<td>Different exercise duration and intensities can transiently affect pulse wave velocity in various ways. Specifically, pulse wave velocity appears to be impacted in an intensity-dependent manner with higher intensity exercise causing greater decreases in pulse wave velocity (Kobayashi et al., 2018; Niebauer et al., 2020; Peres et al., 2018; Sapp et al., 2020; Tordi et al., 2010).</td>
<td>A 30-minute moderate intensity exercise bout was chosen for two reasons: it has previously been shown to transiently decrease pulse wave velocity in young, healthy adults (Heffernan et al., 2007; Kingwell et al., 1997) and if performed every weekday it aligns with the ACSM physical activity guidelines.</td>
</tr>
</tbody>
</table>

**Abbreviations:** ACSM: American College of Sports Medicine, bfPWV: brachial-femoral pulse wave velocity, faPWV: femoral-ankle pulse wave velocity.
**Study Design Considerations**

While several potential study designs were considered to answer the research question, ultimately a crossover design was chosen. The crossover design was chosen as it allows for each participant to undergo both conditions, letting each participant serve as their own control. This limits any confounding variables that may exist when comparing two different people. A randomized controlled trial (RCT) was also considered as it is the gold-standard in determining if an intervention has a significant effect on an outcome. However, because RCTs consist of two groups that are each randomized to a single treatment arm (e.g., condition), it requires a larger sample size. Therefore, the crossover design was chosen as a small size is more practical for the given study, despite the increased time it will require for each participant.

**Arterial Stiffness Measurement Considerations**

Arterial stiffness is a condition marked by reduced elasticity and distensibility due to changes in structure and function. The gold standard of measuring arterial stiffness is measuring carotid-femoral pulse wave velocity (cfPWV) (Laurent et al., 2006). However, there are a variety of arterial segments that can be measured. For this study, bfPWV was chosen in place of cfPWV as it does not require a neck cuff and therefore is less invasive but still represents central arterial stiffness (Keehn et al., 2014). FaPWV was also included to provide a peripheral measure to understand how different arterial segments respond to prolonged sitting and exercise. The measurement for pulse wave velocity is the speed it takes for a pulse wave to travel from a proximal arterial segment to the distal point of the arterial segment. Arterial stiffening causes an increase in PWV because distensibility in the arterial wall allows the forward wave to move quicker through the artery. This relationship can be modeled by: \[ \text{distensibility} = \left(\frac{3.67}{\text{PWV}}\right)^2 \] (Biswas et al., 2015).
While many devices exist that can measure PWV, two popular devices include the Vicorder device and the SphygmoCor XCEL. The Vicorder is an oscillometric device which detects the proximal and distal pulse waves via two cuffs. It has been shown to produce valid measures when compared against magnetic resonance imaging (MRI) and the SphygmoCor XCEL (Hickson et al., 2009; Parikh et al., 2016) The measures have also been shown to be repeatable between and within observers (Hickson et al., 2009). The SphygmoCor XCEL is a tonometry device that has been shown to be highly reliable and valid in measuring PWV (Hwang et al., 2014). While both devices provide high quality data, the SphygmoCor XCEL requires training with the tonometer device to obtain waveforms, which can be technically challenging and time consuming. The Vicorder is much simpler to use and less invasive as cuffs can be placed over clothing. Therefore, the Vicorder is the ideal device for this study.

Exercise Bout Considerations

In choosing the exercise mode, duration, and intensity for this study, previous research was thoroughly analyzed starting with exercise protocols that had previously shown success in decreasing central and peripheral PWV in healthy, young populations. These studies indicated that moderate intensity exercise of approximately 30 minutes was a very successful duration and intensity (Heffernan et al., 2007; Kingwell et al., 1997; Tordi et al., 2010). Although other studies have shown that high intensity interval training is more successful than moderate intensity continuous exercise in lowering PWV post-exercise (Peres et al., 2018; Sapp et al., 2020; Tordi et al., 2010), moderate intensity continuous exercise was chosen as it more closely aligns with the ACSM physical activity guidelines. Lastly, the exercise mode was chosen. Although most studies utilized a cycle ergometer, this study decided to utilize the treadmill as it utilizes more muscle mass and therefore promotes increased blood flow in all arterial segments.
rather than having a greater focus on the legs. Therefore, the exercise protocol chosen for this study is a moderate intensity (55-65% of heart rate reserve [HRR]) walk/jog at a grade on the treadmill.

