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Improving Human Cerebrovascular Function Using Acute Intermittent Hypoxia
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The following document is the IRB-approved Protocol document for the *AIHvasc* Clinical Trial, which contains a statistical analysis plan, uploaded by Principal Investigator Dr Molly Bright.

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PROTOCOL TITLE: Improving Human Cerebrovascular Function Using Acute Intermittent Hypoxia

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STUDY SUMMARY:

Investigational Agent(s) (Drugs or Devices)	Acute Intermittent Hypoxia, RespirAct-MR™
IND / IDE / HDE #	
Indicate Special Population(s)	☐ Children ☐ Children who are wards of the state ☐ Adults Unable to Consent ☐ Cognitively Impaired Adults ☐ Neonates of Uncertain Viability ☐ Pregnant Women ☐ Prisoners (or other detained/paroled individuals) ☐ Students/Employees
Sample Size	40
Funding Source	National Institutes of Health (NINDS)
Indicate the type of consent to be obtained	☒ Written ☐ Verbal/Waiver of Documentation of Informed Consent ☐ Waiver of HIPAA Authorization ☐ Waiver/Alteration of Consent Process
Site	☐ Lead Site (For A Multiple Site Research Study) ☐ Data Coordinating Center (DCC)
Research Related Radiation Exposure	☐ Yes ☒ No
DSMB / DMC / IDMC	☒ Yes ☐ No

OBJECTIVES:

Vascular dysfunction contributes to the pathology of numerous neurological conditions, however current treatment options to address these factors are insufficient. The overall objective of this project is to test the potential of a novel intervention to improve the brain's blood supply, thereby mitigating the vascular contributions to neurodegeneration and functional impairment. Acute Intermittent Hypoxia (AIH) is emerging as a powerful therapy to improve overall cardiovascular health and facilitate neural plasticity, however its impact on human cerebrovascular function is unclear. Hypoxia is known to generally influence angiogenesis and blood flow, and exposures to

brief hypoxic stimuli can "precondition" the vasculature of other organs to be more resilient to severe hypoxic threat. This study tests the hypothesis that a 3-week daily AIH intervention will drive beneficial vascular plasticity in the human brain, increasing blood flow and blood flow responsiveness, more than a sham intervention.

Healthy participants will be recruited to complete a randomized sham-controlled crossover trial to assess the impact of daily AIH versus a sham intervention. The impact of AIH on brain physiology will be determined through comparison of pre- and post-intervention MRI scans in two specific aims:

Aim 1. Quantify the change in resting cerebral perfusion resulting from a 3-week daily acute intermittent hypoxia intervention. We will use phase-contrast MRI and an extended arterial spin labeling MRI protocol to quantify whole-brain and regional perfusion and arterial transit times PRE and POST intervention. We hypothesize that perfusion will increase and transit times will decrease following the daily AIH intervention.

Aim 2. Test whether cerebrovascular reactivity to hypoxia and carbon dioxide challenges is enhanced following a 3-week daily acute intermittent hypoxia intervention. In the PRE and POST intervention MRI scans, we will administer gas inhalation challenges to induce vasodilatory responses to: 1) sustained mild hypoxia, and 2) sustained mild hypercapnia, targeting +6 mmHg end-tidal CO_2. Quantitative whole-brain perfusion and perfusion maps will be calculated during the plateau of the vasodilatory response, and cerebrovascular reactivity calculated for both challenges (ΔCBF/mmHg). We hypothesize that the daily AIH intervention will enhance cerebrovascular reactivity.

BACKGROUND:

Vascular plasticity in the adult brain. Healthy brain function requires successful coupling of neural activity and local vascular supply. These systems develop in concert throughout early development[1], and the cerebrovasculature is highly responsive to environmental changes: animals raised in stimulating environments demonstrate increased capillary surface area and branch points[2], whereas sensory deprivation can rapidly decrease vascular density[3]. Even in the fully developed brain, such environmental factors and repetitive activities (e.g., exercise) may also stimulate local angiogenesis[4-6], allowing for ongoing plasticity of vascular architecture and function through adulthood. The cerebrovasculature can also adapt to the threat of damage and, at least in part, attempt to recover from injury. However, these changes may not always be beneficial long-term. Following traumatic brain injury that acutely reduces vessel density, new capillaries are observed as early as two days later[7] and some metrics of vascular architecture normalize in 2-3 weeks[8]. Unfortunately, this does not necessarily lead to typical *perfusion* of the tissue[9], indicating uncoupling between vascular structure and function. New vessels are often malformed, increasing the risk of delayed stroke and poor overall outcome[10]. Detrimental vascular plasticity also occurs after ischemic stroke, when vasoconstrictor receptors of arterial smooth muscle cells are upregulated, leading to decreased perfusion[11]. ***Interventions to improve vascular function are actively sought to mitigate damage to overall brain function and improve health outcomes.***

Aerobic exercise is an established tool to achieve such benefits: it can induce angiogenesis, ameliorate age-related decline in cerebral perfusion, improve cerebrovascular reactivity, and

increase tolerance to ischemic stroke[12]. However, exercise interventions are challenging to implement, and some will find aerobic exercise impossible to sustain due to physical or cognitive impairments. For example, 61% of heart failure patients report that adherence to an exercise regimen is more difficult than any other behavioral change (e.g., diet modification, medication adherence, smoking cessation)[13]. While we acknowledge and appreciate the benefits of exercise on cerebrovascular and overall health, alternative interventions are needed to successfully treat diverse patients.

Acute Intermittent Hypoxia. Hypoxia is another potent driver of angiogenesis and vascular plasticity[14], primarily through regulation of hypoxia inducible factor (HIF) and its effect on vascular endothelial growth factor (VEGF)[15]. Although it may appear straightforward to deliver hypoxia to human subjects via gas inhalation, capitalizing on hypoxia as an intervention is challenging: whether hypoxia protocols are therapeutic or detrimental depends heavily on the *dose* of exposure[16]. *Intermittent* hypoxia protocols, in which one alternates between breathing mildly hypoxic air and normal air, are being rigorously examined for their potential in improving human physiology, but here, too, the precise dose matters. Intermittent hypoxia doses can mimic obstructive sleep apnea, leading to increased blood pressure, decreased vascular reactivity, and increased risk of stroke[17]. Conversely, *therapeutic* doses of Acute Intermittent Hypoxia (AIH) can *reduce* blood pressure and *protect* tissue from ischemic damage[18]. AIH increases exercise capacity in coronary artery disease[19] and chronic obstructive pulmonary disease[20], and reduces blood pressure in hypertensives[21]. We propose that AIH will also have benefits on *cerebrovascular* physiology. If true, it is a promising tool for evoking beneficial cerebrovascular plasticity, with the potential for mitigating neurodegeneration and tissue damage in a broad spectrum of neurological conditions.

AIH is regularly administered by our neurorehabilitation partner institution and has been demonstrated to improve voluntary ankle flexion strength, lower-limb electromyography activity, walking speed and endurance in individuals with chronic spinal cord injury[22-24]. These methods are currently being tested for the first time in stroke survivors, establishing feasibility and safety guidelines for administering AIH in high-risk individuals with enhanced susceptibility to hypoxia. In all of these trials, the focus has been on the ability of AIH to evoke *neural* plasticity, driving increased synaptic strength. Recent evidence in spinal cord injury animal models suggests that AIH may also improve motor function through *vascular* mechanisms, as inhaled hypoxia improves oxygen delivery to perilesional spinal cord tissue with a timeframe consistent with observed functional improvements[25]. These findings motivate the evaluation of AIH as a clinical tool for improving the function and coordination of *both neural and vascular systems* in humans.

Clinical targets for improving vascular function. Vascular function is known to be altered and related to disease severity in numerous neurological diseases. Small vessel disease is linked to reduced cerebral perfusion and cerebrovascular reactivity (CVR), preceding the formation of white matter hyperintensities and cognitive impairment[26,27]. Similar impairments are observed in Multiple Sclerosis, even at very early disease stages[28-31], and CVR correlates with motor symptom severity in Parkinson's Disease[32]. Although mechanisms linking vascular impairment with symptom progression are unclear, these studies collectively support the premise that ***modifying vascular physiology may mitigate neurodegeneration and functional impairments***.

Dementia is another exciting potential target for vascular interventions. Preclinical studies show chronic vessel hypercontractility in Alzheimer's Disease (AD) models, observed as reduced CVR in human studies[33]. Young asymptomatic ApoE-ε4 carriers at risk of developing AD demonstrate

early CVR impairment[34], suggesting that subtle vascular dysfunction may accumulate over a lifetime to contribute to significant late-stage cognitive impairment. ***Feasible interventions to improve perfusion and CVR are therefore an important ingredient in any approach to mitigate the progression of dementia***. AIH can prevent vascular rarefaction and normalize Aβ-related perfusion impairments in AD models[35]. In individuals with mild cognitive impairment, AIH improves cognitive test scores, decreases Aβ expression, reduces blood pressure, and improves CVR[36,37]. There is also a recent publication demonstrating that one dose of AIH increases dilatory responsiveness of the carotid artery of healthy young adults, suggesting that vascular improvements may be observable even in the absence of pathology[38]. Our study uses MRI to directly probe the vascular plasticity suggested by these pilot data; if successful, AIH should be systematically assessed for therapeutic merit in cognitive aging and dementia. Building on the results of this pilot study, we will develop a clinical trial ascertaining the value of AIH in older individuals with and without vascular risk factors, to determine how to safely and effectively translate this intervention to improve brain health.

STUDY ENDPOINTS:

The primary endpoint of this study relates to achieving recruitment goals, as we anticipate recruiting and successfully collecting (in a randomized order) the AIH and sham AIH interventions and all associated MRI scanning in 18 healthy participants.

STUDY INTERVENTION(S) / INVESTIGATIONAL AGENT(S):

Acute Intermittent Hypoxia

AIH will be administered within a clinical research laboratory (ABC laboratory of Dr. Farzaneh Sorond, located in the Galter Pavillion (7-240) of Northwestern Memorial Hospital). If qualified clinical support is present (e.g., a licensed Emergency Medical Technician, nurse, or physician), the intervention may also be administered in the laboratory of the Principal Investigator (Applied Neuro-Vascular Imaging Lab, 8th floor of 645 N. Michigan Ave). AIH will be administered using the Hyp-123 system from Hypoxico, Inc. (Gardiner, NY, USA).

The Hyp-123 is not an FDA approved device, though the internal mechanisms are similar to a household oxygen concentrator (SeQual Integra 6323-OM with FDA 510(k) approval, K042262). The Hyp-123 is intended to provide, at its output, air with lower O_2 concentrations for high altitude training purposes. This is accomplished using a pressure swing adsorption (PSA) process where room air is filtered through multiple zeolite crystals, allowing only O_2 to pass through. The oxygen-deprived air is then reintroduced to the PSA system. Oxygen concentrations at the output of the Hyp-123 are measured continuously in-line using a Maxtec Handi+ Oxygen analyzer (MAX 250E, Maxtec, Salt Lake City, UT), which has FDA 510(k) approval, K040484. The output of the system is connected to generic pressure tubing (FDA BYX 868.5860) with two, 2-liter reservoir bags (FDA BTC, 868.5320).

Abbreviated IDE: We request an abbreviated IDE for this device as it is neither intended as an implant nor purported or represented to be used for supporting or sustaining human life, is of no substantial use for diagnosing, curing, mitigating, or treating a disease, and presents no potential for serious risk to the health of a subject. A description of the device use, intervention procedures,

risks and mitigation steps are provided, followed by a summary justifying the description of the Hyp-123 as non-significant risk.

Participants are fitted with a neoprene head strap (FDA, BTK 868.5560) and a non-rebreathing face mask (FDA, KGB 868.5570), the face mask being designed to couple with a universal mask circuit connecting to the air generating system. Participant arterial oxygen saturation and pulse rate are continuously monitored using a Nonin Model 7500 (Nonin Medical Inc, Plymouth, MN). This device has been cleared by FDA for measuring, displaying, and recording O_2 saturation of arterial hemoglobin (SpO_2) and pulse rate under 510(k) K071285 (DQA, 870.2700). By continuously measuring SpO_2 it is possible to accurately and consistently monitor hypoxic air administered to the participant.

