Investigating Functional Optical Coherence Tomography and Hypercapnia to Diagnose and Treat Neurogenic Orthostatic Hypotension

Principal Investigator:

Satish R. Raj, MD MSCI FRCPC

Professor of Cardiac Science

Libin Cardiovascular Institute of Alberta

Cummings School of Medicine

Phone: 403-210-6152 Fax: 403-210-9444

Email: satish.raj@ucalgary.ca

Co-Principal Investigator:

Richard Wilson PhD

Co-Investigators:

Robert S Sheldon MD PhD FRCPC

Carlos Morillo MD FRCPC

Jacquie Baker PhD

Sponsor:

Libin Cardiovascular Institute

1.0 BACKGROUND

1.1 Neurogenic Orthostatic Hypotension

Regulation of tissue blood supply to vital organs such as the brain is met in large part by local adjustment of the microvasculature (autoregulation) and autonomic nervous system control of the cardiovascular system. Neurogenic Orthostatic Hypotension (NOH) is a cardinal example of when these systems fail, specifically, the reflexive responses mediated by the sympathetic branch of the autonomic nervous system. NOH is a debilitating condition associated with reduced quality of life, impaired function and is also an independent predictor of mortality[1,2]. NOH is clinically defined as a sustained reduction in systolic blood pressure (SBP) ≥20 mmHg or diastolic blood pressure of ≥10 mmHg within 3 minutes of standing or head-up tilt performed at 60° without an appropriate compensatory postural tachycardia[3].

Significant and persistent orthostatic hypotension can lead to inadequate cerebral blood flow, reduced cerebral perfusion pressure and orthostatic symptoms including light-headedness, pre-syncope, and in some cases, syncope[4]. As cerebral blood flow and perfusion pressure are crucial factors in normal brain health and function, failure to adequately control these variables may significantly impact the functionality of essential brain networks.

1.2 Physiology: The Normal Orthostatic Response

Upon assuming the upright posture, there is an instantaneous shift of 500 to 1000 mL of blood to the capacitance vessels in the lower extremities and splanchnic circulation[5]. Venous return to the heart is decreased[6] and therefore stroke volume and arterial pressure decrease[7]. These changes trigger the baroreflex via unloading of high-pressure arterial baroreceptors in the carotid sinus and aortic arch. This causes a reflex increase in sympathetic efferent nerve activity that increases heart rate and systemic vascular resistance, and concomitantly suppress parasympathetic activity to the heart, which also causes an increase in heart rate[7]. Hormonal mechanisms (e.g. activation of renin-angiotensin and endothelin systems) and skeletal muscles are also engaged during prolonged standing to maintain blood pressure (BP)[8]. In a healthy individual, HR initially increases by 10-20 bpm, and the systolic BP decreases by approximately 5 mmHg[7]. Any abnormality in these autonomic or neurohumoral reflex pathways can result in altered hemodynamic responses during standing.

Baroreflex is a homeostatic mechanism responsible for short-term regulation of arterial blood pressure, but the chemoreflex is also an important modulator of sympathetic activity. It has been shown that the interplay between these two mechanisms is a complex issue, and baroreflex both influences[9–11] and is influenced by chemoreflex activation[12]. It has been demonstrated that changes in baroreceptor activity modulate ventilatory responses to chemoreceptor stimulation[13,14].

1.3 Chemoreflex

The respiratory chemoreflex is an important modulator of sympathetic and parasympathetic activity, and consists of two types of receptors: central and peripheral. Central chemoreceptors

are located primarily in the medulla, and respond primarily to increases in $PaCO_2$ (hypercapnia) and concomitant acidosis. The peripheral chemoreceptors are located in the aortic and carotid bodies, and respond to arterial hypoxia and hypercapnia[14,15], with synergistic effects.

Respiratory chemoreceptors therefore modulate the autonomic nervous system via changes in blood O_2 and CO_2 concentration. The inhibition of K^+ channels by hypercapnia, hypoxia, or acidosis, depolarizes chemosensitive neurons, and increases their firing rate[16,17]. The activation of either the hypoxic or hypercapnic chemoreflex elicits increased ventilation, parasympathetic and sympathetic activation.

1.4 The interactions between the Chemoreflex and Baroreflex

There is a wealth of research demonstrating interactions between the chemoreflex and baroreflex. Baroreceptors can modulate chemoreflex activity and ventilation. For example, ventilatory responses to chemoreceptor stimulation are augmented by arterial baroreceptor unloading (during hypotensive stimuli) and are depressed by baroreceptor loading (during hypertensive stimuli).

The opposite relationship also exists, where chemoreflex activation can modulate the baroreflex-mediated cardiovascular responses to orthostasis. In 2001, Shoemaker et al.[18] observed that mild hypercapnia caused an attenuated sympathetic and vascular response to head-up tilt in seven healthy individuals. However, BP was better maintained during hypercapnia than hypocapnia, via elevated cardiac output. Despite attenuated sympathetic activity, the authors proposed the improved BP response was attributed to enhanced venous return related to blood volume redistribution from splanchnic capacitance vessels. Furthermore, Howden et al.[19], in 2004, demonstrated that hypercapnic conditions in healthy subjects induced an increase in peak heart rate and time to peak heart rate, which combined to improve tolerance to orthostatic stress simulated by lower body negative pressure.

