THE EFFECTS OF PROLONGED SITTING ON CEREBRAL PERFUSION AND EXECUTIVE FUNCTION

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ABSTRACT

Quentin Willey: The Effects of Prolonged Sitting on Cerebral Perfusion and Executive Function
(Under the direction of Lee Stoner)

The study purpose was to determine if prolonged (3-hr) sitting impaired (a) cerebral perfusion and executive function, (b) systemic vascular function, and (c) if heel raise exercises prevent impairments.

Subjects (n=20) participated in a control (CON) and experimental heel-raise (HEEL) study. Near Infra-red Spectroscopy was used to measure cerebral perfusion and venous pooling in the legs. A Stroop Task was used to assess executive function. Vascular health was measured using pulse wave velocity and pulse wave analysis.

Cerebral perfusion and Stroop was not significantly changed. However, venous pooling did occur in the legs (p<0.05) and systemic vascular health was negatively affected (p<0.05) in both days.

Prolonged sitting may not acutely affect cerebral perfusion or executive function in young, healthy individual like it negatively effects vascular health. The link between vascular interruption and brain function during prolonged sitting is unclear and future research should address alternate assessments of cerebral autoregulation and additional measures of cognition be examined.
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LIST OF ABBREVIATIONS

Aix – Augmentation Index

APL – Applied Physiology Laboratory

AS – Arterial Stiffness

BF – Blood Flow

BP – Blood Pressure

cBP – Central Blood Pressure

CO – Cardiac Output

CV – Cardiovascular

CVD – Cardiovascular Disease

FMD – Flow Mediated Dilation

Hb – Hemoglobin

MAP – Mean Arterial Pressure

NIRS – Near-infrared Spectroscopy

NO – Nitric Oxide

PWA – Pulse Wave Analysis

PWV – Pulse Wave Velocity

ROI’s – Regions of Interest
Chapter 1. Introduction

Prolonged sitting may pose a public health risk through its effects on the vascular system and may lead to a reduced ability to process cognitive tasks, thereby negatively effecting the work or school day. Recent evidence indicates that sitting for three hours results in decreased blood flow (BF) to the legs and subsequent local (leg) vascular dysfunction\textsuperscript{36-38}. This previous work did demonstrate that short bouts of walking\textsuperscript{106} or fidgeting\textsuperscript{38} prevented the decline in BF and shear stress, and subsequently local (lower extremity) vascular dysfunction. However, it is currently unknown whether (1) prolonged sitting effects the brain’s executive function, or whether (2) sitting impairs perfusion of the prefrontal cortex of the brain.

Executive function (EF) is required to turn sensory input into an actionable output and is necessary to carry out simple daily tasks. This EF may rely on cerebral perfusion to the prefrontal cortex of the brain. Previous works have shown that EF declines with chronic sedentary behavior,\textsuperscript{16-20} however, few studies examining prolonged sitting have been conducted. Of the studies that have examined prolonged sitting, there is evidence suggesting that EF may have declined after a period of prolonged sitting; though they lacked objective evidence as the data consisted of questionnaires and the mechanisms were not investigated.\textsuperscript{90} Arguably, EF may become impaired as a function of decreased perfusion to the frontal cortex.\textsuperscript{68} While the association between cerebral perfusion and EF has not been examined during prolonged sitting the reverse has been examined; that is acute exercise has been shown to enhance cerebral perfusion, and this enhancement is positively associated with EF.\textsuperscript{109}
Cerebral perfusion may become impaired as a function of blood pooling in the lower extremities during a bout of prolonged sitting. For example, when an individual stands up with locked knees for too long, blood pools in the calf as gravity pulls down and the muscles of the legs are not being repeatedly relaxed and contracted. This can result in fainting and light-headedness from a lack of adequate blood supply. Though similar mechanisms occur while sitting, it is unknown how brain function is effected by a bout of prolonged sitting. Indeed, one of the previous studies examining the effects of prolonged sitting reported increased circumference of the calf indicating an accumulation of blood in the lower limbs. The effects of hydrostatic pressure may be compounded by decreased muscle activity, which would normally act as a venous “pump”. Blood is then more easily pooled in the lower extremities resulting in less venous return from these regions which may lead to fluctuations along the systemic vascular tree.

Decreased blood flow – and subsequent shear stress – throughout the vascular system may also result in impaired vascular function, including the cerebrovascular. Impaired vascular function may compromise the ability to regulate blood flow to the brain. Though it is unknown how impaired vascular function is linked to brain blood flow, studies have found that a 3-hour sitting intervention is sufficient to impose changes in vascular function. Therefore, a 3-hour sitting protocol will likely be enough time to identify if there is a potential link between changes in vasculature and cerebral perfusion. To determine underlying mechanisms that may link vascular health and cerebral perfusion, vascular measurements that estimate what is happening at the level of the aorta may be helpful as it is thought that stiffness, pressure, and waveforms in the aorta may be an accurate representation of what is happening throughout the peripheral vascular tree.

If the mechanism is venous pooling in the lower extremities, simple activities such as raising one’s heels up and down while sitting could be introduced to engage the “muscle pump”
and ensure adequate venous return. Maintaining the flow of blood may thereby prevent decrements to local vascular health and prevent fluctuations in cerebral perfusion leaving executive function unaltered. If intermittent calf contractions caused by raising the heels up and down prevent blood from pooling in the legs and thereby maintain adequate cerebral perfusion, then heel raise exercises may be a viable solution to prevent deleterious effects of prolonged sitting on EF.

**Purpose**

The purpose of the current study is to examine the acute effects of sitting on executive function and the associated mechanisms in the cardiovasculature. Findings from this study may identify a simple strategy such as performing calf raises while being seated for offsetting the negative consequences of sitting and may contribute to public health policy pertaining to sedentary behavior.

**Research Questions**

*Primary*

1. Does prolonged (3 hr) sitting impair: (a) measures of cerebral perfusion and (b) executive function?

2. Do intermittent heel raise exercises, to engage the muscle pump, prevent venous pooling in the lower extremities and thereby prevent changes in (a) cerebral perfusion and (b) executive function?

3. Are changes in executive function associated with cerebral perfusion?

*Secondary*

1. Are changes in systemic vascular measures associated with changes in (a) cerebral perfusion and (b) executive function
2. Are changes in calf muscle perfusion associated with changes in (a) cerebral perfusion and (b) executive function?

Research Hypothesis

Primary

1. Prolonged (3 hr) sitting does impair (a) measures of cerebral perfusion and (b) executive function.

2. Intermittent heel raise exercises, to engage the muscle pump, prevents venous pooling in the lower extremities and thereby prevent changes in (a) cerebral perfusion and (b) executive function.

3. Changes in executive function are associated with cerebral perfusion.

Secondary

1. Changes in systemic vascular measures are associated with changes in (a) cerebral perfusion and (b) executive function.

2. Changes in calf muscle perfusion are associated with changes in (a) cerebral perfusion and (b) executive function.

Significance of Study

As the work force and general public sit for large portions of the day, it is important to understand the health implications of this inactivity. Chronic prolonged sitting has been linked to a multiplicity of negative effects, including vascular dysfunction and diminishing executive function. If it is found that executive function and cerebral perfusion decline during a bout of prolonged sitting and that systemic vascular measures are associated, better prescription with respect to prolonged sitting may be given. These guidelines would help prevent chronic CV complications due to accumulating effects of acute bouts of prolonged sitting leading to CVD.
Intermittent calf raises are an easily applied, cost-effective intervention that can be done in a work-place or school setting to counteract the negative implications associated with sedentary behaviors like working on a computer and sitting in a lecture hall.
Chapter 2. Literature Review

Introduction

The way in which westernized societies interact with the world has changed dramatically over the past several decades. Communication, transportation, and entertainment technologies as well as work-place and educational environments now favor sedentary behavior. Individual’s definition of sedentary behavior is often altered because of societal norms and demands such as driving, sitting at a desk at work or school, and screen time for leisure. However, these sedentary lifestyle activities may pose a risk to public health through their effects on cognitive function and cardiovascular health. For example, it is known that cognitive function declines over time with sedentary behavior and that cardiovascular health is negatively affected both chronically and acutely from sedentary behavior such as prolonged sitting. These data lend support to the possibility of cognitive function being augmented during short, acute bouts of prolonged sitting. Though the mechanisms are not fully understood, chronic and acute declines in cognitive function and cardiovascular health during prolonged sitting may be affected by decreases in blood flow (BF). Fortunately, several cardiovascular measures, such as BF, can be measured noninvasively with high reliability and validity. For this reason, if cognitive dysfunction does occur with an acute bout of prolonged sitting, a relatively simple study may provide a partial explanation for dysfunction. In addition, interventions to mitigate cognitive dysfunction may be further explored and become useful for populations with lifestyle demands favoring sedentary behaviors such as prolonged sitting.
**Sedentary Behavior**

Although, driving and working are often productive and can make defining sedentarism murky when individuals are still carrying out tasks. However, sedentary behavior is defined as any waking behavior characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture.² Sedentary behavior such as prolonged sitting may be effecting a large portion of the population because of occupation, transportation and leisure activity. For example, one study reported that an average of 77% of occupational time was spent sitting.¹¹ Also, another study with a population of 6,329 from the US showed that 54.9 percent of their waking time was spent in sedentary behavior.⁹ This is further supported by multiple national surveys where respondents reported an average of 7 hours/day sitting at work.¹² Though survey responses typically underestimate their sitting time, these conservative data still present a large problem. For example, the US Department of Labor conducted a survey in 2015 where an average 3 hours was dedicated to TV watching and was spent as leisurely, sedentary time.¹³ These reports may be shocking, but according to the American Heart Association, there has been an 83% increase in sedentary jobs since 1950.¹⁰ Therefore, it should be no surprise that sedentary behavior effects a large population in westernized societies.

