# The Combined Effects of Prolonged Sitting and Mental Stress on Vascular and Cerebrovascular Function in Middle-Aged Adults

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**MASTER PROTOCOL**

# The Combined Effects of Prolonged Sitting and Mental Stress on Vascular and Cerebrovascular Function in Middle-Aged Adults

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| PROTOCOL SYNOPSIS | |
|---|---|
| Study Title: | The Combined Effects of Prolonged Sitting and Mental Stress on Vascular and Cerebrovascular Function in Middle-Aged Adults |
| Funding | UNC Department of Exercise & Sport Science |
| Clinical Phase | Pilot Testing |
| Study Rationale | Prolonged sitting may pose a public health risk through its effects on the cardiovascular system, and may lead to impaired whole-body cardiovascular health, which includes both vascular and cerebrovascular function. These effects may interact with other environmental variables, such as stress. However, no study has investigated the combined effect of a mental stressor and prolonged sitting on vascular and cerebrovascular function. The combined effect of prolonged sitting and mental stress may lead to an exacerbated effect on vascular, cerebrovascular, and executive function. We hypothesize that mental stress with the addition of prolonged sitting [SMS] will result in a greater increase in peripheral, central and cerebral arterial stiffness and elicit a decrease in cerebral perfusion, total blood flow to the brain, middle cerebral artery velocity and executive function, compared to mental stress without prolonged sitting [CON]. The findings from this study may result in a public health message regarding sedentary behavior and stress, and will help elucidate the mechanisms behind acute vascular, cerebrovascular, and cognitive dysfunction during prolonged sitting. |
| Study Objective(s) | To examine the cardiovascular response to mental stress with or without previous exposure to a bout of prolonged sitting. |
| Study Design | Randomized Crossover Design, with two experimental conditions: without prolonged sitting + stress [CON] and with prolonged sitting + stress [SMS]. |
| Participant Inclusion and Exclusion Criteria: | Inclusion<br>• Male or female, aged 35 to 59 years<br>Exclusion<br>• Any known cardio-metabolic disorders<br>• Taking medications known to affect cardiovascular function<br>• Report cigarette smoking<br>• Pregnant women |
| Number of Participants | 20 |
| Study Duration | 1 year |
| Study Phases | Familiarization: to all devices<br>CON: mental stress without prolonged sitting<br>SMS: mental stress with prolonged sitting |
| Design | Multi-visit randomized crossover trial |

| | |
|---|---|
| Primary & Secondary Outcomes | **Cerebrovascular**<br>Primary: Brain PWV<br>Secondary: Executive Function & Neurovascular Coupling<br>**Vascular**<br>Primary: Brachial-Femoral PWV<br>Secondary: Carotid-femoral PWV, femoral-ankle PWV, augmentation index, central blood pressures (cSBP, cDBP) |
| Descriptive/ Mechanistic Outcomes | **Cerebrovascular:**<br>Cerebral Perfusion<br>Middle Cerebral Artery Velocity<br>Total Brain Blood Flow<br>Static Cerebral Autoregulation<br><br>**Vascular:**<br>Peripheral blood pressure<br>Heart rate<br>Baroreflex Sensitivity<br>Heart Rate Variability<br>Total hemoglobin<br>Tissue saturation index<br>Accelerometer |
| Statistical and Analytic Plan | All statistical procedures will be competed with SPSS Statistics version 25.0 (SPSS, IN., Durham, NC, USA) and Jamovi v0.9 (Jamovi, UK). The α-level will be set a prior at 0.05 for all statistical procedures. Descriptive statistics and mechanistic outcomes will be collected to compare similarities amongst the sample group and to eliminate the potential for extraneous covariates. Answering the research question will require a linear, multi-level mixed model. |
| Data and Safety Monitoring Plan | No participants will be identified in any report or publication of this study. Participants will be identified via identification code for data collection purposes. All electronically collected data will be stored on a computer that only team members Onyen password will allow access to study information. This computer will be located in the Graduate Student Office of the Applied Physiology Laboratory at the University of North Carolina at Chapel Hill. Data analysis procedures will be performed on secure computers within Fetzer Hall, with which password is required to access. |

# CONTENTS

## ABBREVIATIONS

| ABBREVIATION | DEFINITION |
|---|---|
| ADRD | Alzheimer's Disease and Related Dementia |
| AIx | Augmentation Index |
| APL | Applied Physiology Laboratory |
| BF | Blood Flow |
| bfPWV | Brachial-Femoral Pulse Wave Velocity |
| BP | Blood Pressure |
| CBP | Central Blood Pressure |
| cfPWV | Carotid-Femoral Pulse Wave Velocity |
| cm | Centimeters |
| CON | Control Experimental Session |
| CONSORT | Consolidated Standards of Reporting Trials |
| CV | Cardiovascular |
| CVD | Cardiovascular Disease |
| CVH | Cardiovascular Health |
| CVS | Cardiovascular System |
| cwNIRS-SRS or NIRS | Continuous Wave Near-Infrared Spectroscopy |
| DBP | Diastolic Blood Pressure |
| ECG | Electrocardiogram |
| EF | Executive Function |
| faPWV | Femoral-Ankle Pulse Wave Velocity |
| HIPAA | Health Insurance Portability and Accountability Act |
| HR | Heart Rate |
| hr | Hours |
| HRV | Heart Rate Variability |
| ID | Identification Number |
| IRB | Institutional Review Board |
| kg | Kilograms |
| L | Straight-line length or distance |
| m/s | Meters per second |
| MAT | Mental Arithmetic Test |
| MCA | Middle Cerebral Artery |
| MCAv | Middle Cerebral Artery Velocity |
| min | Minutes |
| mmHg | Millimeters of Mercury |
| MS | Mental Stress or Mental Stressor |
| N/A | Not Available |
| NIBP | Non-Invasive Blood Pressure |
| PA | Physical Activity |
| PCA | Posterior Cerebral Artery |
| PHI | Personal Health Information |

| | |
|---|---|
| PI | Principal Investigator |
| PPG | Photoplethysmography or Photoplethysmography |
| PS | Prolonged Sitting |
| PWA | Pulse Wave Analysis |
| PWV | Pulse Wave Velocity |
| PWV/A | Pulse Wave Velocity and Analysis |
| RDM | Research Database Management |
| SBP | Systolic Blood Pressure |
| SD | Standard Deviation |
| sec or s | Seconds |
| SMS | Sitting & Mental Stress Experimental Session |
| TCD | Transcranial Doppler |
| tHb | Total Hemoglobin |
| TMT | Trails Mental Tests or Trails A + B |
| TSI | Tissue Saturation Index |
| TT or ΔT | Transit Time |
| UNC | University of North Carolina at Chapel Hill |
| US | Ultrasound |
| VFT | Verbal Fluency Test |
| VPN | Virtual Private Network |
| X | Mean or Average |

# 1 BACKGROUND AND RATIONALE

## Introduction and Rationale

Prolonged sitting (PS) has gained attention over the past decade due to the sitting epidemic that takes place in the workplace.[1,2] Participating in a sedentary lifestyle is common due to occupation, technology and vehicle transportation. Sedentary behavior (SB) has been shown to be a risk factor for cardiovascular disease (CVD) and all-cause mortality. Additionally, a sedentary lifestyle has been linked to chronic cognitive dysfunction, thus playing a role in the appearance of Alzheimer's Disease and Related Dementia's (ADRD)[3]. The available research demonstrates that chronic SB is associated with a reduction in brain volume[4] and a decline in cognition, in particular executive function (EF)[5,6]. Although, there is a level of uncertainty in the mechanism(s) connecting repeated acute SB exposure to chronic cerebrovascular complications.

In addition to cerebrovascular implications as a result of sedentarism and stress, central and peripheral cardiovascular function and structure are disrupted[7–9]. In contrast to our understanding of cerebrovascular function, the physiological mechanisms in which a mental stressor or a sedentary behavior like PS[10–12] impact systemic cardiovascular function is better understood. Pooling of blood in the lower-limbs is the first cardiovascular disruption as a result of PS that eventually leads to failures in regulatory mechanism that maintain healthy function[11,13–15]. Repeated activation of neurohormonal pathways due to mental stress is known to impair cardiovascular health[16,17]. It is less clear how these negative stimuli impact the CVS acutely when experienced in combination. Developing our knowledge on the topic of cerebrovascular and vascular health is necessary to develop societal and behavioral solutions for the sedentarism and stress epidemics that significantly burden our healthcare system[18–20] and economic productivity[21]. This study will provide new insights on the physiological mechanisms these health behaviors take to negatively change the responses of the CVS.

## 1.1 Objective(s)

To examine the cardiovascular response to mental stress with or without previous exposure to a bout of prolonged sitting.

## 1.2 Aims

Two primary aims will be addressed:

1. Explore the effects of mental stress with and without a bout of prolonged sitting on cerebrovascular function.
2. Explore the effects of mental stress with and without a bout of prolonged sitting on vascular function.

# 2 STUDY PROCEDURES

This study will be reported in accordance with Consolidated Standards of Reporting Trials (CONSORT) guidelines[22].

## 2.1 Participants

We will recruit 20 adult (35-59 y) male and female participants. A healthy population sample will be recruited to mitigate the risk of age- or disease-related influences on the study outcomes.

**Inclusion Criteria**

- Male or female participants will be between the ages of 35-59 years old.

**Exclusion Criteria**

- Any known cardio-metabolic disorders
- Taking medications known to affect cardiovascular function
- Report drug or alcohol abuse
- Report cigarette smoking
- Pregnant women

## 2.2 Allocation to Treatment Groups and Blinding

The randomization procedure will be performed by a research assistant. Participants will be randomized into either CON or SMS intervention for the first visit. This will be done during the familiarization session using an online randomization software (www.randomizer.org).

## 2.3 Experimental Design

**Study Design**

This study is a multi-visit (three in total) randomized crossover trial.

**Overview**

During a single familiarization session, the research team will obtain informed consent, and will describe to participants the purposes and procedures of this study. They will then be exposed to all experimental devices on in a quiet, dimly lit and environmentally controlled room. Participants will return to the same location for two experimental conditions following these pre-assessment guidelines:

- Fasted (> 12 hours), consuming only water.
- No caffeine consumption 12 hours prior to testing.
- No vigorous exercise 24 hours prior to testing.
- No alcohol consumption 24 hours prior to testing.