Kazimierska et al.[20] recently investigated baroreflex sensitivity during repeated transitions between squatting and standing in normocapnia compared to hypercapnia with hypoxia, in a group of 40 healthy young volunteers. Their results showed that hypercapnia with hypoxia was associated with slightly improved BP control during rapid transitions between body positions, which could indicate alterations in hemodynamic adaptation and autonomic control arising from chemoreflex activation. Therefore, chemoreflex activation might constitute an attractive, and novel, therapeutic option for NOH patients.

1.6 Neurogenic Orthostatic Hypotension and CO_2

In healthy individuals, hypercapnia helps improve orthostatic BPs by augmenting stroke volume and improving cardiac output. Improved venous return may be facilitated by increased ventilation and changes in intrathoracic pressures, and/or the resulting improved central circulation. The latter mechanism is particularly important in autonomic failure as the splanchnic-mesenteric capacitance bed represents a large volume, low resistance reservoir that

constitutes 25-30% of total blood volume. Maintenance of postural normotension is clinically important and a common therapeutic target in orthostatic hypotension[21].

Although the literature surrounding the effects of CO_2 in autonomic failure are sparse, Lipp et al.[22] found the ventilatory responses to hypercapnia and hypoxia were preserved in patients with Multiple System Atrophy; a sporadic and progressive pre-ganglionic disorder characterized by loss of medullary sympathoexcitatory neurons. Patients demonstrated a SBP increase of 18 mmHg in response to hypercapnia.

In addition to improving orthostatic BP, hypercapnia may serve an equally important role in symptomatic management, cerebral autoregulation and maintenance of cerebral perfusion pressures in patients with NOH.

1.5 Cerebral Autoregulation

Regulation of tissue blood supply to vital organs, such as the brain, is met in large part by local adjustments of the microvasculature (autoregulation). In the brain, cerebral blood flow (CBF) is maintained over a wide range of systemic blood pressures via multiple overlapping autoregulatory processes, collectively known as cerebral autoregulation. Cerebral autoregulation is commonly modeled as static and dynamic, which describes the response of the brain to long-term gradual and rapid alterations in BP, respectively[23,24].

In response to variations in perfusion pressure (mean arterial pressure minus intracranial pressure), an adaptation in cerebrovascular resistance will cause CBF to return to its baseline[24]. For instance, postural changes challenge cerebral autoregulation by reducing arterial pressure, cardiac output, and cerebral perfusion pressure. In response to reduced systemic pressures, cerebral vessels will dilate to maintain a relatively constant CBF. Standard orthostatic challenges are a common method to challenge cerebral autoregulation and better understand its role. Similarly, alterations in arterial blood gas partial pressures also influence the cerebral circulation. CO_2 is a very potent cerebral vasodilator, and hypercapnia causes a 3% to 5% increase in CBF per mmHg rise in end-tidal CO_2 ($ETCO_2$). Hypocapnia induces vasoconstriction and can reduce CBF by 2% to 3% per mmHg reduction in $ETCO_2$ [25].

Understanding the role of cerebral autoregulation in autonomic failure remains elusive. For example, patients with NOH experience orthostatic light-headedness, nausea, pre-syncope and even syncope, suggesting impaired cerebrovascular autoregulation and hypoperfusion. Some studies have reported impaired cerebral autoregulation in autonomic failure[26], while other have found preserved autoregulation[27–31] and both preserved and impaired autoregulation, depending on the underlying diagnosis[32–34]. The likely reason for these disparate findings is, in part, due to the considerable variation in techniques to quantify sympathetic activity and cerebral autoregulation. Techniques to measure cerebral autoregulation vary considerably, including the use of different collection tools, quantification methods and various algorithms to interpret and analyze different variables. Similarly, routine measurements of the microvasculature and sympathetic activity are lacking, or avoided as current methods are inaccurate (heart rate variability), lack temporal resolution (plasma norepinephrine), or are finicky and invasive

(microneurography)[35]. Microvascular techniques are equally impoverished. Functional MRI devices are costly for widespread use and have relatively poor temporal and spatial resolutions, and are often insufficient to directly resolve the microvasculature[36]. Laser Doppler Flowmetry provides non-invasive assessment of tissue microvascular hemoconcentration; however, the amount and depth of tissue penetration is not adequate for cerebral assessments. Therefore, calculation of total tissue flow is not possible[37]. Overall, the development of a clinically relevant method to assess cerebral autoregulation, sympathetic nerve activity and microvascular activity/reactivity is needed.

1.6 Functional Optical Coherence Tomography

To better understand the mechanistic underpinnings of NOH, and to explore the use of elevated Fractional Inspired CO_2 ($FiCO_2$) to treat it, we need a better way to monitor sympathetic activity and cerebrovascular perfusion. The imaging of cerebral tissue allows clinical investigation into a tissue bed, which is exquisitely sensitive to various vaso-active stimuli[38]. The eye is an extension of the brain with two distinct vascular beds: 1) The epiretinal vascular arcade supplies the superficial retinal layers resembling the circulation of the brain[39,40]. 2) The choroid, on the other hand is largely sympathetically regulated via α- and β-adrenergic receptors[41,42], resembling peripheral vasculature.

The imaging of vascular beds with such diverse regulation enables the assessment of microvascular autoregulatory and sympathetic mediated regulation, enabling a readout of microvascular and sympathetic activity. Optical coherence tomography (OCT) is an advanced imaging technology using near-infrared laser light providing images with a spatial resolution of 4μm and scanning depth of 500μm[43,44] within 1.5 seconds[43,45,46]. The fine resolution of OCT imaging parameters enables a resolution necessary to detect sensitive microvascular changes in response to sympathetic provocations. While OCT provides useful structural characteristics, Functional Optical Coherence Tomography (fOCT) provides information about tissue dynamics such as blood flow, oxygenation and vascular perfusion, expanding OCT's clinical relevance. fOCT of the retinal and choroid vascular beds of the eye is being explored in Calgary to allow physiological monitoring of these essential variables.