**Executive Function**

It is important to note here that sedentary workers are still working. However, the working capacity of these individuals is largely affected by their cognitive function. Cognitive or Executive Function is an umbrella term for the neurologically-based skills involving processes that all have to do with managing oneself and one's resources in order to achieve a goal.¹⁴ Although largely responsible for productivity in every-day living, Executive Function may be decreased by the sedentary behaviors adopted due to lifestyle demands. However, declines in
executive function would decrease the work capacity of individuals in sedentary jobs ranging from students to full-time employed persons.

**Chronic Associations**

The deleterious effects of chronic sedentary behavior and inactivity have been well documented by many studies. It is clear that sedentary behavior increases risk factors for all-cause mortality and is positively associated with cardiometabolic diseases. Other negative effects of sedentary behavior, like declines in Executive function, have become of recent interest. For example, recent evidence suggests that chronic sedentary behavior is associated with declines in EF and is true for both young and old populations. In addition, individuals with various diseases and mood disorders are negatively affected regarding cognitive measures by sedentary behavior. As expected, chronic sedentary behavior is bad for human health and executive function is no exemption.

**Acute Associations**

In contrast to chronic behaviors, less is known about the effects of acute behaviors concerning Executive Function. However, it is well understood that Executive Function improves with acute bouts of physical activity and exercise. For this reason, interest has mounted whether the opposite might be true; that acute bouts of inactivity, or sedentary behavior, could lead to declines in Executive Function. How the duration of an acute bout is defined may be up for debate; however, several studies could help narrow the definition of acute. For example, bed rest studies ranging from 5-60 days of bed rest have shown to have negative physiological effects including cardiovasculature and executive function. These same effects have been found to occur in shorter durations such as a single day. There is little information concerning the effects of Executive Function alone in shorter amounts of time, though
physiological dysfunctions are seen in as little as 2-3 hours. However, the same complications may not occur in less 2-3 hours as one study showed no significant changes in Executive Function over the course of 1 hour of sedentary behavior. In short, the exact definition of an acute bout has not been well defined, though any longer than 2-3 hours may likely have deleterious effects including Executive Function.

Mechanisms

The evidence presented thus far provides a foundation for research concern regarding the effect of chronic, and more particularly, acute sedentary behavior on Executive Function. One of the aims of the current research study is to discover if Executive Function declines with acute sedentary behavior such as prolonged sitting of 3 hours. If declines in Executive Function are found following an acute bout of prolonged sitting, this study could provide potential mechanisms by which this decline occurs. A better understanding of the mechanisms leading to decline in Executive Function in an acute bout of sedentary behavior may additionally offer an explanation as to why Executive Function declines in chronic bouts of sedentary behavior. Potential mechanisms that may contribute to these effects are presented in Figure 1., and findings among these mechanisms may further strengthen the foundation for further research.
**Figure 1.** Mechanism pathway for declines in EF due to acute sedentary behavior like prolonged sitting.

**Blood Flow**

The purpose of this section is to briefly discuss and define each of the four mechanisms in column 2 of Figure 1 which will then be discussed in the context of chronic (section 2.5.1) and acute (section 2.5.2) bouts of sedentary behavior followed by a discussion of how these mechanisms may be contributing to declines in executive function. First, it is known that these mechanisms are related to one another and changes in one will often have effects on another. Blood flow, for example, is essentially the amount of blood that passes through a portion of a vessel in any given period of time. The magnitude of blood flow is the product of pressure in the vessel divided by the amount of resistance. With the exception of during exercise, blood pressure is relatively constant at rest and does not change drastically over time. For this reason, changes in blood flow are mostly due to changes in resistance to flow. By applying Poiseuille’s law to blood vessels, there are three main factors of resistance to blood flow: vessel diameter, vessel length, and blood viscosity. Since vessel length and blood viscosity changes very little over long periods
of time and is mostly unchanged from one day to another, resistance to blood flow is mostly attributed to changes in vessel diameter. In this way, blood flow is connected to changes in vessel diameter and function.

**Vascular Function**

How well the vessel diameter changes in response to different stimuli is known as vascular function. The vessel’s responses are largely dependent on the second inner-most lining of the vessel walls known as the endothelium and on smooth muscles which receive input from the nervous system. First, the endothelium is made up of endothelial cells that release nitric oxide (NO) in response to shear stress in the vessel. Shear stress is the force produced by blood moving along the endothelial layer and is proportional to blood flow. As blood flow and shear stress increase, NO is released and the vessel dilates to keep blood flow and shear stress at healthy levels. This is one way that vascular function is maintained.

**Autonomic Nervous System**

A second way is through the smooth muscle tone, or tension controlled by part of the central nervous system called the autonomic nervous system. The autonomic nervous system is divided into two parts called the sympathetic and parasympathetic which control bodily function subconsciously. As the body experiences stress, sympathetic tone increases and stimulates smooth muscles in blood vessels to constrict. This occurs throughout the entire body except in vessels that supply the brain and the heart. In contrast, parasympathetic tone stimulates smooth muscles in blood vessels to relax thereby increasing vessel diameter. As diameter of the vessels change, so will shear stress, vascular function and blood flow. It is important to note here that the vasculature in the body is all connected and that effects in one area could be having effects in another. In particular, all the vessels in some way lead to or away from the heart, which is also
effected by sympathetic and parasympathetic tone. Heart rate (HR), contractility, rate of relaxation and rate of conduction are mainly controlled by the autonomic nervous system and these factors can be described in part by Heart Rate Variability (HRV). Why this information is important will be further discussed in section 2.6.

**Brain-derived Neurotrophic Factor**

This last portion will discuss Brain-derived Neurotrophic Factor (BDNF) which is a growth factor protein related to the Nerve Growth Factor and are found in the brain and periphery. BDNF has been shown to increase in acute bouts of exercise and mediates improvements in EF.\(^{28}\) For this reason, it may be of interest to explore how sedentary behavior effects BDNF in chronic and acute bouts of sedentary behavior.

**Chronic Sedentary Behavior and Mechanisms**

As discussed previously, EF declines in response to chronic sedentary behavior. The mechanisms contributing to this phenomenon are not fully understood, however evidence regarding BF, VF, the ANS and BDNF may provide potential explanations. First, the effects that chronic sedentary behavior has on BF and VF may be of greatest interest. As individual’s age, their vascular health, including cerebrovascular health, has been shown to decline;\(^{59}\) however, physical inactivity further exacerbates their decline. As discussed previously, strong evidence links sedentary behavior to cardiovascular disease and is the leading cause of death.\(^{4-8}\) Furthermore, evidence suggests that physical inactivity diminishes the body’s ability to regulate the cardiovascular system.\(^{58}\) One pathway by which BF and vascular function diminish is through Arterial Stiffness (AS). AS refers to the compliance or distensibility of arteries which is required to maintain BF and is a determinant in how hard the heart is working to push blood through circulation. Additionally, AS has been shown to increase with sedentary behavior.
independent of age and metabolic diseases making it a meaningful outcome when considering the effects of chronic sedentary behavior. For example, as individuals are sedentary over time, AS will increase. Increasing AS will decrease blood flow due to an added amount of resistance which will ultimately result in elevated blood pressures and vascular dysfunction. This would lead to less blood reaching working brain tissue and may be a potential mechanism as to why EF declines with chronic sedentary behavior.

This may be the case because as VF is already declining with age and sedentary behavior is intensifying the same effects. However, the negative effects from sedentary behavior appear to be controllable and have positive effects on EF and VF. For instance, evidence has shown that as older individuals make the effort to substantially reduce their blood pressure, EF is maintained over time. Furthermore, older adults with lower sedentary time and higher levels of physical activity have been shown to have better vascular function. In fact, the physiology of vessels seems to change with chronic sedentary behavior as seen in mice models where the endothelial layer and surrounding layers thickened, stiffened and became dysfunctional. Whether these effects are found in cerebrovascular regions is less understood, but systemic effects may be reaching the brain thereby decreasing EF.

