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Impact of Prolonged Sitting on Cognitive Function: Implications for Cardio-metabolic Risk

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IMPACT OF PROLONGED SITTING ON COGNITIVE FUNCTION: IMPLICATIONS FOR CARDIO-METABOLIC RISK

by

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ABSTRACT

Purpose: To determine if prolonged sitting negatively impacts cognitive function, cerebral perfusion, and central cardiovascular hemodynamics; and secondly, to test whether weight or physical activity status alters this response. **Methods:** Participants (N=20, age=26±7; BMI=30±7 kg/m^2; 7 female) were taken through 3 hrs of sitting followed by a 10-min walk (treadmill). Cognitive function was assessed during sitting (10, 60, 120, and 180 mins) and following the walk using a color/word Stroop test. Cerebral perfusion was measured via near infrared spectroscopy (total hemoglobin—tHb). Central cardiovascular hemodynamics and aortic stiffness (pulse wave velocity—PWV) were measured using the SphygmoCor XCEL device before, during and after sitting. Body mass index, %-bodyfat, and accelerometry data were used to characterize weight and physical activity status. **Results**: Following sitting, there was no change in Stroop completion time; however, both Color and Text times significantly decreased following the walk (e.g., Color Time: 10 mins sitting, 19±3 sec vs. Post walk, 16.7±3.6 sec, $p<0.001$). A similar finding was noted for change in reaction time ($p=0.036$). Cerebral perfusion did not change during sitting, but increased during the walk (180 mins sitting=415±38 μM vs. 10 min walk=432±42 μM; $p<0.001$). There was a significant increase in PWV for normal weight (Pre-sit=5.7±1 vs. Post-sit=6.1±1.1 m/s, $p=0.009$), but not in participants classified obese ($p=0.02$). **Conclusion:** These findings suggest that prolonged sitting does not alter cognitive function or cerebral perfusion, but sitting does increase aortic stiffness in normal weight individuals. Walking can improve cognitive function, an effect possibly related to increases in cerebral perfusion.

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I would like to formally thank Dr. Daniel Credeur for his assistance and guidance with this thesis project. I would also like to extend my appreciation for Dr. Stephanie McCoy, Dr. David Dolbow, and Dr. Scott Piland for their support, mentorship and service as members on my thesis committee. This research was funded by the American Kinesiotherapy Association through grant number GR05488.

DEDICATION

I would like to dedicate this to my family and friends, whose support and encouragement were helpful in completing this thesis project.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AP	Augmentation Pressure
BMI	Body Mass Index
BP	Blood Pressure
cDBP	Central Diastolic Blood Pressure
cMAP	Central Mean Atrial Pressure
cPP	Central Pulse Pressure
cSBP	Central Systolic Blood Pressure
CVD	Cardiovascular Disease
deoxyHb	Deoxygenated Hemoglobin
ECG	Electrocardiogram
Hb	Oxygenated Hemoglobin
HR	Heart Rate
MRI	Magnetic Resonance Imaging
NIRS	Near-Infrared Spectroscopy
Pb	Backward Pressure Wave
Pf	Forward Pressure Wave
PWA	Pulse Wave Analysis
PWV	Pulse Wave Velocity
RM%	Refection Magnitude
SRS	Spatially Resolved Spectroscopy
tHb	Total Hemoglobin

CHAPTER I – INTRODUCTION

In society today, there are several circumstances, which may lead someone to adopt a sedentary lifestyle; such as occupation, convenience of personal devices, televisions, self-automated home appliances, and vehicle transportation. Consequently, a majority of adults in the U.S. do not meet the current physical activity recommendations (i.e., a minimum of 150 mins of moderate intensity physical activity per week [1,2,3,4]). Importantly, national maps created by the Centers for Disease Control and Prevention demonstrate that levels of daily physical activity appear to negatively correlate with the prevalence of obesity, type-2 diabetes mellitus and cardiovascular disease (CVD) [5]. This link between sedentary behavior and cardiovascular risk has been documented for several years. In 1966, Morris conducted a study that examined the impact of work-related physical activity on ischemic heart disease complications and mortality by observing individuals who worked for the double-decker bus system in London. Unique to this time, these data demonstrated that sedentary drivers were more prone to having a fatal myocardial infarction as compared to the more active conductors [6].

With technological advances, several occupations are performed by sitting for extended periods of time in front of a computer [7,8]. Further, it is now well-documented that individuals who remain in the seated position for prolonged periods of time develop complications, such as increased blood pressure (BP), increased heart rate (HR) and other risk factors of CVD [7,9,10]. The underlying physiological mechanisms mediating this response are unclear; however, recent evidence has purported that prolonged sitting can also negatively affect peripheral vascular health, specifically, vasodilatory capacity of large arteries in the legs [8,11] which appears to be the result of blood pooling, with

subsequent reductions in blood flow-induced shear stress on the artery wall [12]. From a public health standpoint, these findings are important because more than 8 million Americans suffer from peripheral artery disease which is largely the result of a sedentary lifestyle with too much sitting [13]. Thus, it is imperative for researchers to study the cardiovascular responses to prolonged sitting, both acutely and chronically.

Recent evidence demonstrates that fidgeting or heating of a limb, or interrupting prolonged bouts of sitting with a brief walk can revert some of the negative peripheral vascular responses associated with inactivity [10,13]. Currently, it is unknown whether these sitting-induced impairments in vascular endothelial health and venous pooling in the legs contributes to alterations in hemodynamics of other important organ systems (i.e., heart and brain).

Several studies demonstrate that there is a direct correlation between physical activity and cognitive function [14,15,16]. Low to moderate intensity physical activity has been shown to increase cognitive function and cerebral blood flow [17,18,19]. Cognitive function or more specifically, executive function, is the ability to process information provided by a stimulus and come to a decision based on information provided, which is a regular daily task [20]. There seems to be a correlation between increases in cerebral perfusion and improvements in cognitive function [21]. In light of this, one study demonstrated, in elderly women from France (ages 60-77 years) deemed to have high aerobic fitness, that increases in perfusion and oxygenation to the pre-frontal cortex during executive functioning tasks were greater than matched individuals with lower aerobic fitness [20]. Importantly, the higher fit group also showed greater overall activation of the brain when performing increasingly difficult executive functioning tasks

[20]. With the abundant research on the relation of physical activity and cognitive function, it is still unclear whether inactivity, such as prolonged sitting or sedentary behavior, could have an opposing effect.