2.0 SIGNIFICANCE

The effects of hypercapnia on CBF and the cardiovascular system, under orthostatic stress conditions, have been tested in a limited number of healthy subjects, with the net effect of improving orthostatic tolerance. Therefore, a novel solution to counter the acute effects of NOH is to transiently increase sympathetic activity by stimulating the peripheral and central respiratory chemoreceptors with elevated $FiCO_2$[47,48].

The results of this study will: 1) improve our understanding of the effects of hypercapnia in patients with NOH, and 2) provide robust evidence to support or reject the use of gas trapping devices in the management of NOH patients, which could translate to clinical settings adding options to manage symptoms and improve quality of life for patients.

Additionally, to better understand the cerebral autoregulatory underpinnings of NOH, and to explore the use of elevated $FiCO_2$ to treat it, we need a better way to monitor sympathetic activity and cerebrovascular perfusion. Application of the recently developed fOCT techniques provides a completely novel method to measure sympathetic activity in this unique cohort, gain valuable insight into cerebral autoregulation and potentially provide diagnostic value.

3.0 OUTCOMES, OBJECTIVES and HYPOTHESES

This is a proof-of-concept study to evaluate hypercapnia as a novel therapeutic intervention to improve blood pressure and orthostatic tolerance in patients with NOH. In addition, we will aim to evaluate functional OCT as an advance, non-invasive tool to measure sympathetic and metabolic cerebrovascular control.

The specific objectives of the current proposal are to apply hypercapnia during fOCT monitoring in patients with NOH and healthy controls to: (a) evaluate and compare the effects of hypercapnia on cardiovascular and cerebrovascular responses to better understand basic chemoreflex and baroreflex physiology in patients with NOH, (b) determine if a device that transiently increases FiCO2 in response to postural changes will have efficacy as a non-drug therapeutic and (c) evaluate fOCT as a novel advanced tool to measure sympathetic and metabolic components of cerebral autoregulation in patients with autonomic failure.

We hypothesize that: a) increasing $FiCO_2$ will improve the cerebrovascular and cardiovascular response to standing in patients with NOH, b) we will be able to translate the results of the current study to a clinical setting and c) fOCT will reliably distinguish between patients with NOH and healthy controls adding value as a novel tool to measure sympathetic control.

4.0 INCLUSION/EXCLUSION CRITERIA:

Inclusion Criteria (for all participants):

- Age ≥18 years
- Male and Female
- Non - smokers.
- Able and willing to provide informed consent.
- Ability to travel to Libin Cardiovascular Institute Autonomic Testing Lab at the University of Calgary, Calgary, AB.

Inclusion Criteria for <u>Patients</u>

- Physician diagnosis of Neurogenic Orthostatic Hypotension

Exclusion Criteria (for all participants):

- Medical therapies or medications which could interfere with testing of autonomic function
- Participants with somatization or severe anxiety symptoms will be excluded
- Pregnant or breast-feeding females

• Inability to tolerate mask for the duration of the study
• Subjects who require portable oxygen at rest or with exercise
• Subjects with chronic heart failure or severe pulmonary disease who are unable to climb one flight of stairs due to shortness of breath.
• Presence of failure of other organ systems or systemic illness that can affect autonomic function or the participant's ability to cooperate. These include: dementia, alcohol and/or drug abuse, cerebrovascular disease, kidney or liver disease, surgical procedures where the nerves of the sympathetic nervous system have been cut.
• Other factors which in the investigator's opinion would prevent the participant from completing the protocol, including poor compliance during previous studies.

Additional Exclusion Criteria for <u>Healthy Controls</u>

• The presence of any dysfunction relating to the autonomic nervous system.

In addition, in accordance with the University of Calgary requirements for in-person activities, all students, faculty, staff and visitors to campus, including research participants, are required to show proof of full vaccination status against COVID-19.

5.0 INTERVENTION

The RespirAct™ system (Thornhill Research Inc., Toronto, Canada) is a computer-controlled gas blender providing CO_2, O_2 and nitrogen for a subject to inhale while breathing for the purpose of controlling the concentrations of the respective blood gases. The RespirAct™ records inspired and exhaled gas concentrations, which can be recalled, analyzed and graphed. Data is collected breath-by-breath including end tidal O_2 and CO_2, the length of inspiration and expiration for the breath, the respiration rate and the tidal volume per breath. The RespirAct™ system is an investigational use device, distributed by Thornhill Research to collaborators for use in IRB/REB approved research studies, and has been utilized in several publications in healthy individuals and clinical populations[49,50]. Subjects will be fitted with a facemask, which will be connected to a tube supplied with gas from RespirAct™ system.

6.0 ENROLLMENT, RANDOMIZATION and BLINDING

6.1 Recruitment

Patients will be recruited from the Calgary Autonomic Investigation and Management Clinic, and the practices of Drs. Satish R. Raj, Carlos A Morillo, and Robert S. Sheldon, including past research study participants. We will also recruit participants from the community who contact us directly about their interest in our studies.