To verify the exercise stimulus was able to decrease central and peripheral pulse wave velocity, pilot testing was completed on 4 participants (50% female). Immediately following exercise, faPWV was seen to drop by -0.35 ± 0.41 m/s while bfPWV increased by 0.025 ± 0.31 m/s (Figure 4). These results are supported by previous literature, with peripheral PWV immediately decreasing while central PWV increases (Mutter et al., 2017; Saz-Lara et al., 2021). However, throughout the 90-minute lying bout faPWV did not continue to decrease as seen in previous literature but rather increased on average by 1.54 ± 0.66 m/s. bfPWV on the other hand decreased by -0.55 ± 0.60 m/s on average. Ultimately, these results indicate that the exercise does cause an initial drop in faPWV before it raises and drops bfPWV across the entire bout. However, it must be mentioned that these values were taken while the participants were in a supine position and we do not know how the PWV values may respond while in a seated position.
Figure 4: Response of central (bf-PWV) and peripheral (fa-PWV) arterial stiffness to 30-minutes of moderate-intensity treadmill walking across a 90-minute bout of lying supine. Riley et al., unpublished data.

Postural Considerations

To ensure a valid control to the exercise bout, a variety of postures were considered. The first of which was a 30-minute bout of sitting. However, given that it is known that prolonged sitting causes increases in pulse wave velocity having the participants sit for an extra half hour may only exacerbate the increase seen over the prolonged sitting bout. Therefore, laying and standing were seen as better options. As the participants will be in a standing position while performing their 30-minute bout of exercise, standing is a better posture control than laying. By matching the participants posture at each visit, it will allow for better elucidation of the impact that acute exercise has on pulse wave velocity.

Internal Validity

Internal validity is the extent to which the observed results in the dependent variables represent the truth of what is being studied, without outside influence. This project aimed to maintain internal validity through pre-assessment guidelines and standardized procedures, to
eliminate confounders to the best of our ability. Therefore, the testing took place in the most consistent conditions possible, with the humidity and ambient temperature of the Applied Physiology Lab (APL) or Exercise Oncology Research Laboratory (EORL) being noted at each visit to ensure no uncomfortable conditions are present. Pre-assessment guidelines were also maintained before each visit (Table 3), with researchers interviewing the participants to ensure the guidelines had been followed. To limit confounding variables, stimulants, depressants, food, and exercise were restricted prior to the visit. Each visit took place at a similar time in the morning and experimental visits took place within 1 week of each other to limit variations in stress, hormones, and other physiological factors. Lastly, female participants completed their experimental visits during the follicular phase of their menstrual cycle to minimize any hormonal impact of estradiol on vascular response.

**Table 3: Procedures to control internal validity in participant recruitment and testing.**

<table>
<thead>
<tr>
<th>Consideration</th>
<th>Explanation</th>
<th>Control Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity prior to sitting may improve endothelial function.</td>
<td>Prevent physical activity prior to testing to control for changes in vascular function.</td>
<td>Reminder sent to all participants to refrain from exercising 24 hours prior to testing.</td>
</tr>
<tr>
<td>(Morishima et al., 2017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine and alcohol consumption can impact heart rate and hydration.</td>
<td>Prevent consumption of caffeinated and alcoholic drinks.</td>
<td>Reminder sent to all participants to refrain from caffeine/alcoholic consumption 12 hours prior to testing.</td>
</tr>
<tr>
<td>(Mahmud &amp; Feely, 2001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variability in meal consumption alters physiology. Insulin can induce NO</td>
<td>Ensure no data confounding by diet or mealtimes.</td>
<td>Reminder sent to all participants to fast for 8-10 hours prior to testing (except for protein bar 2 h prior).</td>
</tr>
<tr>
<td>production and controls ET-1 expression.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Amiri et al., 2004)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol levels impacts NO production and thus vasodilatory response.</td>
<td>Prevent confounding by changes in hormone levels, such as during ovulation.</td>
<td>Test female participants during follicular phase of menstrual cycle. Ensure both visits occur during this phase.</td>
</tr>
<tr>
<td>(Adkisson et al., 2010)</td>
<td></td>
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</tr>
</tbody>
</table>
Population/Sampling