The ANS also has systemic, or whole-body effects in that it’s signals are non-specific and will create global effects. As resistance to BF occurs by AS and vascular dysfunction, the heart has to work harder and increase cardiac output (CO) in order to keep the same amount of blood moving through circulation. One of the principle responsibilities of the sympathetic division of the ANS is to increase CO and is achieved by increasing HR and contractility. However, because the sympathetic tone is relatively non-specific, vessels throughout the body in non-active tissues can become constricted thereby increasing resistance in the periphery. Blood pressure will also
then be effected which will keep the sympathetic tone at an elevated state. Figure 2 may be helpful in making connections between the ANS and responses in the body. Well-being in the ANS and sympathetic tone can be measured by HRV and will be further discussed in section 2.6. However, it is important to note here that as the sympathetic tone increases and remains increased, the ANS becomes dysfunctional and the body is less able to respond to stress appropriately.\textsuperscript{47,48} The combination of factors contributing to ANS dysfunction may be another potential explanation for EF decline with chronic sedentary behavior.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{cardiovascular_control_mechanisms.png}
\caption{Scheme of the cardiovascular control mechanisms responsible for the main periodic fluctuations in heart rate.\textsuperscript{49}}
\end{figure}

Lastly, decreased BDNF could be a possible mechanism by which chronic sedentary behavior negatively affects EF. This is because BDNF has been shown to be one of the key pathways in which brain health and function can be improved through physical activity.\textsuperscript{50-53} Also, physical activity has beneficial effects on the regulation of insulin and cytokines which regulate BDNF. This suggests that BDNF is likely part of a central mechanism that physical activity plays
a role in as BDNF has also been shown to mediate the effects of physical activity on EF. However, chronic sedentary behavior may have a negative effect on EF through the dysregulation of insulin and cytokines that in-turn may dysregulate BDNF. Finding a study evidencing this mechanism would be rare, although the recent findings mentioned here are provoking in context of the link between insulin and cytokines to the regulation of BDNF. If BDNF is decreased with chronic bouts of sedentary behavior, this might stimulate a new research avenue previously untapped.

**Acute Sedentary Behavior and Mechanisms**

The effects of acute sedentary behavior on EF is relatively recent area of research interest. However, there is a large body of evidence to support that the proposed mechanisms in column 2 of Figure 1 do occur. Whether or not these mechanisms are the same mechanisms that could lead to declines in EF has not been fully investigated. Although, when considering the all the evidence together in context of one another, there is reason to believe that EF could be effected by acute bouts of sedentary behavior. Discussed below are the findings related to acute sedentary behavior starting with BF and ending with BDNF similar to section 2.3.1.

First, during a bout of acute sedentary behavior such as prolonged sitting, BF was shown to be reduced by 40% in just 1.5 hours. One potential explanation for this large decrease in BF in a short time period could be caused by a decrease in muscle ‘pump’ activity. As the muscles in the lower extremity become inactive while sitting for long periods of time, blood will pool in the lower extremities. Several studies have shown this to occur and calf circumference has been shown to increase due to fluid accumulation. When the blood pools in the veins of lower limbs, blood can become backed-up and there is an increased resistance to local blood flow. The local resistance is also added to by increased clotting factors accumulating in these regions due
to slow moving blood. Blood then becomes more viscous and resistance continues to increase.\textsuperscript{41} As discussed previously, increased resistance will reduce BF and thus BF decreases in the lower extremities.

Second, the decreases in BF cause decreased amounts of shear stress which results in vascular dysfunction. Endothelial cells have been shown to become dysfunctional in response to a bout of prolonged sitting lasting 3 hours.\textsuperscript{36-38} The combination of decreased BF and VF results in a decrease in venous return. According to the Frank Starling Law of the Heart, less blood filling the heart results in weaker contractions and a lower stroke volume (SV). Drops in venous pressure, such as when a person stands up too fast and becomes light-headed, has obvious effects on EF. This happens because there is temporarily less blood being ejected from the heart, meaning less blood reaches and perfuses brain tissue causing temporary executive dysfunction. Therefore, if VF is compromised, there is reason to believe that there could be a connection between prolonged sitting of 3 hours and EF as one study has shown.\textsuperscript{32}

Third, the ANS responds to changes in BF, VF, and BP\textsuperscript{49} and from Figure 2 it is shown that several of the above factors influence the ANS. Measures of ANS activity may be an additional tool in analyzing the mechanisms behind declines in EF due to sedentary behavior. The ANS may further be of interest when determining the acute effects of sedentary behavior because the ANS can respond rapidly to changing environments. The ability of the ANS to change quickly is due in part by the two divisions, sympathetic and parasympathetic always having some influence on the body and the balance of the two can shift rapidly as one increases and the other decreases.\textsuperscript{43} Imbalances in these two divisions can lead to a variety of pathological conditions that affect EF.\textsuperscript{44} Therefore, changes in EF could be partially described by changes in the ANS with BF, VF and BP as underlying mechanisms.
Finally, BDNF could be used to better understand effects of sedentary behavior on EF and in light of current research, might be more useful in studying acute bouts versus chronic. This is because most of what is known about the associations between BDNF and physical activity come from acute bouts of exercise or physical activity.\textsuperscript{50-52} As mentioned in section 3.1, BDNF is regulated by insulin and cytokines. These can be altered in acute bouts of sedentary behavior and may contribute to BDNF declines leading to diminishing EF.

Implications

Sedentary behavior is known to be hazardous to health as has been demonstrated throughout this review. However, it is important that organizations with the ability to impact large populations recognize the evidence and findings regarding sedentary behaviors such as prolonged sitting. A recent example of this happening is the American Medical Association announced in their June 2013 annual meeting that sitting down for too long is hazardous to health. In addition, more audiences are recognizing that simply meeting physical activity guidelines each day does not prevent the negative effects of prolonged sedentary behavior.\textsuperscript{15} Mounting evidence of this nature should promote change in policy and guidelines in corporations and public health initiatives.

Prolonged sitting may pose a public health risk through its effects on the vascular system, and may lead to a reduced ability to process cognitive tasks. Decreases in EF will have negative effects such as lowered productivity at work or poor learning outcomes at school. One of the aims of this study is to show how acute (3 hr) bouts of prolonged sitting can almost immediately have detrimental effects on health such as declines in EF. If it can be shown that there are immediate consequences of sedentary behaviors like prolonged sitting, it may have a greater public impact than potential consequences that happen long-term.
Chapter 3. Methods

Subjects

A relatively homogenous cohort of 20 young (19–35 year) and healthy but sedentary participants was recruited to participate in the study. To be eligible, subjects could not have been meeting current American College of Sports Medicine activity guidelines to be characterized as sedentary. However, because subjects had no other cardiovascular risk factors outside of activity levels, they were considered to be a homogenous, healthy sample. Their sedentary behavior more appropriately represents populations that are frequently sitting for long periods of time. Further reason for selecting sedentary subjects is explained in the discussion. Because this is the first study of its kind and because elderly and diseased populations have different vascular sensitivity, a young, healthy, homogenous population is ideal.

It should also be noted that the effects of estrogen levels on vascular measurement may cause differing levels of vessel reactivity to changes in blood flow and effect cognitive function. Special considerations for female subjects were accounted for such that they were not tested if pregnant and, if not amenorrhoeic, they were tested during the first 7-10 days of the follicular phase of the menstrual cycle when hormone levels are relatively constant in comparison to the luteal phase. However, all female subjects were using birth control and were, as a result, amenorrhoeic. For this reason, it could not be determined if female subjects were tested during the first 7-10 days of the menstrual cycle. Although, by having greater control over hormones from medication, it should be considered that the females in this study were more closely regulated than if they had not been using birth control.
**Study Design**

This study was a randomized crossover design with two experimental conditions (control [CON] and heel raises [HEEL]). The experimental conditions took place on separate days and were preceded by a familiarization session. Each experimental testing session was separated by no more than 7 days. Data collection began between 6:00 am-10:00 AM in the Applied Physiology Laboratory (APL). Prerequisite to participation, subjects took part in an overnight fast while abstaining from alcohol and exercise 24 hours prior to experiment. In addition, they were not allowed caffeine or other supplements the morning of the visit. All subjects were emailed, texted, or personally contacted the day before testing to be reminded of test visit.

**Pre-assessment**

Prior to subject participation, ethical approval was obtained through the IRB and the Office of Human Research Ethics at the University of North Carolina-Chapel Hill. All subjects reported to the APL to fill out general health questionnaires (Appendix A) and gave informed consent (Appendix B).

**Familiarization**

Subjects reported to the APL to review documentation, all study procedures and consent forms. After obtaining height and weight, subjects completed a minimum of 7 Stroop Word and Stroop Color Tasks and continued until completion time plateaued to avoid a learned effect, as previously reported. Following Stroop, heel raises were practiced with a metronome (Pro Metronome Xiao Yixiang ©2016 EUMLab, Xanin Tech) set to 20 beats/minute. Following familiarization, subjects were given a supplement bar (Pure Protein©, Worldwide Sport Nutritional Supplements Inc, United States) to consume 2 hours prior to arrival in the APL to prevent blood glucose from dropping and provide sustenance for subjects’ comfort. Upon APL
arrival, subjects were required to confirm protein bar ingestion and were reminded via email or text message prior to testing day to consume the bar 2 hours prior to arrival.