**Table 1.** Study Variables

| Type | Measure |
|---|---|
| **Independent (Conditions)** | Control (CON): Exposure to MS without PS |
| | Sitting-Mental Stress (SMS): Exposure to MS after period of PS |
| **Primary Outcomes** | Brain PWV |
| | Brachial-femoral PWV |
| **Secondary Outcomes** | Executive function |
| | Neurovascular coupling |
| | Carotid-femoral PWV |
| | Augmentation index |
| | Femoral-ankle PWV |

## Experimental Set-Up

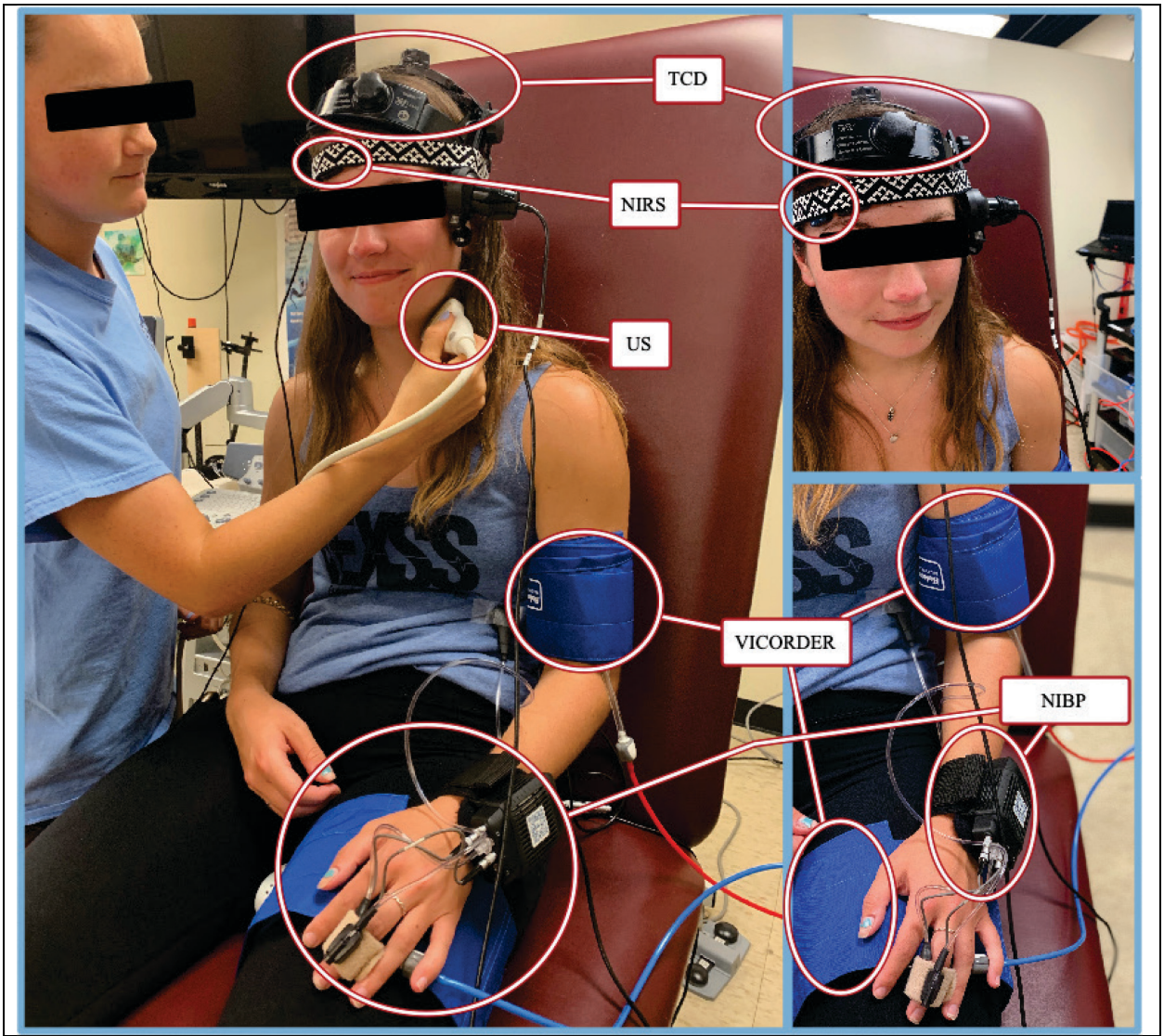



**Figure 1.** Experimental setup for to assess full body cardiovascular health. Each experimental device responsible for collecting data is shown in this picture and close ups.

## Familiarization

Participants will be familiarized with all experimental procedures and then given the opportunity to ask any questions prior to providing written consents. Subjects will be 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. If feet cannot touch the ground, a platform will be used. The angle of recline of the chair will be measured, as well as the distance from the bottom of the chair to the ground to ensure equivalent chair placement for the second visit.

**Experimental Visits**

Refer to both *Table 1 and Figure 1* for a breakdown of when experimental measures will be taken through both visits.

Participants will arrive to the Applied Physiology Laboratory (APL) between 6:00 and 10:00 AM fasted (for Visit 3: 2-5 days following the Visit 2). Participants will be fasted and refrain from caffeine intake for at least 12 hours, and alcohol and strenuous physical activity for at least 24 hours prior to arrival. Upon arrival, height and weight will be recorded followed by 10 minutes of quiet rest in the supine position (white solid boxes in **Figure 1**). During these 10 minutes, the subject will be fitted with a NIRS probe on the prefrontal cortex and medial gastrocnemius. The NIBP, TCD, and VICORDER® devices will also be affixed to the participant during this time period during this time. Sitting periods (solid gray boxes in **Figure 1**) will begin for both conditions once all devices are attached to the participant, the subject is shifted to an upright seated position, and at least 10min of supine rest has been recorded. During the CON visit, subjects will undergo a brief 20min sitting period. For the SMS condition, subjects will be asked to sit still and quietly for 2 hours while watching a non-stimulating documentary. Each condition will receive the MS (black boxes in **Figure 1**) at the conclusion of the sitting periods in both CON and SMS. After exposure to the MS in both conditions, data collection procedures for this protocol will be completed for VICORDER® immediately after the MS and every 5 minutes for 25 minutes (See **Figure 1** for visual details on how and when data is being collected). Brain BF will be assessed by US 20 minutes post-completion of the MAT. Finally, a battery of cognitive tests (Verbal Fluency Test and Trails A+B tests) will be administered to the participant (boxes with diagonal shades in **Figure 1**).

## 2.4 Experimental Procedures

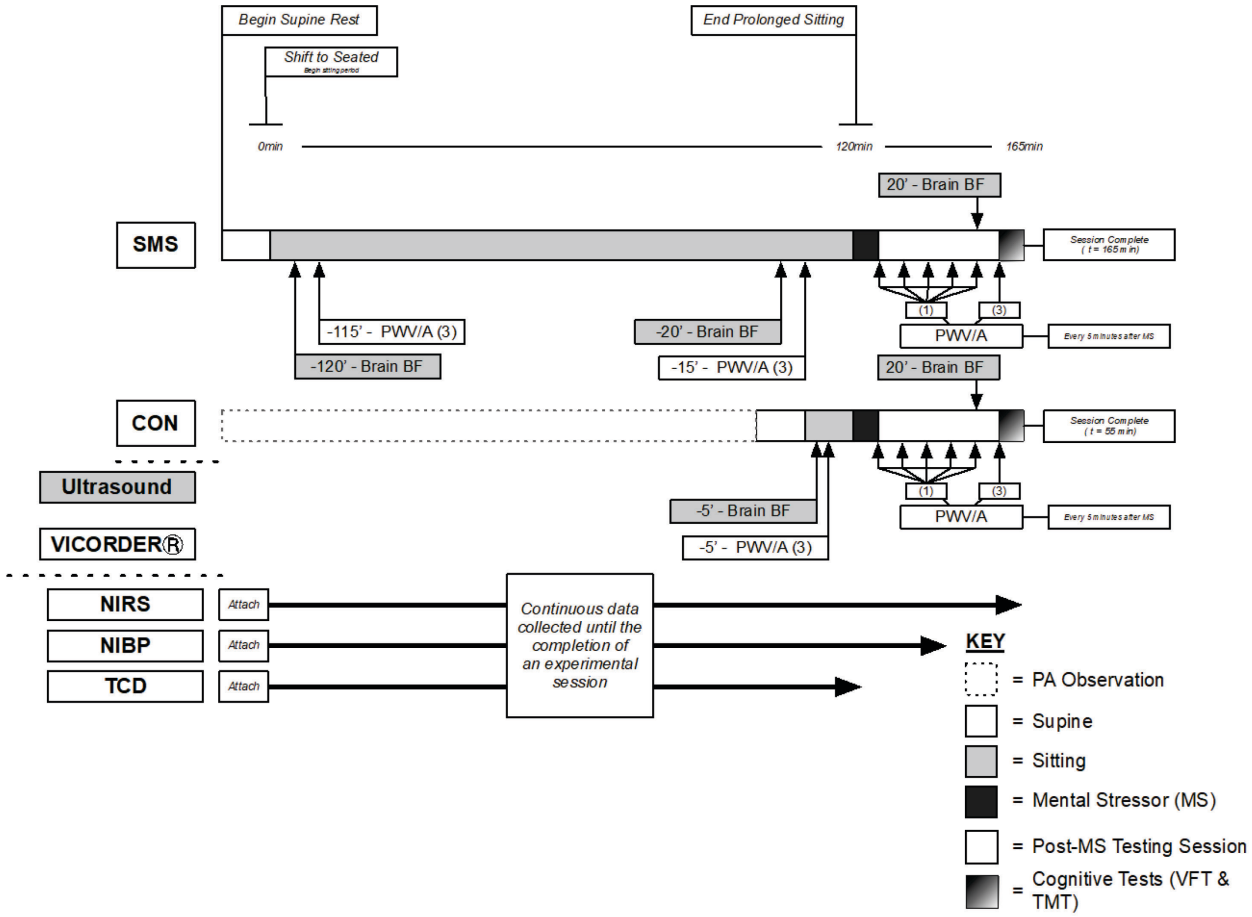
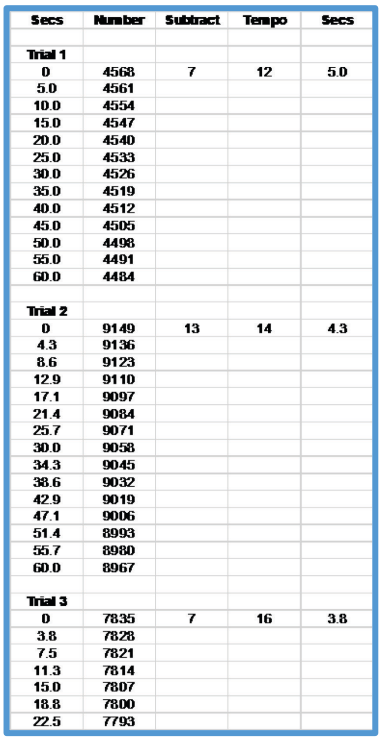



**Figure 2.** A visualization of the experimental protocol. Maintenance of external validity for this study will require exposure of the MS to be time-matched between both experimental conditions. The use of accelerometry will allow investigators to observe physical activity and sleep patterns to ensure uniformity in pre-assessment behaviors. All time points are expressed relative to the time of completion of the mental stressor. ***Abbreviations*:** BF, blood flow; PWV/A, Pulse wave velocity & analysis; (1), collection of PWV/A for the primary outcome, bfPWV/A will be collected at this time point only; (3) collection of PWV/A for bfPWV, cfPWV, and faPWV; PA, Physical activity; t, time; NIRS, Near-infrared spectroscopy; NIBP, Non-invasive blood pressure; TCD, Transcranial Doppler.