Participants will be asked to report activities that may modulate physiological responses to hypoxia exposure and any changes in medication or medical diagnoses. Once fitted with the mask, initial recordings of heart rate, blood pressure, and arterial oxygen saturation (SpO_2) will be taken. The sequence of hypoxia will consist of 30–60 seconds of 9-10% O_2 (FiO_2 0.09), alternating with 60-90 seconds of 21% O_2 (normoxic air FiO_2 0.21). The delivery of hypoxic and normoxic air mixtures will be repeated up to 15 times per session each, for a total of up to 30 minutes, targeting SpO_2 mimima of 80-90%. Heart rate and pulse oximetry will be continuously monitored and regularly recorded throughout. Blood pressure will be taken upon completion of the total sequence, and participants will complete the intervention questionnaire to report any side effects experienced during the intervention. If the participant's breathing rate is very slow, they may be paced to breathe at a slightly faster rate during the intervention using visual cues; preliminary data from studies at the Shirley Ryan AbilityLab indicate that very slow breathing rates prevent target SpO2 levels from being reached in the one-minute period allowed.

An FDA-approved pulse oximetry unit, the Nonin Model 7500, will provide continuous monitoring of heart rate and peripheral arterial oxyhemoglobin saturation (SpO_2). SpO_2 levels of 80-90% will be targeted during the hypoxic bouts, however, SpO_2 as low as 75% will be accepted. If the SpO_2 drops below 75%, we will terminate the hypoxia exposure immediately.

Any subjective symptoms will be recorded and the subject will be monitored throughout the study period for any signs of cardiopulmonary distress.

If there is a dangerous alteration in the cardiopulmonary parameters being assessed, the experiment will be stopped and appropriate care will be provided by the physician on call or nursing or other clinically qualified staff.

Risks and risk-management when using the Hyp-123 device

There are several potential risks involved with the delivery of gas mixtures to humans. We outline potential risks, as well as how our study protocol mitigates these risks. The Users' Manual from Hypoxico Inc. is attached to this protocol as supplementary information.

1. Risk Associated with Inhaled Hypoxia

    a. Risk: moderate to severe hypoxia: Severe hypoxia can have damaging effects on brain function and systemic metabolism.

i. Control: The Hyp-123 generates hypoxic gases from room air. The minimum fractional O2 level it can produce is 8.9% O_2 (as per the users' manual). Instead of relying on these estimated specifications based on flow rates, an FDA-approved oxygen sensor attached to the Hyp-123 outlet is used in all experiments to quantify the amount of oxygen in the gas the Hyp-123 produces. Our Hyp-123 system is measured to produce a minimum of 9.5% O_2. This was known at the time of purchase from Hypoxico Inc, and is intentional to ensure safe delivery of oxygen at or above this minimum at all times.

ii. Control: Additional participant monitoring will occur whenever the Hyp-123 is being used. An FDA-approved finger/ear sensor in conjunction with the Nonin Model 7500 Pulse Oximeter will continuously monitor SpO2 levels to ensure safe limits of blood oxygenation saturation are maintained. **The intervention is designed to target SpO2 of ~85% during bouts of hypoxia.** Substantially lower oxygen saturations of 65%–85% safely occur in healthy people driving to the top of mountains (e.g., Mount Evans in Colorado), and brief profound hypoxia (SaO2 50–70% for 10–30 minutes) is well tolerated by healthy humans and not accompanied by systemic acidosis or circulatory impairment.[40]

iii. Control: **The intervention is terminated and safety procedures triggered if SpO2 drops below 75%.** Note, this has never occurred in any study from our lab using the Hyp-123 system and all SpO2 data are regularly reviewed by the Independent Medical Monitor of this trial.

iv. Control: In the Intermittent Hypoxia protocol, if the target SpO2 is not reached, we deliver hypoxia for a maximum duration of 1 minute.

v. Control: The experimenter is in constant contact with the participant during the Hyp-123 AIH intervention, and will monitor for any of the following symptoms (which may precede a serious adverse response and would result in immediate cessation of the intervention).

- Nausea or vomiting
- Headache
- Confusion, disorientation
- Abnormal or slurred speech
- Difficulty with comprehension
- Numbness or weakness in arm/leg/face, particularly on one side
- Pain or discomfort in arms, back, neck, jaw, or stomach
- Loss of vision
- Chest pressure, fullness, or pain
- Severe shortness of breath
- Cold sweats

vi. Control: The experimenter will go through these symptoms with the participant during informed consent procedures and prior to the start of interventions. This will ensure the participant is aware of possible adverse

reactions to hypoxia and instruct them to alert the study team member administering the intervention if any symptoms are experienced.

vii. Control: If SpO2 drops below 75%, terminating the intervention, and does not rapidly recover to baseline, supplemental oxygen will be available for delivery via nasal cannula to improve oxygen saturation until emergency medical assistance arrives. A clinical study team member will be present for all AIH interventions to direct this response (although not necessarily present for sham interventions).

b. Risk: claustrophobia or anxiety associated with wearing face mask and breathing hypoxic gas mixtures

i. Control: Participants are able to experience the intervention during the initial study visit practice session, to determine if they experience claustrophobia associated with wearing a mask.

ii. Control: Participants are able to immediately terminate the intervention at any time, for any reason, including anxiety or claustrophobia. This may be achieved by simply “unplugging” the gas tubing from their face mask, removing their face mask, or by alerting the research team member.

iii. Control: The study team will record self-reported participant experiences, as well as anticipated mild side effects of gases, in a post-intervention questionnaire to monitor the frequency of these occurrences and adjust the protocol as needed in consultation with the Independent Medical Monitor of this trial.

NSR justification

We contend that the Hyp-123 device does not pose significant risk to the participant. The device produces a minimum inspired fraction of oxygen of 8.9% (or 9.5% in the case of our system), ensuring sufficient minimum oxygen delivery to sustain human function at all times. Furthermore, by having a study team member constantly monitoring the participant’s vitals (including SpO2), we are able to immediately stop a bout of hypoxia that does not stay within our conservative blood oxygen targets. We have demonstrated that these protocol procedures have prevented SpO2 from dropping below our safety limit of 75% in all cases to date, as monitored by this trial’s Independent Medical Monitor (relevant data from our IMM reporting is attached as supplemental material). The participant can stop the intervention at any time by alerting the study team member administering the intervention (speaking or hand gestures) or by unplugging the breathing circuit from the face mask. They will be reminded that the intervention can stop for any reason, including adverse reactions to hypoxia, discomfort, or simply a desire to stop participating.

Abbreviated IDE Compliance

We will comply with the requirements for an Abbreviated IDE as set forth by the FDA. Specifically, we attest that

- The Hyp-123 device is not a banned device
- The Hyp-123 device is labeled “CAUTION – Investigational Device. Limited by US law to investigational use.”
- This study will be monitored in accordance with 21 CFR 812.46. Unanticipated adverse effects will be evaluated, and if presenting an unreasonable risk to the participant the investigation will be terminated within 5 working days. Furthermore, this study is subject to additional Independent Medical Monitoring as per NINDS requirements.
- The PI will ensure study records are maintained in accordance with 21 CFR 812.140. These records will be accurate, complete, and current. They will include:
    - Protocol
    - Name and address of study team members
    - Details of Hyp-123 device provided by manufacturer (Hypoxico Inc.)
    - Signed and dated consent forms of all participants
    - Case Report Forms (including adverse events and debriefing questionnaires)
    - Device disposition (faults, repairs, etc)
- The PI will report to the IRB as required by 21 CFR 812.150. This will include:
    - un-anticipated adverse effects (within 10 working days)
    - progress reports (at least yearly)
    - recall notices or device disposition changes (e.g., repair, within 30 working days)
    - final report (within 6 months of study completion)
- The PI will ensure all members of the study team obtain and document consent from each participant.
- The study will comply with the prohibitions against promotion and other practices, as described in 21 CFR 812.7.

Respiratory challenge (RAMR™)

The RespirAct-MR (RAMR)™ is a computer-controlled gas blending system that facilitates delivering gases to people in the MRI environment. We are employing the RAMR™ device as a research tool (not for any medical diagnosis or treatment) to deliver hypoxic and hypercapnic respiratory challenges to our participants during scanning. This device is not FDA approved, however we are not evaluating this device.

The RAMR™ will be used during MRI scanning at the CTI facilities. It may also be tested outside of the imaging center, in the department of Physical Therapy and Human Movement Sciences, located at 645 N. Michigan Avenue (8th floor), Chicago, Illinois 60611.

The RAMR™ consists of a computerized gas blender that delivers precise, repeatable, gas mixtures to the participant via a face mask while monitoring inhaled and exhaled gas concentrations. This system is described in greater detail in the literature [17,26–28]. Over 130 peer reviewed publications have been published using the RespirAct™.

Description of RAMR™ apparatus

1. A face mask is positioned on the participant and skin tape (Tegaderm™) is used to seal the edges where the mask touches the skin to maintain the mask positioning and prevent leaks.
2. Three distinct "source" gases are supplied to the RAMR™ with different contents of CO_2, O_2, and N_2. All gases have a minimum of 5% O_2 to prevent severe levels of hypoxia.
3. The operator enters into the RAMR™ computer the participant parameters and the target "sequence"–the amplitude and timing of the intended $P_{ET}CO_2$ and $P_{ET}O_2$ stimulus.
4. The RAMR™ computer models the correct gas blending to achieve the target sequence in the individual participant, and delivers the correct gas blend to the participant's face mask. There is an automatic set-up period where the RAMR™ tests physiological parameters and the individual participant's ventilation (approximately 5 minutes).
5. Gas exhaled by the participant into the face mask is sampled by the RAMR™ and displayed on the monitor in real-time. The investigator can observe the participant's $P_{ET}CO_2$ and $P_{ET}O_2$ as well as breathing rate at all times during the experiment.
6. The gas blender unit and the gas sampling analyzer are MRI-safe and can be positioned adjacent to the scanner (beside the participant during scanning). Keeping this distance short results in more accurate and better synchronized gas sampling.
7. There is no discernable resistance in the system, meaning that inhalation is not any more difficult than when breathing without the mask.
8. The system is open to the environment, so that failure in gas delivery (line blockage, faulty regulator, empty gas cylinder, etc.) will result in the participant breathing room air.

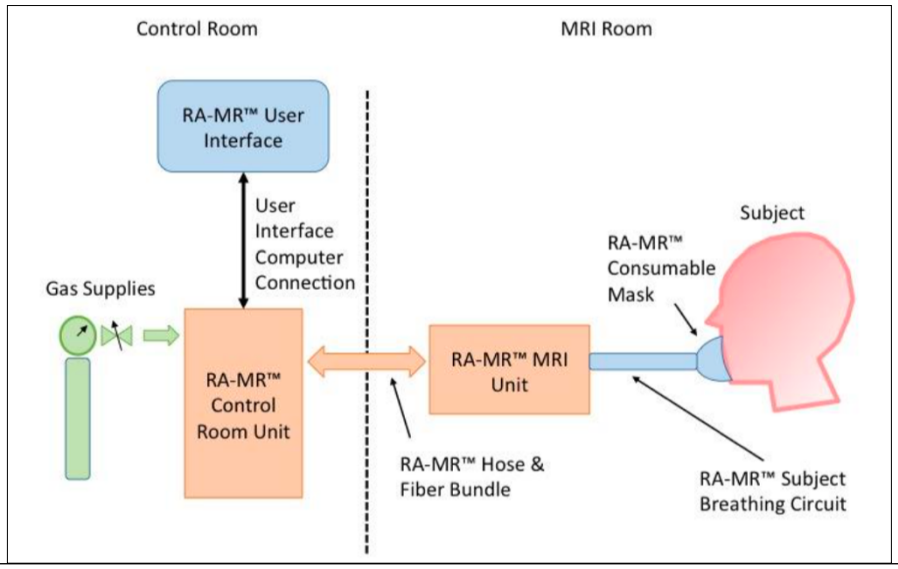


Figure 1. RAMR™ schematic in an MRI environment
Control room: Source gases are supplied to the RAMR™ control room unit. The Investigator enters study and participant details and sets the respiratory challenge protocol using the touch screen monitor (User Interface). A hose and fiber bundle connect the Control Room Unit to the MRI Unit through a wave-guide opening in the scanner room wall.
MRI scan room: The RAMR™ MRI Unit sits in the scanner room adjacent to the scanner bed. The MRI Unit continuously adjusts the blend of gases to achieve the desired respiratory challenge protocol, and outputs the resulting gas mix through the breathing circuit into a disposable face mask worn by the participant.