Healthy adults aged 18- to 35-years-old, will be recruited via flyers, email, social media, word of mouth, and class presentations from the University of North Carolina at Chapel Hill campus and surrounding areas. The variety of media will aim to target a wide range of individuals. To be eligible a participant must be between the ages of 18 to 35 years as arterial stiffness has been shown to increase with age (Mitchell et al., 2010). To limit the possibility of other confounders, additional exclusion criteria has been established (Table 4). Individuals who have cardiometabolic diseases or those who take medications that affect the cardiovascular system will be excluded. Along with individuals who use nicotine products (e.g. cigarettes and e-cigarettes) as they have been shown to increase arterial stiffness and blood pressure (Chaumont et al., 2018). Lastly, as fluctuations in estrogen have been shown to impact vascular outcomes (Adkisson et al., 2010), women who do not currently have a menstrual cycle due to pregnancy, breastfeeding, or hormonal intrauterine device will be excluded.

<table>
<thead>
<tr>
<th>Table 4: Eligibility and exclusion criteria.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criteria</td>
</tr>
<tr>
<td>Age 18-35 y</td>
</tr>
<tr>
<td>Known cardiometabolic disorders</td>
</tr>
<tr>
<td>Take any medications known to affect cardiovascular health</td>
</tr>
<tr>
<td>Smokes cigarettes or e-cigarettes</td>
</tr>
<tr>
<td>Pregnancy or plan to become pregnant</td>
</tr>
</tbody>
</table>
Participants who use hormonal intrauterine devices, use OC but skip their placebo week, or who are currently breastfeeding.

Screening interview.

Lack of menstrual cycle does not allow for tracking to identify the follicular phase.

**Abbreviations:** ACE: angiotensin converting enzyme, AS: arterial stiffness, BP: blood pressure, OC: oral contraceptives.

**Biological Factors**

This study will not be exclusionary to any individual based on sex nor race/ethnicity during recruitment. The impact of the menstrual cycle on outcomes will be limited by testing females in the early follicular phase, only.

**External Validity/Generalizability**

External validity is the ability for associations, trends, or significant conclusions from the study sample to be applied to a general population. This project does maintain a homogenous sample to emphasize internal validity, limiting the generalizability to only those who meet the recruitment criteria. The selected sample of healthy participants aged 18-35 years was chosen as it will eliminate confounding factors such as age and hypertension which ultimately would distort arterial stiffness measures. As this is the first study to look at the effects of acute aerobic exercise performed prior to prolonged sitting on arterial stiffness measures, starting with a healthy population will help to better clarify the relationship between these variables. While this model does not allow the generalizability of the results to older individuals or to those with vascular disease, they will provide a good foundation for future studies in differing populations.

**Statistical Considerations**

Several analytical processes were considered for the data. To account for the varying conditions applies to the participants in the study, a mixed model Analysis of Variance (ANOVA) would allow for examination of within-subjects and between-subjects data. However,
an ANOVA would not allow for adjustments of covariates which are essential when looking at PWV. Linear mixed models on the other hand can be adjusted for covariates and include fixed and random effects. Therefore, linear mixed models will be used to analyze differences within-subjects for bfPWV and faPWV. Furthermore, descriptive statistics will be collected to allow for comparison of similarities amongst the sample.
CHAPTER III: METHODS

Study Design

This study used a randomized crossover design with two experimental conditions (exercise [EX] and standing [STAND]) preceded by a familiarization session, with a 2-7 day washout period between experimental conditions (Figure 5). All testing took place in the APL at the University of North Carolina at Chapel Hill. Upon arriving for their familiarization visit, participants filled out an informed consent, followed by a medical history questionnaire, physical activity readiness questionnaire (PAR-Q), and physical activity questionnaire (IPAQ-S). Block randomization was used to ensure an equal number of participants began in the control (STAND) and exercise condition (EX). The study was approved by the University of North Carolina at Chapel Hill’s institutional review board.

Pilot Testing

In order to verify the exercise stimulus, pilot testing was completed. Participants underwent the 30-minute moderate intensity (55-65% HRR) bout of brisk walking at a grade. Prior to the exercise bout bfPWV and faPWV were measured in the supine position after a 10-minute resting period. After the exercise bout, the PWV measures were repeated 0-, 30-, 60-, and 90-minutes post-exercise to track the PWV response to acute aerobic exercise.
**Participants**

Healthy men and women ages 18-35 years who meet the ACSM physical activity guidelines (150 min/wk of moderate-to-vigorous physical activity) were recruited between January 15th, 2022 and April 3rd, 2022 through flyers, emails, and short presentations to classes at the University of North Carolina at Chapel Hill. Participants gave written consent and were screened for exclusion criteria which includes: any known cardiometabolic disorders, taking any medications known to affect cardiovascular health, pregnant women, and smoking. As fluctuations in estrogen can impact cardiovascular health, women were tested during the follicular phase (days 1-10) of their menstrual cycle (Adkisson et al., 2010). Menstrual cycle was self-reported by female participants.