Visit 2 & 3

To begin, subjects were fitted for NIRS and then rested, lying supine for 10 minutes under quiet, controlled conditions (20-24 degrees Celsius) while NIRS signals stabilized. Then, PWA was measured, taking approximately 10 minutes, followed by PWV measurement which took approximately 3-5 additional minutes in the supine position (20-25 min total). Subjects were then brought to an upright, seated position using an Armedica AM353 Hi-lo Treatment Table (Tiger Medical, TIGER#TM83695) with feet flat on the ground with approximately 90 degrees of knee flexion. Subjects were instructed to not fidget during 180-minute protocol and a Grand Designs program was started. Grand Designs is an English architecture television series that was used as a low-stimulus control for cognitive alertness during the prolonged sitting protocol with the same episodes being shown to each subject but not the same from the first to the second day. The purpose of showing this program was to prevent large fluctuations in cognitive stimulation that homework, cell-phones, or games may have caused. Grand Designs provided a minimal stimulus to maintain wakefulness and prevent subjects from falling asleep. While sitting, PWA and Stroop were collected at 10, 90, and 170 minutes, and NIRS signals were collected continuously. Immediately prior to and following the sitting protocol, NIRS signals were marked to acquire pre-to-post change scores. Throughout HEEL and CON subjects were notified at 10-minute intervals, but only HEEL performed 10 heel raises to a metronome (Pro Metronome Xiao Yixiang ©2016 EUMLab, Xanin Tech) set to 20 bpm to activate the muscle pump as it was thought that this would be frequent enough to prevent any potentially
negative effects of prolonged sitting. After 180 minutes, subjects were transferred back to the supine position to conclude with PWA and PWV.

Blood volume may change throughout the course of the sitting protocol due to filtration of the blood in the kidneys and insensible water loss through perspiration and respiration. This can cause between 100-250 ml of water loss in a period of 3 hours. For this reason, and at the request of the IRB, water intake was monitored during both testing sessions and subjects were given 40 mL of water every 30 minutes. In addition, subjects were instructed to refrain from using the restroom during the study because standing and walking to the restroom would alter CV mechanisms. There were no instances of subjects getting up to use the restroom at any point during the study.

**Measurements**

*Executive Function:*

To assess if EF was affected by prolonged sitting and perhaps declines in cerebral perfusion, the Stroop Word-Color Task (Stroop) and was administered on a laptop computer. For both the word and color portion of the Stroop Task, 32 iterations were performed and time till completion was used as the primary outcome. Familiarization consisted of a minimum of seven tasks, or until total completion time plateaued for both the word and color portions of the Stroop as has been done previously. Stroop has been widely used for the testing of EF and is considered a valid and reliable test including young, healthy populations.

*Cerebral Perfusion:*

To determine if prolonged sitting led to a decrease in cerebral perfusion, a continuous wave near infra-red spectroscopy (NIRS, Portalite, Artinis Medical Systems, The Netherlands) probe was used with path-length correction factors set for the forehead (Appendix C). NIRS is one of
several non-invasive measures and other works’ have shown its effectiveness in measuring oxygen uptake and delivery in the brain. For this reason, a NIRS probe was placed on the forehead to measure relative changes in total hemoglobin as has been described in previous works and as shown in Appendix C. By placing a NIRS probe on the forehead, over the prefrontal cortex, the total amount of Hb (tHb), and thereby perfused blood, was measured over the course of the study. In addition, the total saturation index (TSI) was measured, which is an index represented as a percentage of oxygenated Hb (O2Hb) versus deoxygenated (HHb) found in the tissue of interest.

The deepest probe depth where the infrared light would travel the furthest into the tissue (or T3, meaning the third infrared light emitter) for tHb measurement was determined to be the best measurement output for capturing Hb within the prefrontal cortex. TSI, however, uses an average saturation of all three NIRS light infra-red-light sources and produces a single percentage making probe placement very important. For this reason, the tHb and TSI were measured with careful consideration to avoid any large vessels and ultrasound was utilized to verify probe placement. Snap-shots of ultrasound capture can be found in Appendix D.

The NIRS probe emits infrared light which can easily pass through skin, muscle and bone tissue. However, there is always some amount of light that is scattered and then reflected by bodily tissues including blood. The NIRS probe is programmed to detect the wavelength of reflected light from oxygenated hemoglobin (Hb), and deoxygenated hemoglobin (deoxyHb) in red blood cells. According to the manufacturer’s guidelines, the NIRS probe can emit and retrieve light up to 4 cm under the skin and a high-speed camera on the probe detects this reflected light in real time. In this way, blood perfusion can be measured in any tissue within 4 cm under the skin. For outcome assessment, data was averaged over 30 seconds at the beginning and end of each sitting protocol, at the three time points (10,90,170) following vascular measures, and at the beginning and end of
each bout of heel raise exercise. Because the live NIRS data feed rises and falls slightly with each heartbeat and breath, selecting a single data point is less accurate. A 30 second average has been previously used in studies utilizing NIRS and was determined appropriate for this study.\textsuperscript{107}

\textit{Venous Pooling in Lower Extremities:}

To measure the amount of blood pooling in the lower extremities, the muscle belly of the medial gastrocnemius was found using ultrasound to avoid large vessels. A NIRS probe was placed on the medial gastrocnemius of the right leg as shown in Appendix C and blood pooling was monitored using NIRS set to a pathlength correction factors for the calf (Appendix F). To test if cerebral perfusion and calf perfusion changed as a result of prolonged sitting, tHb and TSI were the dependent variables and were first normalized relative to baseline by using change scores instead of absolute outputs.

\textit{Vascular Health:}

To obtain a comprehensive overview of systemic vascular health, and to see if changes may be related to possible declines in cerebral perfusion and EF, several non-invasive methods were used, including PWV and PWA. Both measurement techniques have high reliability, validity and provide quantified data for AS and VF.\textsuperscript{64,65} If AS is shown to increase, there is reason to believe that the burden on the heart will increase.\textsuperscript{101} Any general relationships between systemic vasculature, cerebral perfusion and EF may be helpful in determining underlying mechanisms for the effects of prolonged sitting.

First, pulse wave velocity (PWV) was used to measure arterial stiffness (AS) before and after a bout of prolonged 3 hour sitting. Measurement from carotid pulse to sternal notch and from inguinal crease to the top of the thigh pressure cuff was done with a tape measurer. However, a novel method of measuring distance from sternal notch to the top of the thigh cuff was performed
by the use of a large, hand-made caliper so as to avoid measuring the contours of the body and strictly measure the length of the descending aorta (Appendix E).

PWA was obtained by using oscillometric pressure waveforms recorded on the left arm using a brachial cuff following standard manufacturer guidelines.\(^6^2\) An aortic pressure waveform was generated using a validated transfer function based off of the pressure waveform derived at the arm.\(^9^9\) The AIx is the augmentation pressure (AP), expressed as a percentage of central pulse pressure. AP is defined as the peak systolic pressure minus the pressure at the inflection point (Fig. 1). The aortic wave can be separated (bottom panel) into its forward (Pf) and backward (Pb) waves, and reflection magnitude (RM) can be computed (Pb/Pf).\(^9^6\)

![Aortic pulse wave analysis](image)

**Fig. 3 Aortic pulse wave analysis.** Using the generated aortic pressure waveform (top panel), the augmentation index (AIx) is calculated by expressing augmentation pressure (AP) as a percentage of the central pulse pressure (cPP). The AP is the additional pressure added to the forward wave by the reflected wave and is defined as the maximum central systolic pressure minus the pressure at the inflection point. Using a physiologic flow waveform (middle panel), the aortic wave can be separated (bottom panel) into its forward (Pf) and backward (Pb) waves, and reflection magnitude (RM) can be computed (Pb/Pf).\(^9^6\)
after assuming a triangular flow wave, and reflection magnitude (RM) is given by Pb/Pf.\textsuperscript{64,96-98} A1x@75 is simply a measure of A1x after being corrected for heart rate deviations. However, because they responded nearly identically, only A1x will be discussed moving forward.

**Power Calculation**

Sample size calculations were based on the primary central vascular health outcomes, aortic pulse wave velocity (PWV). While the effects of prolonged sitting on central vascular health have not been investigated, previous studies have reported that prolonged sitting reduces leg vascular health between 57-80\%.\textsuperscript{36-38,73} Based on a PWV of 6.6 m/s, which is expected for healthy participants <30 y (Reference Values for Arterial Stiffness’ Collaboration 2009), a 57\% decrease in PWV would be 3.8 m/s. For the current study, it was opted to sample based on a conservative change score of 1 m/s. We also opted to use a conservative typical error of 1 m/s.\textsuperscript{74} Using magnitude-based inference, to estimate the sample size required to detect the smallest detrimental (or beneficial) effect in a cross-over study, with the maximum chances of a type 1 and 2 error set at 5\% (i.e. very unlikely), approximately 12 participants are required. However, over-sampling became necessary here because there is no current representative study involving executive function to use as a reference for sample size.

**Statistical Analysis**

All statistical analyses were performed using SPSS 21. First, to test the hypothesis that prolonged sitting results in decreased executive function, data were analyzed using general linear modelling with repeated measures for the 2x3 ANOVA crossover design using the two conditions (CON and HEEL) and three time points throughout the sitting protocol (10,90,170 mins) as the independent variables. The absolute time until completion for both the Word and Color Stroop Tasks were the dependent variables.
To test the hypothesis that cerebral perfusion trends downwards and calf perfusion trends upwards over the course of a prolonged sitting protocol the same statistical model used for executive function was repeated to test perfusion changes in the prefrontal cortex and the calf. Secondary outcomes were also tested using the same model as previously described.