**Protocol 1: Mental Arithmetic Test**

| Test Structure | Application |
|---|---|
|  |  |
| • Test duration: Approximately 5min<br>• Participant is in seated upright position<br>• At the beginning of the test, the researcher will call out a four digit number and ask the participant to subtract either 7 or 13<br>• Correct responses will not be recorded as an outcome for this study | • A metronome will play at a pre-set tempo. Subjects will be instructed to subtract 7 or 13 for one minute from the original number continuously<br>• Each minute, a new four digit number will be called out and the participant must subtract the 7 or 13 from the number |



| Secs | Number | Subtract | Tempo | Secs |
|---|---|---|---|---|
| | | | | |
| Trial 1 | | | | |
| 0 | 4568 | 7 | 12 | 5.0 |
| 5.0 | 4561 | | | |
| 10.0 | 4554 | | | |
| 15.0 | 4547 | | | |
| 20.0 | 4540 | | | |
| 25.0 | 4533 | | | |
| 30.0 | 4526 | | | |
| 35.0 | 4519 | | | |
| 40.0 | 4512 | | | |
| 45.0 | 4505 | | | |
| 50.0 | 4498 | | | |
| 55.0 | 4491 | | | |
| 60.0 | 4484 | | | |
| | | | | |
| Trial 2 | | | | |
| 0 | 9149 | 13 | 14 | 4.3 |
| 4.3 | 9136 | | | |
| 8.6 | 9123 | | | |
| 12.9 | 9110 | | | |
| 17.1 | 9097 | | | |
| 21.4 | 9084 | | | |
| 25.7 | 9071 | | | |
| 30.0 | 9058 | | | |
| 34.3 | 9045 | | | |
| 38.6 | 9032 | | | |
| 42.9 | 9019 | | | |
| 47.1 | 9006 | | | |
| 51.4 | 8993 | | | |
| 55.7 | 8980 | | | |
| 60.0 | 8967 | | | |
| | | | | |
| Trial 3 | | | | |
| 0 | 7835 | 7 | 16 | 3.8 |
| 3.8 | 7828 | | | |
| 7.5 | 7821 | | | |
| 11.3 | 7814 | | | |
| 15.0 | 7807 | | | |
| 18.8 | 7800 | | | |
| 22.5 | 7793 | | | |

The MS used for this protocol is the Mental Arithmetic Test (MAT), a commonly used exam to impose a small psychological stimulus on the participant. This test will approximately last for five minutes. The researcher will call out a four-digit number and ask the participant to subtract either seven or 13. Correct responses will not be recorded as an outcome for this study. During this test a metronome will play at a pre-set tempo. The subjects will be required to conduct continuous subtraction with the tempo to the best of their ability. Every minute, a new four digit number will be called out and the participant must subtract the seven or 13 from the number.

## 2.5 Measurements

**Table 2.** Summarization of measures used for the protocol, "The Combined Effects of Prolonged Sitting and Mental Stress on Vascular and Cerebrovascular Function in Middle-Aged Adults". Each experimental variable is listed under the "Outcomes" column with its designation as either a primary, secondary, or mechanistic measure listed under the "Role Classification" column. The "Device" column names the equipment used to collect the data. **Abbreviations:** TCD, Transcranial Doppler; CON, Control condition; SMS, Sitting mental stress condition; VFT, Verbal fluency test; TMT, Trail making test; NIRS, Near-infrared spectroscopy; cwNIRS-SRS, Continuous-wave near-infrared spectroscopy. Minute values are expressed relative to the administration of the MAT.

| Outcomes | Device | Unit | | Time Period (min) | | Role Classification |
|---|---|---|---|---|---|---|
| | | | Condition | Time Periods (min) | Measurements Taken | |
| **Brain Pulse Wave Velocity** | TCD | meters per second (m/s) | CON | *Continuous* | 1 | Primary |
| | | | SMS | *Continuous* | 1 | |
| **Brachial-femoral Pulse Wave Velocity (PWV)** | VICORDER® | meters per second (m/s) | CON | -5, 0, 5, 10, 15, 20, 25 | 7 | Primary |
| | | | SMS | -115, -15, 0, 5, 10, 15, 20, 25 | 8 | |
| **Executive Function** | VFT/ TMT | Cognitive performance: measured in seconds (s) | CON | 25 | 1 | Secondary |
| | | | SMS | 25 | 1 | |
| **Neurovascular Coupling** | NIRS/ TCD | Unitless: relationship between local neural activity and subsequent changes in cerebral blood flow. [CITE] | CON | *Continuous* | 1 | Secondary |
| | | | SMS | *Continuous* | 1 | |
| **Augmentation Index (AIx)** | VICORDER® | Percentage. It is defined as the difference between the second and first systolic peaks (P2-P1) expressed as a percentage of the pulse pressure[24,25] | CON | -5, 0, 5, 10, 15, 20, 25 | 7 | Secondary |
| | | | SMS | -115, -15 0, 5, 10, 15, 20, 25 | 8 | |
| **Carotid-femoral PWV** | VICORDER® | meters per second (m/s) | CON | -5, 25 | 2 | Secondary |
| | | | SMS | -115, -15, 25 | 3 | |
| **Central Blood Pressures (CBP)** | VICORDER® | Millimeters of Mercury (mmHg) | CON | -5, 0, 5, 10, 15, 20, 25 | 7 | Secondary |
| | | | SMS | -115, -15 0, 5, 10, 15, 20, 25 | 8 | |
| **Femoral-ankle PWV** | VICORDER® | meters per second (m/s) | CON | -5, 25 | 2 | Secondary |
| | | | SMS | -115, -15, 25 | 3 | |
| **Physical Activity** | ActiGraph | Minutes. Activity data will be classified as either "Light", "Moderate", or "Vigorous". | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Sleep Duration** | ActiGraph | Minutes. | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Cerebral Perfusion** | cwNIRS-SRS | Relative changes in total hemoglobin concentration (tHb). Expressed as a percentage. | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Cerebrovascular Blood Flow** | Doppler Ultrasound | meters per second (m/s) | CON | -5, 20 | 2 | Mechanistic |
| | | | SMS | -120, -5, 20 | 3 | |
| **Middle Cerebral Artery Velocity** | TCD | Centimeters per second (cm/s) | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Static Cerebral Autoregulation** | TCD | Unitless. Refers to MAP and CBF under steady state conditions that can be observed over a period of time[26] | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Baroreflex Sensitivity** | NIBP | Sensitivity: BRS is defined as the change in heart rate variability in milliseconds per unit change in BP | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Heart Rate Variability (HRV)** | NIBP | milliseconds (ms) | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Heart Rate** | NIBP | Beats per minute | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Peripheral blood pressures (SBP, DBP)** | VICORDER® | Millimeters of Mercury (mmHg) | CON | -5, 0, 5, 10, 15, 20, 25 | 7 | Mechanistic |
| | | | SMS | -115, -15 0, 5, 10, 15, 20, 25 | 8 | |
| **Tissue Saturation Index** | cwNIRS-SRS | Relative changes in TSI. Data will be presented as percent changes. | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |
| **Venous Pooling in the Gastrocnemius** | cwNIRS-SRS | This is a physiological response to prolonged sitting. It is quantified using the NIRS-SRS device by recording levels of tHb at the gastrocnemius | CON | *Continuous* | 1 | Mechanistic |
| | | | SMS | *Continuous* | 1 | |

**DEVICE 1: VICORDER®**

PWV is calculated by dividing arterial path length (L) by the pulse transit time (TT) between a proximal and distal arterial segment. Measurements for L are acquired by recording the straight line distance between the proximal and distal cuff, per manufacturer guidelines. For PWV measures including the carotid artery, the straight line distance from the carotid artery to the sternal notch is included in calculations for L.

Transit time is calculated by the Vicorder software's proprietary algorithm that measures the time between the foot of the proximal pressure waveform to the foot of the distal pressure waveform. Pressure waveforms will be simultaneously captured using volume displacement cuffs. A small balloon cuff is placed over the carotid artery. The balloon used to generate the carotid pulse wave does not cause discomfort as it inflates to sub-diastolic blood pressure (~50 mmHg).

**DEVICE 2: Transcranial Doppler**

Brain PWV and Middle cerebral artery velocity (MCAv) will be measured using continuous bilateral TCD (ST3, Spencer Technologies). MCAv accounts for 70-80% of the brain's total perfusion[25]. The data will be acquired continuously (50 Hz) using an analog-to digital convertor (PowerLab 30, ADInstruments).

**DEVICE 3: Non-Invasive Blood Pressure (NIBP)**

The PowerLab data acquisition system (PowerLab 30 series, ADInstruments, CO, USA) will be used to simultaneously acquire signals from each device and from ECG. A standard Lead II ECG will be recorded from three electrodes placed on the right and left shoulders, and just below the left rib. The signals from the ECG and two test devices will be sampled a frequency was 1000 Hz, providing a temporal resolution of 1ms.

**DEVICE 4: Doppler Ultrasound**

A commercial ultrasound device equipped with 11-3 MHz linear array probe will be used. Experimenters will assess blood flow in three cerebral arteries: common carotid, internal carotid, and vertebral.

**DEVICE 5: Continuous Wave Near-Infrared Spectroscopy with Spatially Resolved Spectroscopy (cwNIRS-SRS or NIRS)**

A continuous, non-invasive system to measure cerebral perfusion. The near-infrared spectroscopy probe used in this protocol emits a single frequency of light (cwNIRS) to monitor relative change in total hemoglobin. Using the spatially-resolved spectroscopy (SRS) technique, cwNIRS can estimate absolute measures of perfusion (e.g. total hemoglobin, tissue saturation index). The cwNIRS-SRS device will be positions on the forehead, approximately 3cm to the right of the center, directly over the eyebrow. An additional probe will be placed over the medial gastrocnemius to observe venous pooling of blood in the lower-limbs. The location of both probes will be marked to ensure identical placement for the next experimental session. The device will be covered with a dark opaque cloth to prevent signal contamination by ambient light as per manufacturer recommendations.

## DEVICE 6: ActiGraph

An ActiGraph accelerometer will be distributed to the research participant at the conclusion of the familiarization session along with a sleep journal packet. The subject will be asked to record on the timeline when he/she began to sleep and awaken each day until the conclusion of his/her participation in this study. The device will be worn on the individual's non-dominant wrist according to manufacturer guidelines. The data collected from this device will be used for one purpose:

- Validate the subject's compliance with pre-testing behavioral restrictions for the purpose of internal validity:
    - Waking up at similar times on the mornings of both experimental session

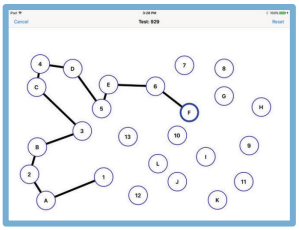
## DEVICES 7 & 8: Data Acquisition (PowerLab) and Data Processing (LabChart)

For the TCD and NIBP, devices, data acquisition and processing will be automated using the accompanying software.

For the two test devices, the PowerLab data acquisition system (PowerLab 30 series, ADInstruments, CO, USA) will be used to simultaneously acquire signals from each device and from ECG.

LabChart 8 (ADInstruments) will record and process the data acquired using PowerLab. The ECG signal does not require any post-processing, the software filters ensure a distinct R peak. The foot of the pressure waveforms from the NIBP and PPG devices will be identified using a standard second derivative function. Offline, a custom macro will identify the peak of the R wave and the peak of the second derivative of each pressure waveform, then calculate the time between said peaks.