**Familiarization**

Participants arrived for their familiarization visit after abstaining from vigorous exercise for 24 h prior and caffeine for 12 h prior. Pre-assessment guidelines were verbally confirmed. After reviewing documentation and signing the informed consent, anthropometric measurements and resting vitals were collected. Participants were then familiarized with the Vicorder device. After which, participants underwent a treadmill protocol to determine the speed needed to elicit...
the desired heart rate (HR) for a moderate-intensity bout (55-65% of HRR). The protocol began with a 2-min warm-up at a self-selected speed between 2-4 miles per hour (mph) at 0% grade. After which grade was increased by 2% every 90 seconds until the target HR was achieved. Once the target HR was achieved participants continued to walk at that speed and grade for 3 minutes to ensure their HR did not continue to increase. Pre-assessment guidelines were reviewed and participants were sent home with a supplement bar (Pure Protein, Bohemia, NY, USA) to consume 2 h prior to their first visit to reduce hunger distractions. The amount of supplement bar each participant received was based upon their estimated total daily energy expenditure, which was calculated using the Harris-Benedict equation and the Katch-McArdle activity multipliers (Harris & Benedict, 1918). As the 50g bar is a single serving based off of a 2,000 calorie diet, if a participant’s TDEE is 3,000 calories they will receive 75g of the supplement bar.

Visit 1 & 2

Prior to each visit, it was verbally confirmed that participants were 8-10 h fasted, with the exception of the supplement bar provided to them, have not vigorously exercised in the last 24 h, have not used alcohol, caffeine, or tobacco, in the last 12 h, and took passive transportation to the lab. Participants were then fitted for an accelerometer (wGT3X-BT; ActiGraph LLC, Fort Walton Beach, FL) on their right hip to covary for spontaneous movement. They then rested quietly for 10 minutes, followed by supine baseline measurements of bfPWV and faPWV on both sides of the body (Figure 2). Afterwards, 30 min of moderate intensity treadmill exercise or 30 min of standing was performed. The exercise session included 30-min of moderate intensity treadmill walking at the predetermined workload from the familiarization visit. This exercise protocol was chosen for three reasons: the treadmill utilizes large amounts of muscle mass
throughout the body potentially allowing for increased shear stress in all arterial segments, 30 min of moderate-intensity exercise aligns with the ACSM physical activity guidelines if performed each weekday, and 30-minutes of moderate intensity has been shown to transiently decrease arterial stiffness in healthy, young adults (Heffernan et al., 2007; Kingwell et al., 1997). During EX and STAND HR was monitored with a Polar monitor, if during the last minute of each 5-minute interval HR was above or below the 55-65% HRR prescribed treadmill speed was adjusted (Polar Electro Inc., Lake Success, NY). Participants underwent body mass measurements directly before and after each condition to allow for accurate fluid replacement, for each pound the participant lost they were given 16 fluid oz. of water to consume (Roy, 2013).

Immediately after the EX or STAND condition each participant underwent a standardized 5-min protocol to allow for HR recovery, during which they were weighed and given their determined amount of water. Following this, participants began their prolonged seated bout.

Participants remained seated for 2.5 hours and completed desk-based work. While sitting the participant’s position allowed their knees to bend approximately 90 degrees and the table height was adjusted to that their feet touched the floor. To account for differences in autonomic nervous system activation, which can alter vascular responses, participants were asked to refrain from listening to heavy music, watching TV (other than pre-approved non-stimulatory television programs), and perusing social media (Credeur et al., 2019). Additionally, participants were asked to stop all desk-based work 10-minutes prior to any measurements. Study personnel monitored participants to ensure no lower extremity movements took place as it can prevent endothelial dysfunction associated with changes in PWV (Bellien et al., 2010; Morishima et al., 2016). Participants underwent bfPWV and faPWV measurements again prior to the sitting bout (Pre) and at the end of the sitting bout (Post).
Representative timeline of one visit. Participants will eat a protein bar two hours prior to the start of the visit. They then underwent baseline measures of bfPWV and faPWV. Following this they either completed 30 min of moderate-intensity exercise or 30 min of standing. Both conditions were followed with 5 min of standing and a 2.5 h prolonged sitting bout. BfPWV and faPWV measurements were taken prior to the sitting bout (Pre) and upon termination of sitting bout (Post).