6.2 Randomization:

Each study participant will complete 5 Active Stand Tests on a single day. Subjects will be fitted with a facemask connected to a tube supplied with gas from the RespirAct™ system:

a) Free breathing: Normal (not coached) breathing (no $ETCO_2$ clamp)

b) Baseline: Normal not coached breathing with $ETCO_2$ clamped at baseline levels
c) Low Hypercapnia: Normal (not coached) breathing with $ETCO_2$ clamped at +5 mmHg above eucapnia
d) High Hypercapnia: Normal (not coached) breathing with $ETCO_2$ clamped at +10 mmHg above eucapnia
e) Hypercapnia + Hypoxia: Normal (not coached) breathing with $ETCO_2$ clamped at +10 mmHg above eucapnia and ETO_2 clamped at 50 mmHg.

The study will involve a partial randomization. The order of interventions b-d will be randomized to account for any possible effects associated with repeated Active Stand Tests. Randomization will be performed using a pre-prepared randomization table. Protocol A will always be performed first to determine individual eucapnic levels. Protocol E will always be performed last to due to the long lasting hemodynamic and sympathetic changes associated with hypoxia.

7.0 STUDY PROCEDURE

7.1 Informed Consent

The informed consent will be sent by email to the patients in advance. We will give each patient a chance to ask questions about the protocol and have these questions answered to their satisfaction. Written informed consent will be documented prior to engaging in study-related procedures.

7.2 Holding Pre-Existing Medications

Participants will be asked to hold their clinical medications if possible, but this will not be mandatory. We will make note of the medications used by each participant at the time of the study.

7.3 Study Day

7.3.1 Instrumentation

- Patient will be instrumented at least 2h post-prandial or fasting on an empty bladder. Participants will be asked to abstain from alcohol, caffeine and exercise for a period of 12 hours prior to testing. Participants will be allowed to drink water on the morning of study.
- Skin electrodes will be applied for continuous ECG and HR monitoring.
- BP will be monitored continuously using a finger volume clamp method (using beat-to-beat blood pressure monitoring) and calibrated with intermittent brachial cuff measurements.
- From the continuous BP waveform, we can get an estimate of stroke volume, cardiac output, and systemic vascular resistance (Modelflow, FMS, Amsterdam, Netherlands).
- Middle cerebral artery blood flow velocity (CBFv) will be assessed using a digital transcranial Doppler system (PMD150 Unilateral digital transcranial Doppler, License number 73603).

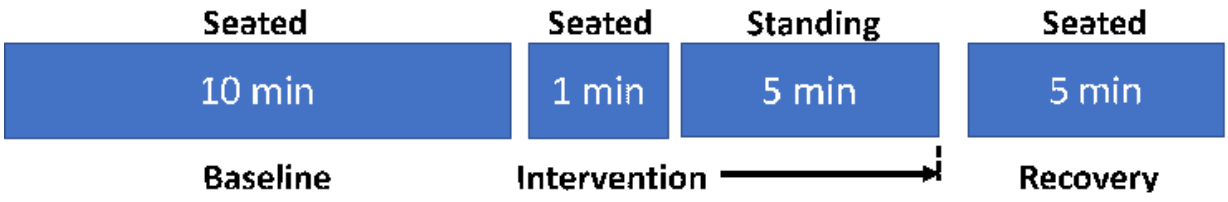
- Subjects will be fitted with a facemask. The facemask will be connected to a tube supplied with gas from a programmable gas mixing system (RespirAct™; Thornhill Research Inc., Toronto, Canada).
- Oxygen saturation and End-tidal CO_2 will be monitored by the RespirAct™ system.
- Data will be digitally sampled and collected digitally for offline analysis

7.3.2 Study Design

- Review procedure and reconfirm consent.
- The participant will be asked about co-morbid disorders, and current pharmacological and non-pharmacological treatments for NOH.
- The participant will be instrumented as discussed above
- Participant will be seated comfortably
- The participant will rest comfortably in a seated position for 10 minutes prior for baseline data collection
- Each study participant will complete all 5 Active Stand Test in a single day.

7.3.3 *For each Active Stand Test (AST)*

- Physiological signals will be digitally sampled throughout the study
- Up to 10-minute baseline data collection (seated position)
- Subjects will be introduced to each gas condition while seated 1 min prior to the AST.
- Participants will actively stand and remain upright for up to 5 minutes
- Standing will be terminated early if systolic blood pressure falls below 70 mmHg, SpO_2 levels drop below 80%, or the patient requests the test be stopped.
- At least 5-minute recovery data collection after returning to the seated position, or until normalization of $ETCO_2$ to baseline rest level.
- We will document hemodynamic parameters (BP, cardiac output, HR and CBFv) and respiratory parameters (tidal volume, respiratory rate, ETO_2, and $ETCO_2$) during seated position and each Active Stand Test.
- We will assess acute orthostatic tolerance using the Vanderbilt Orthostatic Symptoms Score (VOSS) at the end of each Active Stand Test.



7.3.4 $ETCO_2$ Baseline Levels

The baseline $ETCO_2$ levels will be defined during the first 10 min baseline, and will be calculated from the mean $ETCO_2$ over the last 5 min of first baseline phase.