Purpose

Given this background, the purpose of this study was two-fold: 1) to determine whether a single bout of prolonged, uninterrupted sitting (3 hours) affects cognitive function and cerebral perfusion; and 2) to determine whether pre-existing morbidities (e.g., presence of obesity or low levels of daily physical activity) amplifies the negative cardiovascular and cognitive health effects associated with prolonged sitting. Using a pre-test, with multiple post-tests study design, we hypothesized that prolonged sitting would increase central BP and vascular stiffening, decrease cerebral perfusion, and subsequently, reduce cognitive function. Secondly, we hypothesized that this response would be greater in participants classified as obese, and physically inactive.

CHAPTER II - METHODOLOGY

Subjects

Twenty volunteer subjects (7 female; age 26±7 years) participated in this study. All participants were recruited on the University of Southern Mississippi Campus and surrounding Hattiesburg, Mississippi area. A goal was to recruit a heterogeneous cohort that was representative of the U.S. demographic; consisting of men and women, and individuals of different ethnic and racial backgrounds (US Census 2016: 50% Female, 17% Hispanic or Latino, 13% Black, 5% Asian, 61% White [22]). All participants provided written informed consent after being presented a detailed explanation of experimental procedures and measures prior to participation in any data collection. Inclusion for participation consisted of the following: ages between 18-55 years old, Body Mass Index (BMI) >30 and less than 150 min of physical activity per week for individuals classified as obese and inactive. Obesity was determined by a combination of BMI and percent body fat (bioelectrical impedance analysis). An accelerometer was used to determine daily physical activity levels over the course of one week. Exclusion for participation was elevated resting blood pressure (>140/90), possible arrhythmias and any diagnosed cardiovascular, metabolic or neurological diseases. All experimental procedures and protocols were reviewed and approved by the University of Southern Mississippi Interstitial Review Board (Protocol Approval #16061301).

Experimental Measures

Cognitive Function Testing. A Stroop Word-Color Test was administered on a laptop computer to assess overall cognitive function (Xavier Educational Software, Bangor, Gwynedd). Specifically, the Stroop test is a widely used assessment of executive

function, through measuring selective attention [21, 23, 24, 25, 26, 27]. Executive function assessments, such as the Stroop, have been shown to be sensitive to changes in physical activity status (e.g., acute exercise) [15]; therefore, we posited that Stroop performance would also be equally sensitive to prolonged sitting. Previous reports have demonstrated strong correlations between test-retest Stroop scores (i.e., Pearson correlations for completion times between trials 1-2 and 2-3 were 0.91 and 0.94, respectively), such that a learning effect, while small, appears to plateau after only 3 trials [28]. In light of this evidence, the participants of the present study were required to perform 2 practice trials prior to the experimental protocol to lessen the learning effect across study time points.

For each Stroop trial, the words blue, red, yellow, and green appeared on a laptop computer screen, and the participants were asked to identify them as quickly as possible using the mouse pad. Color identification was either congruent (Color Version) or incongruent (Text Version) with the word. For instance, in the *Text* version, the participant would select the color that the word signifies, not the actual color (e.g., if word red is printed as blue text, then participant selects red from color cog-wheel); whereas, in the *Color* version, participants selected the actual color of the word, independent of the text (e.g., word red printed as blue text, participant selects blue from color cog-wheel).

Cerebral Perfusion. Cerebral perfusion was measured using a continuous-wave near infrared spectroscopy (NIRS) device (Portalite, Artinis, Netherlands). The NIRS probe measures perfusion and relative changes in total hemoglobin [20]. To do this, the probe emits an infrared light, which passes through skin, adipose tissue, muscle and bone.

A receiver registers the absorbance of light waves passed through the adjacent tissues continuously in real-time. The probe is programmed to determine the light wavelength absorbance spectra for oxygenated hemoglobin (Hb), deoxygenated hemoglobin (deoxyHb), and total hemoglobin (tHb). These wavelengths are 770, 848, and 901 nm that are then applied to the Lambert-Beer Law, which allows for calculations of optical density [29]. However the signal is vulnerable to light scattering due to the tissue being a non-homogenous medium. For this reason, we chose to use the spatially resolved spectroscopy (SRS) signal for our calculations. SRS takes in to account the light scattering and from this can obtain an absolute hemoglobin concentration [30]. For the experimental protocol, the NIRS probe was positioned on the forehead, approximately 3 cm to the right of center, directly over the eyebrow. The rationale for this placement was based off the international Electroencephalography 10-20 system for brain mapping and placement of electrodes for measuring the pre-frontal cortex [20]. Importantly, perfusion changes within this region have been shown to correlate to executive functioning, as denoted by perfusion changes via MRI scanning during Stroop testing [31].

Central Cardiovascular Hemodynamics. Central cardiovascular hemodynamics and aortic vascular stiffness were measured via automated sphygmanometry and applanation tonometry, respectively (SphygmoCor XCEL, AtCor Medical, Itasca, Illinois). To do this, a standard blood pressure cuff was positioned around the subject's left upper arm at heart level. The XCEL system first performs a standard blood pressure measure, and then subsequently inflates to the sub-systolic pressure to determine the brachial pressure waveform (oscillometric method), and then, wave decomposition is performed via pulse wave analysis (details outlined in *Data Analysis* below). Carotid-

femoral artery pulse transit time (sec) was determined via applanation tonometry performed on the left common carotid artery, coupled with the oscillometric method performed over the left upper thigh. Transit time (Δt) was calculated as the time interval between the diastolic foot of the pressure wave from the tonometer probe and the thigh cuff arterial waveform recording. Prior to this, distance measurements (m) between the carotid pulse site and sternal notch (*L1*), and sternal notch to proximal edge of the thigh cuff (*L2*) were obtained using a specialized caliper. Along with pulse transit time, distance was applied to the following equation to calculate aortic Pulse Wave Velocity (aPWV) which is considered the gold standard for measuring arterial stiffness [32]:

$$\text{aPWV (m/s)} = \textit{Length (L2 – L1)} / \Delta t$$

Venous Pooling. Venous pooling was estimated by examining calf circumference changes over the course of the sitting intervention. To do this, a weighted tape measure was stretched and positioned around the largest portion of the right calf. A small mark was made at this site to ensure consistency between measurements performed during the study.