To test the hypothesis that prolonged sitting results in reduced cerebral perfusion, before and after data was analyzed using general linear modelling with a repeated measures 2x2 ANOVA crossover design using the two conditions (CON and HEEL) and two time-points (pre- and post) as the independent variables. This same design was also used to test the hypothesis that vascular health measures would significantly become worse as a result of prolonged sitting.

To test the hypothesis that EF, cerebral perfusion and vascular function are related, Pearson’s correlation tests were used to first assess if changes in EF were associated with changes in cerebral perfusion. A Pearson coefficient ($r$) would be evaluated by using $r$ values of 0.1-0.3 being a small association, 0.3-0.5 being medium, and 0.5-1.0 being large. Since it hypothesized that EF will decline as cerebral perfusion declines, we would expect these values to be negative but of the same magnitude from interpretation.

Then, to test secondary hypotheses, Pearson’s correlation tests will again be performed to assess if important vascular health variables such as PWV, Central Blood Pressures, and Augmentation Index are associated with cerebral perfusion and EF. Each vascular variable and either cerebral perfusion or EF will be tested separately and $r$ values will be evaluated in the same way as stated previously. In addition, to determine if venous pooling in the calf is related to cerebral perfusion and EF, Pearson’s correlation tests will be again performed between venous pooling and both cerebral perfusion and EF.
Lastly, partial eta squared effect sizes were considered in the statistical interpretation of all relevant significant (p<0.05) outcomes where $\eta^2 = .02$, 0.13 and 0.26 represent a small, medium and large effect respectively. Partial eta squared effect sizes were used because partial eta squared is the variance explained by a given variable of the variance remaining after excluding variance explained by other predictors. In this way, the size of the effect of time or of day can be interpreted after the variance explained by one or the other has been factored into the remaining variance. For example, if data between days was different, this variance will be accounted for and statistical analysis will be performed on the variance explained by different time points throughout the experiment.
Chapter 4. Results

Subjects

There was some ethnic diversity, though predominantly Caucasian (n=13). The remaining subjects were African American (n=4), Hispanic (n=1), Middle-eastern (n=1), and Asian (n=1).

Executive Function

There were no interaction effects for executive function as measured by the use of a Stroop Color and Word task. In addition, there was no effect of day on Stroop scores (Table 2).

Table 1. Subject Characteristics

<table>
<thead>
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<th>Mean</th>
<th>SD</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
<td>BMI</td>
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<td>5.3</td>
</tr>
<tr>
<td>Sex (%) Female</td>
<td>70</td>
<td>N/A</td>
</tr>
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</table>

Table 2 – Stroop Completion Time

<table>
<thead>
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<th>Stroop Task</th>
<th>Time (min)</th>
<th>Interaction</th>
<th>Time</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>90</td>
<td>170</td>
<td>P</td>
</tr>
<tr>
<td>Color</td>
<td>39.4</td>
<td>38.6</td>
<td>36.5</td>
<td>0.348</td>
</tr>
<tr>
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<td>9.3</td>
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<td>0.186</td>
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<td>39.4</td>
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<td>38.3</td>
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</tr>
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<td></td>
<td>6.9</td>
<td>5.9</td>
<td>5.3</td>
<td>0.322</td>
</tr>
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<td>Word</td>
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<td>31.7</td>
<td>0.892</td>
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<td>HEEL</td>
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<td>33.1</td>
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<td></td>
<td>5.0</td>
<td>4.9</td>
<td>3.5</td>
<td>0.152</td>
</tr>
</tbody>
</table>

Con Control, Exp Experimental, X mean, SD standard deviation, P p-value, ES effect size
* represents statistically significant main effect (p<0.05).
** represents statistical trend toward a significant main effect (p<0.010).
Cerebral Perfusion

There were no interaction effects for cerebral perfusion (Table 3). Also, cerebral perfusion was not shown to significantly change over the course of the bout of sitting and we fail to reject the null hypothesis that prolonged sitting does not result in decreased cerebral perfusion. However, tHb in the HEEL day was found to be significantly (p=0.009) different from the control day with a mean difference of 2.685 ± 0.919, [CI @95% 0.76, 4.609]. This, and a large effect size, may partially explain the trend (p=0.073) towards significant interaction effects. Although there was some effect of day, because tHb changed in the opposite direction, we fail to reject the null hypothesis that calf raises prevent changes in cerebral perfusion.

Table 3 – Normalized NIRS Forehead Data Trend

<table>
<thead>
<tr>
<th></th>
<th>Forehead</th>
<th>Time</th>
<th>Interaction</th>
<th>Time</th>
<th>Day</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10</td>
<td>90</td>
<td>170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tHb</td>
<td>CON X</td>
<td>1.6</td>
<td>1.1</td>
<td>1.3</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.6</td>
<td>3.7</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HEEL X</td>
<td>0.0</td>
<td>-1.6</td>
<td>-2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>3.3</td>
<td>6.0</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>TSI</td>
<td>CON X</td>
<td>0.2</td>
<td>0.0</td>
<td>0.4</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.5</td>
<td>2.1</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HEEL X</td>
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<td>1.0</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.4</td>
<td>2.6</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>

Con Control, Exp Experimental, X mean, SD standard deviation, P p-value, ES effect size
* represents statistically significant main effect (p<0.05).
** represents statistical trend toward a significant main effect (p<0.010).

Venous Pooling in Lower Extremities

There was no interaction effect for tHb or TSI in the calf. However, blood was shown to accumulate in the calf on both days as there were significant (p=0.002) within-day differences found between the first and second (p=0.012) as well as the second and third (p=0.046) time points for tHb in the calf for CON and HEEL with a mean increase of 2.577 ± 0.922 [CI 95% 0.646,
and 1.655 ± .774 [CI 95% 0.36, 3.275] respectively. In addition, within-day TSI for CON and HEEL was found to be statistically different (p<0.001) from first to second (p<0.001) and first to third time point (p<0.001) with a mean decline of 2.399% ± 0.402% [CI 95% 1.557, 3.24] and 2.863% ± 0.473% [CI 95% 1.872, 3.854] respectively(Table 4, Figures 2-3).

Table 4 – Normalized NIRS Calf Data Trend

<table>
<thead>
<tr>
<th></th>
<th>Time (pre-post)</th>
<th>Interaction</th>
<th>Time</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>90</td>
<td>170</td>
<td>P</td>
</tr>
<tr>
<td>tHb</td>
<td>CON</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>11.4</td>
<td>14.6</td>
<td>12.5</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>6.6</td>
<td>8.5</td>
<td>9.7</td>
</tr>
<tr>
<td>HEEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>10.0</td>
<td>12.0</td>
<td>10.7</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>7.0</td>
<td>7.6</td>
<td>8.5</td>
</tr>
<tr>
<td>TSI</td>
<td>CON</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>-4.3</td>
<td>-7.2</td>
<td>-7.8</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>3.6</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>HEEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>-3.7</td>
<td>-5.6</td>
<td>-5.9</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>2.4</td>
<td>2.9</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Table 4 represents mean change scores over time points 10, 90, 170 mins into sitting for Forehead and Calf.

* represents statistically significant main effect (p<0.05).

** represents statistical trend toward a significant main effect (p<0.010).

In addition, the effect size suggests a large effect of time on both tHb and TSI. For this reason, we fail to reject the null hypothesis that calf raises prevent venous pooling. However, CON declined significantly more than the HEEL from pre- to post and the effect size suggests a large effect of time on TSI (Table 5). These trend data can be found in.

Table 5 – Normalized NIRS Pre-Post

<table>
<thead>
<tr>
<th></th>
<th>Pre-Post</th>
<th>Con</th>
<th>Exp</th>
<th>P</th>
<th>ES</th>
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<tr>
<td>tHb</td>
<td>Forehead</td>
<td></td>
<td></td>
<td>0.483</td>
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<tr>
<td></td>
<td></td>
<td>1.6</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.7</td>
<td>9.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSI</td>
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<td>0.440</td>
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<td></td>
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<td>2.0</td>
<td>8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tHb</td>
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<td></td>
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<td>0.215</td>
<td>-0.345</td>
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<td></td>
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<td>11.2</td>
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<td></td>
<td></td>
<td>10.2</td>
<td>11.6</td>
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<tr>
<td>TSI</td>
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<td>4.9</td>
<td>3.2</td>
<td></td>
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</tr>
</tbody>
</table>

Table 5 represents change scores between the beginning of the sit trial and end.

* represents statistically significant main effect (p<0.05).

** represents statistical trend toward a significant main effect (p<0.010).
Figure 4 – tHb Calf Trend

This graph represents change scores for tHb (µmols y-axis) in the calf over three time points (x-axis). Time point 1, 2, and 3 are approximately 10, 90, 170 minutes into sitting bout.
Vascular Health

There were no interaction effects of any vascular measures over the course of the three time points (10, 90, 170 mins) (Table 6.1) nor from pre-post sitting (Table 6.2). Although, Augmentation Index (AIx) declined over time on each day from 10 minutes in to 90 minutes in by an average of 9.638 ± 1.863 (p<0.001, [CI 95% 5.739, 13.536]) and from 10 to 170 minutes in by 8.236 ± 2.224 (p=0.001, [CI 95% 3.608, 12.917].) The same effect was seen from pre- to post trial with a decrease of 9.14 ± 1.574 (p<0.001, [CI 95@ 5.846, 12.434]). Effect sizes for both pre-post and trend over the course of the study indicates large effects of time on AIx.