7.3.5 Specific Active Stand Test Protocols (AST):

1. **Free breathe: AST with Normal Breathing (no $ETCO_2$ clamp):**
   - $ETCO_2$ will not be clamped (i.e. participants will breathe freely); they will stand for up to 5 minutes
2. **Baseline: AST with Normal Breathing ($ETCO_2$ clamped at baseline):**
   - The $ETCO_2$ will be clamped at baseline levels. Participants will then be asked to stand for up to 5 min
3. **Hypercapnia: AST with Normal (Not Coached) Breathing and Low Hypercapnia**
   - 1 min before Active Stand Test, the $ETCO_2$ will be ramped up to 5mmHg above eucapnic levels, the participant will then stand for up to 5 min
4. **Hypercapnia: AST with Normal (Not Coached) Breathing and High Hypercapnia**
   - 1 min before Active Stand Test, the $ETCO_2$ will be ramped 10mmHg above eucapnic levels, the participant will then stand for up to 5 min
5. **Hypoxia and Hypercapnia**
   - 1 min before Active Stand Test, the $ETCO_2$ will be ramped up to 10mmHg above eucapnic levels and ETO_2 will be ramped down to 50mmHg. Participant will then stand up for up to 5 min

The study will be conducted in a human physiology research and procedure room. The study will take approximately 2.5 hours.

8.0 STUDY OUTCOME MEASURES

The primary outcome measures will be:

1) The magnitude of the hemodynamic response (ΔHR/ΔBP difference between HR/BP from seated to peak HR/BP during standing) for each intervention.
2) Choroid and retinal (surrogates for peripheral sympathetic activity and cerebral autoregulation, respectively) perfusion density and reactivity
3) Comparison of NOH with control to determine the potential of fOCT to distinguish NOH patients from controls

Secondary outcome measures will be the ΔCBFv upon standing and the VOSS symptom score for each intervention. Additional outcome measures will include ventilatory rate and tidal volume at different levels of $ETCO_2$, and the changes when moving from seated to standing.

- Seated parameters will be calculated as the mean value (HR, $ETCO_2$, CBFv) between the 8^{th} and 9^{th} minute of seated baseline.
- Peak parameters during standing will be defined as the mean value during the first min and between the 2^{rd} and 5^{th} min (mean at each 30 seconds)
- VOSS Symptom Rating at the end of each 5 min Active Stand Test

9.0 STATISTICAL CONSIDERATIONS

9.1 Primary Analysis

The effects of $ETCO_2$ level and posture on all measured variables will be assessed using a repeated measures two-way analysis of variance (ANOVA) procedure and post-hoc analysis.

To determine diagnostic potential of fOCT, OCT scans (choroid and retinal perfusion densities) will be compared between NOH patient and healthy controls using an independent t-test.

9.2 Secondary Analysis

The secondary analysis will compare the effects of $ETCO_2$ level and posture on CBFv using a repeated measures two-way analysis of variance (ANOVA) procedure and post-hoc analysis. VOSS Symptom Rating will be completed at end of each 5 min Active Stand Test protocol and compared between $ETCO_2$ levels in a similar manner.

Other secondary analyses will include the magnitude of $\Delta ETCO_2$ from seated to standing, and peak standing $ETCO_2$ in the no gas intervention (Protocol D), during first min and between the 2^{rd} and 5^{th} min (mean at each 30 seconds)

9.3 Sample Size Calculation

There are limited existing data regarding the effect of hypercapnia in NOH patients. Therefore, we can only estimate the sample size for the current pilot study. Based on the limited number of previous studies in similar patient populations, whose sample sizes ranged from 7 to 16, we aim to recruit 40 patients with NOH, and 40 healthy controls. This sample size will allow for additional sex-based comparisons

10.0 RISKS and INCONVENIENCES

BP cuff: Some may find the arm or finger cuffs uncomfortable, or to keep their arm in a relatively fixed position.

Electrodes: Sticky electrodes will be put on the chest and the limbs to record electrical activity from your heart. This can occasionally cause a rash.

***Active Stand Test*:** There might be light-headedness, tremor, headache, nausea or feelings of faintness during active standing. These symptoms usually resolve rapidly upon return to the seated position. To ensure patient safety, hemodynamic (BP, HR, stroke volume) will be continuously monitored throughout the entire study, and standing will be terminated early if systolic blood pressure falls below 70 mmHg, SpO_2 levels drop below 80%, or the patient requests the test be stopped. Expert personnel will be present at all times.

Transcranial Doppler (TCD): Ultrasound gel will be applied which may cause a rash. The Doppler probe will be in direct contact with the head, which may be uncomfortable. The headband/apparatus will be snug, but there will be opportunities to loosen it off during the recovery periods, and the headband is adjustable.

RespirAct: Subjects will be fitted with a facemask, which might be uncomfortable to the participant. The mask will be connected to a tube supplied with gas from an automated, non-invasive programmable gas mixing system (RespirAct™ Thornhill Medical). Briefly, the RespirAct employs an automated gas blender to adjust the composition and flow of CO_2 and O_2 to a sequential gas-delivery mask and breathing circuit. The RespirAct™ system is an investigational use device, distributed by Thornhill Research to collaborators for use in IRB/REB approved research studies, and has been utilized in several publications in healthy individuals and clinical populations.

Breathing Protocol: The subjects will breathe three different inspiratory gas mixtures, which are not typically encountered in day-to-day life. Hypercapnia is similar to re-breathing air but not to a point where the oxygen is exhausted. The levels we will use are the same as in expired air during moderate exercise. Rebreathing will be a combination of both hypoxia (low oxygen) and hypercapnia as mentioned above. Hypoxia is similar to high altitude and affects cardiovascular and cardiorespiratory indices similarly, including increased heart rate and breathing. Some participants have described the sensation of a mild headache; however, this is diminished quickly upon cessation of the breathing exercise. Syncope is rare and uncommon in response to the composition of gases employed within this study. Hyperventilation may induce a light-headed sensation. This dissipates quickly upon return to normal ventilatory rate. To ensure patient safety, hemodynamic (BP, HR, stroke volume) will be continuously monitored throughout the entire study, and we will have expert personnel present at all times.