## Cognitive Measurement 1: Trails A+B Tests

| Test Structure & Application | |
|---|---|
| The Trail-Making Test – Part A, the circles are numbered 1 – 25, and the subject will draw lines to connect the numbers in ascending order. Part B presents numbers and letters placed in a semi-random fixed order, in such a manner to avoid overlapping lines being drawn. The subject will connect 25 encircled numbers and letters in numerical and alphabetical order, alternating between the numbers and letters. For example, the first number ''1'' is followed by the first letter ''A'' followed by the second number ''2'' then second letter ''B'' and so on[23] Results for both TMT A and B will be reported in seconds. |  |

## Cognitive Measurement 2: Verbal Fluency Task

| Test Structure | Application |
|---|---|
| Tests of verbal fluency evaluate an individual's ability to retrieve specific information within restricted search parameters[24]. The two subtests are (1) semantic fluency, and (2) phonemic fluency. The total number from each test will be summed and equate to the subjects' cognitive performance. | *Verbal Fluency Test* — *Instructions*<br>Phonemic fluency — Rapid generation of words beginning with a specific letter. Typically, F, A, and S are used as stimulus letters.<br>Semantic fluency — Rapid generation of words from within a semantic category. "Animal names" and "supermarket items" are commonly used.<br>Alternating fluency — Rapid generation of words by alternating between two semantically unrelated categories. "Fruit and furniture" are often used.<br>Excluded-letter fluency — Rapid generation of words that do not contain a specified vowel (e.g., the letter "A"). |

## Cognitive Measurement 3: Memory Recall

Participants will be read word lists from the Hopkins Verbal Learning Test at the rate of 1 word every 2 seconds pre-test and post- cognitive testing period. The Hopkins Verbal Learning test word lists contain 12 words, 4 words from 3 semantic categories.

## Cognitive Measurement 4: Brain Fog

Brain fog will be subjectively measured using a modified Borg Rate of Perceived Exertion (RPE) scale as there is no known reliable and valid objective measure of brain fog. This will be administered pre-test and post-cognitive testing period.

## Data Reduction

The data collection periods are listed in Table 3. Minute values are noted in **Figure 1** and are expressed relative to the administration of the MAT.

**Table 3.** Data reduction methods for experimental protocol

| Device | Outcomes | CON (min) | SMS (min) | Reduction |
|---|---|---|---|---|
| **VICORDER ®** | bfPWV | -5, 0, 5, 10, 15, 20, 25 | -115, -15, 0, 5, 10, 15, 20, 25 | Measures done in triplicate. Closest 2 will be averaged for mean and variance reported |
| | cfPWV | -5, 25 | -115, -15, 25 | |
| | faPWV | -5, 25 | -115, -15, 25 | |
| | CBPs | -5, 0, 5, 10, 15, 20, 25 | -115, -15, 0, 5, 10, 15, 20, 25 | |
| | Peripheral BPs | -5, 0, 5, 10, 15, 20, 25 | -115, -15, 0, 5, 10, 15, 20, 25 | |
| | Augmentation index | -5, 0, 5, 10, 15, 20, 25 | -115, -15, 0, 5, 10, 15, 20, 25 | |
| **TCD** | Brain PWV | Continuous | Continuous | 30s selections made by following a |
| | MCA velocity | Continuous | Continuous | |

| | | | | |
|---|---|---|---|---|
| | Static cerebral autoregulation | Continuous | Continuous | prearranged protocol. Data for the outcome in the time epoch will be averaged and SD will be reported |
| | Neurovascular coupling | Continuous | Continuous | |
| **NIBP** | Baroreflex sensitivity | Continuous | Continuous | 30s selections made by following a prearranged protocol. Data for the outcome in the time epoch will be averaged and SD will be reported |
| | Heart rate variability | Continuous | Continuous | |
| | Heart rate | Continuous | Continuous | |
| **Ultrasound** | Cerebrovascular BF | -5, 25 | -120, -5, 20 | |
| **NIRS** | Cerebral perfusion | Continuous | Continuous | 30s selections made by following a prearranged protocol. Data for the outcome in the time epoch will be averaged and SD will be reported |
| | Tissue saturation index | Continuous | Continuous | |
| | Venous pooling | Continuous | Continuous | |
| **ActiGraph** | Physical activity | Continuous | Continuous | |
| | Sleep duration | Continuous | Continuous | |
| **Cognitive Tests** | Executive Function | 25 | 25 | A single score for each cognitive test is received from each participant from each experiental visit |

## 2.6 Timeline

### Study Time Commitment

| Stage | Time (mins) | Description |
|---|---|---|
| Recruitment | 10 | Via email, flyer and/or class presentation |
| Familiarization Session | 35 | Familiarize test subject with the purpose of the study, all experimental devices and the timeline of each experimental condition |
| Experimental Visits | 255 | All measurements, single visit |
| **Total** | 300 | |

**Familiarization Session**

| Time (mins) | Action |
|---|---|
| 0:00-5:00 | Share adult **consent form** with participant. Questions and concerns will be heard on the protocol and purpose of this study. |
| 5:00-10:00 | **Discuss the underlying physiology** and purpose that lead to the creation of this study. Discuss how this study will answer the research questions. |
| 10:00-30:00 | The researcher will attach each device to the participant to **familiarize him/her to the protocol**. No data will be collected for later analysis during this period. |
| 30:00-35:00 | Subject and researchers **will schedule the date and time** for CON and SMS. ActiGraph will be attached to the research participant. A document will be provided detailing all behavioral restrictions (e.g. abstinence from caffeine, alcohol) and instructions on wearing the ActiGraph will be provided. |
| **Session End – Total time spent in APL: 35 minutes** | |

**CON Experimental Session**

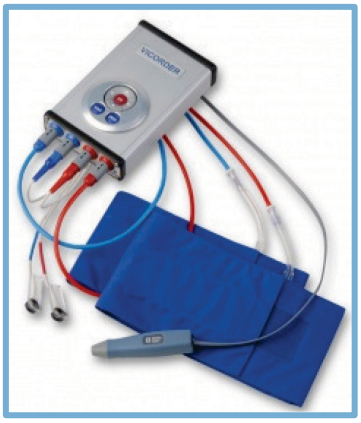
| Time (mins) | Action | Measurement Order | EXAMPLE START TIME |
|---|---|---|---|
| -30:00 - -25:00 | Anthropometrics | Weight (kg)<br>Height (cm)<br>BMI (km/m$^2$) | 10:15 AM |
| -25:00 | *Begin Supine Rest* | N/A | 10:20 AM |
| -25:00 - -15:00 | Affix devices to participant. | Path lengths (cm) | 10:20 AM |
| -15:00 | *End Supine Rest*. Begin continuous data collection from devices. *Begin sitting period* | TCD measures<br>NIRS measures<br>NIBP measures | 10:30 AM |
| -15:00 - -5:00 | Ultrasound data collection | Cerebrovascular blood flow (common carotid, internal carotid, and vertebral arteries) | 10:30 AM |
| -15:00 - -5:00 | VICORDER® data collection | bfPWV/A<br>cfPWV/A<br>faPWV/A | 10:30 AM |
| -5:00-0:00 | Mental Arithmetic Test | N/A | **10:40 AM** |
| 00:00-1:00 | VICORDER® data collection | bfPWV/A immediately after MAT | 10:45 AM |
| 5:00-20:00 | VICORDER® data collection | bfPWV/A every 5m post-MAT for addition 20m | 10:50 AM |
| 25:00-30:00 | VICORDER® data collection | All measures of PWV/A 10m post-MAT | 11:10 AM |
| 30:00-40:00 | Cognitive tests | Trails A+B<br>VFT | 11:15 AM |
| Test End – Total time spent in APL: 75 minutes | | | |

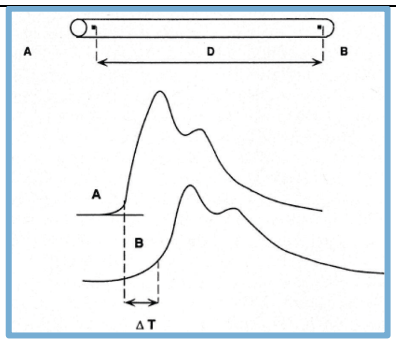
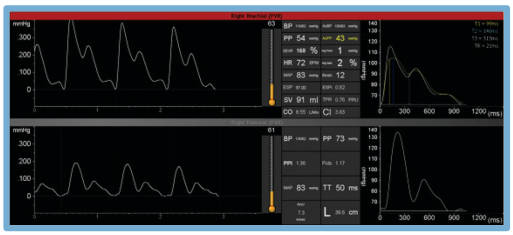
**SMS Experimental Session**

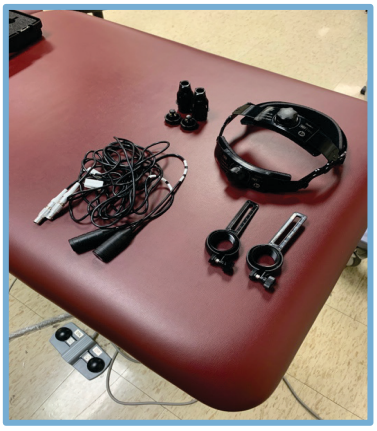
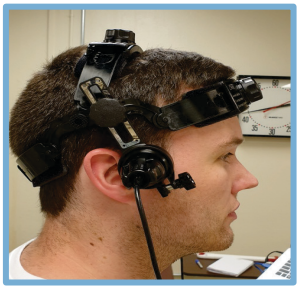
| Time (mins) | Action | Measurement Order | EXAMPLE START TIME |
|---|---|---|---|
| -140:00 - -135:00 | Anthropometrics | Weight (kg)<br>Height (cm)<br>BMI (kg/m$^2$) | 8:25 AM |
| -135:00 | ***Begin Supine Rest*** | N/A | 8:30 AM |
| -135:00 - -125:00 | Affix devices to participant. Collect path lengths required for measures from PWV/A | Path lengths (cm) | 8:30 AM |
| -125:00 | ***End Supine Rest***. Begin continuous data collection from devices. ***Begin prolonged sitting period*** | **TCD** measures<br>**NIRS** measures<br>**NIBP** measures | 8:40 AM |
| -120:00 -115:00 | **Ultrasound** data collection | Cerebrovascular blood flow (common carotid, internal carotid, and vertebral arteries) | 8:45 AM |
| -115:00 - -110:00 | **VICORDER®** data collection | bfPWV/A<br>cfPWV/A<br>faPWV/A | 8:50 AM |
| -20:00 - -15:00 | **Ultrasound** data collection | Cerebrovascular blood flow (common carotid, internal carotid, and vertebral arteries) | 10:25 AM |
| -15:00 - -5:00 | **VICORDER®** data collection | bfPWV/A<br>cfPWV/A<br>faPWV/A | 10:30 AM |
| -5:00 | ***End prolonged sitting period*** | N/A | 10:40 AM |
| -5:00 – 0:00 | Mental Arithmetic Test | N/A | **10:40 AM** |
| 00:00-1:00 | VICORDER® data collection | bfPWV/A immediately after MAT | 10:45 AM |
| 5:00-20:00 | VICORDER® data collection | bfPWV/A every 5m post-MAT for addition 20m | 10:50 AM |
| 25:00-30:00 | VICORDER® data collection | All measures of PWV/A 10m post-MAT | 11:10 AM |
| 30:00-40:00 | Cognitive tests | Trails A+B<br>VFT | 11:15 AM |
| **Test End – Total time spent in APL: 180 minutes** | | | |

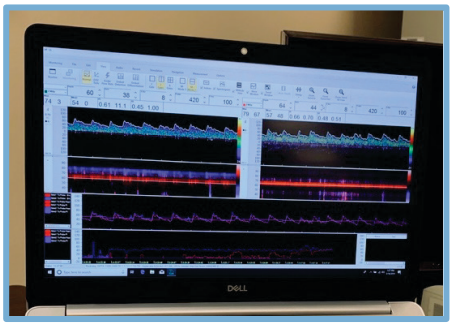
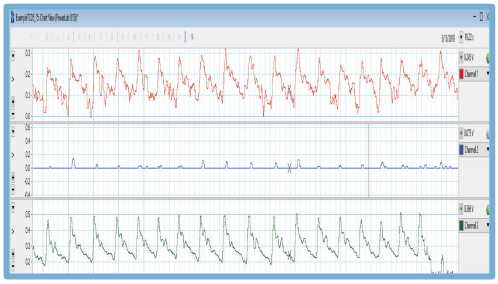
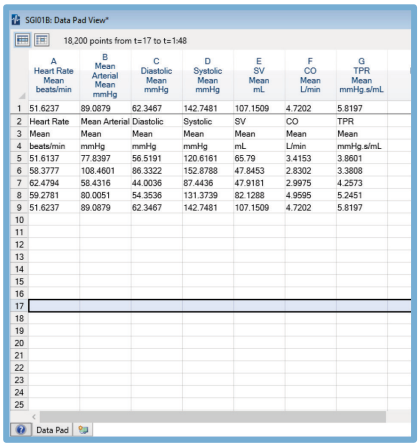
# 3 MEASUREMENT DEVICES