Risks and risk-management when using the RAMR™ device

There are several potential risks involved with the delivery of gas mixtures to humans. It should be noted that the primary risks are associated with gas challenges, rather than the gas blending system. We outline potential risks, as well as how the RAMR™ and our study protocol mitigate these risks. A summary of the RAMR™ (also known as RespirAct™ Gen 4), the complete RAMR™ manual, and additional information about risk control assessment (Design and Failure Mode Analysis) from Thornhill Research Inc. are attached to this protocol as supplementary information.

2. Risk Associated with Gas Challenges

    a. Risk: moderate to severe hypoxia: Severe hypoxia can have damaging effects on brain function and systemic metabolism.

        i. Control: All source gases connected to the RAMR contain O_2 to ensure a minimum level of oxygen is always delivered. The composition of the three "source" gases (medical grade) are:
            - Gas A — 5% O_2, 90% N_2
            - Gas B — 10% O_2, 90% CO_2
            - Gas C — 100% O_2

            In this protocol we **do not target any $P_{ET}O_2$ levels below 55 mmHg**. This degree of hypoxia occurs naturally when people are resting at high altitudes (~5km)[39].

        ii. Control: Additional monitoring will occur for all studies involving hypoxic gas challenges. An FDA-approved finger sensor will continuously monitor SpO2 levels to ensure safe limits of blood oxygenation saturation are maintained (Invivo Precess 360 MRI Patient Monitoring System, 510(k) K053462). **The gas challenges will be adjusted or terminated to prevent SpO2 from being below 85% during an MRI experiment.** Substantially lower oxygen saturations of 65%–85% safely occur in healthy people driving to the top of mountains (e.g., Mount Evans in Colorado), and brief profound hypoxia (SaO2 50–70% for 10–30 minutes) is well tolerated by healthy humans and not accompanied by systemic acidosis or circulatory impairment.[40]

        iii. Control: During a practice session outside of the MRI environment (required before an MRI experiment involving gas challenges), the experimenter will be continuously monitoring and communicating with the participant. Specifically, they will be monitoring PETO2 and SpO2 to ensure these parameters stay within the intended safety limits (see Table 1). The experimenter will also periodically converse with the participant and look for any of the following symptoms (which may precede a serious adverse response and would result in immediate cessation of the hypoxia stimulus).

            - Nausea or vomiting
            - Headache
            - Confusion, disorientation

- Abnormal or slurred speech
- Difficulty with comprehension
- Numbness or weakness in arm/leg/face, particularly on one side
- Pain or discomfort in arms, back, neck, jaw, or stomach
- Loss of vision
- Chest pressure, fullness, or pain
- Severe shortness of breath
- Cold sweats

iv. Control: The experimenter will go through these symptoms with the participant prior to the start of the MRI scan involving the sustained hypoxia challenge. This additional stage will ensure the participant is aware of possible adverse reactions to hypoxia and instruct them to alert the study team with the emergency squeeze ball if any symptoms are experienced. The experimenters will continue to monitor vital signs and the video feed of the participant's face and body during the scan session (Table 1).

v. Control: The study team is trained in Basic Life Support and there is a ***study SOP*** (see accompanying document) for responding in the event that a person experiences these symptoms, becomes non-responsive, or SpO2 levels drop below 85% and do not recover or drop further after the gas challenge is terminated. Supplemental oxygen will be available outside of the scan room for delivery via nasal cannula to improve oxygen saturation until emergency medical assistance arrives.

b. Risk: moderate to severe hypercapnia: In the extreme, severe hypercapnia over the level of about 80 mmHg (much in excess of 54 mmHg, which we will maximally implement), may cause drowsiness or even loss of consciousness. Indeed, even 2-3 times this level—250 mmHg or greater—is not known to cause persistent harm if not accompanied by low oxygen. In other words, there is full recovery even from such extreme levels when normal CO_2 levels are restored. This speaks to the benign aspect of this intrinsic gas. However, high levels of CO_2 can cause transient discomfort such as feeling of shortness of breath, elevated heart rate, elevated blood pressure, and panic.

i. Control: In this protocol, we **do not target any CO_2 values above +6 mmHg over the individual's baseline $P_{ET}CO_2$ level or 54 mmHg**, whichever is lower. Specifically, we are targeting a +6mmHg challenge with respect to their baseline levels. For reference, a +10 mmHg increase in $P_{ET}CO_2$ is approximately the effect of a 20-second breath hold[41], or playing a wind instrument. A $P_{ET}CO_2$ of 54 mmHg occurs in divers when exercising at depth[42], and in most people during sleep. Even greater levels can occur when undergoing periodic sleep apneas, which many otherwise healthy humans do during the night. If $P_{ET}CO_2$ exceeds 70 mmHg for 10 seconds (~2-4 breaths), the system will alarm and stop delivering the gas mixture. Normal CO_2 levels are then quickly re-established within 2-3 breaths of normal air. Participants can always disconnect their mask or alert the investigator to turn off the gas delivery if they experience any discomfort below this threshold, at any point in the study.

3. Risk Associated with the Gas Blending and Delivery System

    a. Risk: insufficient air delivery (blockage in the lines)

        i. Control: The breathing circuit of the RAMR™ is open to the environment. If insufficient gas is delivered to the mask, the participant can breathe normally from room air.

    b. Risk: high pressure gas delivery to face mask (malfunction of cylinder or regulator)

        i. Control: The RAMR™ is open to the environment, and thus cannot reach pressures greater than atmospheric pressure.

    c. Risk: claustrophobia or anxiety associated with wearing face mask in the MRI scan environment

        i. Control: Participants are able to experience the “mock scanner” environment at CTI while wearing the RAMR™ face mask to determine if they experience claustrophobia associated with wearing a mask in the enclosed space.

        ii. Control: Participants are able to immediately terminate the session at any time, for any reason, including anxiety or claustrophobia. This may be achieved by simply “unplugging” the gas tubing from their face mask or by alerting the research team via the squeeze alert. The participant will practice the unplugging action prior to scanning.

        iii. Control: The study team will record self-reported participant experience of claustrophobia and anxiety, as well as anticipated mild side effects of gases, in a debriefing questionnaire to monitor the frequency of these occurrences and adjust the protocol as needed.

Our protocol provides additional risk-management.

1. All investigators using the RAMR™ will be trained to use the device by Dr. Molly Bright, the PI on this study. Training will be reviewed yearly. They will also undergo First Aid/CPR/AED training (for example, a course offered by the Red Cross).
2. All MRI studies involving the RAMR™ will involve *at least* two individuals: one will operate the scanner and a different person will operate the RAMR™. This will ensure proper focus is given to both MRI and respiratory challenge safety. The two people will be in constant communication during the session to coordinate the scanning with the respiratory challenge.
3. We will target “moderate” respiratory challenges only: $P_{ET}CO_2$ of +6mmHg and $P_{ET}O_2$ of 55 mmHg.
4. Clear stopping criteria (*see Table 1 later in this protocol*) based on continuous end-tidal gases, SpO2, heart rate, breathing rate, video monitoring, and periodic communication with the participant will be used to stop gas challenges safely.
5. Participants will experience (“practice”) the respiratory challenge prior to an MRI scan visit to ensure they are comfortable and to adjust the challenges to improve comfort as needed.

6. Participants will be instructed to alert investigators if at any time they want to stop the scan and respiratory challenge. During scanning, this is achieved by squeezing the scanner buzzer alert. They may also disconnect the breathing circuit from the face mask manually (it is press-fit and easy to detach), resulting in instant breathing of room air.

An SOP for responding to mild and (rare) severe adverse responses to the gas challenges has been provided with this protocol, outlining the actions of the study team to ensure the safety of the participant. In brief, indication of severe adverse responses will result in the immediate termination of the gas challenge and scan, removal of the participant from the scan room environment, and calling of 911 for emergency medical assistance. If a person is non-responsive, basic life support (CPR) will be initiated. If a person is responsive, they will be monitored (SpO2, BP). If SpO2 does not normalize or drops further, supplemental oxygen will be administered via nasal cannula. This SOP was prepared with clinical co-investigator Dr. Farzaneh Sorond, MD PhD, who will regularly review the clinical safety of our procedures.

NSR justification

We contend that the RAMR gas blending device does not pose significant risk the participant.

We have regularly used the RAMR™ to administer gas challenges during scanning in other approved studies at Northwestern University, both safely and effectively. As per discussion with the IRB, we acknowledge the rare but potential risk for adverse responses to gas challenges and have developed an SOP for responding in these situations.

There is well documented evidence of safety using the RAMR™ to blend and deliver gases during MRI. Across the lifespan (ages 9–88 years) and in diverse pathologies (including moyamoya and atherosclerosis of the carotid or intracranial arteries), no serious adverse events were reported in 434 examinations (Spano et al. 2013 Radiology). Transient mild side effects associated with the gases themselves were indeed reported (11% of participants), as would be anticipated with or without the RAMR blending system. These include breathlessness, claustrophobia, anxiety, dizziness, nausea, and headache. In our three current studies at Northwestern, we administer a post-scan debriefing questionnaire to gauge the incidence of these side effects in our participants: we have documented mild adverse side effects (scoring 5 or higher out of 10) in 4 out of 35 participants (11.4%), with a very low average overall discomfort (1.5 out of 10). We provide these data in an attached document, and a description of how we responded to these side effects in a safe and rapid manner, following the stopping criteria and communication policies in our protocol.

The RAMR is currently used at 36 institutions in North America with a Non-Significant Risk determination by the local IRB, including Mayo Clinic, Cedars-Sinai Medical Center, Duke University, Johns Hopkins University, University of Illinois Chicago, University of Chicago, and others (a complete list is attached to this protocol). It is currently approved for use in three IRB-approved studies at Northwestern University (***STU00207665, STU00210575, STU00212894***) with an NSR determination by this IRB. Dr. David McLeod has used the RAMR in over 8 IRB-approved studies at Duke University, and has shared IRB documentation for one study involving the RAMR and hypoxia challenges with the NSR determination (attached to this submission).

Furthermore, we do not rely on the RAMR for safety monitoring: we employ an FDA-approved SpO2 monitor (Invivo Precess 3160 MRI Patient Monitoring System; 510(k) K053462) to continuously measure oxygen levels during all gas challenges inside and outside the scanner.

We use this information as our primary measure of participant well-being and as our primary stopping criterion during gas challenges.

Summary: We are not evaluating the RAMR™ device, but rather using it as a research tool to improve our measurement of cerebrovascular reactivity by blending gases in the MRI environment. The risk in our study is associated with gases breathed by the participant rather than the device for blending these gases. Due to the mild gas levels targeted by this protocol, and the highly conservative stopping criteria based on continuous multi-modal monitoring of the participant during all procedures (Table 1 in subsequent section), we feel that the risk to healthy adults is not significant.

Additional Comments:

- The induced changes in PCO_2 are in the same range as experienced by most people in day to day living.
- The induced changes in PO2 are typically less than those experienced by people driving up a mountain in Colorado.
- There is no chance of asphyxia (insufficient gas to breathe) as the system is open to air
- The system has undergone ISO 13485 design and failure mode analysis which is available from the company (*attached to this protocol*)
- The system has an emergency stop switch, and will also abort gas challenges if the participant removes or disconnects their mask or the investigator presses a button.
- The system is designed to fail safe
- Demonstrated safety: well over 1,500 studies over 13 years, including with earlier models of the device, without incident.