Physical Activity Status. To determine physical activity status, an accelerometer (Actigraph, wGT3X-BT, Pensacola, Florida) was worn by each participant. The Actigraph is a motion-censored device that records frequency, intensity, and duration of activity throughout the day while also monitoring time [33]. Following the study, participants wore the device for at least 8 waking hours on 7 consecutive days. A log was provided to record any time the device was removed during those waking hours. Due to

technical issues, one participant had only 3 days' of data obtained, and one subject had only one days' worth of accelerometer data.

Additional Cardiovascular Measures. Heart rate (HR), rhythm, and R-R variability were monitored continuously throughout the study using a 3-lead surface electrocardiograph (Lead II) (Powerlab Bioamp, AD-Instruments, Dunedin, Otago).

Experimental Protocol

Each experimental study visit lasted ~5 hours. All participants arrived to the lab within the School of Kinesiology in the morning (between 8:00-11:00 am). Participants were asked to eat a light breakfast and refrain from caffeine intake for at least 2 hours, and alcohol and strenuous physical activity at least 24 hours prior to arrival. After providing written informed consent, participants completed a detailed a medical health history form, and leisure activity questionnaire, and then performed 2 practice trials for the Stroop test familiarization. Height, weight, and body composition (%-fat and lean mass) were measured prior to baseline testing using a stadiometer and InBody-720 bioelectrical impedance monitor (InBody, South Korea). Following these initial measurements, the participants were positioned supine on the examine table and instrumented for the experimental measurements (see **Figure 1** for study protocol timeline). Following 15 mins of quiet rest, lights were dimmed and baseline testing commenced. After the initial baseline measurements, subjects were repositioned upright in a chair next to the examine table. Laboratory timers were started and the participants remained in this positioned for 3 hours. Within the first 10 mins of sitting, cardiovascular (ECG and PWA), calf circumference, cerebral perfusion (NIRS) and baseline cognitive function testing (Stroop; One Text and Color trial performed, randomized by chance) was

performed, and repeated every hour during sitting (60, 120, and 180 mins). Following the 180 min sitting time point, participants were then hoisted using a mechanical lift (Invacare Reliant 450, Invacare, Elyria, Ohio) and repositioned supine on the exam table. Posting sitting measurements were performed, proceeded by a 10 min walk on treadmill (3 mph, 0 grade) in an adjacent lab, and finally, one last cognitive function assessment (performed again in seated position).

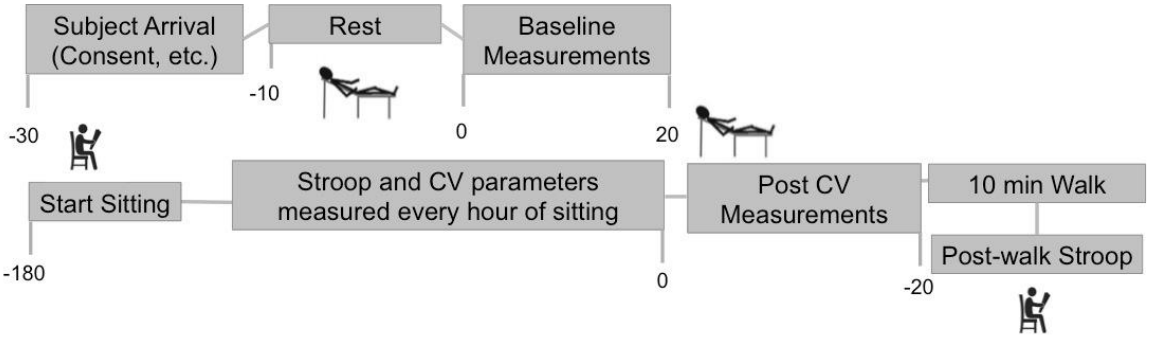

Figure 1. Protocol timeline for experimental measurements.

Data Analysis

Following data collection, all information was recorded and compiled on a spreadsheet (Excel, Microsoft). The Stroop test records accuracy, mean reaction time, and total test time. These absolute data values were compared between pre- and post-sitting time points, and also over the course of the sitting intervention (10-180 mins). For cerebral perfusion, these data were analyzed first as a temporal profile over the course of the entire protocol (i.e., before, during, and after sitting, and 10 min walk). Secondly, cerebral perfusion was averaged during each Stroop version (~50 sec). Lastly, the change in cerebral perfusion was calculated as the difference in tHb immediately prior (30 sec

pre-stimulus average), subtracted from the peak tHb response during each Stroop version. All NIRS data were analyzed off-line using Oxysoft software (Portalite, Artinis, Netherlands).

For pulse wave analysis, oscillometric pressure waveforms were recorded and analyzed by the SphygmoCor XCEL device [34]. Each measurement cycle (pre- and post-sitting, and during each sitting time point) lasted ~60 sec, consisting of a brachial BP recording and then a 10-sec sub-systolic recording. An aortic pressure waveform was then generated by the device using a validated transfer function, [35] from which the following central hemodynamic indices were derived: central systolic BP, diastolic BP, pulse pressure, augmentation pressure, augmentation index, forward and backward pressure components (Pf and Pb), and reflection magnitude (RM%). The Augmentation Index is defined as augmentation pressure (AP) expressed as a percentage of total pulse pressure, where AP is defined as the maximum systolic pressure, minus the pressure at the inflection point. The generalized aortic pressure waveform is then decomposed into its forward—Pf and backward—Pb wave components by assuming a triangular flow wave [36,37]. This methodology generates a triangular-shaped wave by matching start, peak, and flow wave end to the timings of the foot, inflection point, and the incisura of aortic pressure wave. The Pb and Pf are then constructed with the following equations $Pf = [P + Zc \times Q]/2$ and $Pb = [P - Zc \times Q]/2$, respectively, where P is the aortic pressure wave, Q is the approximated pseudo-flow wave, Zc is the characteristic impedance. The RM% was calculated as Pb/Pf*100.

All other cardiovascular data, i.e., heart rate and heart rate variability—HRV, were acquired and analysed using Powerlab and LabChart software (AD-Instruments,

Colorado Spring, CO.), respectively. For HRV, both time (e.g., standard deviation of R-R intervals) and frequency domain indices (power spectral analysis) were examined to provide an index of overall autonomic balance (i.e., sympathetic and vagal input to heart rate), and performed in accordance with published guidelines [38].