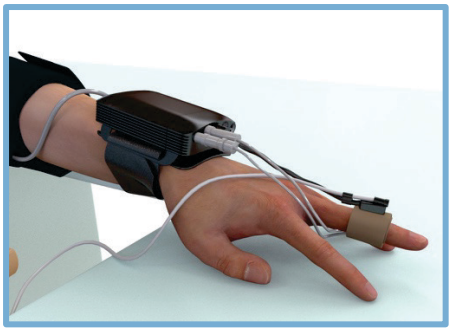
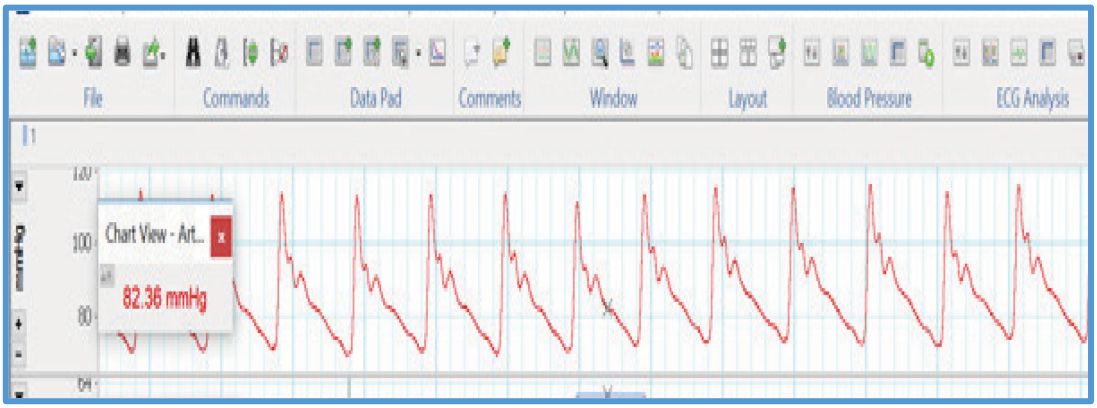
## Device 1: VICORDER®

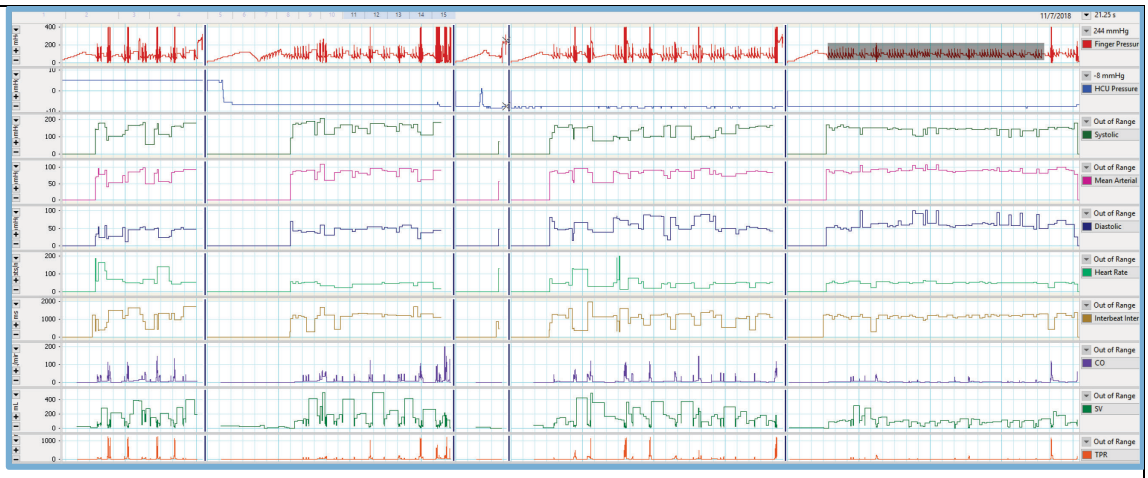
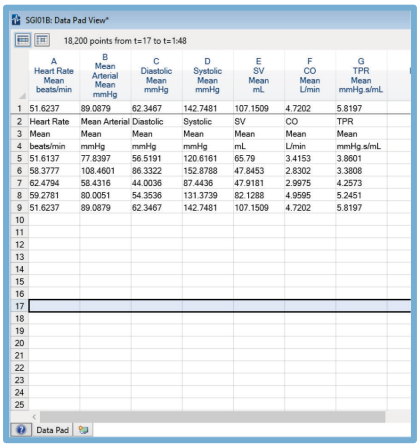
| | | |
|---|---|---|
| **Device** |  | • **Company:** SMT Medical<br>• **Model:** VICORDER®: Arterial Stiffness Model<br>• **Location:** Wurzburg, Germany<br>• Oscillometric cuffs inflate at sub-systolic pressures to detect changes in the pressure waveform.<br>• Different sized cuffs are available. |
| **Example Set-up** |  | • The cuffs can be placed on any 2 arterial segments.<br>• In the figure to the left, pressure cuffs placed over the brachial and femoral arteries.<br>• A small balloon is placed over the carotid artery to obtain a measurement for cfPWV. A low pressure (50 mmHg) is used for this site with minimal discomfort. |
| **Raw Data** |  | • Top display = pressure waveform from proximal arterial segment<br>• Bottom display = pressure waveform from distal arterial segment<br>• Intrinsic software automatically detects the foot of the waveform of each arterial segment |
| **Data Analysis** | | • A = Site of proximal artery<br>• B = Site of distal artery<br>• **Pulse wave velocity (PWV)** = distance (D) / transit time (TT)<br>• **Straight line distance (L)** = Distance between two cuffs as measured according to manufacturer guidelines |

| | | |
|---|---|---|
| |  | • **Transit Time (ΔT or TT)** = Time between foot of proximal and distal waveforms.<br>• Oscillometric pressure waveforms will be determined at the brachial artery of the non-dominant arm using the VICORDER® in order to determine brachial and central arterial blood pressures. |
| **Data Reduction** |  | • L is plugged into software prior to measurement.<br>• TT is averaged across 3 waveforms, and used to calculate one PWV<br>• Process repeated 3 times, for 3 PWVs per measurement cycle.<br>• Closest 2 PWVs averaged and used as outcome for measurement cycle. |

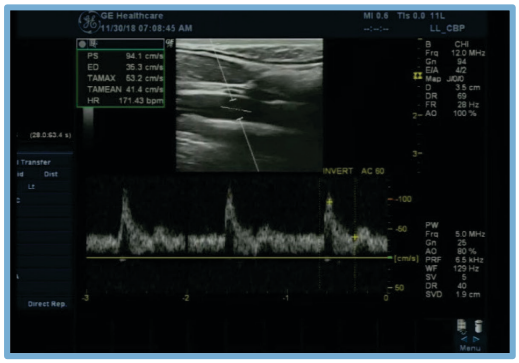
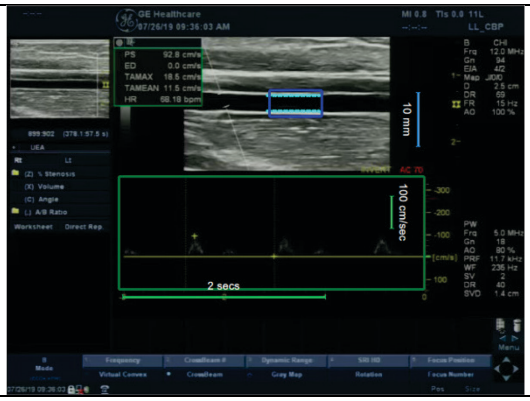
| **Device 2: Transcranial Doppler (TCD)** | | |
|---|---|---|
| **Device** |  | • **Company:** Compumedics DWL<br>• **Model:** Transcranial Doppler Sonography TCD<br>• **Location:** Charlotte NC, USA<br><br>• DiaMon headset can be adjusted in 3 ways to accommodate each individual<br>• Connection between rear band and side panel sits behind the ear, below the occipital bone to ensure signal<br>• TCD probes should be in contact with head if headset has been fitted correctly |
| **Example Set-up** |  | • Ensure probe is positioned over the temporal window<br>• Apply ultrasound gel to probe and/ or temporal window<br>• Move probe until faint trace appears and then make further fine movements to increase trace of artery velocity |

| | | |
|---|---|---|
| **Raw Data** |  | • Signal quality is indicated by color: the more yellow, the stronger the signal<br>• Gain and depth adjust can be made in the signal window. Gain should sit between 38-44 for optimal signal output<br>• Typical velocities for middle cerebral artery (MCA) are ~80 cm/s-1, and ~60 cm/s-1 in older populations<br>• Typical depth for MCA is 45-55 |
| **Data Analysis** |  | • Using a custom macro, the software (Labchart) identifies the peak of the ECG R- wave and the peak of the second derivative of each pressure waveform (corresponds to foot), then calculate the transit time (TT) between said peaks.<br>• Pulse wave velocity (PWV) = distance (L) / TT |
| **Data Reduction** |  | • Measures of brain PWV, MCA velocity, static CA and neurovascular coupling will be collected from this device are continuously recorded during a testing session<br>• Data reduction for this protocol will require the selection of 30s epochs at specific time points to observe how PS and MS are impacting measures of CVH<br>• In the picture to the left, a researcher selected a time period to report the data collected during this time.<br>• A strict protocol will be followed to ensure that selection of 30s epochs are synchronized amongst all measures and research participants to prevent bias. |

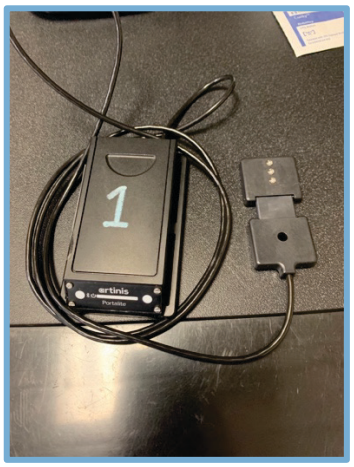
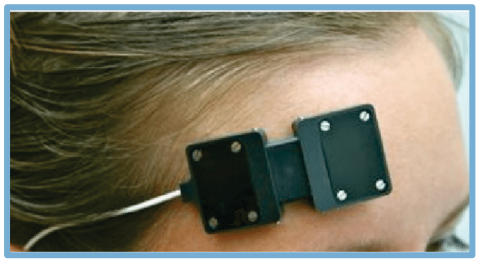
| Device 3: Non-Invasive Blood Pressure (NIBP) | | |
|---|---|---|
| **Device** |  | • **Company:** ADInstruments<br>• **Model:** Human NIBP Nano System, Finapress<br>• **Location:** Sydney, Australia<br>• Single cuff contains an infrared photoplethysmographic (940 mmHg) sensor and inflatable cuff.<br>• PPG sensor continuously captures the pulse waveform.<br>• Inflatable cuff used to calibrate waveform<br>• Height correction unit corrects for hydrostatic pressure changes due to change in hand position relative to the heart. |
| **Example Set-up** |  | • Device placed between distal and proximal inter-phalangeal joint of the index finger.<br>• Wrist apparatus is strapped to the individual's non-dominant wrist |
| **Raw Data** |  <br>• Data captured using a data acquisition system (PowerLab).<br>• Also displayed are electrocardiogram (ECG) signals captured by PowerLab. | |

| | | |
|---|---|---|
| **Data Analysis** |  <br>• LabChart 8 can perform real time or offline function, including data averaging, peak detection, derivates etc. | |
| **Data Reduction** |  | • All measures collected from this device are continuously recorded during a testing session<br>• Data reduction for this protocol will require the selection of 30s epochs at specific time points to observe how PS and MS are impacting measures of CVH<br>• In the picture to the left, a researcher selected a time period to report the data collected during this time.<br>• A strict protocol will be followed to ensure that selection of 30s epochs are similar amongst all measures and research participants to prevent bias. |