Dr. Molly Bright, the PI of this study, is extremely familiar with the RespirAct™, having used it for studies in the MR environment in previous institutions over the last decade in the United Kingdom, including the University of Nottingham Sir Peter Mansfield Imaging Center. Dr. Bright's lab has successfully used this device in three other IRB protocols of healthy participants (***STU00207665, STU00210575, STU00212894***). In this protocol, we are only including healthy participants: we do not expect any additional risk beyond the transient mild side effects reported in the literature[43] and observed in our other ongoing studies. All of our participants are healthy, and they will be informed of potential mild and severe side effects of the respiratory challenges; they can end the study at any time and switch to breathing room air if they become uncomfortable.

<u>Abbreviated IDE</u>

Having been using the RAMR in other IRB-approved studies at Northwestern University under an Abbreviated IDE, we will continue to comply with the requirements for an Abbreviated IDE as set forth by the FDA. Specifically, we attest that

- The RAMR™ device is not a banned device
- The RAMR™ device is labeled "CAUTION – Investigational Device. Limited by US law to investigational use."
- This study will be monitored in accordance with 21 CFR 812.46.
  Unanticipated adverse effects will be evaluated, and if presenting an unreasonable risk to the participant the investigation will be terminated within 5 working days. Furthermore,

this study is subject to additional Independent Medical Monitoring as per NINDS requirements.

- The PI will ensure study records are maintained in accordance with 21 CFR 812.140. These records will be accurate, complete, and current. They will include:
    - Protocol
    - Name and address of study team members
    - Details of RAMR™ device provided by manufacturer (Thornhill Research Inc.)
    - Signed and dated consent forms of all participants
    - Case Report Forms (including adverse events and debriefing questionnaires)
    - Device disposition (faults, repairs, etc)
- The PI will report to the IRB as required by 21 CFR 812.150. This will include:
    - un-anticipated adverse effects (within 10 working days)
    - progress reports (at least yearly)
    - recall notices or device disposition changes (e.g., repair, within 30 working days)
    - final report (within 6 months of study completion)
- The PI will ensure all members of the study team obtain and document consent from each participant.
- The study will comply with the prohibitions against promotion and other practices, as described in 21 CFR 812.7.

PROCEDURES INVOLVED:

Study design

In this sham-controlled crossover study, up to 40 healthy participants between the ages of 21 and 50 years will be recruited to participate for approximately 5 months to determine whether daily AIH has beneficial impact on cerebrovascular function.

Potential participants will be initially screened over the phone or in person, to ensure compliance with inclusion/exclusion criteria. If the participant qualifies for the study, they will be scheduled for a practice session. Informed consent, safety screening, and gas inhalation practice will all take place in the participant's first study visit. The first visit will take place at a convenient time at the Center for Translational Imaging (CTI) of Northwestern University (Olsen Pavilion, 710 N. Fairbanks Ct., Lower Concourse, Chicago, Illinois 60611). They will then be randomly enrolled in either the daily AIH intervention or the sham intervention. This will involve an extensive MRI (PRE) scan in the first week (at CTI), followed by three weeks of regular visits to the ABC lab (Galter Pavillion, 7-240) to receive the intervention. Note, that if clinically qualified personnel are present for the session (i.e., a licensed Emergency Medical Technician, nurse, or physician), intervention visits may also be held at the Applied Neuro-Vascular Imaging Lab in 645 N. Michigan Avenue to facilitate visit scheduling. The study team will endeavor to have all intervention visits for a given participant be in the same location. 1–3 days following the last intervention visit, the participant will have a second MRI (POST) scan at CTI. The participant will then wait 3 months to allow any effects of the first intervention to normalize, in line with the washout periods used by our colleagues at the Shirley Ryan AbilityLab, who are testing a similar 4-week intervention in spinal cord injury. After this delay period, the participant will begin the second intervention, following a similar pattern of MRI (PRE), 3 weeks of intervention visits, and a repeated MRI (POST) scan. A schematic of the study design is provided in **Figure 2**.

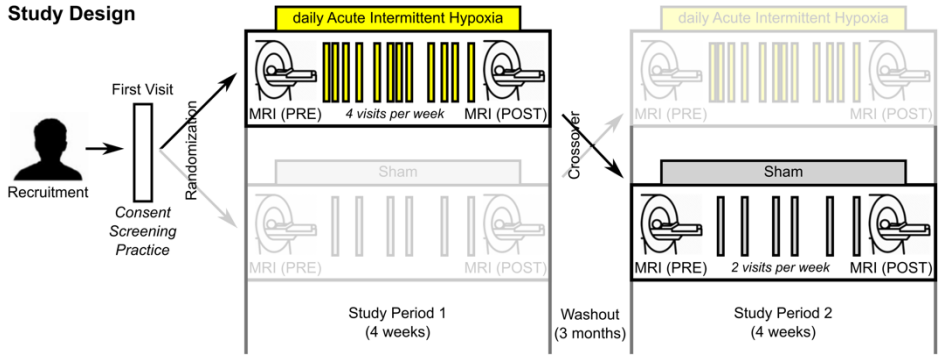


Figure 2. Schematic of overall study design showing randomization, crossover, washout.

Daily AIH Intervention: Participants will visit the lab 4 times per week for 3 weeks.

Sham Intervention: Participants will visit the lab 2 times per week for 3 weeks.

Participants will be told the two interventions are different *doses* of AIH that may have different effects on physiology. Hypoxia does not stimulate breathing rate in the same way as hypercapnia, thus we do not expect participants to note major differences.

All scanning will take place at Northwestern University's Center for Translational Imaging (CTI). Scans will include whole-brain phase-contrast measures of baseline cerebral blood flow (**CBF**) and quantitative arterial spin labeling CBF maps. The MR-compatible RAMR™ gas blending system will administer mild sustained hypoxic and hypercapnic challenges to the participant during repeated CBF scans in order to measure the perfusion response, a measure known as cerebrovascular reactivity (**CVR**). Upon study completion, we will ascertain if daily AIH achieved significant improvements in CBF or CVR. **Figure 3** shows a schematic of all intervention visits and the details of MRI scan visits. Details of the Intervention and MRI visits are provided in a section below.

First Visit:

1) Consent: All procedures and requirements of the study will be explained by a member of the study team, encouraging the participant to ask questions to ensure comprehension of the risks and sequence of events. Participants will have one primary contact person on the study team, and they will also have the ability to speak with the Principal Investigator if at any time they are uncomfortable or need further information about the study.
2) Safety Screening; They will complete both a study and MRI safety screening, including information on current prescription medications, and have a blood pressure reading (and pregnancy test when indicated (uncertain or >28 days since last period) or requested), to ensure it is appropriate for them to participate in the study. Dr. Sorond or another clinician on the study team will determine the suitability of each participant for enrolling in the study, including review of medications.
3) Participant Information; Contact details for the participant, their primary care physician details, and payment setup information will be collected.
4) Practice sessions; After consent and screening, participants will practice using both systems involved with delivering the intermittent hypoxia intervention and sustained hypoxia challenge to ensure they are comfortable with both procedures and improve data quality and consistency throughout the study.
    a. Intermittent hypoxia will be administered via a hypoxia generator system (Hyp-123, Hypoxico Inc., New York, NY, USA). The Hyp-123 is commonly used for

altitude training, and is currently used to administer intermittent hypoxia interventions in our collaborations at the Shirley Ryan AbilityLab. The participant will be fitted with a latex-free full non-rebreathing mask with custom neoprene head strap. The mask is designed to couple with a universal circuit connecting to the air mixture system, and minimizes room air entrainment. The mask has an attached reservoir bag where the controlled air mixture (FiO_2 = 0.09) that the generator fills in between breaths, and a valve that largely prevents the inhalation of room or exhaled air. Delivery of air mixtures will be done through adjustment of one-way valves attached to the delivery lines. Tubing will extend from the reservoir bag directly to the machine. Practice: Once fitted with the mask, initial recordings of heart rate, blood pressure, and arterial oxygen saturation (SpO_2) will be taken. The sequence of hypoxia will consist of 30-60 seconds of 9% O_2 (FiO_2 0.09), alternating with 60-90 seconds of ~21% O_2 (normoxic air, FiO_2 0.21) for a 2-minute period. The delivery of hypoxia and normoxic air mixtures will be repeated at least 5 times and up to 15 times in this practice session. Heart rate and pulse oximetry will be continuously monitored throughout. Blood pressure will be taken upon completion. The participant can indicate to the researcher if they are not comfortable at any time, pausing or ending the session. If the participant is not comfortable with the intermittent hypoxia intervention, or if their physiological parameters change beyond safe limits, then they will no longer continue with the study. If they are comfortable with the intermittent hypoxia intervention and have typical physiologic responses, they will continue with the next practice.

b. Sustained hypoxia and hypercapnia will be administered via the MRI-compatible RAMR™ system (Thornhill Medical, Toronto, Canada). The RAMR™ is an MRI-safe device for delivering respiratory challenges that is currently used by the Principal Investigator in multiple MR imaging studies of brain physiology. It is a computer-controlled gas blending system that delivers gas mixtures on a breath-by-breath basis. Practice: While comfortably seated in a reclined position, the participant will be asked to position the RespirAct-MR™ face mask over their nose and mouth, adjusting it until it is comfortable. At this time, they will be breathing room air through the mask. Once the position is set, the researcher will use Tegaderm™ tape to affix the mask and seal off any leaks around the edges. The researcher will then initiate the set-up procedures of the RespirAct-MR™ system (the system records exhaled gas content while subtly modulating the delivered gas blend to ensure the baseline physiological parameters are accurately set). The researcher will note the baseline parameters. Next, the participant will practice a sustained hypoxia challenge. After informing the participant of the challenge, the RespirAct-MR™ operator will target gas levels of $P_{ET}O_2$=55 mmHg and $P_{ET}CO_2$=+0 mmHg above baseline levels for at least 10 minutes. Following this, the participant will practice a short hypercapnia challenge, targeting $P_{ET}O_2$=+0 mmHg and $P_{ET}CO_2$=+6 mmHg above baseline levels. If the participant is not comfortable with the challenge, they can stop immediately at any time. Alternatively, they can agree to try again after adjustments are made for their comfort. If the participant is not comfortable with the respiratory challenge, the session will end and the participant will no longer continue with the study. If they are comfortable with the respiratory challenge, they will continue with the scheduling the rest of the study.

5) Scheduling of Experimental Protocol: Each participant will be assigned to an order of interventions (active and sham) at random. A member of the study team will discuss the scheduling of the first intervention to identify appropriate times for the PRE and POST

MRI visits, and draft the timings of intervention visits. The participant will be told that nail polish must be removed for all study visits.

MRI visits: MRI scanning in this project will take place on a Siemens 3 Tesla scanner at the Center for Translational Imaging (CTI) of Northwestern University (Olsen Pavilion, 710 N. Fairbanks Ct., Lower Concourse, Chicago, Illinois 60611). At least two people will oversee the MRI visit: one will operate the scanner (either a qualified member of the study team or a qualified MR technologist from the CTI staff) and one will operate the RAMR™ respiratory challenge (with documented yearly training/re-training from the PI on safe running of the RAMR™ system).

Participants will undergo study and MRI safety screening upon arrival at CTI to ensure no changes have occurred since a prior screening encounter. The participant will be asked to change into scrubs and leave all personal belongings (including all metal jewelry, watches etc.) in a secure locker. The participant's blood pressure will be measured using a standard brachial cuff. While comfortably seated, the participant will be asked to position the RAMR™ face mask over their nose and mouth, adjusting it until it is comfortable. At this time, they will be breathing room air through the mask. Once the position is set, the researcher will use Tegaderm™ tape to affix the mask and seal off any leaks around the edges. The participant will then be escorted into the scan room by the MR technologist; there are metal detectors at the door to the scan room as a final safety check that no metal is brought into the MRI environment.

Ear plus and/or headphones will be supplied to protect participant hearing during scanning. Soft pads will be used to stabilize head position inside of the head-coil. Passive physiological monitoring equipment will be affixed to the participant, including an SpO_2 fingertip monitor. A squeeze-ball alert will be held in one hand, and pads, pillows and blankets will be used to maximize participant comfort. The participant will be moved slowly into the scanner bore, and the intercom and alert systems will be tested.