**Pulse Wave Velocity**

Two measures of PWV were taken, bfPWV and faPWV. These were chosen as they cover the central and peripheral arterial segments, respectively. PWV measurements are calculated by dividing arterial path length (D) by pulse transit time (PTT) between a proximal and distal arterial segment. PTT was measured using the Vicorder device (Skidmore Medical, Bristol, UK), which has previously been shown to be valid and reliable (Hickson et al., 2009; Kracht et al., 2011). Cuffs were placed around the arterial segments of interest and inflated to a sub-diastolic pressure simultaneously. The Vicorder software automatically calculated the time between the foot of the proximal pressure waveform to the foot of the distal pressure waveform, giving PTT. To determine D, each path was measured using a custom caliper to strictly measure length of artery and avoid measuring body contours. For bfPWV, D was measured from the suprasternal notch to the umbilicus. For faPWV, D was measured from the mid-point of the cuff.
at the superficial femoral artery to the mid-point of the cuff at the ankle. All measurements were taken in triplicate, on both sides of the body, and the closest two were averaged.

**Power Calculation**

The sample size calculation was based on previous data that showed an average increase of the combination of bfPWV and faPWV of 0.45 m/s during a prolonged sitting bout, with a conservative typical standard deviation of 0.5 m/s (Kelsch et al., 2021). Basing the sample size calculation on the ability to detect this change and setting the type I error at 5% and type II error at 20%, a sample size of 21 is required (Hopkins et al., 2009). The number was inflated to 22 to allow for an even number of males and females to be recruited.

**Statistical Analyses**

Data were analyzed with jamovi version 2.0 (The jamovi project, Sydney, Australia). The α level was set *a priori* α=0.1 for interaction effects and α=0.05 for all other statistical procedures. Descriptive statistics were used to summarize subject characteristics and all data were presented as mean ± standard deviation (SD). To evaluate the impact of prolonged sitting on central and peripheral PWV with and without prior aerobic exercise, data were analyzed using a linear mixed model with condition (EX vs. STAND) and time (baseline, pre, post) as fixed factors and subjects as a random effect. Group x time interactions were resolved using simple effects to examine group responses at each time point. Analyses were performed separately for bfPWV and faPWV. Time-by-condition interactions were evaluated with mean arterial pressure as a potential covariate. MAP was only shown to be significant for faPWV and therefore was not used for bfPWV. Additionally, percent change was calculated as 100 x [(final – original)/(original)] where baseline or pre were the original for comparison purposes. Effect sizes
were calculated using Cohen’s $d$ such that 0.2, 0.5, and 0.8 represent small, medium, and large differences, respectively.
CHAPTER IV: RESULTS

Participants

Fifteen participants were screened for eligibility, but two could not meet the scheduling demands of the study. Participant characteristics are reported in Table 5. Participants met ACSM physical activity guidelines for adults, self-reporting 230 (194) minutes of MVPA each week. On average, participants also engaged in 6.0 (1.4) hours of sedentary behavior each day.

<table>
<thead>
<tr>
<th>Table 5. Participant Characteristics (n = 13)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22 (3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.3 (7.8)</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>78.3 (17.6)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 (4.1)</td>
</tr>
<tr>
<td>RHR (bpm)</td>
<td>68 (6)</td>
</tr>
<tr>
<td>Physical Activity Levels (min/wk)</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>274 (223)</td>
</tr>
<tr>
<td>Moderate</td>
<td>141 (106)</td>
</tr>
<tr>
<td>Vigorous</td>
<td>320 (223)</td>
</tr>
<tr>
<td>Sedentary Behavior (hrs/day)</td>
<td>6.0 (1.4)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; RHR, resting heart rate; SBP, systolic blood pressure; SD, standard deviation.

At baseline, bfPWV, faPWV, and all other hemodynamic measures were consistent between conditions (Table 6).
Table 6. Baseline differences between hemodynamic measures in young healthy men and women.