Statistical Analysis

Assuming a moderate-large effect for changes in primary dependent variables (e.g., Stroop scores, cerebral perfusion and central blood pressure), two-tailed alpha ≥ 0.05, and 80% power, 20 subjects were required for the following analyses: 1) To test the hypothesis that prolonged sitting increases central blood pressure and vascular stiffening, and decreases cerebral perfusion and cognitive function, separate one-way analysis of variances (ANOVA) were performed on dependent variables; 2) To test the exploratory hypotheses, that participants classified as obese and physically inactive would exhibit a larger negative cardiovascular health and cognitive function response to sitting, separate two-way (time*group—active vs. inactive, and normal weight vs. obese) repeated measures ANOVAs were performed on dependent variables. In the event of a significant interaction, Bonferroni post-hoc testing was performed. Normality of was checked using a Shapiro-Wilk test. If non-normal data distributions occurred, non-parametric testing was performed using the Friedman Repeated Measures ANOVA on Ranks test, with Tukey's pairwise multiple comparison procedure selected as a post-hoc test. All statistical analyses were performed using Sigma-Plot Analysis software (Version 12.0, San Jose, CA) and SPSS (IBM, Watson Analytics). Statistical significance was set *a priori* at $p<0.05$. Data are presented as mean±standard deviation or as specified otherwise.

CHAPTER III - RESULTS

Subjects

Subject characteristics are summarized in **Table 1**. Average age and BMI for subjects were 26±7 years and 30±7 kg/m^2, respectively. Within this cohort, 65% of the subjects were males, 35% females, 35% identified themselves as black, 55% white, and 10% Hispanic or Latino.

Table 1 *Subject Characteristics.*

(N= 20 Male 13, Female 7)	
Age (yrs)	26 ± 7
BMI (kg/m^2)	30 ± 7
Body Fat (%)	28 ± 11
Lean Mass (%)	72 ± 11
Body Fat (lbs)	57.4 ± 35.5
Lean Mass (lbs)	139 ± 31

Cognitive Function

At start of sitting, average Stroop test completion time, reaction time for each trial, and accuracy for the Color and Text versions were 19±3 and 19±4 sec, 1.19±0.19 and 1.2±0.24 sec, and 98.3±4.5 and 99.1±2.8%, respectively. Over the course of sitting, there was no significant change in Stroop test completion time for either version; however, both Color and Text completion times significantly decreased from sitting following the 10-min walk (e.g., Color Time: 10 mins sitting, 19±3 sec vs. Post walk, 16.7±3.6 sec, $p<0.001$) (**Figure 2A**). A similar finding was noted for changes in mean reaction time on the Color Version ($p=0.036$), but a non-significant tendency for improvement in reaction time for the Text version from sitting to following the 10 min

walk (p=0.094) (**Figure 2B**). No significant changes were noted on performance accuracy for any time point (**Figure 2C**).

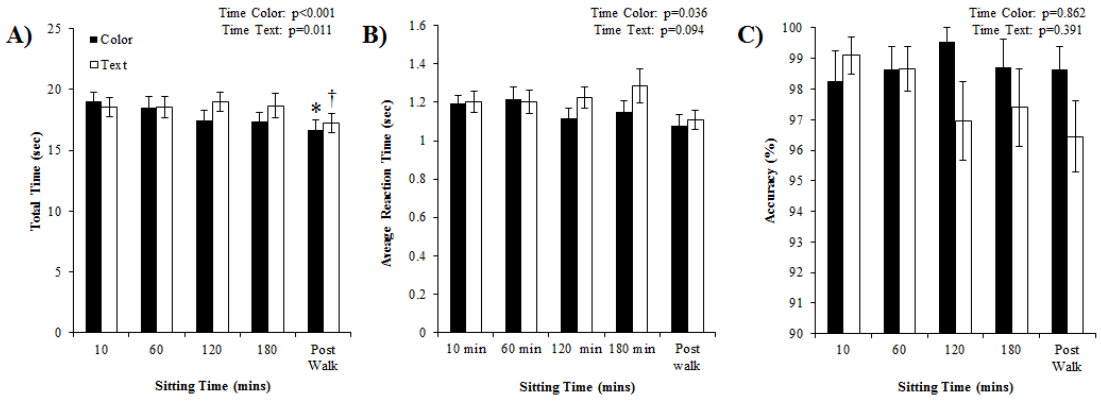

Figure 2. Impact of Sitting on Cognitive Function.

Total time to complete each Stroop version (Panel A), the average reaction time of trials (Panel B), and accuracy (Panel C) through the sitting intervention and post walking period are illustrated. **Denotes p<0.05 vs. 10 min sitting time point; †p<0.05 vs. 120 min sitting time point.*

Cerebral Perfusion

Prior to sitting, cerebral perfusion (i.e., tHb from SRS signal measured supine) was 391±49 μM. At start of sitting, perfusion increased (p<0.05) and was maintained throughout the sitting intervention and into the post study time point. During the 10 min walk, cerebral perfusion experienced a secondary increase (180 mins of sitting=415±38 μM vs. 10 min walk=432±42 μM; p<0.001), a level that was maintained into the post walking time point (**Figure 3A**). During cognitive function testing, there was no difference in cerebral perfusion when averaged over each version (Color and Text) (**Figure 3B**). When expressed as a change score (i.e., difference in peak response during Stroop version vs. rest immediately prior), there was a significant increase in cerebral

perfusion from sitting following the 10 min walk (e.g., Text version: 180 mins of sitting Δ=1.1±8 μM vs. post walk Δ=9.2±9.4 μM, p=0.008) (**Figure 3C**).

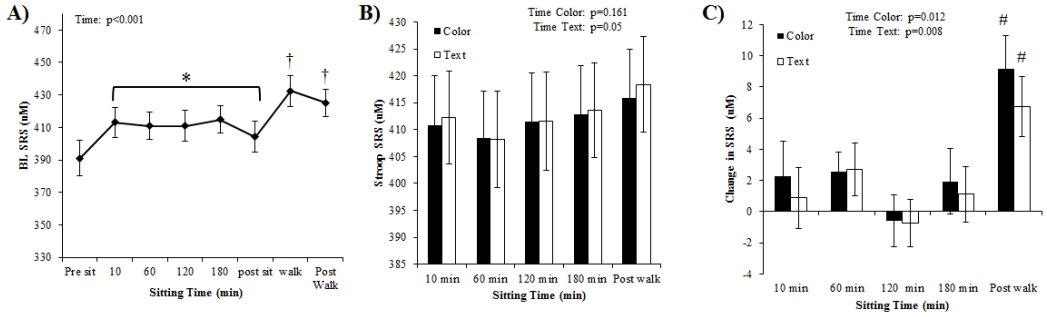

Figure 3. Impact of Sitting on Cerebral Perfusion.