The scan will be divided into three sections: baseline (25 minutes, $P_{ET}O_2$=+0 mmHg, $P_{ET}CO_2$=+0 mmHg), hypercapnia (16 minutes, $P_{ET}O_2$=+0 mmHg, $P_{ET}CO_2$=+6 mmHg), and hypoxia (24 minutes, $P_{ET}O_2$=55 mmHg, $P_{ET}CO_2$=+0 mmHg). Note, these targets may be slightly altered *(less hypoxic* or *less hypercapnic*) depending on the initial practice session (more mild targets may be used to improve participant comfort or keep the participant SpO2 at or above ~85%). The duration of these sections may be shortened if all scans can be achieved in a shorter period of time. A series of ASL and ASL calibration scans will be acquired during the final ~14 minutes of the baseline, hypoxia, and hypercapnia periods. Structural and diffusion-weighted scans will take place at the beginning of the scan session. At the end of all scanning, the participant will return to breathing normal air as they are moved out of the scanner. The tubing is then disconnected from the face mask, and peripheral monitoring equipment is removed. After exiting the scan room, the face mask is carefully removed and disposed of, and the participant will be asked to complete a debriefing questionnaire to reflect on any discomfort or unusual experiences during the gas inhalation part of the experiment. A second blood pressure reading will be made. Finally, the participant can change back into their original clothing, collect their belongings, and work with a member of the study team to finalize reimbursement details before exiting the center.

The total time in the MRI scanner will last no more than 1.5 hours to complete this 1-hour protocol, and the total visit to CTI will last approximately 2 hours. The minimum hypoxia targeted during the RespirAct-MR™ gas challenge is 55 mmHg, and the challenge will be aborted if the end-tidal levels drop below 52 mmHg. This is approximately what levels occur at rest while at high altitudes (~5km)[39]. SpO_2 will be monitored throughout the experiment (FDA-approved MRI-

compatible Invivo 3160 sensor), and the gas inhalation challenge will be aborted if SpO_2 drops below 85%. The hypoxia stimulus will not be allowed to exceed 26 minutes.

After the final MRI visit, the participant will be informed of the true study design (see Final Study Debriefing Script), specifically explaining that one of the study periods involved a sham intervention.

Figure 3 provides a schematic of both the intervention visits and details of the MRI scan visits. **Figure 4** shows test data for a sustained hypoxia challenge using the RAMR™.

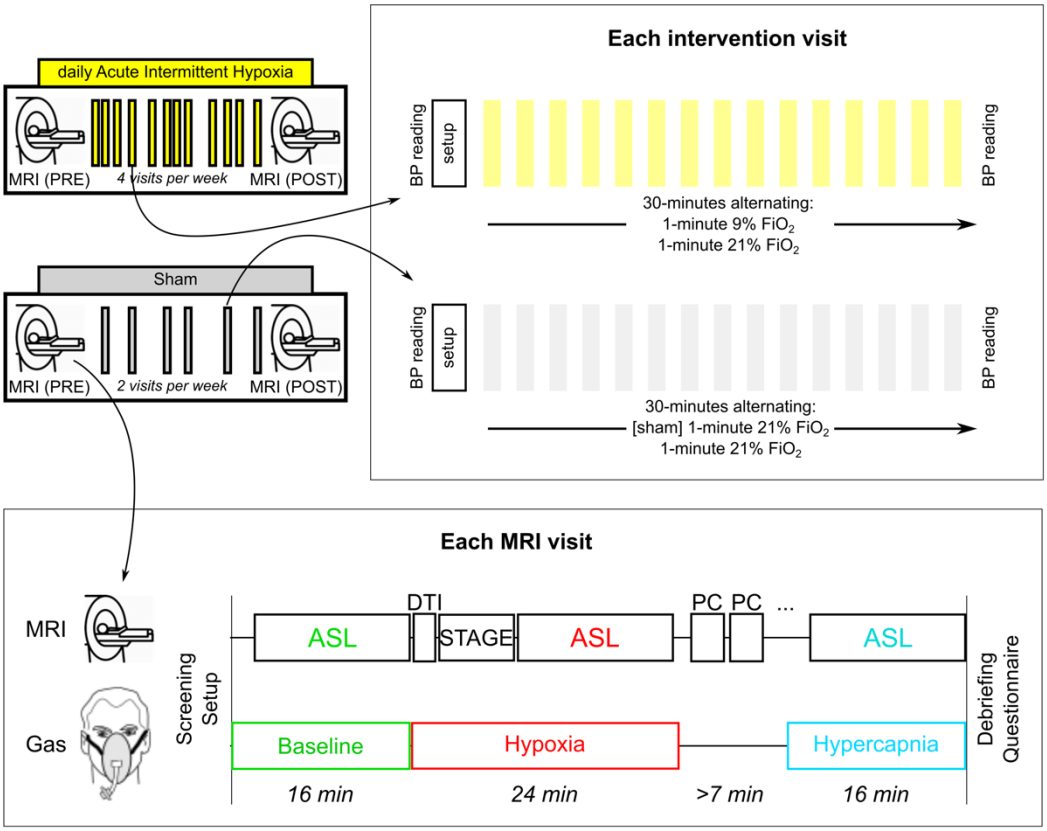


Figure 3. Schematic of the intervention and MRI experimental design

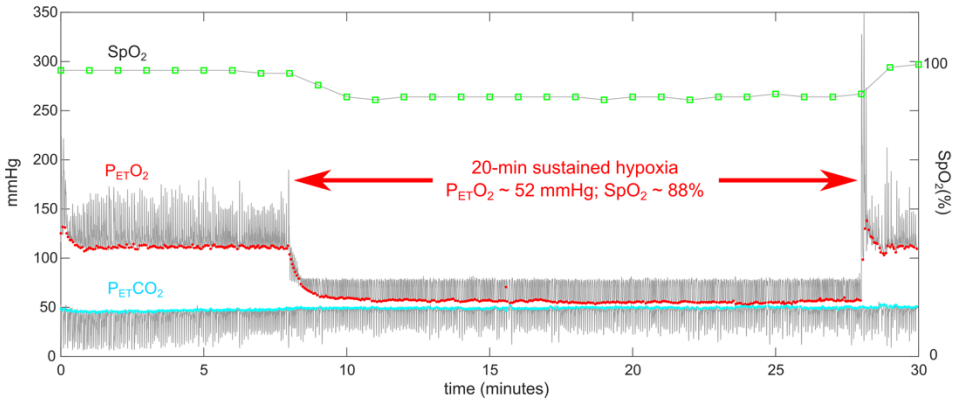


Figure 4. Examples of sustained hypoxia challenges achieved using the RespirAct-MR™ system at Northwestern University, including the independent SpO2 monitoring data.

Intervention visits: All intervention visits (for either the "active" intermittent hypoxia or the sham intervention) will take place in the aging brain circulation (abc) laboratory space of Dr. Farzaneh Sorond, MD, in the Galter Pavilion (7-240) of Northwestern Memorial Hospital, 675 N. St. Clair St. Chicago, IL 60611. As noted above, the intervention visits may also be performed at the laboratory of the Principal Investigator, the Applied Neuro-Vascular Imaging Lab, (rm 851) in 645 N. Michigan Ave, only if clinically qualified study team personnel are present (for example, a licensed Emergency Medical Technician, nurse, or physician). Upon arrival, participants will be asked to report activities the participant engaged in that may alter their physiological response to hypoxia exposure, as well as to report any changes in medication or medical diagnoses (questions 1-4 of the *Intervention Questionnaire)*. Changes in medication or medical diagnoses will be assessed by the clinical co-investigator before administration of hypoxia. If the clinical co-investigator cannot be reached via phone or email within 15 minutes, the intervention visit will be postponed. After recording blood pressure and connecting the physiologic monitoring equipment (end-tidal gases and SpO_2 monitoring), the research assistant or another qualified member of the study team will administer the intervention using the Hyp-123 (Hypoxico, New York, NY). Both interventions last 30 minutes; the active intermittent hypoxia intervention involves alternating between 30-60 seconds of inhaling 9% O_2 and 60-90 seconds of inhaling 21% O_2, for a period of 2 seconds, and the sham intervention involves alternating between 1 minute of inhaling (sham) 21% O_2 and 1 minute of inhaling 21% O_2. Visual cues may be shown to pace the participant's breathing during the intervention to improve consistency across visits. (Preliminary data at Shirley Ryan AbilityLab indicates that extremely slow breathing rates limit the ability of the intervention to achieve the targeting SpO2 level in the one-minute period allowed.) The participant and physiologic recordings will be observed throughout the intervention period to monitor for adverse effects as described in the safety section below. If SpO2 drops below 75%, the intervention will be terminated early. If this occurs in more than 2 consecutive intervention visits or in more than 3 nonconsecutive intervention visits, the participant will be removed from the study (Table 2). Upon completing the 30-minute protocol, a second blood pressure reading will be recorded and the participant will be asked to complete the intervention questionnaire to assess any side effects experienced during the intervention (questions 5-7 of the *Intervention Questionnaire)*. The participant will be free to go after confirming the next visit timing with the research coordinator. **If blood pressure is elevated by more than 15mmHg**, the participant will be withdrawn from the study. If systolic blood pressure drops below 90mmHg, the participant will be withdrawn from the study.

Safety Monitoring and Risk Management

All medical diagnoses and prescription medications, including changes reported at follow-up visits, will be reported to and reviewed by a clinical co-investigator on the study team.

The initial practice session, practicing both the AIH intervention and the RAMR™ sustained hypoxia and sustained hypercapnia challenges, will occur at CTI outside of the MRI scanner. This is done to ensure that the participant is comfortable with all gas challenges being used throughout the study in an environment where the study team can appropriately observe their response.

All study visits will take place at CTI or in the clinical laboratory space of Dr. Farzaneh Sorond (the aging brain circulation "abc" lab) located within Northwestern Memorial Hospital or in the PI's lab space if clinically qualified personnel are present. Blood pressure (BP) readings will be taken before and after the intervention, and SpO_2 will be recorded throughout all practice, intervention, and scanning sessions. Participants who demonstrate a systolic BP increase of >15mmHg from

initial levels, or that drops below 90mmHg, will be withdrawn from the study. An intervention will be abbreviated if SpO_2 drops below 75%.

In the practice session and in the intervention visits, participants will be asked to report any side effects experienced, and the experimenter will continuously monitor the participant for any signs of adverse effects of the gas inhalation challenges and communicate regular to ensure the participant is comfortable. Specifically, they will be monitoring for signs of the following symptoms, which may indicate that a serious adverse response (such as heart attack or stroke) may occur: dizziness, nausea, lightheadedness, vomiting, headache, confusion, disorientation, abnormal or slurred speech, difficulty with comprehension, numbness or weakness in arm/leg/face, particularly on one side, pain or discomfort in arms, back, neck, jaw, or stomach, vision impairment, chest pressure, fullness, or pain, severe shortness of breath, cold sweats.

During MRI scan sessions, where sustained hypoxia and hypercapnia challenges are delivered while the participant is inside the MRI scan room environment, the experimenter will communicate with them between scans and monitor the video feed and physiologic recordings to identify any signs of participant distress. They will also remind the participant of possible adverse reactions to the gas challenges immediately prior to their commencement, and the mechanisms for pausing or stopping the scan at any point via emergency alert squeeze ball or by detaching the breathing circuit from the face mask.

During gas challenges, we will continuously monitor end-tidal gases, SpO2, pulse, and breathing. While in the MRI scanner, a video feed of the participant's face and body will also be monitored for signs of distress. The participant will have constant access to an intercom/alert system to communicate with the researchers throughout the scan session. Communication may also be achieved through hand gestures viewed through the video feed. Stopping criteria include:

Table 1 Monitoring during MRI experiments and stopping criteria

Monitoring	Stopping Criteria
Pulse	< -10 bpm below baseline Moderate tachycardia is safe and anticipated
Breathing rate	< 6 bpm Moderately elevated ventilation is safe and anticipated
SpO2	< 85%
End-tidal O2	< 52 mmHg

End-tidal CO2	> 54 mmHg or > +10 mmHg above baseline
Video feed	Distress, no visual response to prompts (e.g., thumbs up)
Communication via intercom	No response to prompts between scans or as gas challenge is initiated

If these thresholds are met, the gas challenge will be immediately terminated. The participant may also terminate the gas challenge *at any time for any reason*, either by simply “unplugging” their face mask themselves or alerting the study team via an emergency alert buzzer.