<table>
<thead>
<tr>
<th></th>
<th>STAND</th>
<th>EX</th>
<th>P-value</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>123 (7)</td>
<td>119 (11)</td>
<td>0.098</td>
<td>0.50</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>56 (8)</td>
<td>56 (9)</td>
<td>0.944</td>
<td>0.02</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>79 (6)</td>
<td>77 (7)</td>
<td>0.151</td>
<td>0.24</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>59 (7)</td>
<td>61 (8)</td>
<td>0.204</td>
<td>0.27</td>
</tr>
<tr>
<td>bPWV (m/s)</td>
<td>5.2 (0.8)</td>
<td>5.2 (0.7)</td>
<td>0.970</td>
<td>0.01</td>
</tr>
<tr>
<td>fPWV (m/s)</td>
<td>7.1 (1.1)</td>
<td>7.0 (1.0)</td>
<td>0.841</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Data are mean (SD).
Abbreviations: bPWV, brachial-femoral pulse wave velocity; DBP, diastolic blood pressure; ES, effect size; EX, exercise condition; fPWV, femoral-ankle pulse wave velocity; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure; STAND, stand condition.

Central Arterial Stiffness

No interaction effect was found for bPWV (0.2 m/s, 95% CI -0.2, 0.6, d = 0.4, p = 0.667, Figure 5) and no condition (0.1 m/s, 95% CI -0.1, 0.3, d = 0.1, p = 0.315) nor time (0.1 m/s, 95% CI -0.1, 0.3, d = 0.1, p = 0.547) effects were present.

Figure 7. Acute changes in bPWV (m/s) at baseline and with prolonged sitting with and without prior exercise. Abbreviations: bPWV, brachial-femoral pulse wave velocity; EX, 30-minutes of moderate-intensity aerobic exercise; Pre, immediately post-exercise or standing but prior to prolonged sitting; Post, after 2.5 hours of uninterrupted sitting; STAND, 30-minutes of standing.
Peripheral Arterial Stiffness

After covarying for MAP, a condition x time interaction was present for faPWV (p < 0.001, Figure 6). From baseline to pre, EX decreased by 6.5% (-0.6 m/s, 95% CI -0.9, -0.3, \( d = 0.43 \), p < 0.001) and STAND increased by 6.1% (0.4 m/s, 95% CI 0.1, 0.7, \( d = 0.37 \), p = 0.018). Therefore, at pre faPWV was 14.2% difference between conditions (1.0 m/s, 95% CI 1.3, 0.7, \( d = 0.78 \), p < 0.001). From pre to post, EX increased by 14.0% (1.0 m/s, 95% CI 0.6, 1.4, \( d = 0.85 \), p < 0.001), while STAND remained unchanged (0.1 m/s, 95% CI -0.2, 0.4, \( d = 0.37 \), p = 0.383). These changes resulted in no difference between conditions at post (0.2 m/s, 95% CI -0.2, 0.6, \( d = 0.14 \), p = 0.304).

![Figure 8. Acute changes in faPWV (m/s) at baseline and with prolonged sitting with and without prior exercise.](image)

**Figure 8.** Acute changes in faPWV (m/s) at baseline and with prolonged sitting with and without prior exercise. Abbreviations: faPWV, femoral-ankle pulse wave velocity; EX, 30-minutes of moderate-intensity aerobic exercise; Pre, immediately post-exercise or standing but prior to prolonged sitting; Post, after 2.5 hours of uninterrupted sitting; STAND, 30-minutes of standing.

*\( p < 0.05 \) vs. Prior timepoint

#\( p < 0.05 \) vs. STAND

Accelerometry

There was no interaction effect for step count during prolonged sitting (\( p = 0.811 \), Figure 3A) and no condition (\( p = 0.259 \)) nor time (\( p = 0.869 \)) effect was present. On average, participants spent 98.3% (1.3) of their sitting time sedentary, independent of condition.
Specifically during the 30-minutes of walking (EX), substantially greater step counts were reported (3678 steps, 95% CI 3085, 4272, $d = 5.32$ $p < 0.001$, Figure 3B), with participants only taking ~10 steps during STAND. On average, participants spent 11% (27) of their time in the EX condition in light physical activity, 27% (38) in moderate physical activity, and 62% (42) in vigorous physical activity. During the STAND condition, 98% (1.7) of the time was spent in sedentary behavior and 2% (1.7) in light physical activity.