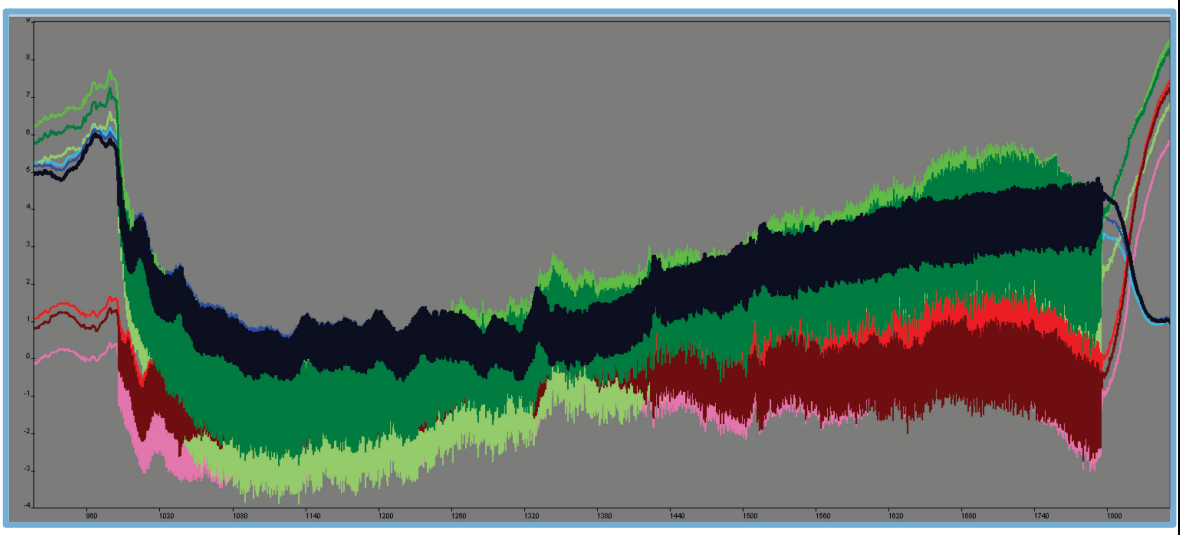
| **Device 4: Doppler Ultrasound** | | |
|---|---|---|
| **Device** |  | • **Company:** GE Healthcare<br>• **Model:** Logiq P6<br>• **Location:** Chicago, IL<br>• An 11L linear probe will be used for this protocol |

| | | |
|---|---|---|
| **Example Set-up** |  | • Measurement of blood flow through the carotid artery using a linear probe<br>• Probe is placed parallel to the blood vessel<br>• 10s videos of the common carotid, vertebral, and internal carotid arteries will be collected at each designated time point in the protocol<br>• Participants will be instructed to hold one's breath for the duration of the recording to ensure a clear video will be captured of the observed artery |
| **Raw Data** |  | • Ultrasound videos are recorded at 30 Hz using an external video capture system<br>• Debut Professional (v4.0, NCH Software®) is used to record the present image on the Ultrasound device for later analysis<br>• Image to the left shows an example video captured by this software |
| **Data Analysis** |  | • The captured videos will be analysed offline using specialized image analysis software (FMD Studio®,) shown to the left.<br>• The software measures and exports second-by-second diameters and blood velocities<br>• Cardiovascular Suite (Quipu, v3.5.3) analyzes raw ultrasound footage<br>• Coordination with ECG will produce measures of blood flow |
| **Data Reduction** | | |

| | |
|---|---|
| |  <br>• Lab-designed macro cleans raw data output generated by the video analysis software<br>• Macro is capable of calculating blood flow along with other measures that define the flow profile of the observed artery |

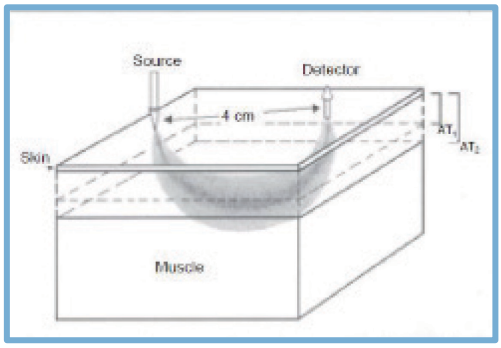
| **Device 5: Continuous Wave Near-Infrared Spectroscopy with Spatially Resolved Spectroscopy (cwNIRS-SRS or NIRS)** | | |
|---|---|---|
| **Device** |  | • **Company:** Artinis Medical Systems<br>• **Model:** Portalite<br>• **Location:** Amsterdam, Netherlands<br>• Two probes will be used in this protocol<br>  ○ Cerebral perfusion: placed over the frontal cortex<br>  ○ Lower-limb perfusion: placed over the muscle belly of the gastrocnemius |
| **Example Set-up** |  | • Researchers will follow manufacturer guidelines on appropriate probe placement<br>• An opaque cloth (not shown) will be wrapped over the probe to prevent the influence of ambient light on infrared signals<br>• For NIRS probe placement over the gastrocnemius: An 11L linear probe from the Logiq 6 ultrasound device will be used to ensure the NIRS device is not placed over a blood vessel.<br>• The ultrasound device is only be used to ensure the probe is being placed only over the muscle belly of the gastrocnemius |

**Raw Data**



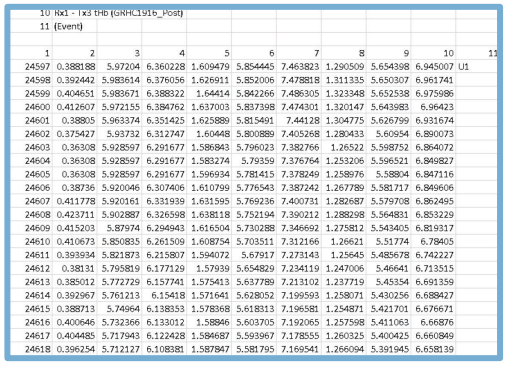
- Oxysoft, a proprietary software system developed by Artinis Medical Systems, will be used to collect and display raw data
- Displayed are three signals each for total hemoglobin (greens), oxygenated hemoglobin (reds), and deoxygenated hemoglobin (blues)

**Data Analysis**




- The NIRS devices emits three separate wavelengths to capture different depths of the
- The spatially resolved signal working with the probe is capable of estimating absolute values for each variable used in this protocol

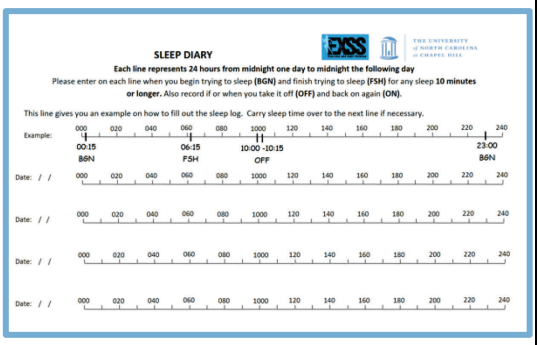
**Data Reduction**

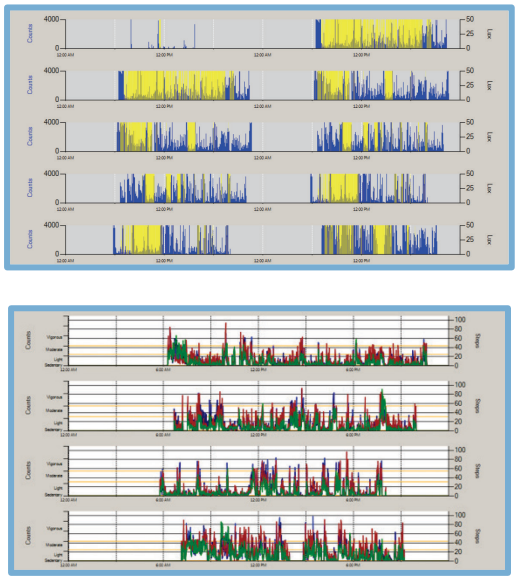
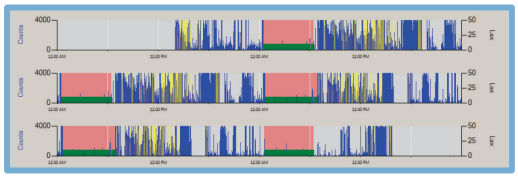


10 Rx1 - Tx3 THb (GNHC191b_Post)
11 (Event)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 24597 | 0.388188 | 5.97204 | 6.360228 | 1.609479 | 5.854445 | 7.463823 | 1.290509 | 5.654398 | 6.945007 | U1 | |
| 24598 | 0.392442 | 5.983614 | 6.376056 | 1.626911 | 5.852006 | 7.478818 | 1.311335 | 5.650307 | 6.961741 | | |
| 24599 | 0.404651 | 5.983671 | 6.388322 | 1.64414 | 5.842266 | 7.486305 | 1.323348 | 5.652538 | 6.975986 | | |
| 24600 | 0.412607 | 5.972155 | 6.384762 | 1.637003 | 5.837398 | 7.474301 | 1.320147 | 5.643983 | 6.96423 | | |
| 24601 | 0.38805 | 5.963374 | 6.351425 | 1.625889 | 5.815491 | 7.44128 | 1.304775 | 5.626799 | 6.931674 | | |
| 24602 | 0.375427 | 5.93732 | 6.312747 | 1.60448 | 5.800889 | 7.405268 | 1.280433 | 5.60954 | 6.890073 | | |
| 24603 | 0.36308 | 5.928597 | 6.291677 | 1.586843 | 5.796023 | 7.382766 | 1.26522 | 5.598752 | 6.864072 | | |
| 24604 | 0.36308 | 5.928597 | 6.291677 | 1.583274 | 5.79359 | 7.376764 | 1.253206 | 5.596521 | 6.849827 | | |
| 24605 | 0.36308 | 5.928597 | 6.291677 | 1.596934 | 5.781415 | 7.378249 | 1.258976 | 5.58804 | 6.847116 | | |
| 24606 | 0.38736 | 5.920046 | 6.307406 | 1.610799 | 5.776543 | 7.387242 | 1.267789 | 5.581717 | 6.849606 | | |
| 24607 | 0.411778 | 5.920161 | 6.331939 | 1.631595 | 5.769236 | 7.400731 | 1.282687 | 5.579708 | 6.862495 | | |
| 24608 | 0.423711 | 5.902887 | 6.326598 | 1.638118 | 5.752194 | 7.390212 | 1.288298 | 5.564831 | 6.853229 | | |
| 24609 | 0.415203 | 5.87974 | 6.294943 | 1.616504 | 5.730288 | 7.346692 | 1.275812 | 5.543405 | 6.819317 | | |
| 24610 | 0.410673 | 5.850835 | 6.261509 | 1.608754 | 5.703511 | 7.312166 | 1.26621 | 5.51774 | 6.78405 | | |
| 24611 | 0.393934 | 5.821873 | 6.215807 | 1.594072 | 5.67917 | 7.273143 | 1.25645 | 5.485678 | 6.742227 | | |
| 24612 | 0.38131 | 5.795819 | 6.177129 | 1.57939 | 5.654829 | 7.234119 | 1.247006 | 5.46641 | 6.713515 | | |
| 24613 | 0.385012 | 5.772729 | 6.157741 | 1.575413 | 5.637789 | 7.213102 | 1.237719 | 5.45334 | 6.691359 | | |
| 24614 | 0.392967 | 5.761213 | 6.15418 | 1.571641 | 5.628052 | 7.199593 | 1.258071 | 5.430256 | 6.688427 | | |
| 24615 | 0.388713 | 5.74964 | 6.138353 | 1.578368 | 5.618313 | 7.196581 | 1.254871 | 5.421701 | 6.676671 | | |
| 24616 | 0.400646 | 5.732366 | 6.133012 | 1.58846 | 5.603705 | 7.192065 | 1.257598 | 5.411063 | 6.66876 | | |
| 24617 | 0.404485 | 5.717943 | 6.122428 | 1.584687 | 5.593967 | 7.178555 | 1.260325 | 5.400425 | 6.660849 | | |
| 24618 | 0.396254 | 5.712127 | 6.108381 | 1.587847 | 5.581795 | 7.169541 | 1.266094 | 5.391945 | 6.658139 | | |