Function Optical Coherence Tomography (fOCT): We will use a state-of-the-art commercially available OCT machine (Zeiss Cirrus 5000) that is used by ophthalmologists around the world. The OCT uses a weak and harmless near infra-red laser beam to illuminate the retina and choroid. Participants need to rest their chin and forehead on support bars which can be uncomfortable. However, we have added padding to support the chin and reduce discomfort. During scans, participants may also experience mild pain or eye fatigue equivalent to looking into a bright light. These sources of discomfort will be mitigated by positioning participants appropriately using back supports, placing the machine at the appropriate height using a table with fine height adjustment and limiting each scan to ~3-5 seconds.

Tape allergy reaction: A tape will be applied to fix the mask to the participant's face, which may cause a rash or mild discomfort when removing.

Privacy Risks: Patient identifiers will be used in this study. The investigators will comply with the patient privacy guidelines of the University of Calgary and applicable provincial and federal rules.

COVID-19: This study involves in-person interactions, and therefore will require increased time within a health care facility and increased exposure to other people

We cannot foresee any other risks, but there may be previously unknown or unforeseen risks.

11.0 DATA and SAFETY PLAN

11.1 Adverse Event (or Unanticipated Problem) Reporting

Any adverse events of a serious nature will be reviewed immediately with the principal investigators. Serious adverse events will be reported in writing to the CHREB within 10 days of the PI's notification of the event. All study adverse events will be summarized once a year, during the annual review reporting, for the CHREB. The research coordinator will be responsible for tracking adverse events in this study.

The adverse event will be described with the following information: description of the event, outcome of the event, how long it lasted, whether the event required treatment or intervention, and the outcome.

The definition of events is as follows:

Mild – transient and mild in nature, with no treatment necessary.
Moderate – some intervention and treatment necessary, but participant completely recovers.
Severe – an event that results in hospitalization, disability, death or is life threatening.

11.2 Data & Safety Monitor

There will be no external Data & Safety monitor for this study.

12.0 STUDY WITHDRAWAL or DISCONTINUATION

12.1 Principal Investigator Initiated Withdrawal

The principal investigator reserves the right to withdraw the participant from the study after they have provided informed consent, but before study completion. This could occur for one of many reasons, which include, but are not limited to: non-compliance with the protocol, a concern for participant safety, the availability of new knowledge that might affect continued participation in the study, or study termination.

12.2. Study Participant Initiated Withdrawal

Participants are free to withdraw from this study at any time. Withdrawal of consent or refusal to participate will not prejudice their health care.

12.3 Data Post-Withdrawal

If the participant is taken out of the study, or if the participant chooses to no longer be in the study, then we will stop collecting any information on the participant. With the participant's permission we will keep the data already collected, and this will still be used for analysis.

13.0 COMPENSATION

We will cover the parking costs near TRW for participants during their study day. Participants will not be financially compensated for their time involved in this study. We are doing assessments that might generate knowledge about their medical condition and may indirectly be of benefit to them.

14.0 PRIVACY and CONFIDENTIALITY ISSUES

Protected Health Information will be used in this study. The investigators will comply with the patient privacy guidelines of the University of Calgary and applicable provincial and federal rules.

The research team is comprised of experienced research nurses and research assistants who are aware of the importance of confidentiality of health information. Paper research records will be stored in a locked office. Digital records will be stored on password-protected University of Calgary computers/servers and in the University of Calgary Clinical Research Unit REDCap Database.

Every effort will be made to publish and present the data from this study. At no time will any participant be identified in any such publication.

15.0 FOLLOW-UP AND RECORD RETENTION

15.1 Follow Up

There is no follow-up in this study. Once the physiological study day has been completed, the participant will be finished in this protocol. We may approach participants who participate in this study for further studies related to NOH.

16.0 STUDY SPONSOR

Departmental Funds and the start-up package of Dr. Satish R Raj will support this study.

17.0 BIBLIOGRAPHY

1. Bendini C, Angelini A, Salsi F, et al. Relation of neurocardiovascular instability to cognitive, emotional and functional domains. *Arch Gerontol Geriatr*. 2007;1:69-74.
2. Rose KM, Eigenbrodt ML, Biga RL, et al. Orthostatic hypotension predicts mortality in middle-aged adults: the Atherosclerosis Risk In Communities (ARIC) Study. *Circulation*. 2006;114(7):630-636.
3. Freeman R, Wieling W, Axelrod FB, et al. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clin Auton Res*. 2011;21(2):69-72.
4. Gibbons CH, Freeman R, Kaufmann H. The recommendations of a consensus panel for the screening, diagnosis, and treatment of neurogenic orthostatic hypotension and associated supine hypertension. *J Neurol*. 2017;264(8):1567-1582.
5. Smith JJ, Porth CM, Erickson M. Hemodynamic Response to the Upright Posture. *J Clin Pharmacol*. 1994;34(5):375-386. doi:10.1002/j.1552-4604.1994.tb04977.x.
6. Ocon AJ, Medow MS, Taneja I, Clarke D, Stewart JM. Decreased upright cerebral blood flow and cerebral autoregulation in normocapnic postural tachycardia syndrome. *Am J*

Physiol Circ Physiol. 2009;297(2):H664-H673. doi:10.1152/ajpheart.00138.2009.