Total perfusion profile (Panel A), the perfusion during Stroop versions (Panel B), and change in perfusion (Panel C) throughout the sitting intervention and post walking period are illustrated. **Denotes p<0.05 vs. Pre sit; †p<0.05 vs. Pre sit, and all sitting time points; #p<0.05 vs. 180 min sitting time point.*

Central Cardiovascular Hemodynamics

Central cardiovascular hemodynamic data are presented in **Table 2.** From pre- to post-sitting time points (supine position) there was a significant decrease in resting heart rate, augmentation pressure, and augmentation index (p<0.001). There was a significant increase in aortic PWV (Pre-sit=5.7±1 vs. Post-sit=6.1±1.1 m/s, p=0.009). No significant changes were noted for any cardiovascular parameter over course of sitting intervention (i.e., 10-180 mins of sitting). When examining heart rate variability, there was a significant increase in time domain indices (SDNN and RMSSD, p<0.05) and decrease in low frequency power, all suggestive of a shift in autonomic balance, possibly an increase in vagal tone [38]. Sitting also resulted in a significant increase in calf circumference, indicative of venous pooling (10 min sitting=40.41±5.05 vs. 180 mins sitting=41.58±5.03, p<0.001).

Table 2 Impact of prolonged sitting on central cardiovascular hemodynamics.

	HR (bpm)	cSBP (mmHg)	cDBP (mmHg)	cPP (mmHg)	cMAP (mmHg)	AP (mmHg)	Pf (mmHg)	Pb (mmHg)	PWV (m/s)
Pre sit	65±8	113±13	76±10	37±6	91±11	5±6	30±6	15±3	5.7±1
10	69±8	112 ±13	81±11	31±5	93±12	3±5	26±4	13±2	
60	69±10	113±14	83±11	31±6	95±12	2±4	26±5	12±3	
120	66±12	113±14	82±11	32±6	94±12	2±4	28±6	13±3	
180	67±11	115±12	83±10	32±6	96±10	2±5	28±4	13±3	
Post sit	60±10*	113±14	77±10	36±8	90±11	1±5*	29±6	13±3	6.1±1*

Influence of Weight and Physical Activity Status on Cognitive Function, Cerebral Perfusion and Cardiovascular Responses to Sitting

Weight Status. There was no significant influence of weight status on cognitive function or cerebral perfusion during the sitting intervention. Compared to normal weight participants, those classified as obese had significantly higher blood pressure values at rest (pre-sit) for cSBP (diff in means=13.5 mmHg, p=0.009), cPP (diff in means=5.7 mmHg, p=0.036), cMAP (diff in means=10.2 mmHg, p=0.02), AP (diff in means=5 mmHg, p=0.026), Pb (diff in means=2.8 mmHg, p=0.013), and a significantly greater aPWV at rest (diff in means=1.2 m/s, p=0.019). In response to prolonged sitting, normal weight subjects appeared to exhibit an increase in aPWV (p=0.028), an effect that was not apparent in the obese subjects (p=0.135) (**Figure 4**). Participants classified obese also had a greater calf circumference at the start (10 mins) and for each time point during sitting ($p<0.001$).

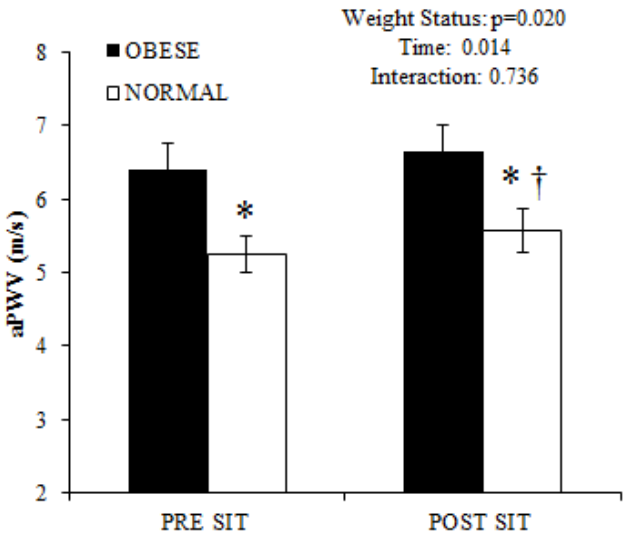

Figure 4. Impact of Weight Status of Vascular Stiffness Response to Sitting.

Aortic Pulse Wave Velocity (aPWV) was higher in participants classified as obese (black bars) as compared to normal weight (white bars). In response to sitting, there was an increase in aPWV for normal weight subjects, an effect that was not apparent for obese participants. **Denotes $p<0.05$ vs. obese participants; $^{\dagger}p<0.05$ vs. PRE SIT.*

Physical Activity Status. There was no significant influence of physical activity status on cognitive function, cerebral perfusion, or central cardiovascular hemodynamics at rest or in response to the sitting intervention ($p>0.05$).

CHAPTER IV – DISCUSSION

The primary purpose of this study was to determine whether or not prolonged, uninterrupted sitting could negatively impact cognitive function and central cardiovascular hemodynamics. In opposition to our first hypothesis, there was no significant impact of sitting of cognitive function, as quantified by performance measures obtained from a computerized Stroop test. Importantly, and in agreement with other reports, our data demonstrate that a brief bout of physical activity (i.e., 10 min walk on treadmill) does significantly improve cognitive function, which may be the result of an increase in perfusion to the prefrontal cortex of the brain. Uniquely, our data also demonstrate that central cardiovascular hemodynamics are significantly impacted by prolonged sitting, characterized as a reduction in augmentation pressure, an effect that may be mediated by an increase in venous pooling. Contradictory to our second hypothesis, weight and physical activity status did not appear to alter the cognitive and cerebral vascular response to sitting. However, people of normal weight status exhibited an increase in vascular stiffening in response to prolonged sitting, an effect that was not apparent in those classified as obese. Collectively, these data highlight the importance of engaging in physical activity as a means to improve cognitive function and cerebral vascular health.