**Figure 9.** A) Step count comparisons between condition over prolonged sitting bout and B) between condition. Abbreviations: EX, 30-minutes of moderate-intensity aerobic exercise; Pre, immediately post-exercise or standing but prior to prolonged sitting; Post, after 2.5 hours of uninterrupted sitting; STAND, 30-minutes of standing.

#p<0.05 vs. STAND
CHAPTER V: DISCUSSION

Sedentary behavior leads to arterial stiffening, which is associated with CVD. Currently, interruption strategies have been the focus to combat these changes with varying results. Aerobic exercise has been shown to acutely decrease arterial stiffness and therefore may serve as an alternative strategy to attenuate arterial stiffening during prolonged sitting. Yet, the impact of aerobic exercise performed prior to uninterrupted sitting on arterial stiffness is unknown. Therefore, this was the first study to examine if moderate-intensity aerobic exercise mitigates increases in central and peripheral PWV across prolonged sitting. The main findings show that 30-minutes of moderate-intensity walking failed to prevent increases in central and peripheral PWV when performed prior to a prolonged sitting bout. These results indicate that prior aerobic exercise does not appear to be an alternative option to mitigate the negative impact of sedentary behavior on arterial stiffness.

Strengths and Limitations

To contextualize the findings, the limitations and strengths of this study are presented next. First, a homogenous group of healthy, active, young adults were recruited to reduce confounding influence of age and cardiometabolic diseases. While this strengthened internal validity of our measures, it reduced external validity. Therefore, our findings may not be generalizable to populations who may be at higher risk for CVD. Another limitation is the total
sample size. While this interim analysis was pre-planned, the current data set includes only part of the total sample size as data collection is on-going. However, our interim analysis appears not to lack statistical power, as the post-hoc power analysis revealed a $\beta = 0.99$. Due to constraints of the measurement (i.e., must be taken lying down), pulse wave velocity data collection was only performed at pre- and post-sitting measures. This limited our ability to elucidate how pulse wave velocity was changing during smaller increments of time across the sitting bout. Strengths include the novelty of being the first study (that we are aware of) to examine the effects of prior aerobic exercise on arterial stiffness across a prolonged sitting bout, a crossover design which allowed for participants to serve as their own control, and minimizing the effects of the menstrual cycle by testing in the early follicular phase only.

**Comparison to Literature**

This is the first study, to our knowledge, examining the effects of prior aerobic exercise on central (bfPWV) and peripheral (faPWV) arterial stiffness prior to prolonged sitting. In contrast to our hypothesis, 30-minutes of moderate-intensity aerobic exercise did not prevent increases in bfPWV across 2.5 hours of sitting. Despite the lack of significance, it was interesting to note that bfPWV did not change across the sitting bout in EX, while there was a 0.2 m/s increase with STAND. The 0.2 m/s rise follows a similar pattern in comparable literature but to a lesser degree. In two separate studies looking at the impact of 3 hours of sitting in inactive and recreationally active adults, bfPWV increased by 0.3 m/s (Evans et al., 2019) and 0.36 m/s (Kelsch et al., 2021). The difference in magnitude of change may be related to population, with our study focusing on a more active population.

Currently, there is no direct comparison using prior exercise to offset changes in bfPWV associated with sedentary lifestyles. Despite the lack of direct comparisons, a meta-analysis
showed that acute aerobic exercise resulted in no change in central arterial stiffness (Sardeli et al., 2018), which is consistent with our findings. However, the studies included in this analysis did not take into account the effect exercise may have across a prolonged sitting bout, as all recovery was in the standard supine position. Performing physical activity prior to a prolonged sitting bout is an area of increasing interest. 45 minutes of moderate-to-vigorous intensity treadmill exercise prevented impairment of femoral artery endothelial function (Ballard et al., 2017), while 30-minutes of vigorous-intensity cycling did not lower blood insulin levels (Engeroff et al., 2022). Collectively, investigations that utilize outcomes related to cardiometabolic health may identify alternative approaches to combat the negative effects of sedentary behavior in the future.