7. Raj SR. The postural tachycardia syndrome (POTS): Pathophysiology, diagnosis & management. *Indian Pacing Electrophysiol J*. 2006;6(2):84-99.

8. Nilsson D, Sutton R, Tas W, Burri P, Melander O, Fedorowski A. Orthostatic Changes in Hemodynamics and Cardiovascular Biomarkers in Dysautonomic Patients. *PLoS One*. 2015;10(6):e0128962. doi:10.1371/journal.pone.0128962.

9. Halliwill JR, Morgan BJ, Charkoudian N. Peripheral chemoreflex and baroreflex interactions in cardiovascular regulation in humans. *J Physiol*. 2003;552(1):295-302. doi:10.1113/jphysiol.2003.050708.

10. Bernardi L, Gabutti A, Porta C, Spicuzza L. Slow breathing reduces chemoreflex response to hypoxia and hypercapnia, and increases baroreflex sensitivity. *J Hypertens*. 2001;19(12):2221-2229. doi:10.1097/00004872-200112000-00016.

11. Somers VK, Mark AL, Zavala DC, Abboud FM. Influence of ventilation and hypocapnia on sympathetic nerve responses to hypoxia in normal humans. *J Appl Physiol*. 1989;67(5):2095-2100. doi:10.1152/jappl.1989.67.5.2095.

12. Cooper VL, Pearson SB, Bowker CM, Elliott MW, Hainsworth R. Interaction of chemoreceptor and baroreceptor reflexes by hypoxia and hypercapnia - A mechanism for promoting hypertension in obstructive sleep apnoea. *J Physiol*. 2005;568(2):677-687. doi:10.1113/jphysiol.2005.094151.

13. Heistad DD, Abboud FM, Mark AL, Schmid PG. Effect of baroreceptor activity on ventilatory response to chemoreceptor stimulation. *J Appl Physiol*. 1975;39(3):411-416. doi:10.1152/jappl.1975.39.3.411.

14. Marshall M. Peripheral chemoreceptors and cardiovascular regulation. *Physiol Rev*. 1994;74(3):543-594.

15. Schultz HD, Li YL, Ding Y. Arterial Chemoreceptors and Sympathetic Nerve Activity. *Hypertension*. 2007;50(1):6-13. doi:10.1161/HYPERTENSIONAHA.106.076083.

16. Wilson RJA, Teppema LJ. Integration of Central and Peripheral Respiratory Chemoreflexes. 2016;6(April):1005-1041. doi:10.1002/cphy.c140040.

17. van Beek AHEA, Olde Rikkert MGM, Pasman JW, Hopman MTE, Claassen JAHR. Dynamic Cerebral Autoregulation in the Old Using a Repeated Sit-Stand Maneuver. *Ultrasound Med Biol*. 2010;36(2):192-201. doi:10.1016/j.ultrasmedbio.2009.10.011.

18. Shoemaker JK, O'Leary DD, Hughson RL. Pet CO2 inversely affects MSNA response to orthostatic stress. *Am J Physiol Circ Physiol*. 2001;281(3):H1040-H1046. doi:10.1152/ajpheart.2001.281.3.h1040.

19. Howden R, Lightfoot JT, Brown SJ, Swaine IL. The effects of breathing 5% CO2 on human cardiovascular responses and tolerance to orthostatic stress. *Exp Physiol*. 2004;89(4):465-471. doi:10.1113/expphysiol.2004.027250.

20. Kazimierska A, Placek MM, Uryga A, Wachel P, Burzyńska M, Kasprowicz M.

Assessment of Baroreflex Sensitivity Using Time-Frequency Analysis during Postural Change and Hypercapnia. *Comput Math Methods Med*. 2019;2019. doi:10.1155/2019/4875231.

21. Low P a, Singer W. Management of neurogenic orthostatic hypotension: an update. *Lancet Neurol*. 2008;7(5):451-458.

22. Lipp A, Schmelzer JD, Low PA, Johnson BD, Benarroch EE. Ventilatory and cardiovascular responses to hypoxia and hypercapnia in multiple system atrophy. *Arch N*. 2010;67(2):211-216. doi:10.1001/archneurol.2009.321.

23. Franco Folino A. Cerebral Autoregulation and Syncope. *Prog Cardiovasc Dis*. 2007;50(1):49-80. doi:10.1016/j.pcad.2007.01.001.

24. Van Beek AHEA, Claassen JAHR, Rikkert MGMO, Jansen RWMM. Cerebral autoregulation: An overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab*. 2008;28(6):1071-1085. doi:10.1038/jcbfm.2008.13.

25. Claassen JAHR, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol*. 2006;102(3):870-877. doi:10.1152/japplphysiol.00906.2006.

26. Castro PM, Santos R, Freitas J, Panerai RB, Azevedo E. Autonomic dysfunction affects dynamic cerebral autoregulation during Valsalva maneuver: Comparison between healthy and autonomic dysfunction subjects. *J Appl Physiol*. 2014;117(3):205-213. doi:10.1152/japplphysiol.00893.2013.

27. Asahina M, Sato J, Tachibana M, Hattori T. Cerebral blood flow and oxygenation during head-up tilt in patients with multiple system atrophy and healthy control subjects. *Parkinsonism Relat Disord*. 2006;12:472-477.