Our results from the Stroop test indicate that while 3 hours of sitting does not appear to negatively impact cognitive function, there is a significant improvement following a brief bout of light physical activity. This finding is in agreement with other reports in regards to the acute effects of physical activity and executive function. For

instance, one study examining the impact of aerobic and strength training exercises on cognitive function found that both modalities demonstrate a significant beneficial effect, as defined by a reduction in time to completion for two, of three conditions, i.e., color word, and non-color word, for the Victoria version of the Stroop test, as compared to a non-exercising control group 23]. The authors speculate that one potential mechanism mediating this improvement in cognitive function following exercise is due to an increase in cerebral blood flow, with a subsequent increase in oxygen delivery to central processing areas of the brain, such as the pre-frontal cortex.

Our study was the first attempt to characterize temporal responses of cerebral perfusion to prolonged sitting while performing cognitive function testing. Our data demonstrate that cerebral perfusion, for the most part, is maintained relatively well over the course of sitting, but significantly increases following a simple, light bout of physical activity. This was at first surprising, given previous evidence demonstrating that cerebral perfusion is maintained constant across a wide range of conditions, including light-moderate exercise, due to cerebral autoregulation [39]. It is important to note that previous work examining changes in brain blood flow in response to physical activity have utilized gross estimates of blood flow, with a majority of the classic work relying on Doppler velocity (i.e., obtained over middle cerebral artery through temporal window), which presents a limitation in that vessel diameter is not always obtained and factored into the calculation. More recently, due to advancements in imaging, higher resolution (temporal and spatial) methodologies, such as spatially resolved spectroscopy and duplex-Doppler ultrasound, facilitate more accurate and reliable estimates of perfusion and blood flow. Importantly, it has been shown more recently that light walking actually

increases blood flow to the brain, as quantified through duplex-Doppler ultrasound image of the internal carotid artery [40]. The authors of this work point out that changes in aortic retrograde pressure waves originating in the foot during heel strikes may facilitate the increase in blood flow. Given this evidence, we postulated that prolonged sitting would have the opposite effect, but contrary to this, no change in perfusion during sitting was observed, only during the 10 min walk period.

Uniquely, our study demonstrated a significant reduction in augmentation pressure ($p<0.001$), but an increase in aortic stiffening as determined by the increase in PWV ($p=0.009$). This would suggest that both changes in arterial wave reflection and stiffening of the aorta may occur independently of one another. This is in agreement with previous reports in cardiovascular disease patients, which indicate that these two measurements do not necessarily correlate [41]. The reduction in augmentation pressure may likely be due to venous pooling, which has been shown to dampen wave reflection [42,43]. The overall clinical significance of a reduction in augmentation pressure in response to sitting is unclear and will require additional future work. What is most surprising is the increase in aortic stiffening, a measurement which is traditionally thought of as structural and something that changes chronically, as would occur with a prolonged sedentary lifestyle. The finding that obese subjects exhibited no change in PWV may suggest that people with greater PWV at rest are less likely to exhibit stiffening in response to a prolonged period of sitting. Importantly, our data show that normal weight subjects are more prone to developing aortic stiffening in response to sitting. One limitation of our study was that PWV was not measured following the 10 min walk, thus, we can no definitely state whether breaking-up prolonged bouts of sitting

with light activity, such as walking, can revert some of the negative cardiovascular health effects of sitting in healthy low-risk individuals. It is plausible that reductions in endothelial function of large arteries in the leg (e.g., femoral arteries) in response to sitting [44] may be contributing to some of the changes in PWV in response to sitting. Future studies will be needed to fully elucidate this mechanism.

CHAPTER V – CONCLUSION

These preliminary findings suggest that prolonged, uninterrupted sitting does not significantly alter cognitive function and cerebral perfusion. However, sitting does appear to decrease aortic augmentation pressure, as well as increase aortic stiffness, the latter, which is apparent only in healthy, normal weight individuals. Light physical activity, such as a brief 10-min walk can improve cognitive function, an effect that may be related to increases in cerebral perfusion. These data emphasize the importance of breaking-up sitting with brief bouts of physical activity as a means to improve cognitive function and cerebral vascular health.

APPENDIX A –IRB Approval Letters (Original Approval and Modification Resubmission)

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI.

INSTITUTIONAL REVIEW BOARD
118 College Drive #5147 | Hattiesburg, MS 39406-0001
Phone: 601.266.5997 | Fax: 601.266.4377 | www.usm.edu/research/institutional.review.board

NOTICE OF COMMITTEE ACTION

The project has been reviewed by The University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the "Adverse Effect Report Form".
- If approved, the maximum period of approval is limited to twelve months.
  Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: CH2-16061301
PROJECT TITLE: Impact of Prolonged Sitting on Cardiovascular Health: Role of Intermittent Pneumatic Compression Therapy
PROJECT TYPE: Change to a Previously Approved Project
RESEARCHER(S): Daniel Credeur, Ph.D.
COLLEGE/DIVISION: College of Health
DEPARTMENT: School of Kinesiology
FUNDING AGENCY/SPONSOR: N/A
IRB COMMITTEE ACTION: Expedited Review Approval
PERIOD OF APPROVAL: 09/22/2016 to 09/21/2017
Lawrence A. Hosman, Ph.D.
Institutional Review Board

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

INSTITUTIONAL REVIEW BOARD
118 College Drive #5147 | Hattiesburg, MS 39406-0001
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NOTICE OF COMMITTEE ACTION

The project has been reviewed by The University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the "Adverse Effect Report Form".
- If approved, the maximum period of approval is limited to twelve months.
  Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: CH3-16061301
PROJECT TITLE: Impact of Prolonged Sitting on Cardiovascular Health: Role of Intermittent Pneumatic Compression Therapy
PROJECT TYPE: Change #3 and Renewal to a Previously Approved Project
RESEARCHER(S): Daniel P. Credeur, Ph.D.
COLLEGE/DIVISION: College of Health
DEPARTMENT: School of Kinesiology
FUNDING AGENCY/SPONSOR: N/A
IRB COMMITTEE ACTION: Expedited Review Approval
PERIOD OF APPROVAL: 09/22/2017 to 09/21/2018
Lawrence A. Hosman, Ph.D.
Institutional Review Board

APPENDIX B - Long Form Consent

ORI | Office of Research Integrity

INSTITUTIONAL REVIEW BOARD

LONG FORM CONSENT

LONG FORM CONSENT PROCEDURES

This completed document must be signed by each consenting research participant.

- The Project Information and Research Description sections of this form should be completed by the Principal Investigator before submitting this form for IRB approval.
- Signed copies of the long form consent should be provided to all participants.