- A lab-designed macro will export the raw data to an Excel sheet
- Column 1 represents the time point
- A researcher will then select 30s sections for specific time points during the experimental conditions (e.g. immediately post-MAT, 5 minutes post-MAT)

| Device 6: ActiGraph | | |
|---|---|---|
| **Device** |  | • **Company:** ActiGraph<br>• **Model:** wGT3X-BT<br>• **Location:** Pensacola, FL |
| **Example Set-up** |  <br>  <br>SLEEP DIARY<br>Each line represents 24 hours from midnight one day to midnight the following day<br>Please enter on each line when you begin trying to sleep (BGN) and finish trying to sleep (FSH) for any sleep 10 minutes or longer. Also record if or when you take it off (OFF) and back on again (ON).<br>This line gives you an example on how to fill out the sleep log. Carry sleep time over to the next line if necessary.<br>Example: 00:15 BGN; 06:15 FSH; 10:00 - 10:15 OFF; 23:00 BGN<br>Date: / / | • Device is worn on the non-dominant wrist<br>• To maintain internal validity subjects will be asked to complete a sleep journal that details when the ActiGraph device is being worn, when the participant goes to sleep, and when he/she awakens.<br>• This information will only be used to validate the adherence of the participant to pre-test behaviors: Waking up at relatively the same time of the morning, no vigorous exercise 24hr prior |

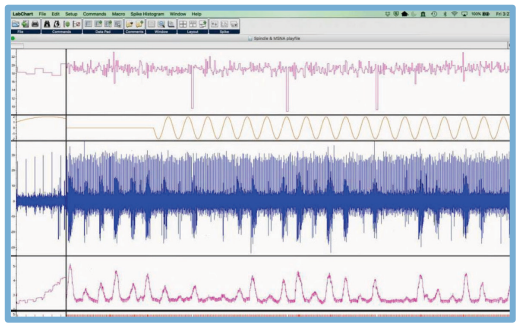
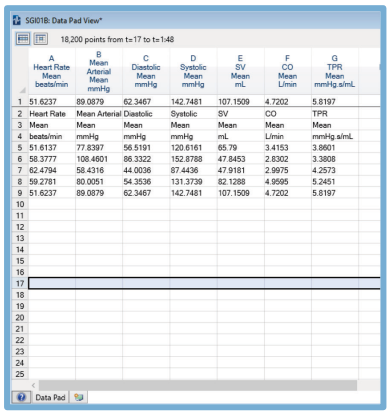
| | | |
|---|---|---|
| **Raw Data** |  | • Upper image: 24 hour observation of counts of physical activity and ambient light throughout several days<br>• Lower image: More detailed view of physical activity counts. |
| **Data Analysis** |  | • Trained researchers will use the returned sleep journal to set cut points for the beginning and end of sleep periods of the research participant<br>• This process allows more accurate reports of ambient physical activity before and during experimental sessions |
| **Data Reduction** | | |

| Filename | Sedentary | Light | Moderate | Vigorous | Total MVPA | % in MVPA | Time | Time Unfiltered | Calendar Days | Calendar Days Unfiltered |
|---|---|---|---|---|---|---|---|---|---|---|
| TEST (2019-03-17)60sec.agd | 1253 | 743 | 403 | 0 | 403 | 16.8 | 2399 | 2880 | 2 | 2 |
| TEST (2019-03-17)60sec.agd | 739 | 497 | 204 | 0 | 204 | 14.2 | 1440 | 1440 | 1 | 1 |
| TEST (2019-03-17)60sec.agd | 735 | 455 | 250 | 0 | 250 | 17.4 | 1440 | 1440 | 1 | 1 |
| TEST (2019-03-17)60sec.agd | 605 | 641 | 194 | 0 | 194 | 13.5 | 1440 | 1440 | 1 | 1 |
| TEST (2019-03-17)60sec.agd | 686 | 532 | 222 | 0 | 222 | 15.4 | 1440 | 1440 | 1 | 1 |
| TEST (2019-03-17)60sec.agd | 728 | 634 | 78 | 0 | 78 | 5.4 | 1440 | 1440 | 1 | 1 |
| TEST (2019-03-17)60sec.agd | 749 | 445 | 115 | 0 | 115 | 8.8 | 1309 | 2880 | 1 | 2 |

- Data can be reduced on an hourly or daily basis
- Accelerometry data separates daily ambulation into different categories: sedentary, light, moderate, and vigorous

## DEVICES 7 & 8: Data Acquisition (PowerLab) and Data Processing (LabChart)

| | | |
|---|---|---|
| **Device** |  | • Company: ADInstruments<br>• Model: PowerLab 8/35<br>• Location: Sydney, Australia<br>• Device will work in conjunction with LabChart software and NIBP cuff |

| | | |
|---|---|---|
| **Example Set-up** |  | • Example set-up shown to an in the image to the left. Shown in this example:<br>  ○ Upper left: EKG<br>  ○ Bottom PowerLab Unit: TCD inputs for eventual display on LabChart |
| **Raw Data** |  | • Shown is LabChart receiving the signals interpreted by the PowerLab system |
| **Data Analysis** | • LabChart 8 can perform real time or offline function, including data averaging, peak detection, derivates etc. | |
| **Data Reduction** |  | • After the completion of a data collection set, summarized data is calculated by the software<br>• Data will be exported to lab Excel files for comparison amongst subjects and final analysis |

# 4 STATISTICAL CONSIDERATIONS

## 4.1 Study Outcomes

The study outcomes are listed below.

**The primary outcomes are:**

- Brain PWV
- Brachial-femoral PWV

*Note: All power calculations for this protocol are based on measures from PWV*

**The secondary outcomes are:**

- Executive function
- Neurovascular coupling
- Carotid-femoral PWV
- Augmentation index
- Femoral-ankle PWV

**Mechanistic and descriptive outcomes are:**

- Cerebral perfusion
- Total brain blood flow
- Middle cerebral artery velocity
- Static cerebral autoregulation
- Baroreflex sensitivity
- Heart rate variability
- Heart rate
- Peripheral diastolic blood pressure
- Peripheral systolic blood pressure
- Tissue saturation index
- Venous pooling in the gastrocnemius
- Bouts of physical activity
- Sleep duration

## 4.2 Statistical Analysis

All statistical procedures will be competed with SPSS Statistics version 25.0 (SPSS, IN., Durham, NC, USA) and Jamovi v0.9 (Jamovi, UK). The α-level will be set a prior at 0.05 for all statistical procedures. Descriptive statistics and mechanistic outcomes will be collected to compare similarities amongst the sample group and to eliminate the potential for extraneous covariates. Answering the research question will require a linear multi-level mixed model. Statistical models will observe for changes in the primary and secondary outcomes within conditions (Baseline, Post-PS, Post-MS) and between conditions (CON vs. SMS at similar time points). Mechanistic outcomes will also be used to assist in discussions of physiological responses to MS and PS. Effect sizes will be calculated as Cohen's d, where <0.20 is considered to be a small, > 0.20 to < 0.50 a moderate, and > 0.60 a large effect. For the mixed models Cohen's d will be calculated as the effect of condition (β) or time (β) from linear mixed models divided by the baseline SD. Raw data will be presented as mean [standard deviation] and mixed model data are presented as mean [95% confidence interval].

## 4.3 Tabulation of Results

Mean and variance will be tabulated after raw measures taken in triplicate are entered into a lab-created template.

**Table 4.** Methods to describe result summarization for this experimental protocol.
**Abbreviations:** X, Mean/SD will be calculated for this result; 1, Measure is reported as a single value from each experimental visit; n/a, Measure is not being taken at this timepoint.

| | | Baseline | | Post-PS | | Post-MS | |
|---|---|---|---|---|---|---|---|
| **Device** | **Outcomes** | **X** | **SD** | **X** | **SD** | **X** | **SD** |
| **VICORDER ®** | bfPWV | X | X | X | X | X | X |
| | cfPWV | X | X | X | X | X | X |
| | faPWV | X | X | X | X | X | X |
| | CBPs | X | X | X | X | X | X |
| | Peripheral BPs | X | X | X | X | X | X |
| | Augmentation index | X | X | X | X | X | X |
| **TCD** | Brain PWV | X | X | X | X | X | X |
| | MCA velocity | X | X | X | X | X | X |
| | Static cerebral autoregulation | X | X | X | X | X | X |
| | Neurovascular coupling | X | X | X | X | X | X |
| **NIBP** | Baroreflex sensitivity | X | X | X | X | X | X |
| | Heart rate variability | X | X | X | X | X | X |
| | Heart rate | X | X | X | X | X | X |
| **Ultrasound** | Cerebrovascular BF | 1 | X | 1 | X | 1 | X |
| **NIRS** | Cerebral perfusion | X | X | X | X | X | X |
| | Tissue saturation index | X | X | X | X | X | X |
| | Venous pooling | X | X | x | X | x | X |
| **Cognitive Tests** | Executive Function | n/a | n/a | n/a | n/a | 1 | X |

## 4.4 Missing Values

Subjects with missing data will be included in the analysis. The linear mixed-effects model is well-designed for coping with incomplete longitudinal data. Reasons for non-complete data sets will be recorded, and available values will be compared against the population estimates to ascertain risk of bias.

## 4.5 Quality Control

For a given outcome all measurement and analysis will be conducted by a single observer. At the start of the study, the first three sets of data collected by the first three research participants will be checked by an independent observer.

At the conclusion of the study, a random selection of 10% of the data sets (e.g., all data from 2 participants) will be re-scored by an independent observer and used to calculate inter-observer reliability.