One study team member, identified before the start of the session, will be responsible for the safe administration of the gas challenge. This will involve verifying pertinent safety screening forms, monitoring of all continuous physiologic recordings during the visit, and terminating the gas challenges when necessary. During practice sessions outside the scanner, they will communicate regularly with the participant. During scan visits, the scan operator will be responsible for communicating regularly with the participant over the intercom system. ***No gas challenge will be initiated without a positive indication from the participant.***

These stopping criteria do not inherently indicate an adverse response. However, if any stopping criteria are met, all activities will be stopped and the participant will be brought out of the scanner and then the study team will assess whether a mild or serious adverse event is in progress.

If stopping criteria are met, if the participant squeezes the alert buzzer, if the study team identifies any adverse responses, or if the participant does not communicate when prompted, the gas challenge and scan will be immediately stopped and the SOP for responding to adverse responses will be followed (see attached document). The adverse response must be documented in the CRF, and serious or unanticipated adverse events must undergo additional reporting to the Independent Medical Monitor, the IRB, and the sponsor.

The MRI scanner will be run by a trained operator on the research team or a member of the technical staff at the Center for Translational Imaging. All researchers involved in the study will undergo the CTI safety training; the PI is responsible for yearly review of any new CTI policies and communicating them to the team, and all other research staff and students must have their training recertified yearly. The scanner performs calculations to prevent running scans that may cause heating (specific absorption rate, SAR) or peripheral nerve stimulation.

RAMR™ Risk management:

We have detailed our risk management strategy for the controlled respiratory challenges in the above section on Study Interventions / Investigational Agents (see the subsection on RAMR™). Here is a summary of our approach:

- One person will operate the RAMR™ while another person operates the MRI scanner, to ensure sufficient focus on both aspects. These two people will be able to communicate with each other at all times.
- Only trained operators may run the RAMR™, and training will be reviewed annually.

- The participant can get the attention of the researchers at any time to discontinue the gas delivery and return to breathing room air.
- The participant can disconnect the face mask at any time to return to breathing room air.
- The face mask and breathing circuit is open to the environment, ensuring sufficient room air is available to breathe if the RAMR™ lines become blocked.
- All gases supplied to the RAMR™ have safe Oxygen content to prevent severe hypoxia.
- Only moderate CO_2 and O_2 levels will be targeted, and exhaled gas content and SpO2 will be continuously monitored for any deviations (see Table 1 on previous page for stopping criteria).
- Participants will practice the respiratory challenge prior to the MRI scan to ensure they are comfortable.
- A detailed SOP for responding safely and effectively to adverse events is attached.
- Supplemental oxygen will be available outside of the MRI scan room for delivery via nasal cannula to a participant who experiences low SpO2 levels that do not normalize upon termination of the gas challenge.
- All subjects will complete a post-scan questionnaire to report their experience of mild side effects of gas challenges. The study will periodically assess the rate of mild anticipated adverse responses to ensure it remains in line with the literature.

Study data

Unidentifiable demographic information (age, sex) and baseline physiological parameters, such as blood pressure, will be recorded. Exhaled gas content will be monitored continuously by the RAMR™ system, and these data are automatically saved in an anonymized format. All MR imaging data will be anonymized and transferred from the scanner to the Northwestern University Research Image Processing System (NURIPS) for secure long-term storage. Physiological and imaging data will be analyzed by the research team using computers (and associated peripheral devices) encrypted by Northwestern University. Communication records, screening and demographic details, and participant information from the CRF, and the debriefing questionnaire data will be entered into REDCap.

Data collection paper forms will be kept in a locked, secure file cabinet with the Clinical Research Study Coordinator at Northwestern University. Electronic data will be secured in a protected study folder stored on a server provided by the medical school; access to the server is regulated and only the PI and study team will have access to the study folder. The link between anonymized study identification numbers and personal identifying information (e.g., name, address, age, and contact details) will be kept separate from the dataset. Only the research study team members that need these details will have access to this linking information, to facilitate check-ins and scheduling of the intervention and MR visits. Data analysis will be completed on Northwestern University computers and computing resources, with rigorous security protections and secure backup regiments.

There are no plans for long-term follow-up of study participants, although participants can be asked if they are interested in participating in future studies (an optional item on the consent form).

DATA AND SPECIMEN BANKING

Upon enrollment into the study, participants will be assigned an anonymous identification code. All MRI and physiologic recording data will only use this anonymous code, and never use the

participant's name or other identifiable information. These physiological data and imaging data will be stored on the FSMResFiles server in a fully anonymized format. Data will be stored in a subdirectory only accessible to members of the research team when connected to the Northwestern VPN.

Imaging data will be archived on the Northwestern University Research Image Processing System (NURIPS) in a fully anonymized format. NURIPS is an XNAT-based data repository for capturing clinical, cognitive, and imaging data on NURIPS. NURIPS' features include: 1) a range of image upload/download methods, including DICOM, FTP, and web interface; 2) incorporation of new data types by automatically generating the necessary databasing components; 3) quality control modules and audit trails; 4) a secure web-based user interface; 5) a sophisticated search engine; 6) an online image viewer; and 7) automatic image processing pipeline support. In particular, data security and integrity features involve the following: data are transmitted over secure channels using SSL and stored on a RAID 5 device with backup; data access is restricted to authorized users with specific privileges (create, delete, read, edit) according to their role in the study.

As described in a later section, when results from this study are published, we will also publish anonymized imaging and physiologic data on an open access platform to facilitate knowledge sharing (e.g., Open Science Framework, Neuroimage Data-In-Brief). Prior to this point, fully anonymized data may be shared with collaborators on an ad-hoc approval basis.

SHARING RESULTS WITH PARTICIPANTS

The images obtained in this study will not be reviewed for diagnostic purposes, and individuals will not be informed of any results coming specifically from their scan. However, we will inform participants that amalgamated results from the ongoing methods development work will be announced on the lab website: http://www.brightlab.northwestern.edu

In addition, we will follow the CTI standard procedures for dealing with any incidental findings in the MRI scans: if a researcher or their students or staff should notice something that is potentially abnormal in the images, this information will be forwarded to a CTI Tech. The Tech will log the incident in the event log book, which will show the date observed, the study name, the radiologist reviewing the images, and the final outcome and recommendations. The subject will not be informed about the abnormality at the time of scanning in order to eliminate any risk of anxiety for no reason. The CTI staff will have a radiologist review the research images. Information about the subject may be required in order for the radiologist to make an informal reading. This exchange will be facilitated by the Techs. If the suspected abnormality is normal there is nothing else to do. If the finding requires a follow-up, the findings will be reported back to the PI. The PI will either contact the participant or the participant's physician, depending on what the participant desires. An incidental finding letter will be mailed to the participant.

Sequence of events:

1. Images are taken
2. The researcher or their staff, CTI technologist or other Radiology personnel may notice a potential abnormality without disclosing to the subject.
3. Technologist records the incident in the event log book.

4. The de-identified images are forwarded to an appropriate Radiologist for review.
5. A determination is made by the Radiologist, either medically insignificant or medically significant.
6. If the finding is medically significant an incidental letter will be mailed to the subject.

If an incidental finding is observed, the clinical co-investigator on the study team (Dr. Farzaneh Sorond) will make the determination whether the finding should exclude the participant from being a healthy participant. If this is the case, the participant will be removed from the study and paid for their participation in full. It may also occur that the finding would not interact with the study and research question at hand, and the participant will be given the option of continuing their participation.

STU#: STU00215559

STUDY TIMELINES

We conservatively estimate that we will recruit one participant every 4 weeks in the start of the study until the first participants begin the second intervention arm, and then one participant every 3 weeks. Participant dropout is most likely to occur at the time of Intervention B; a new participant will then be scheduled to replace this subject. A timeline for study recruitment and both interventions is provided in **Fig. 5.**

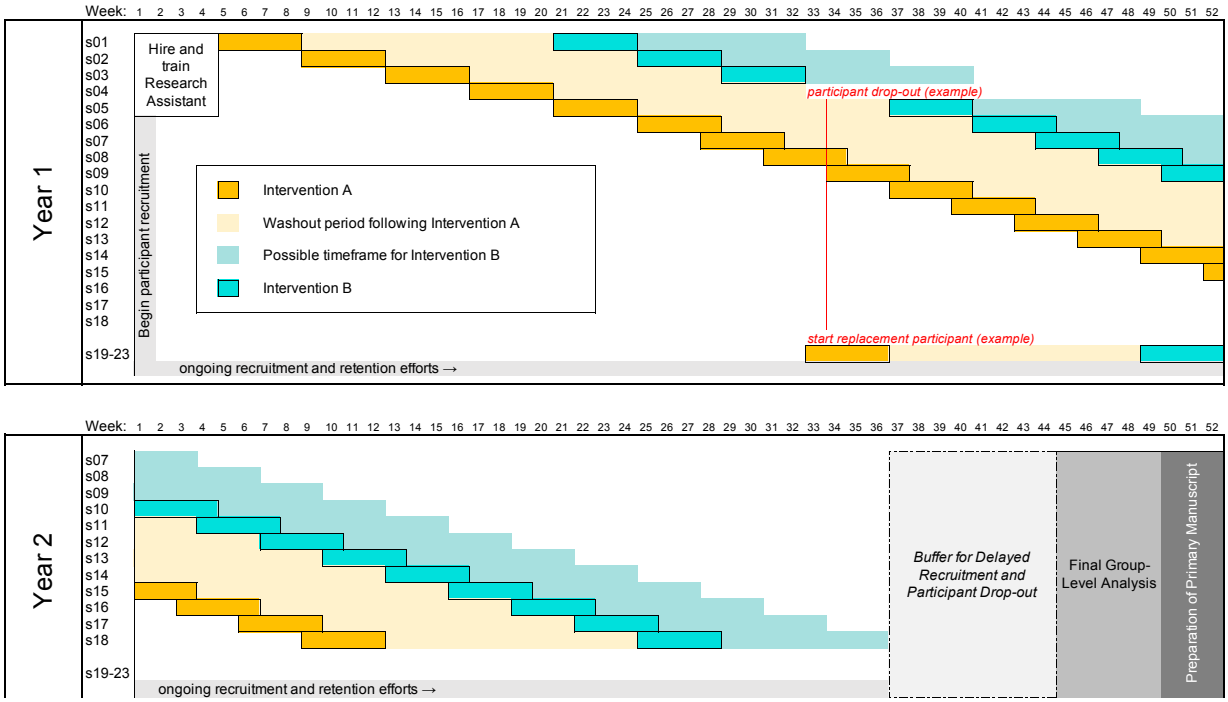


Figure 5 Overall study timeline

Details of the Acute Intermittent Hypoxia and sham intervention timelines are provided in **Fig. 6**. We attempt to balance the need for flexibility in working with each participant's own time restrictions and preferences with the need for fair scientific comparisons between the two intervention arms. Because the sham intervention is not anticipated to evoke vascular plasticity, we reduce the burden on the participant by requiring only 2 visits per week. (Note, they will be told the intervention is a different dose of intermittent hypoxia, rather than mimicking the true intervention.) The Acute Intermittent Hypoxia intervention will require 4 visits per week. All intervention visits can be flexibly scheduled for mornings, evenings, weekdays, or weekends to maximize participant retention and successful completion of all visits in the allotted time. The MRI (PRE) scan will occur any day in the week preceding the first intervention visit. The MRI (POST) scan must occur one day following the last intervention visit, or on the subsequent Monday if one day following would fall on a weekend and no technologist is available. However, all effort will be made to perform the POST scan 1-day following the last intervention visit. Note, **Fig. 6** shows an *example schedule* for a hypothetical participant.