Figure 5 – TSI Calf Trend

This graph represents change scores in TSI (%) decreases in the calf over time. 10 minutes into sitting bout is negative because it has changed from zero by about -4%. “A” represents statistical significance (p<0.001) between 10 and 90 minutes. “B” represents statistical significance (p<0.001) between 10 and 170 minutes.
Table 6.1 – Seated Cardiovascular Measurement Trend

<table>
<thead>
<tr>
<th>Variable</th>
<th>Con Control</th>
<th>Heel Experimental</th>
<th>Interaction</th>
<th>Within Day</th>
<th>Between Day</th>
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<td>SBP</td>
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<td>HEEL</td>
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<td>DBP</td>
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<td>0.109</td>
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<tr>
<td>HEEL</td>
<td>66.7</td>
<td>68.7</td>
<td>68.8</td>
<td>0.109</td>
<td>0.219</td>
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<tr>
<td>MAP</td>
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<td>82.0</td>
<td>82.3</td>
<td>0.109</td>
<td>0.219</td>
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<tr>
<td>HEEL</td>
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<td>68.7</td>
<td>68.8</td>
<td>0.109</td>
<td>0.219</td>
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Con control, Heel experimental, SBP systolic blood pressure, DBP diastolic blood pressure, MAP mean arterial pressure, HR heart rate, cSBP central systolic blood pressure, cDBP central diastolic blood pressure, AIx augmentation index, AIx@75 augmentation index @ 75bpm, Pf pressure wave forwards, Pb pressure wave backwards, HF high Frequency, LF low frequency.

* represents statistically significant main effect of time (p<0.05).

** represents statistical trend for main effect of time (p<0.010).
Table 6.2 Supine Cardiovascular Measurement Pre-Post

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PWV Pulse Wave Velocity, BBI Buckberg Index

* represents statistically significant main effect (p<0.05).

** represents statistical trend toward a significant main effect (p<0.010).
Next, the reflected pressure wave represented by Pb decreased from the first (10 min) and second (90 min) time point on average by $1.45 \pm 0.363$ (p<0.001, [CI 95% 0.69, 2.21]) and from the first to third (170 min) time point on average by $1.388 \pm 0.385$, (p<0.002, [CI 95% 0.0583, 2.192]). Similar to other cardiovascular measures, Pb also decreased within day from pre- to post by $1.3 \pm 0.466$ (p=0.012, [CI 95% 0.325, 2.275]). Other vascular measures that are representative of hemodynamic changes such as HR, Alx@75, and RM were also found to be significant. These data are presented in Table 6.1 where the trend over three time points (10, 90, 170 min) were tested. Then, in Table 6.2, data points from before and after the sitting protocol was undertaken are presented as pre-post averages along with their associated ANOVA results.

Finally, PWV increased in the control and HEEL days with an average increase of $0.3 \pm 0.075$, [95% CI 0.143, 0.457]. In addition, the effect size proposes a large effect of time. However, increases in the control day were not significantly different from increases in HEEL.

From these data, our secondary hypotheses cannot be clearly tested because there were no detriments to EF or cerebral perfusion, and calf raises did not seem to have any preventative effect warranting tests of association with systemic vascular measures.

**Associations**

Tests of association were not conducted following the results of previous tests because no significance was found for cerebral perfusion and EF that warranted testing of association.
Chapter 5. Discussion

Summary of Findings

We aimed to provide evidence as to 1) whether or not prolonged sitting resulted in declines in EF and cerebral perfusion, and 2) if change in cerebral perfusion were found, whether they were associated with blood pooling in the calf, and/or impaired vascular function. It was also hypothesized that doing a set of 10 seated calf raises every 10 minutes could be a minimally effective dose to attenuate negative effects that may arise due to prolonged sitting. Several considerations are discussed below to interpret the results of this study.

Executive Function

This is the first study to our knowledge to investigate the effects of prolonged sitting on EF. In this study, there were no main effects of time on Stroop scores and EF was not shown to change as a result of prolonged sitting. However, several considerations regarding the nature of brain measurement should be considered here including age, level of education, cognitive examination, and the assessment of cerebral blood flow. First, subjects’ young age and their level of education may have resulted in an elevated baseline and perhaps partially helped to prevent changes in cerebral perfusion and executive function. Likewise, when investigating the effects of interventions on cognition, controlling for variables that may interfere with cognitive stimulation is a limitation because stimulation is tampered with simply by administering the test. However, because changes in Stroop scores are predominately reactive and higher-level processing is likely not a factor, the level of education, age and over-stimulation should not be large limitations. For cognitive examination, the Stroop was chosen because of its relative simplicity and has been used
in many studies as a measure of executive function. Even so, it may be too simple to generalize to more complicated tasks in the typical work or school day. Also, the Word portion of the stroop is easier and thus more resistant to change, and though the main effect of day trended towards significance, the effect size of this trend ($\eta^2 = 0.191$) is medium, and is small relative to the effect size of significant outcomes in this study such as the effect sizes of the NIRS trend data and AIx in both the pre-post and trend data. For these reasons, it should be interpreted that Stroop scores remained mostly unchanged and the null hypothesis is failed to be rejected.

**Cerebral Perfusion and Calf Pooling**

Previous research from Restaino et al. showed that shear stress was reduced in the popliteal artery after 3 hours of sitting. Shear stress is the frictional force that is caused by blood cells passing over vessel walls and is indicative of adequate blood flow. When shear stress is decreased and stays low, blood flow has likely been disrupted in some way. This may have been caused in this previous study because of increased pooling in the microvasculature of the lower extremities as it was shown that calf circumference increased with an acute bout of sitting. The findings from the current study are consistent with findings from this previous work and may lend further clarity regarding the effects on the microvasculature during a bout of prolonged sitting.

It should be noted that limited data was collected to measure venous pooling as we only had one probe on one portion of the calf and could not look at the lower limbs in their entirety. However, the amount of total Hb showing up in the measurement, is a combination of both oxygenated Hb and deoxygenated Hb that has accumulated in the microvasculature in the center of the muscle belly. This means that blood has already pooled in the large vessels and has then been perfused into the muscle belly. This indication is further supported by the decline in TSI which is an index of the percentage of $O_2$Hb in a given area. Drops in TSI indicate an increase in
deoxygenated Hb because as blood accumulates or pools in the veins and venules in the muscle belly, the oxygen is offloaded from O$_2$Hb into the tissue and deoxygenated Hb then remains in the area. Though NIRS was the only device used, the evidence is overwhelming that venous pooling does occur as represented by increases in tHb and declines in TSI.

It was hypothesized that blood pooling in the legs would lead to a decrease in cerebral perfusion and that heel raises would prevent blood from pooling and thereby prevent decreases in brain blood flow and cerebral perfusion. This is because the muscle contraction of the calf normally does generate enough venous return to prevent blood pooling such as in walking. Although blood was expected to pool in the calf as has been shown to occur before,$^{37,38}$ it was not expected that HEEL would also express significant blood pooling. However, because HEEL demonstrated significant blood pooling in the calf and there were no main effects of day on tHb, venous return was likely not significantly different between days. Though it cannot be determined for certain why this occurred, one possibility is that calf raises increased oxygen demand in the lower extremities prompting increased BF and may help to explain these unexpected findings.

As mentioned, the calf raises during sitting could have elicited a slight increase in BF to the calf muscles resulting in a similar blood pooling effect to sitting alone. For example, BF was measured in the femoral artery via ultrasound in a pilot study prior to the commencement of the current study. Though unpublished, calf raises did indeed increase BF as manifested in the femoral artery using ultrasound (see Appendix E). However if BF increased to the working muscle, but the calf raises were not frequent enough to remove the increased flow of blood, more blood would be coming in than out and increased BF may partially explain pooling similarities between days. Although, the pilot study was not conducted over a 3-hour sitting protocol and it is possible that
time may be a potent variable when considering the effects of prolonged sitting on BF in the lower extremities.

*Arterial Stiffness and PWV*

Though it is not entirely clear how vascular dysfunction acutely effects cerebral perfusion and EF, it is clear that AS did increase as a result of prolonged sitting and may be one critical consequence that could be contributing to long term detriments of sedentary behavior. Likewise, AS was likely increased at the level of the aorta represented by increases in PWV, though aortic stiffness was not accurately represented by decreases in AIx. In addition, PWV was measured 15 minutes after having returned to the supine position and with the large effect of time on PWV, prolonged sitting likely has effects that may not be quickly reversed. Nonetheless, it should be noted that these repeated increases in AS at the aorta as a result of prolonged sitting may be placing additional burden on the heart while sitting.