## 4.6 Sample Size

Calculations to determine minimum sample size will be based on central vascular health outcome, aortic pulse wave velocity (PWV). We will conduct a power calculation based on reported normal values for the primary outcome, central PWV. Healthy people aged 40-49 maintain an average PWV of 7.5 m/s. For the current study, we will opt to sample based on a conservative change score of 1 m/s. We will also use a conservative usual error of 1.25 m/s based on reported data. 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 eighteen participants will be required.

## 4.7 Statistical Support

Statistical expertise, study coordination and statistical computations for research database management (RDM) and data analysis will be provided by the research team. Advised by Lee Stoner, PhD. If needed, the faculty advisor will submit a request to NCTracs.

## 5 Participant Screening, Withdrawal & Completion5 n failure procedures

Participants will not be able to participate if they do not meet the inclusion criteria. The acceptability of participants' potential recordings from each device will be made during the familiarization visit.

### 5.2 Participant withdrawal

If a participant decides to withdraw from the study all efforts should be made to complete and report study assessments as thoroughly as possible. The investigator will contact the participant by telephone or through a personal visit to establish the reason for the study withdrawal. A complete final evaluation at the time of the patient's study withdrawal should be made with an explanation of why the patient is withdrawing from the study. If the reason for removal of a patient from the study is an adverse event, the principal specific event will be recorded on study data collection forms. If the reason for withdrawal or removal is not an adverse event, the nature of the reason will be recorded on the study data collection forms.

### 5.3 Participant Completion

Upon completion of the study, participants will be thanked, and the results discussed.

# 6 SAFETY MONITORING & MANAGEMENT

## 6.1 Adverse Event Risk

Investigators leading this project are knowledgeable of clinically healthy values for BP, cardiac output, and other experimental outcomes. It is anticipated the collection of values of clinical concern will be rare as those with a diagnosis of cardio-metabolic diseases will be excluded from this study entirely. However, in the event of clinically meaningful cardiovascular values, the participant will be notified of his or her results and consultation with faculty advisor, Dr. Lee Stoner will be completed on further action, if needed. It is ultimately the decision of the subject to follow through with action after gaining knowledge and receiving advice on his or her unfavorable cardiovascular health.

The devices used in this study are noninvasive. There are no known severely adverse events that have occurred with use of the stated devices.

**VICORDER®** - The system requires the placement of pressure cuffs over several arteries for the collection of PWV/A data. Pressure cuffs will only be inflated underneath a level of 65 mmHg. Physical harm or discomfort is unlikely and include, but are not limited to:

- Risk 1: Discomfort/unease: Infrequent (1 – 10%) – Application of a slight pressure over the carotid artery may impose a sense of unease for the participant. However, the light pressure used for this experimental protocol will in no way significantly damage cardiovascular structure or place the participant in danger. Investigators will make certain that communication on the procedures during testing session are clearly conveyed to the participant for comfort and safety.

**Near-infrared spectroscopy (NIRS):** Risk of injury or discomfort is extremely low due to this device. Possible physical harms are, but not limited to:

- Risk 1: Eye damage/irritation: Rare (<1%) – Please do not, at any point, stare into the light emitted from the NIRS probe
- Risk 2: Skin heating and irritation: Rare (<1%) – Wearing the NIRS probe for extended periods of time at once can theoretically lead to a warm feeling at the area where the probe is placed. However, this risk is minimal because the light emitted from this probe is not powerful enough to heat the skin. If you let the investigators know of any discomfort due to the probe, we will follow manufacturer guidelines to ensure the device is functioning correctly.

**Transcranial Doppler (TCD):** Data collection from this system requires the affixation of a headpiece to the participant. Risk of injury due to this device is extremely low. Possible harms may include, but are not limited to:

- Risk 1: Mild headache: Infrequent (1 – 10%) – High quality data from this device requires the placement of the probe over the middle cerebral artery (MCA) and posterior

cerebral artery (PCA). The slight pressure applied to the area may be slightly discomforting and unusual for the participant.

## 6.2 Responding Adverse Events

In the unlikely event that a subject sustains an injury throughout the duration of the study, they will be referred to the appropriate medical personnel on the UNC campus (i.e. Campus Health Services or the UNC Hospitals Emergency Room). 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 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.

## 6.3 Confidentiality

Limiting the number of research team members in the laboratory during any testing session will minimize breach of confidentiality. By needing key card access to the laboratory, we are limiting the number of individuals not on the research team who have access to the lab. Those who do have key card access are exercise physiology professors, Ph.D. candidates, and master's candidates, and selected undergraduate students who are directly associated with the study and have performed all necessary trainings regarding sample handling, laboratory procedures, and confidentiality. All participants within the study are coded with an individual ID and no names will be identified in any document besides a master key document. This master key document will be kept in a locked drawer in the Applied Physiology Research Lab. No subjects will be identified in any report or publication of this study.

## 6.4 Participant Withdrawal

If a subject fails to comply with pre-testing guidelines, their allocated session will be rescheduled. If a subject fails to comply with pre-testing guidelines for a second time, they will be withdrawn from the study. Any subjects included in the study may withdraw without penalty at any point and will be reminded of their right to do so before the commencement of data collection.

# 7 DATA COLLECTION AND MANAGEMENT

Management of the research data for this study will involve collection, entry, processing, storage, retrieval, archival, distribution and documentation of information collected according to a written protocol. The overall strategy for quality assurance in data management will be of a professional level as described in the following sections.

## 7.1 Quality Assurance

The PI will allocate adequate time to monitor the study to ensure quality and integrity of data collected. The PI will review study files, regulatory documents, and consent forms The PI will have overall responsibility for ensuring quality in the data and in the procedures that produce the data. In addition to the master protocol document, written guidelines and detailed procedure manuals may be needed for uniform adherence to the detailed intent of the protocol. The written record of how the study was performed will be completed prior to commencement of recruitment and will be updated during the course of the study. The PI will have overall responsibility for the definition and production of the documents necessary to describe all aspects of the study in sufficient detail to ensure the study can be conducted in a scientifically sound, standardized manner. The PI will be assisted by members of the research team in monitoring adherence to protocol.

## 7.2 Database Security

The database will be created within centralized files and maintained on University approved; password protected, encrypted, shared research drives in the orthopedic surgery department and viewed only on approved devices by approved study personnel. Collected data will be kept in a locked cabinet in the Applied Physiology Lab in Fetzer Hall on the University of North Carolina at Chapel Hill campus. Only members of the research team will have keys for this cabinet. All shared files between the research team will be stored on a UNC file requiring an Onyen Login and either a UNC computer or UNC campus VPN.

## 7.3 Confidentiality

Research data will be identified only by study identification numbers (IDs). These study IDs will be used to maintain relationships in the data between various tables. All consent forms and any paper data collection instruments will be stored in a locked cabinet in a secure location. The list identifying subjects with their contact information will be kept separate from the scientific study data. No participants will be identified in any report or publication of this study. Participants will be identified via identification code for data collection purposes. All electronically collected data will be stored on a computer that only team members Onyen password will allow access to study information. This computer will be located in the Graduate Student Office of the Applied Physiology Laboratory at the University of North Carolina at Chapel Hill. Data analysis procedures will be performed on secure computers within Fetzer Hall, with which password is required to access.

## 7.4 PHI Security

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Procedures to maintain privacy and confidentiality will be followed rigorously. Each research

subject will be assigned a study identification number (ID). The scientific study data will be stored in a digital file that does not include patient identifiers or personal health information (PHI). A separate data file will contain identifiers and PHI. A master file linking patient identifiers to the study data will be kept under lock and key by principal investigator. The database will be password protected and will be stored on servers housed in a secure location.

## 7.5 Risk of Deductive Disclosure

We will minimize the risk of unauthorized persons using the database to figure out a subject's identity and responses.

## 7.6 Data Collection

Data collection will adhere to precise written instructions and will be monitored to ensure adherence to the protocol.

## 7.7 Data Entry

All data entered into the database will be verified by comparing the original source values to the values in the database. We will perform double data entry, where one person will enter data into a spreadsheet under Tab1. A second person will enter data under Tab 2. A third tab (Tab 3) represents the difference in Tab 1 and 2. If values are not 0, the second person will find and fix data.

## 7.8 Data Editing

The PI will be responsible for reviewing data-monitoring results and investigating questions raised (i.e., queries) about remarkable or questionable data values.

## 7.9 Database Documentation

The names of the variables and their valid ranges or categorical values will be listed in a “data book”. Documentation will also include an index of computer programs and an index of reports. All programming for statistical computation will include comments providing internal documentation.

## 7.10 Pilot Testing of Operations

All aspects of data management and project operation will be pilot tested prior to the commencement of the study in order to verify adequacy of the methods, materials and systems prepared. Every clinical study collects such pilot data –either intentionally prior to commencement of the study, or unintentionally after recruitment has begun.

## 8 RECRUITMENT STRATEGYRECRUITMENT STRATEGY

### 8.1 Recruitment Strategies

Participants will be recruited via email, flyer and/ or workplace presentation from working class individuals in the Raleigh-Durham-Chapel Hill area. Given that we are targeting a fairly general population, and only a limited number of participants are being recruited, we believe there is a high likelihood of having access to the projected number of individuals.

### 8.2 Recruitment Personnel

Recruitment will be conducted by the PI or the trained master's students listed on this application. The team will not recruit students who are currently enrolled in a class they are responsible for.

### 8.3 Protection of Privacy

During the recruitment process potential subjects will not be required to confirm their interest in the presence of individuals who are not part of the research team.

### 8.4 Contacting Participants

Following the provision of the study information, subjects will be encouraged to contact the principal investigator via telephone or email to discuss their involvement or arrange for a face-to-face discussion to take place on a separate occasion.

### 8.4 Efforts to ensure equal access to participation among women and minorities

Strong efforts will be made to ensure equal access to participation among all populations. All ethnicities have equal access to these places and will thus be given equal access to volunteer as subjects. Pregnant women will be excluded from the initial study to limit the variation seen within subjects.

# 9 STUDY MANAGEMENT/ CONSENTSTUDY MANAGEMENT/ CONSENT

## 9.1 Consent and Institutional Review Board (IRB) Approval

Before recruitment and enrollment into this study, the patient will be given a full explanation of the study. A member of the research team will cover each section of the consent form in detail with the subject. At the end of each section, the research team will ask the subject if he or she has any questions. Once the entire consent form is described to the subject, the subject will be reminded that they may withdraw from the study at any time without repercussion. After all questions have been exhausted and the subject agrees to participate, the subject will sign the consent form in the presence of a research team member. Data collection will begin only if the subject has signed an informed consent form.

## 9.2 Efforts to Minimize Influencing the Participant's Decision to Participate

During screening and familiarization, the subject will be allowed to ask any question about the study while the consent form is being explained. The subject will sign the consent form after it has been described in full and all study-related questions answered. If subjects need additional time to decide, it will be granted. It will be made clear to the subject that signing of the consent form does not bind the subject to participation in the study and if at any time he or she wishes to withdraw from the study, he or she may do so without repercussion.

## 9.2 Required Documentation

Before the study can be initiated at any site, the following documentation will be placed on file at the University of North Carolina:

- A copy of the official IRB approval letter for the protocol and informed consent
- A copy of the IRB-approved consent form

## 9.3 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

## 9.4 Emergency Modifications

Members of the research team will inform participants of the study protocol and any potential risk. They will then sign an informed adult consent form included in the IRB. As mentioned previously, subjects will be properly treated and cared for in the unlikely event of an emergency or adverse reaction to any of the noninvasive treatments or venous blood draws.

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