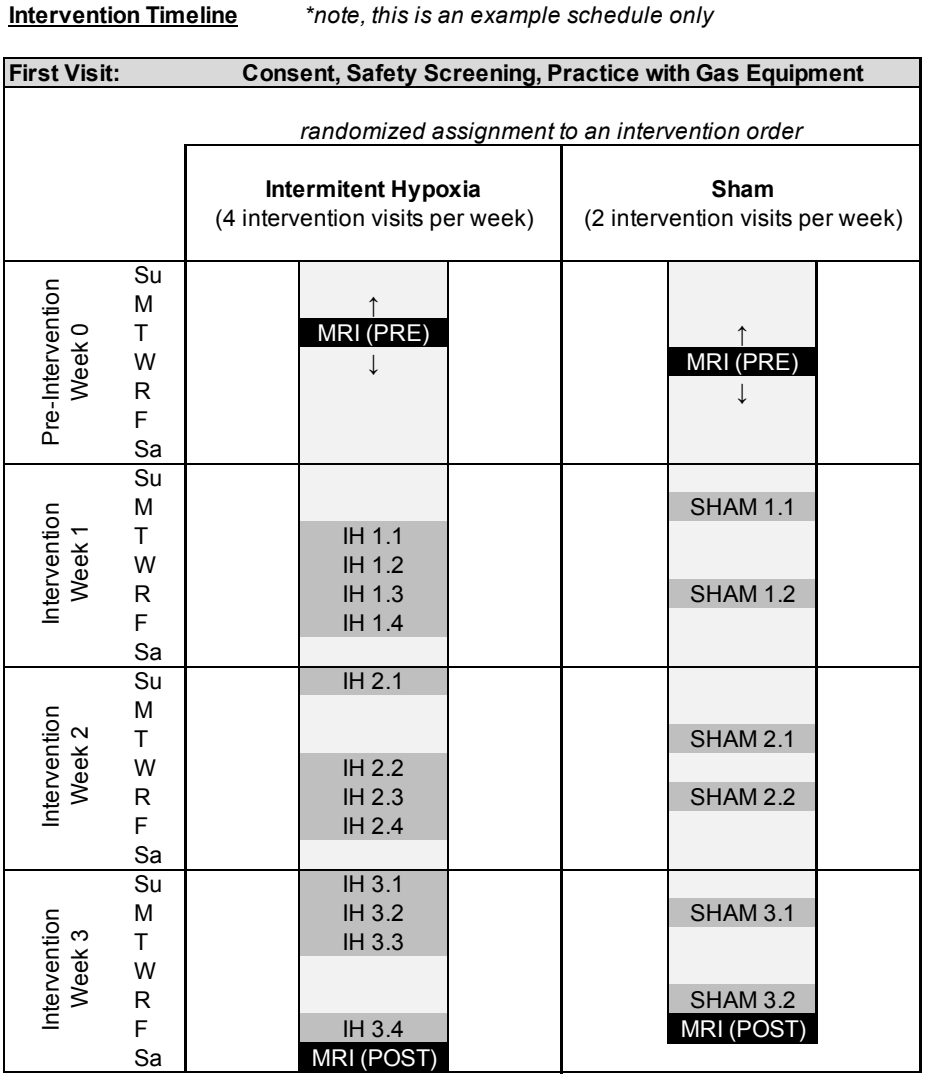


Figure 6. Example schedule for a hypothetical participant.

In total, we are asking participants to volunteer a total of approximately 22.5 hours of their time (exclusive of transportation) over a 5- to 7-month period (**Fig. 7**).

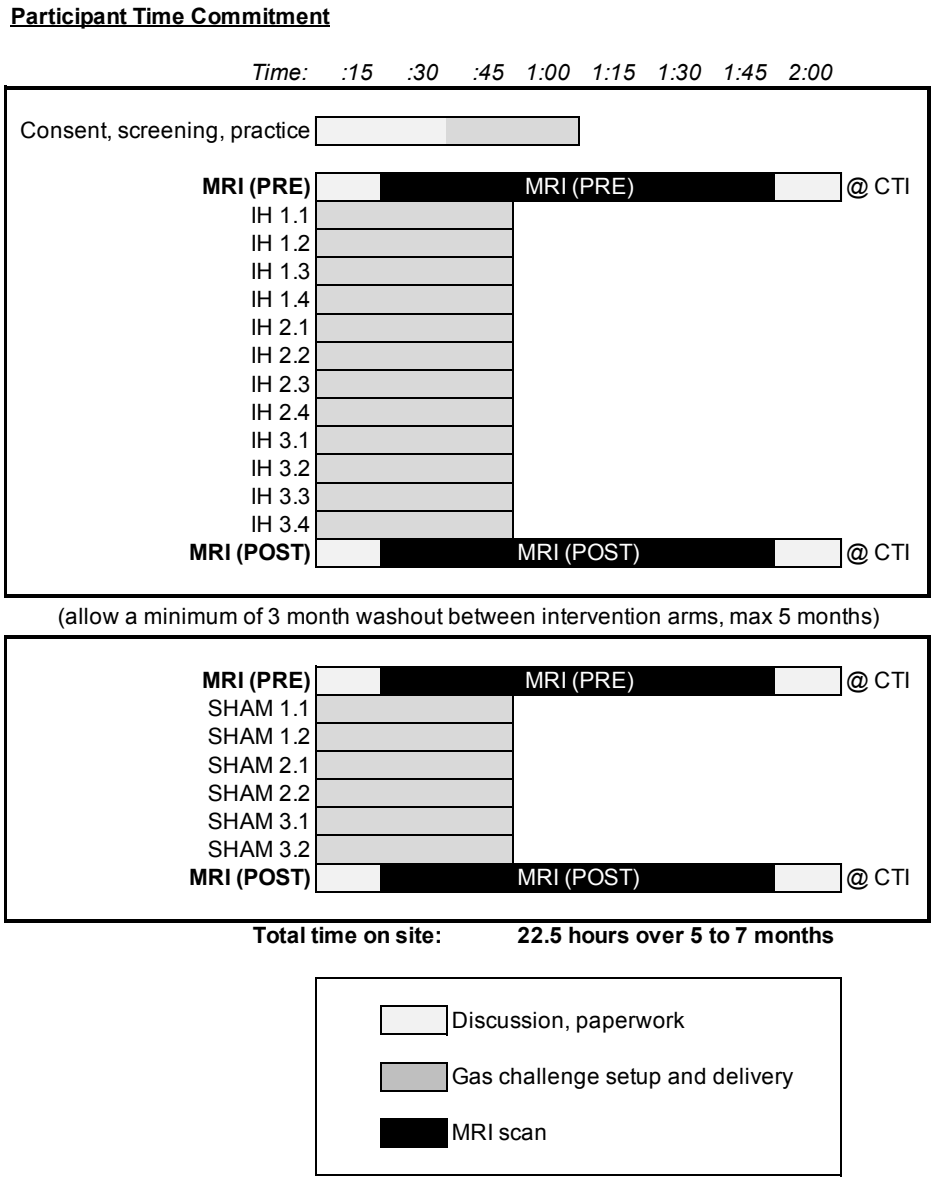


Figure 7. Summary of the time commitment of an individual participant.

INCLUSION AND EXCLUSION CRITERIA

Healthy adults will be verbally screened during the consent process to determine if they meet the inclusion criteria and whether they have any of the exclusion criteria. A screening form, created by the PI and clinical co-investigator, will be used to ensure the participant is appropriately meeting these criteria and is safe to be enrolled in the study.

Inclusion criteria:

- Healthy volunteers between ages of 21 and 50 years
- No known neurological, respiratory or vascular conditions*
- Safe to be scanned using MRI
- Able to communicate in English

Exclusion criteria:

- MRI contraindications as indicated on MRI safety screening form
- Subjects with pacemakers, cochlear (in the ear) implants, or aneurysm clips or subjects who have worked with metal
- Pregnant women*
- Individuals with known neurological or vascular conditions
- Individuals with sleep apnea, emphysema, or other respiratory conditions**
- Individuals with severe claustrophobia
- Subjects unwilling or unable to give written informed consent in English
- Prisoners
- Frequent smoker
- Allergy to Tegaderm
- Blood pressure greater than 140/90 or less than 90/55.
- Individuals who report headaches, feeling dizzy or light-headed, or have heart palpitations on the day of the study.
- Individuals prescribed Aripiprazole and/or Lamotrigine***

* Women who might be pregnant, or women whose last menstrual period was >28 days ago, will be provided with a urine-based pregnancy test during screening.
**Note, mild (well-controlled) asthma is not immediate grounds for exclusion.
***Other prescription medications may be grounds for exclusion; prescription medication information will be acquired during initial screening and at subsequent follow-up visits. These will be reviewed by a clinical co-investigator (Dr. Farzaneh Sorond or other qualified physician) and eligibility determined.

PARTICIPANT POPULATION(S)

Accrual Number:	Category/Group: (Adults/Children Special/Vulnerable Populations)	Consented: Maximum Number to be Consented or Reviewed/Collected/Screened	Enrolled: Number to Complete the Study or Needed to Address the Research Question
Local	Adults	40	18
Study-wide		40	18

Total:		40	18

RECRUITMENT METHODS

Healthy volunteers will be recruited from the local populations of Chicago, Illinois by placing informational posters around the Chicago community (and surrounding suburbs). The poster will also be included on Northwestern University related websites. Social media, email, and messaging platform (e.g. Slack) announcements may also be used in short form, to direct interested readers to the full poster online. Email may be sent to Northwestern University email lists, following approval from the list manager, including the research lists of the Department of Physical Therapy and Human Movement Sciences and the Northwestern University Interdepartmental Neuroscience of which the PI is a member. The study will be listed on pertinent web-based recruitment portals. In all cases, potential participants will be invited to email the lab for further details about the study.

Initial pre-screening may occur via phone or email to confirm that the basic inclusion criteria are met. Participants will be asked for their birthdate to confirm that age criteria are met. This will be followed by a first visit to the Center for Translational Imaging to complete informed consent and safety screening procedures. This visit will also involve a practice session with the gas delivery systems used in the study, as described in greater detail above.

No subjects under the age of 21 years of age, regular smokers, or pregnant women will be included. There will be no exclusion of subjects based solely on race or gender.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES

Participants will receive $45 per MRI visit and $25 per intervention visit as compensation for their time invested in the study and any travel expenses (including discounted parking). However, not all payments will take place immediately in full. The initial screening and practice visit will be compensated with $20 immediately. Following enrolment in the study, 50% of accrued payment will be supplied at the end of the first arm of the crossover trial or final date of participation (up to $195), 50% of subsequent accrued payment will be supplied at the end of the second arm or final date of participation, with the remaining balance supplied if all study visits are achieved (combined total up to $630). Payments are an established way to successfully improve retention in clinical trials; our approach additionally encourages participants to complete both arms of this crossover trial.

Following this schedule, participants will receive payment up to 3 times: the screening and practice visit, the end of the first arm of the cross-over trial (or date of withdrawal, whichever comes first) and the end of the second arm of the cross-over trial (or date of withdrawal, whichever comes first). If a participant withdraws from the study prior to completion of the study, compensation is approximately 50% of what it would be if they completed all elements of the study.

Payments will be made by check, following Northwestern’s Accounts Payable procedures; the participant’s name, address, and social security number will be requested and payment will be considered taxable income.

We will inform participants that amalgamated results from the ongoing methods development work will be announced on a rolling basis on the lab website:
http://www.brightlab.northwestern.edu

WITHDRAWAL OF PARTICIPANTS

Participants may withdraw at any time for any reason. Reasons could include feeling uncomfortable in the MRI scanner, feeling uncomfortable or responding outside of allowable limits during the acute intermittent hypoxia or the sustained respiratory challenges, or some combination of these factors. The participant will still be asked to complete any pertinent debriefing questionnaire, and the reason for withdrawal (if volunteered) will be recorded.

A participant may be withdrawn from the study due to technical difficulties in achieving high-quality imaging data; in these circumstances, if respiratory challenges were not performed, the debriefing questionnaire will not be administered.

Following this, no further data will be collected, but any data previously collected may still be used in analysis.

RISKS TO PARTICIPANTS

MRI

This project uses some advanced imaging techniques that are not currently a part of standard clinical care. Thus, the “software program” being run by the scanner will not be identical to the “programs” being run in standard clinical scans. However, all scans will operate within the safety guidelines set by Siemens as approved by the FDA. (In this operating mode, the scanner will not allow unsafe sequences to run.) No contrast dyes will be used in this study.