28. Blaber AP, Bondar RL, Stein F, et al. Complexity of middle cerebral artery blood flow velocity: effects of tilt and autonomic failure. *Am J Physiol Hear Circ Physiol*. 1997;273:H2209-H2216.

29. Haubrich C, Pies K, Dafotakis M, Block F, Kloetzsch C, Diehl RR. Transcranial Doppler Monitoring in Parkinson's Disease: Cerebrovascular Compensation of Orthostatic Hypotension. *Ultrasound Med Biol*. 2010;36(10):1581-1587. doi:10.1016/j.ultrasmedbio.2010.06.016.

30. Hetzel A, Reinhard M, Guschlbauer B, Braune S. Challenging cerebral autoregulation in patients with preganglionic autonomic failure. *Clin Auton Res*. 2003;13(1):27-35. doi:10.1007/s10286-003-0028-3.

31. Pavy-Le Traon A, Hughson RL, Thalamas C, et al. Cerebral autoregulation is preserved in multiple system atrophy: A transcranial doppler study. *Mov Disord*. 2006;21(12):2122-2126. doi:10.1002/mds.21130.

32. Caronna JJ, Plum F. Cerebrovascular Regulation in Preganglionic and Postganglionic Autonomic Insufficiency. *Stroke*. 1973;4:12-19. http://ahajournals.org. Accessed March 2,

2020.

33. Hesse B, Mehlsen J, Boesen F, et al. Regulation of cerebral blood flow in patients with autonomic dysfunction and severe postural hypotension. *Clin Physiol Funct Imaging*. 2002;22(4):241-247. doi:10.1046/j.1475-097X.2002.00425.x.

34. Novak V, Novak P, Spies J, Low P. Autoregulation of Cerebral Blood Flow in Orthostatic Hypotension. *Stroke*. 1998;29:104-111. doi:10.5005/jp/books/11286_7.

35. Grassi G, Esler M. How to assess sympathetic activity in humans. *J Hypertens*. 1999;17(6):719-734. doi:10.1097/00004872-199917060-00001.

36. Neeman M, Dafni H. STRUCTURAL, FUNCTIONAL, AND MOLECULAR MR IMAGING OF THE MICROVASCULATURE. *Annu Rev Biomed Eng*. 2003;5:29-56. doi:10.1146/annurev.bioeng.5.040202.121606.

37. Rajan V, Varghese B, Van Leeuwen G, Steenbergen W. Review of methodological developments in laser Doppler flowmetry. doi:10.1007/s10103-007-0524-0.

38. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. *Cerebrovasc Brain Metab Rev*. 1990;2(2):161-192.

39. Schmetterer L, Polak K. *Role of Nitric Oxide in the Control of Ocular Blood Flow*.

40. Yoshida A. [Noninvasive analysis of retinal microstructure and function: challenges and a promising future]. *Nihon Ganka Gakkai Zasshi*. 2013;117(3):212-44; discussion 245.

41. Edvinsson L, Lindvall M. *Autonomic Vascular Innervation and Vasomotor Reactivity in the Choroid Plexus*.

42. Steinle JJ, Krizsan-Agbas D, Smith PG. Regional regulation of choroidal blood flow by autonomic innervation in the rat. *Am J Physiol Regul Integr Comp Physiol*. 2000;279:202-209. http://www.ajpregu.org. Accessed June 16, 2020.

43. Manjunath V, Taha M, Fujimoto JG, Duker JS. Choroidal Thickness in Normal Eyes Measured Using Cirrus-HD Optical Coherence Tomography. *Am J Ophthalmol*. 2010;150(3):325-329. doi:10.1016/j.ajo.2010.04.018.

44. Margolis R, Spaide RF. A Pilot Study of Enhanced Depth Imaging Optical Coherence Tomography of the Choroid in Normal Eyes. 2009. doi:10.1016/j.ajo.2008.12.008.

45. Fujimoto JG, Pitris C, Boppart SA, Brezinski ME. Optical Coherence Tomography: An Emerging Technology for Biomedical Imaging and Optical Biopsy 1. *Neoplasia*. 2000;2:9-25. www.nature.com/neo. Accessed June 16, 2020.

46. Poddar R, Kim DY, Werner JS, Zawadzki RJ. In vivo imaging of human vasculature in the chorioretinal complex using phase-variance contrast method with phase-stabilized 1-μm swept-source optical coherence tomography. *J Biomed Opt*. 2014;19(12):126010. doi:10.1117/1.

47. Morgan BJ, Crabtree DC, Palta M, Skatrud JB. Combined hypoxia and hypercapnia evokes long-lasting sympathetic activation in humans. *J Appl Physiol*. 1995;79(1):205-

213. doi:10.1152/jappl.1995.79.1.205.

48. Schultz HD, Li YL, Ding Y. Arterial chemoreceptors and sympathetic nerve activity: Implications for hypertension and heart failure. *Hypertension*. 2007;50(1):6-13. doi:10.1161/HYPERTENSIONAHA.106.076083.

49. Sobczyk O, Battisti-Charbonney A, Poublanc J, et al. Assessing cerebrovascular reactivity abnormality by comparison to a reference atlas. *J Cereb Blood Flow Metab*. 2015;35(2):213-220. doi:10.1038/jcbfm.2014.184.

50. Sam K, Peltenburg B, Conklin J, et al. Cerebrovascular reactivity and white matter integrity. *Neurology*. 2016;87(22):2333-2339. doi:10.1212/WNL.0000000000003373.