*Central Hemodynamics and Augmentation Index*

As for other vascular measures, it is clear that a single bout of prolonged sitting does have an effect on central cardiovascular measures. However, it is not clear whether changes in central hemodynamics had any connection to the brain or whether calf raises had any effect on the central cardiovasculature. Of the vascular health findings, AIx and AIx@75 were the most robust findings as both days evidenced decreases as a result of sedentary behavior. So, from pre to post and as a trend throughout the study, the difference between the peak systolic pressure and the peak found at the inflection point, decreased. AIx was not expected to decrease because this typically signifies that an individual’s CV health has improved when measured over time. However, there is speculation regarding the reliability of large negative AIx in estimating wave reflection magnitudes as was the case in this study. Also, in the case of acute measurement, large acute
changes in AIx are likely not healthy regardless of directionality. Though these large changes cannot explain what occurred in the brain in this study, it should be understood that acute vascular dysfunction from prolonged sitting could be leading to poor chronic outcomes.

**Strengths and Limitations**

Three considerations regarding subjects need to be discussed here including BMI, age, and sex. First, the subjects’ average BMI crossed into the overweight category and being overweight is an independent risk factor for CVD,\(^8^1\) subjects were normotensive and free of disease. By using sedentary, overweight subjects, this study more closely represents the population of interest as it has been shown that only 21.7% of Americans meet the ACSM guidelines\(^8^3\) and sedentary individuals are often overweight or obese.\(^8^2\) Second, age is also thought to be an independent risk factor for CVD and for this reason, participants were all young and relatively homogenous with respect to age. Lastly, the majority of subjects were female which should be considered a strength making sample more homogenous. Additionally, most studies around this topic have used predominantly male participants, which makes this study increasingly novel.\(^3^6^-^3^8\)

Lastly, measuring the amount of blood being perfused into only one area of the brain lacks the breadth that something as complicated as the brain might deserve. We lacked the ability to measure cerebral blood flow through the cerebral artery and did not have a trans-cranial doppler to continuously monitor flow to other regions of the brain. Though a limitation, we are confident that if only one NIRS probe can be utilized, using NIRS over pre-frontal cortex as demonstrated by this and other studies,\(^3^1,^7^9\) is the best way to measure cerebral perfusion. Overall, the objective measures taken in this study during a bout of prolonged sitting are a strength to the study as they may be the first of their kind.
Implications

Moving forward, the data presented here further support the increasing interest and body of evidence that being sedentary for even a few hours negatively affects venous return from the legs and subsequently, negatively affects AS and arterial wave reflections. However, it is still unclear how these changes in cardiovascular health effect other areas such as brain function. If nothing else, the repeated bouts of negative responses to prolonged sitting may summate and largely contribute to the known risks accompanied by chronic inactivity. Though our hypotheses regarding cerebral perfusion and EF were not correct, it is clear that chronic inactivity has been linked to poor cognitive outcomes and there are still questions to be answered.

Clarity on this topic may be found in studying other populations, using different cognitive testing strategies with many options to stimulate venous return and reduce blood pooling in the calf. In addition, one bout of prolonged sitting may be too short of a period to connect the chronic cognitive impairments that result from sedentary behavior and acute outcomes that have only recently begun to be investigated. Future research will need to explore other pathways over differing periods of time to further uncover the consequences and accompanied mechanisms of acute sedentary behavior like prolonged sitting.
Chapter 6. Conclusion

These findings indicate that after 3 hours of sitting, executive function measured by the Stroop Task and cerebral perfusion measured by NIRS remain mostly unchanged. However, it is clear that drainage of venous blood in the leg is significantly impaired during a bout of prolonged sitting. Though AS also increased after a bout of prolonged sitting, it is unclear how these changes occur and how they are related to the prefrontal cortex of the brain. However, it is still unknown whether EF will be affected in other populations for different periods of time using alternative methodology. Still, previous findings, combined with findings supported here, bolster the evidence that breaking up sedentary time will be beneficial, improving blood flow in the legs and potentially ward off some of the deleterious effects of sitting.
Appendix A

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<td>Have you ever had:</td>
<td>Have you ever had:</td>
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| STAFF COMMENTS: |

Have you ever had your cholesterol measured? Yes □ No □ If yes, value ___ Where: ___

Are you taking any prescription (include birth control pills) or nonprescription medications? Yes □ No □
For each of your current medications, provide the following information:

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<th>Years on medication</th>
<th>Reason for taking</th>
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HOSPITALIZATIONS: Please list recent hospitalizations (Women: do not list normal pregnancies)

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<tbody>
<tr>
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Any other medical problems/concerns not already identified?  Yes ☐  No ☐  If so, please list:  ____________

LIFESTYLE HABITS

Do you ever have an uncomfortable shortness of breath during exercise or when doing activities?  Yes ☐  No ☐

Do you ever have chest discomfort during exercise?  Yes ☐  No ☐

If so, does it go away with rest?  Yes ☐  No ☐

Do you currently smoke?  Yes ☐  No ☐  If so, what?  Cigarettes ☐  Cigars ☐  Pipe ☐

How long have you smoked?  _______________ years

How much per day:  <½ pack ☐  ½ to 1 pack ☐  1 to 1½ packs ☐  1½ to 2 packs ☐  >2 packs ☐

Have you ever quit smoking?  Yes ☐  No ☐  When?  _______________

How many years and how much did you smoke?  _______________

Do you drink any alcoholic beverages?  Yes ☐  No ☐  If yes, how much in 1 week? (indicate below)

Beer _____ (cans)  Wine _____ (glasses)  Hard liquor _____ (drinks)

Do you drink any caffeinated beverages?  Yes ☐  No ☐  If yes, how much in 1 week? (indicate below)

Coffee _____ (cups)  Tea _____ (glasses)  Soft drinks _____ (cans)

Are you currently following a weight reduction diet plan?  Yes ☐  No ☐

If so, how long have you been dieting?  _____ months

Is the plan prescribed by your doctor?  Yes ☐  No ☐

Have you used weight reduction diets in the past?  Yes ☐  No ☐  If yes, how often and what type?  ____________

ACTIVITY LEVEL EVALUATION

What is your occupational activity level?  Sedentary ☐  Light ☐  Moderate ☐  Heavy ☐

Do you currently engage in vigorous physical activity on a regular basis?  Yes ☐  No ☐

If so, what type(s)?  ___________________________

How many days per week?  ______________________

How much time per day?  <15 min ☐  15-30 min ☐  31-60 min ☐  >60 min ☐

How long have you engaged in this type of activity?  <3 months ☐  3-12 months ☐  >1 year ☐

Do you engage in any recreational or leisure-time physical activities on a regular basis?  Yes ☐  No ☐

If so, what activities?  ___________________________

On average:  How often? ______ times/week;  for how long? ______ time/session

How long have you engaged in this type of activity?  <3 months 3-12 months >1 year

Your fitness goals and objectives are:  ______________

STAFF COMMENTS:  ______________

44
Subject: __________________________ Telephone: ______________

Address: __________________________________________________________________

Email: ___________________________ Age: __________________________

Patient History

1. How would you describe your general health at present? YES NO
2. Excellent______ Good_______ Fair______ Poor______
3. Do you have any health problems at the present time? _____ _____
4. If yes, please describe: ____________________________________________

5. Have you ever been told you have heart trouble? _____ _____
6. If yes, please describe: ____________________________________________

7. Do you ever get pain in your chest? _____ _____
8. Do you ever feel light-headed or have you ever fainted? _____ _____
9. If yes, please describe: ____________________________________________

10. Have you ever been told that you have high blood pressure? _____ _____
11. If yes, please describe: ____________________________________________

12. Have you ever had difficulty breathing at rest or with exertion? _____ _____
13. If yes, please describe: ____________________________________________

14. Have you ever been treated for infectious mononucleosis, hepatitis, pneumonia, or another infectious disease during the past year? _____ _____
15. If yes, name the disease: ___________________________________________
16. Have you ever been treated for or told you might have diabetes? _____ _____
17. Have you ever been treated for low blood sugar? _____ _____
18. Have you ever experienced heat stroke or heat exhaustion? 

19. If yes, when?

20. Are you now taking any pills, medications, or supplements? 

21. If yes, please list:

22. Have you had any recent (within 1 year) difficulties with your:
   a. Feet
   b. Legs
   c. Back

23. What was the start date of your most recent menstrual cycle?

24. Has anyone in your family (grandparent, father, mother, and/or sibling) experienced any of the following?
   a. Sudden death
   b. Cardiac disease
   c. Marfan’s syndrome

25. Have you ever been treated for Osgood-Schlatter’s disease?

26. Have you ever had any injury to your neck involving nerves or vertebrae?

27. Do you experience pain in your back?

28. Have you ever had an injury to your back?

29. If yes, did you seek the advice of a doctor?

30. Have you ever been told that you injured the ligaments or cartilage of either knee joint?

31. Do you think you have a trick knee?

32. Do you have a pin, screw, or plate anywhere in your body as the result of bone or joint surgery that presently limits your physical capacity?