Last Edited August 28th, 2014

Today's date:		
PROJECT INFORMATION		
Project Title: Impact of Prolonged Sitting on Cardiovascular Health: Role of Intermittent Pneumatic Compression Therapy		
Principal Investigator: Daniel P. Credeur, Ph.D.	Phone: 601-266-6303	Email: daniel.credeur@usm.edu
College: Health	Department: Kinesiology	

RESEARCH DESCRIPTION

1. **Purpose**:

The purpose of this study is to examine how your body, particulary the heart and blood vessels, respond to 3 hours of uninterupted sitting. In addition, we will determine if applying cuff compressions to your legs can prevent any changes in the cardiovascular system while sitting. For people with spinal cord injury, we will determine how 3 hours of limited movement from your wheelchair impacts your cardiovascular health. In addition, for people with Spinal Cord Injury, we will determine how cuuf compressions applied to your legs impact your blood glucose response when given a sugary drink.

2. **Description of Study:**

Your participation in this study will require 1-4 visits, which will last up to 4 hours. You can stop participating at any time, and your decision to withdraw from the study will not affect in any way your medical care and/or benefits.

Below is a detailed description of the procedures to be performed during your visit to the laboratory:

Initial Visit:

You will arrive to the research laboratory, Rm. 125 of the Kinesiology Building, at your scheduled time for a review of inclusion/exclusion criteria, and to discuss the experimental procedures, protocols, and potential risks and benefits of participating in this study. After providing written informed consent, you will be asked to complete a health history and physical activity questionnaire, after which we will determine your height and weight, and familiarize you to the lab and measures to be carried-out during the experimental sessions.

The order of the following experimental sessions will be randomized:

Session 1: 3-hours sitting, followed by 10-minute walk, or rolling your own wheelchair

You will be instrumented with an ECG system to monitor heart rate, and a blood pressure cuff on your left arm and thigh to obtain study measurements. An ultrasound probe will be placed over the skin of your right ankle

to examine the artery and blood flow at rest, and in response to a 5-minute cuff occlusion on your calf muscle. Another device (NIRS probe), will be placed on your forehead to non-invasively determine brain oxygenation. Following 20 minutes of quiet rest, we will collect baseline data. Next you will transferred to a chair where you will remain seated upright for 3-hours. You will be asked to refrain from any excessive leg movement or fidgeting. Following 10 minutes of sitting, we will repeat the blood pressure and blood flow measurements. We will also assess your cognitive function using a computerized Stroop test. In addition, we will have you complete a Stroop test to determine your cognitive function. To do this, you will be asked to identify the colors of a square, then the words associated with colors. You will be timed on these tasks. Every 30 minutes during sitting, we will determine blood pressure, and blood flow. You will repeat the cognitive function test during the final 10 minutes of sitting. Following sitting, you will then be transferred back onto the exam table, and the baseline tests will be repeated. Next you will get up and walk around the building, or wheel yor chair for people with spinal cord injury, for 10-minutes, after which we will repeat the study measurements one last time. This session may take ~4 hours to complete. Following the study, you will be given a monitor to wear for 7 days to determine daily physical activity levels.

Session 2: 3-hours of sitting, with cuff-compressions on your legs

You will be instrumented with an ECG system to monitor heart rate, and a blood pressure cuff on your left arm and thigh to obtain study measurements. An ultrasound probe will be placed over the skin of your right ankle to examine the artery and blood flow at rest, and in response to a 5-minue cuff occlusion on your calf muscle. Another device (NIRS probe), will be placed on your forehead to non-invasively determine brain oxygenation. Following 20 minutes of quiet rest, we will collect baseline data. Next you will transferred to a chair where you will remain for 3-hours. All procedures will be repeated from session 1, except we will apply cuffs to your feet and calves (IPC) that will periodically inflate and deflate to increase blood flow to your legs. You will not perform the 10-minute walk during this session.

Sessions 3 and 4: For subjects with and without spinal cord injury, you will arrive to the research laboratory at your scheduled time (between 6:00-10:00am), in fasted state, and having refrained from caffeine for 12 hours, and strenuous physical activity and alcohol for 24 hours. Following an overview of the experimental procedures and protocols to be performed, you will be positioned supine on the examination table and instrumented with a heart rate and blood pressure monitor, and a blood flow probe secured to the skin on your right thigh. Following 20 minutes of quiet rest, with the lights will be dimmed, and baseline measures will be performed. Next fasting glucose will be measured using a finger stick, then you will consume a 75 gram glucose drink. This is about the same amount of sugar that is in a 20 oz coca-cola bottle. Your blood glucose will also be measured at the 30, 60, and 120 min time points from the left ring/middle finger with the finger stick. The cardiovascular measures will also be examined at the same time points. This study visit will last ~3 hours. Following completion of this protocol, you will be asked to return to the lab at least 48 hours later to repeat this procedure, except we will apply the intermittent pneumatic compression therapy to your legs.

Measures to be performed:

Blood Pressure (BP) - A blood pressure cuff will be placed around your left upper arm to periodically measure peripheral and central blood pressure. An additional cuff will be placed on your left thigh, in conjunction with a pen–like pressure probe to determine the speed of your pulse (pulse wave velocity).

Heart Rate - Electrodes (patches) will be placed on the surface of your chest and sides for heart rate measurements per ECG.

Brain Oxygenation - Will be measured non-invasively via a NIRS probe placed on your forehead and secured with specialized tape and a cover.

Vascular Health Test - The health of the artery in your foot will be examined with an ultrasound machine. Following baslines measures, a BP cuff wil lbe inflated on your right calf for 5-mins. We will continuously monitor the artery during the occlusion, and for 3 minutes following release of the cuff.

3. **Benefits:**

If you agree to take part in this study, there may or may not be a direct medical benefit to you. You may expect to benefit from taking part in this research to the extent that you are contributing to scientific knowledge. Our hope is that the information gained from this study will improve the understanding of

prolonged sitting effects the cardiovascular system.

4. **Risks:**

While in the study, you are at risk for the side effects described below. You should discuss these with the investigator and/or your doctor. There may also be other side effects that we cannot predict:

ECG: Some people may have a skin irritation from the patches that connect the wires on the chest to the computer. Skin and hair are pulled slightly when the patches are removed after the test. Research personnel will attach and remove the patches as carefully as possible.

Blood pressure cuff inflation: The blood pressure cuff will squeeze your arm and or leg tightly; however, any discomfort will be alleviated as soon as the pressure in the cuff is released.