For faPWV, there was an initial 6.5% drop following the exercise bout and a 6.1% increase following STAND. Combined, this difference produced a 1.0 m/s difference in conditions prior to sitting (pre). While 1.0 m/s represents a clinically significant change for central arterial stiffness that is associated with a CV event and CV mortality risk change of 14% and 15%, respectively (Vlachopoulos et al., 2010), we observed this change only in our peripheral measure. Contrary to our hypothesis, these positive changes in faPWV were not sustained throughout the sitting bout. At post, peripheral arterial stiffness measures were the same between conditions. While the current literature supports the initial drop in peripheral arterial stiffness following exercise, how long that decrease persists is undetermined. A meta-analysis looking at the effects of different types of exercise on arterial stiffness in various arterial segments, showed that faPWV had the greatest acute effect from aerobic exercise with decreases lasting up to 24 hours post-exercise (Saz-Lara et al., 2021). However, this meta-analysis included a large variety of aerobic exercise protocols, with differences in intensity, duration, and
modality. The best available comparison to our exercise protocol comes from a study that had participants perform 30 minutes of moderate-intensity cycling (Kingwell et al., 1997). These participants experienced a 0.8 m/s decrease in peripheral PWV immediately post-exercise but values had recovered to baseline by 1-hour post, suggesting that the effects were short-lived.

One possibility as to why 30-minutes of moderate-intensity aerobic exercise did not attenuate increases in central and peripheral arterial stiffness across a prolonged sitting bout may be due to the exercise protocol itself. Although moderate-intensity aerobic exercise has been shown to acutely decrease central and peripheral arterial stiffness (Heffernan et al., 2007b; Kingwell et al., 1997; Kobayashi et al., 2017), exercise protocols of higher intensity may provide greater benefits in terms of arterial stiffness. Multiple studies have shown that high intensity interval exercise leads to greater decreases in central and peripheral arterial stiffness when compared with continuous, lower intensity exercise (Okamoto et al., 2018; Peres et al., 2018; Sapp et al., 2020; Tordi et al., 2010). Specifically, cfPWV decreases immediately post-exercise during the high-intensity protocol that is sustained across 60 minutes of supine recovery, while moderate-intensity exercise had no such effect (Sapp et al., 2020). The authors proposed that this difference may be due to the greater rate of stress on the vessel walls caused by high intensity exercise. Peripheral arterial stiffness exhibited a similar pattern, with high-intensity exercise producing larger decreases in PWV immediately post-exercise when compared to moderate-intensity exercise and sustaining that difference throughout the 30-minute recovery period (Tordi et al., 2010). For the current study, 30-minutes of moderate-intensity aerobic exercise was chosen for its alignment with the current ACSM physical activity guidelines. However, in light of our results and previous work comparing higher vs. lower intensity exercise effects on PWV,
the current exercise bout may not be of sufficient intensity to prevent increases in central and peripheral arterial stiffness during sitting.

**Implications**

For adults engaged in sedentary behavior for more than 7 hours per day, each 1-hour increase is associated with a 5% increase in all-cause mortality risk even after accounting for physical activity (Chau et al., 2013). Those individuals who sit for more than 8 hours per day have to perform 60-75 minutes of moderate-intensity physical activity daily to reduce their CVD risk (Ekelund et al., 2016). Although meeting ACSM physical activity guidelines may mitigate CVD risk caused by sedentary behavior, it does not completely eliminate it (Chau et al., 2013; Ekelund et al., 2016). While meeting ACSM PA guidelines (i.e., 30-minutes of daily moderate-intensity aerobic exercise) may not be a sufficient stimulus to offset the deleterious effects of prolonged sitting, this study is a first step in developing effective countermeasures for instances when prolonged sitting may be unavoidable. Considering that U.S. adults report an increase in sedentary behavior from 7.7 hours per day in 2004 (Matthews et al., 2008) to 9.5 hours per day in 2019 (Matthews et al., 2021), it is critical to identify interventions that successfully negate the deleterious effects of sedentary behavior on cardiovascular health. Further research is warranted to examine these effects, including higher intensity exercise protocols and to directly compare the effects of prior aerobic exercise vs. sitting interruption strategies (e.g., intermittent standing, brief walking bouts) on arterial stiffness.

**Conclusion**

As prolonged sitting leads to arterial stiffening and increasing CVD risk, this study sought to examine if prior aerobic exercise offsets the adverse effects of sedentary behavior. The results of the present study indicate that 30 minutes of moderate-intensity aerobic exercise prior
to 2.5 hours of sitting is insufficient to attenuate increases in central and peripheral arterial stiffness, despite an initial drop in peripheral arterial stiffness directly following exercise. With U.S. adults continuing to increase their daily sitting time, meeting physical activity guidelines in conjunction with effective sedentary behavior disruption strategies (both before and/or during) remain a pressing need to minimize CVD risk and maximize health benefits.
REFERENCES