33. If yes, indicate where:

34. During your early childhood (to age 12) would you say you were:
   Very active _____ Quite active_____ Moderately active_____ Seldom active_____
35. During your adolescent years (age 13-18) would you say you were:
   Very active ____ Quite active____ Moderately active____ Seldom active____

36. Did you participate in:
   a. Intramural high school sports? _______ _______
   b. Community sponsored sports? _______ _______
   c. Varsity high school sports? _______ _______
   d. Active family recreation? _______ _______

37. Since leaving high school, how active have you been?
   Very active ____ Quite active____ Active____ Inactive____

38. Have you previous participated in strength training ____ _____

39. Do you participate in any moderate to vigorous activity at present? ____ _____

40. If yes, please list:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency</th>
<th>Duration</th>
<th>Intensity</th>
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41. Whom shall we notify in case of emergency?
   Name: __________________________________________________________

   Phone: (Home)____________________________ (Work)________________________

   Signature: ________________________________ Date: ____________________
Appendix B

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants

Consent Form Version Date: ____________
IRB Study #: 16-3051
Title of Study: Effects of Prolonged Sitting on Cerebral Perfusion and Executive Function
Principal Investigator: Quentin Willey
Principal Investigator Department: Exercise and Sport Science
Principal Investigator Phone number: (919) 962-0396
Principal Investigator Email Address: qwilley@live.unc.edu
Co-Investigators: Erik Hanson, Claudio Battaglini, William Evans

Faculty Advisor: Lee Stoner
Faculty Advisor Contact Information: (919) 962-0534

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
The purpose of the current study is to examine the acute effects of prolonged sitting on cardiovascular health and cognition. Findings from this study may identify a simple strategy for offsetting the negative consequences of sitting, and may contribute to public health policy pertaining to sedentary behavior.

You are being asked to be in this study because you are between the ages of 18-35, and are not engaging in 90 minutes of moderate intensity activity or 30 minutes of vigorous activity per week.

Are there any reasons you should not be in this study?
You should not be in this study if you are/have:

- Diabetes
- Heart Disease
- Atherosclerosis
- Arrhythmias
- Taking medications known to affect cardiovascular function
- Smoking cigarettes
- Pregnant

How many people will take part in this study?
There will be approximately 20 people in this research study at UNC-Chapel Hill.

How long will your part in this study last?
Should you wish to participate in the study, you will be required to attend Fetzer Hall for three visits. These visits will include an initial 30 minute visit to familiarize you, followed by two additional 4 hour visits.

What will happen if you take part in the study?

During visit one, participants will report to the UNC EXSS Laboratory where we will discuss the study with you. You will be screened for participation in the study which will include a medical history questionnaire. You will be fitted for a small probe and a chair. You will also take a cognitive test 7 to 10 times or until your scores normalize. Lastly, you will sit while measurements are taken during which you perform several sets of 10 calf raises.

During visits two and three a cannula, similar to an IV, will be inserted. You will lie for 20 minutes. After this, vascular health measures will be measured and 30mL of blood will be drawn. You will then be transferred to a sitting position, where you will remain for 180 minutes while watching a low-stimulus nature documentary. A probe will non-invasively measure brain blood flow continuously during this time, and after 10, 90 and 170 minutes of sitting, non-invasive vascular function and executive function will be measured. At 180 minutes, you will then be transferred to the supine position. Following 10 minutes of quiet rest, vascular health will be non-invasively measured again. Although you will use the restroom prior to sitting, if you need to use the restroom, this will be recorded, and you will be asked to repeat this movement for the subsequent visit. Visit
two and three will be the same procedure, but you will be doing 10 calf raises to a metronome every 10 minutes in one of these visits.

**What are the possible benefits from being in this study?**
Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study.

**What are the possible risks or discomforts involved from being in this study?**
While in this study, blood will be collected. This requires an initial needle stick which may be uncomfortable and could cause bruising.

There may be uncommon or previously unknown risks. You should report any problems to the researcher.

A Urine Pregnancy test provided by the study will be obtained for all women of child-bearing potential.

**What if we learn about new findings or information during the study?**
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

**How will information about you be protected?**
Your identity will be confidential and protected through the use of identification numbers. Additionally, all measurements will be collected in a private setting with access to the laboratory behind several secure doors. Identification numbers will be assigned to attached data and stored in a locked filing cabinet in the EORL, which only Quentin Willey and William Evans will have access to. Your identification number and associated data will only be accessible to the research team. All information uploaded to an external hard drive will be encrypted.

Participants will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

**What will happen if you are injured by this research?**
In the occurrence of a rare adverse event, all members of the research team are CPR/AED certified so that they can provide the proper care to the Participant. A member of the research team will be with the Participant the whole time while in the neuromuscular research lab and 1-2 members of the research team will be present during each exercise test. If deemed necessary,
emergency medical services will be contacted.

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. You do not give up any of your legal rights by signing this form.

**What if you want to stop before your part in the study is complete?**

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

**Will you receive anything for being in this study?**

You will be receiving measures of cardiovascular health reports for taking part in this study. Otherwise, there will be no compensation for study participation.

**Will it cost you anything to be in this study?**

If you enroll in this study, you will not have any associated costs.

**What if you have questions about this study?**

You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

**What if you have questions about your rights as a research participant?**

All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.
**Participant’s Agreement:**

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_______________________________  ________________________
Signature of Research Participant   Date

_______________________________
Printed Name of Research Participant

_______________________________  ________________________
Signature of Research Team Member Obtaining Consent   Date

_______________________________
Printed Name of Research Team Member Obtaining Consent
Appendix C

NIRS Optode Positions

**Cerebral:**

- Positioned at FP1 and FP2.
- Place elastic band on head.
- Measure Nz to Lz (approx. 36cm).
- Mark distance of 10% upwards for Nz and Lz, these are Fpz and Iz.
- Move elastic band onto 10% line.
- Measure circumference at 10% line (Fpz to Iz, approx. 56 cm).
- Measure and mark 5% of total circumference to left and right of Fpz and mark.
- These are Fp1 (LEFT) and Fp2 (RIGHT).

*Position probe 1 on Fp1 and probe 2 on Fp2.*

**Gastrocnemius:**

- Positioned bilaterally on the medial gastrocnemius belly.
- Ask patient to stand against bed and move on to tip toes (if possible)
- Identify outer edge of muscle. Identify muscle belly and mark with dot.
  (This is just a preliminary identification to help with initial template placement.)

- Ask participant to sit on a bench or table high enough that their leg is relaxed and suspended with approximately 90° between calf and thigh.
- Next, find the joint line between the femur and the tibia on the medial side. Follow that joint line by palpation laterally towards the patella. Mark the point at which the joint line and the patella first intersect with one dot.
- Then, palpate the medial malleolus and find an approximate center and mark another dot.
• Between the two dots described above, use a flexible meter stick to mark a straight line at the level of the gastrocnemius that if continued would intersect with each dot (Image A).

• The line just drawn will be used as a base for the Calf ROI Stencil. Place the edge of the stencil on the line and make sure the slots of the stencil are parallel to the leg and on top of the medial gastrocnemius muscle belly (Image B).

• Mark the bottom edge of the stencil by making a perpendicular line with the line already marked on the participant’s leg. Then, measure and record the distance (cm) between the line at the bottom of the stencil to the dot placed in the center of the medial malleolus. This distance will be used on this participant in future visits.

• Finally, there will be 10 slots labeled on the stencil to mark where the NIRS probe will be secured. Two or three slots will need to be marked on the first visit in order to find the best NIRS placement. The best placement will be a relatively flat surface on the medial gastrocnemius.

• Note which slots were marked, which slot mark will be used, and on which side of the mark the NIRS will be placed (+ = towards higher number or - towards lower number).

• Remove any slot markings not being used from skin.

*Position probe 3 on left and probe 4 on right.
Appendix D

These are ultrasound snap shots taken at the region of interest as described in Appendix C. The red line represents the point at which the third NIRS probe (T3) is said to retrieve the reflected infra-red light from. The blue lines represent the target muscle belly of the medial gastrocnemius.
Appendix E

The cartoon (below) is a representation of how the caliber was utilized. This measure was taken while subjects lay supine and was conducted before and after the bout of sitting. Pictured below next is the actual caliper used.

FIGURE 1 Diagram showing distance measurements on the body for carotid-femoral pulse wave velocity calculation and the additional segment measured when using a cuff to acquire the femoral pulse \( d_{FTC} \). The linear distances \( d \) were measured between the suprasternal notch (S), the site wherein the carotid pulse could be palpated (C), the site wherein the femoral pulse could be palpated and measured with a tonometer (FT), and the top of the thigh cuff approximately above the location of the femoral artery (FC).
**Appendix G**

**Table 1. BF in Femoral Artery Sitting with Heel Raises**

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<th>SSAve</th>
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**Graphs:**
- Antegrade Shear Stress over Time (minutes)
- Antegrade Shear Stress with time (Minutes)
In the calculation of the TSI, the scattering coefficient of the tissue is needed. The reduced scattering coefficient $\mu_s$ is related to the distance that the photons travel before being scattered. In tissue, $\mu_s$ is found to scale linearly with the applied wavelength. The scattering coefficient is defined as $\mu_s = k(1 - h \lambda)$ and is measured on various kind of tissue by several research groups. The values of $k$ and $h$ found by Matcher et al. (1997) are given in Table 3. In Oxysoft, the scattering coefficient is approximated as being constant over time and the values of $k$ and $h$ can be set.

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<td>Calf</td>
<td>1.63</td>
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REFERENCES


10. http://www.heart.org/HEARTORG/HealthyLiving/PhysicalActivity/StartWalking/The-Price-of-Inactivity_UCM_307974_Article.jsp#.WRYNFIjytPY