Occlusion cuff inflation: The occlusion cuff will squeeze your arm tightly; however, any discomfort will be alleviated as soon as the pressure in the cuff is released.

Intermittent Pneumatic Compression (IPC) - IPC is a non-invasive FDA-approved therapy which includes the administration of brief 120 mmHg cuff compressions (3 sec each) around your calf and foot region performed sequentially over the course of the 3-hour sitting (~3 compressions/min). Blood flow in your ankle and thigh will be measured using the ultrasound machine periodically during the session.

Oral Glucose Tolerance Test (OGTT): The potential risks of performing an OGTT are minimal; however, you may experience a slight change in heart rate and blood pressure, which is a normal response to ingesting any food/drink substance. In rare occasions, mild nausea or light-headedness may occur when high levels of glucose are ingested. However, the volume that will be consumed in this protocol would be similar to the amount of sugar (i.e., glucose) in a 20 oz coca-cola bottle (~65 g). Importantly, blood glucose levels, heart rate, and BP will be monitored during this test.

Autonomic Dysreflexia: For people with SCI, sometimes a sudden change in heart rate and blood pressure may occur. For your safetly, we will continuously monitor your heart rate and blood pressure throughout the study. In the event that an episode occurs, the study would be immediately stopped and the stimulus triggering this event would be discontinued.

5. **Confidentiality:**

Information produced by this study will be stored in the investigator's file and de-identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law. It is possible that your medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the study sponsor and/or federal or state government agencies in the course of carrying out their duties. If your record is inspected or copied by the study sponsor (and/or its agents), or by any of these agencies, the University of Southern Mississippi will use reasonable efforts to protect your privacy and the confidentiality of your medical information. The results of this study may be published in a medical book or journal or used for teaching purposes. However, your name or other identifying information will not be used in any publication or teaching materials without your specific permission.

6. **Alternative Procedures:**

An alternative is to not participate in this research study.

7. **Participant's Assurance:**

This project has been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations.

Any questions or concerns about rights as a research participant should be directed to the Chair of the IRB at 601-266-5997. Participation in this project is completely voluntary, and participants may withdraw from this study at any time without penalty, prejudice, or loss of benefits.

Any questions about the research should be directed to the Principal Investigator using the contact information provided in Project Information Section above.

CONSENT TO PARTICIPATE IN RESEARCH

Participant's Name: _____

Consent is hereby given to participate in this research project. All procedures and/or investigations to be followed and their purpose, including any experimental procedures, were explained to me. Information was given about all benefits, risks, inconveniences, or discomforts that might be expected.

The opportunity to ask questions regarding the research and procedures was given. Participation in the project is completely voluntary, and participants may withdraw at any time without penalty, prejudice, or loss of benefits. All personal information is strictly confidential, and no names will be disclosed. Any new information that develops during the project will be provided if that information may affect the willingness to continue participation in the project.

Questions concerning the research, at any time during or after the project, should be directed to the Principal Investigator with the contact information provided above. This project and this consent form have been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations. Any questions or concerns about rights as a research participant should be directed to the Chair of the Institutional Review Board, The University of Southern Mississippi, 118 College Drive #5147, Hattiesburg, MS 39406-0001, (601) 266-5997.

Include the following information only if applicable. Otherwise delete this entire paragraph before submitting for IRB approval: The University of Southern Mississippi has no mechanism to provide compensation for participants who may incur injuries as a result of participation in research projects. However, efforts will be made to make available the facilities and professional skills at the University. Participants may incur charges as a result of treatment related to research injuries. Information regarding treatment or the absence of treatment has been given above.

_____	_____
Research Participant	**Person Explaining the Study**
_____	_____
Date	**Date**

APPENDIX C - Medical History Form

University of Southern Mississippi, Laboratory of Applied Physiology Medical Health History Form	
All of the information provided in this form is voluntary.	
Date:	Biographical information:
Last Name:	First: MI:
Occupation:	Email:
Home Phone: ( )	Work: ( ) Cell: ( )
Address:	
DOB: / /	Age: Gender M / F Height: Weight:
Highest Education Achieved:	
Race:	What race do you consider yourself to be? Select one or more of the following:
	Hispanic or Latino - A person of Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin," can be used in addition to "Hispanic or Latino."
	American Indian or Alaska Native - A person having origins in any of the original peoples of North, South, of Central America, and who maintain a tribal affiliation or community attachment.
	Asian- A person having origins in any of the originals peoples of the Far East, Southeast Asia, or the Indian subcontinent, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in the previous data collection strategies.)
	Black or African American - A person having either origins in any of the black racial groups of Africa. "Haitian" can be used in addition to "Black" or "African American."
	Native Hawaiian or Pacific Islander - A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.
	White - A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.
Primary Care Physician:	
Name:	Office Phone:
Address:	
Emergency Contact:	
Name:	Relationship: Phone #
Medications: Include over the counter drugs/ oral contraceptive/ dietary supplements	
Name/ Dosage/ How often taken:	
Allergies:	
Smoking History:	
Do you smoke	Cigarettes? Pipe/ Cigar? Other? If you quit, what year did you quit?
# of packs smoked per day _____	For how many years _____

Medical Health History Form Page 2

Alcohol Consumption History:

Do you currently drink alcohol? If you drank alcohol previously, when did you stop?

If you ever did drink alcohol, what is (was) the volume consumed?
_____# ounces / day for _____ # of years

Medical History:

NO	YES	*Please explain any "YES" answers*
		high blood pressure
		chest pain/ history of heart attack
		extra heart beats or racing
		abnormal electrocardiogram (ECG)
		other heart trouble (e.g. murmur, valve problems)
		high cholesterol
		diabetes
		seizures
		stroke
		fainting spells
		anxiety (diagnosed)
		depression (diagnosed)
		recurrent fatigue
		insomnia
		thyroid problems
		difficulty breathing
		emphysema/ asthma/ chronic bronchitis
		tuberculosis
		chronic infection
		stomach/ GI problems
		hepatitis
		bleeding disorder
		kidney/ urinary problems
		joint injuries/ joint pain
		arthritis (rheumatoid or osteoarthritis)
		migraine headaches
		vision problems (exclude corrected near/ far sightedness)
		surgical procedures
		For premenopausal females, Pregnant?
		For premenopausal females, Early follicular phase, i.e., start of menstrual cycle?

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