An MR safety questionnaire will be given to all participants and reviewed by study personnel prior to the scanning procedure to identify any conditions that may pose a risk. Subjects at risk for injury from MR scans are those with pacemakers, aneurysm clips or shrapnel fragments. The questionnaire given to all participants prior to the scan will be used to identify these risk factors.

If the participant undergoes safety screening and is deemed safe to be scanned, the general risks of having an MRI are very small. If the patient notices any heating or discomfort, the technologist will stop the examination. There is a small chance that patients may feel claustrophobic or anxious. If this is the case, again, the technologist may stop the examination. It is not known how MRI directly affects an embryo or fetus. Therefore, women who are pregnant or may be pregnant will be excluded from this study. Communication is maintained with the subjects throughout the MR scanning procedure and a subject may choose to stop the research scan at any time.

Respiratory Challenges during MRI

There are several potential risks involved with the delivery of gas mixtures to humans. We have thoroughly discussed these risks thoroughly in the earlier section “Study intervention/investigational agent” (Pages 7–11), highlighting how these risks are minimized, and summarize this discussion here.

Risk: moderate to severe hypoxia.

Severe hypoxia can have damaging effects on brain function and systemic metabolism.

> Control: All source gases connected to the RAMR™ contain O_2 levels that ensure a safe minimum level of oxygen is always delivered.
> Control: In this protocol we **do not target any $P_{ET}O_2$ levels below 55 mmHg**. This degree of hypoxia occurs naturally when people are resting at high altitudes (~5km)[39].
> Control: SpO2 is monitored continuously via finger clip, and hypoxic gas challenges will be modulated or terminated **to prevent SpO2 from dropping below 85%.**
> Control: Experimenters will communicate regularly with the participant during the practice session to identify adverse responses to hypoxia challenges, and will discuss these symptoms with the participant prior to the scan session to ensure they are aware of these possible adverse responses and able to end the experiment if they experience any of them.
> Control: Supplemental oxygen will be available outside of the MRI scan room, to be delivered to participants when SpO2 drops below 85% (stopping criterion) and does not normalize rapidly after the gas challenge is terminated.

Risk: moderate to severe hypercapnia

Extreme, severe hypercapnia, over the level of about 80 mmHg, may cause drowsiness or even loss of consciousness. However, even hypercapnia even 2-3 times greater than this amount is not known to cause persistent harm if not accompanied by low oxygen. Hypercapnia can cause transient discomfort such as feeling of shortness of breath, elevated heart rate, elevated blood pressure, and panic. Returning to normal CO_2 levels resolves these side effects almost instantaneously.

> Control: In this protocol, we **do not target any CO_2 values above +6 mmHg over the individual’s baseline $P_{ET}CO_2$ level or 54 mmHg**, whichever is lower. For reference, a +10 mmHg increase in $P_{ET}CO_2$ is approximately the effect of a 20-second breath hold[41], or playing a wind instrument. A $P_{ET}CO_2$ of 54 mmHg occurs in divers when exercising at depth[42], and in most people during sleep.
> Control: If $P_{ET}CO_2$ exceeds 70mmHg for 10 seconds (~2-4 breaths), the system will alarm and stop delivering the gas mixture. The participant will breathe room air.

Risk: insufficient air delivery (blockage in the lines) or high pressure gas delivery (malfunction of cylinder/regulator)

> Control: The face mask of the RAMR™ is open to the environment, and thus cannot reach pressures above atmospheric pressure. If insufficient gas is delivered to the mask, the participant can breathe normally from room air.

Risk: high pressure gas delivery to face mask (malfunction of cylinder or regulator)

> Control: The RAMR™ is open to the environment, and thus cannot reach pressures greater than atmospheric pressure.

Risk: claustrophobia or anxiety associated with wearing face mask in the MRI scan environment

> Control: Participants are able to experience the "mock scanner" environment at CTI while wearing the RAMR™ face mask to determine if they experience claustrophobia associated with wearing a mask in the enclosed space.
> Control: Participants are able to immediately terminate the session at any time, for any reason, including anxiety or claustrophobia. This may be achieved by simply "unplugging" the gas tubing from their face mask or by alerting the research time via the squeeze alert. The participant will practice the unplugging action outside of the scanner.
> Control: The study team will record participant experience of claustrophobia and anxiety, as well as anticipated mild side effects of gases, in the debriefing questionnaire to monitor the frequency of these occurrences and adjust the protocol as needed.

We will explain the potential for transient side-effects due to the respiratory challenge. The participant will be able to practice the respiratory challenge and make adjustments to ensure they are comfortable prior to any MRI scanning. The participant may stop the respiratory challenge at any time, for any reason. They can take off or disconnect their mask or alert the investigator to turn off the gas, resulting in breathing room air. We will also detail the risk of rare serious adverse responses to gas challenges during the consent process and immediately prior to practice and MRI sessions to ensure ongoing informed consent and help the participant identify key symptoms that indicate immediate cessation of a challenge.

Finally, an SOP detailing the response of the study team to mild and more serious adverse responses to gas challenges during MRI scanning is attached to this protocol. All study team members will be familiarized with the SOP, it will be posted on the RAMR system for ready reference, and the PI and clinical co-investigator will periodically direct a refresher training on all study procedures pertaining to participant safety.

Acute Intermittent Hypoxia

Hypoxia-associated reduction in inspired oxygen can result in lightheaded sensation, dizziness, reduced vision, or euphoria. The experiment will be stopped immediately if participants experience any of these symptoms, and available nursing staff or physician on call will be notified. Initial screening will include questions regarding cardiopulmonary stability to exclude participants with increased risk of these side effects. We will also monitor heart rate, blood pressure, and oxygen saturation levels to ensure participant safety throughout the intervention. If subject blood pressure increases by more 15 mmHg from the beginning of their participation in the study, they will be withdrawn from the study. If a subject's SpO2 drops below 75%, the intervention will be terminated early. If this occurs more than 2 consecutive intervention visits, or more than 3 non-consecutive visits, they will be withdrawn from the study. A summary of criterion for stopping and participant withdrawal is provided in the table below:

Table 2 AIH intervention monitoring and stopping criteria

Intervention Visit Criterion	Response
Systolic BP > +15 mmHg above initial value (practice session)	Participant withdrawn from study*
Systolic BP < 90 mmHg	Participant withdrawn from study*
SpO2 < 75% (first instance)	Immediate termination of AIH intervention.
SpO2 < 75% (second instance, the visit immediately after the first)	Immediate termination of AIH intervention. Participant withdrawn from study.
End-tidal O2 SpO2 < 75% (second instance, *NOT* the visit immediately after the first)	Immediate termination of AIH intervention.
SpO2 < 75% (third instance)	Immediate termination of AIH intervention. Participant withdrawn from study.

* Note, if BP is elevated >15 mmHg, the study team should repeat the measure after 5 minutes to confirm this stopping criterion is met.

POTENTIAL BENEFITS TO PARTICIPANTS

There is no direct benefit to individuals who participate in this study. We are testing whether the intervention can beneficially alter resting brain blood flow and cerebrovascular reactivity, but this hypothesis is not yet empirically supported.

DATA MANAGEMENT AND CONFIDENTIALITY

This study is a randomized sham-controlled crossover study with a long washout period, using MRI to assess the impact of a daily acute intermittent hypoxia (AIH) intervention on brain physiology. The primary and secondary study outcome measures consist of whole-brain measures of perfusion (CBF) and cerebrovascular reactivity (CVR) acquired using phase-contrast MRI at PRE and POST intervention timepoints, where the intervention is either AIH or Sham. We also collect *maps* of these parameters using complementary MRI techniques in order to characterize regional patterns in the cerebrovascular response to the intervention.

Linear mixed-effects models will be used to assess whether AIH significantly impacts cerebral perfusion (CBF, Aim 1) or cerebrovascular reactivity (CVR, Aim 2) more than Sham. In this model, subjects are considered random effects, intervention (AIH or Sham) and timepoint (PRE

or POST) as within-subject fixed effects, and sex as a between-subject fixed effect. With this approach, whole-brain metrics and imaging maps can be modeled in the same manner, partial data may be included and additional continuous covariates (e.g., age, BMI, blood pressure) can be explored to guide future research.

Sample size and power: Working with Dr. Kwang-Youn Kim, a biostatistician with the Biostatistics Collaboration Center, we performed a simplified and conservative power calculation outlined below using paired *t*-tests and excluding partial data. Because participants will need to be active for a 5-month period to complete the crossover design, we estimate 20% participant dropout and have adjusted the number of subjects recruited into the trial accordingly.

Perfusion decreases ~5% per decade with healthy aging and ~7% in early stages of neuropathology[30,44,45], indicating that perfusion changes of this magnitude would be clinically meaningful. We have therefore completed a power calculation to estimate the sample size needed to detect a 6% change in perfusion after AIH. Extended exercise interventions are known to evoke changes in perfusion 2–3 times this[46], and Aim 1 tests whether a 3-week AIH intervention can have a similar, albeit smaller, beneficial effect.

The 3T phase-contrast MRI method for estimating whole-brain perfusion has an intersession within-subject coefficient of variation of 4%[47]. Covariance between measurements is unknown, but likely positive; as such, we conservatively assume covariance = 0. Using a two-sided paired *t*-test achieves 80% power to infer that the mean ratio is different from a ratio of 1 when n = 18, the significance level is 0.05, the ratio of resting cerebral perfusion change is 6% greater in the AIH intervention group, and the coefficient of variation is 6%. To account for dropout, we will recruit up to 40 patients.

In a small pilot study of individuals with mild cognitive impairment, a similar multi-week AIH intervention caused a 21% increase in CVR (as measured using Transcranial Doppler ultrasonography), which was associated with an improvement in cognition[36]. Although we are not studying cognitive changes in this clinical trial, CVR changes of this approximate magnitude would be deemed clinically meaningful.

Long-term test–retest reliability data are readily not available for 3T phase-contrast MRI CVR using sustained hypercapnic challenges. Spencer and colleagues calculated the 6-month within-subject coefficient of variation of whole-brain CVR equal to 12.1%, using TCD and a sustained +5mmHg hypercapnia stimulus[48]. Phase-contrast CVR measures are more repeatable than TCD CVR measures[49], and thus we will use the TCD value as a conservative estimate for our own work. As above, we assume covariance = 0. Using a two-sided paired *t*-test achieves 80% power to infer that the mean ratio is different from a ratio of 1 when n = 18, the significance level is 0.05, the ratio of cerebrovascular reactivity is 18.4% greater in the AIH intervention group, and the coefficient of variation is 17.1%. We are therefore appropriately powered to infer CVR changes of the magnitude predicted by pilot studies described above.

We will also analyze the impact of the AIH intervention on *maps* of these parameters acquired using Arterial Spin Labeling and BOLD fMRI imaging methods in order to identify regional responses in brain physiology. We will employ a linear mixed-effects modeling approach optimized for neuroimaging data (AFNI software package, 3dLMEr function), noting that we will assess empirically if data should be first log-normalized or otherwise transformed. Voxelwise outlier removal is incorporated into this procedure: any voxels of a given dataset with CBF or CVR values outside of physiological bounds will be treated as missing. *F*-statistics for main effects and interactions will be calculated for every voxel in the brain, and significant clusters identified and corrected for family-wise error. This approach allows for missing data, thus all

data acquired can contribute to the statistical analysis despite participant drop-out or regional data quality issues with select MRI scans (a known concern with Arterial Spin Labeling imaging). This modeling approach will be expanded to further consider age, BMI, and blood pressure as exploratory covariates.

Analyses will take place on local desktop and laptop computers, following all encryption and security guidelines of the Northwestern University Feinberg School of Medicine. No data will contain any identifiable information at any time. An encrypted file linking participant identification numbers and names will be stored on FSMResFiles with access limited to the study team.

Electronic study data will be stored on the FSMResFiles server in a fully anonymized format. Data will be stored in a subdirectory only accessible to members of the research team when connected to the Northwestern VPN.

Imaging data will be stored on the Northwestern University Research Image Processing System (NURIPS) in a fully anonymized format. NURIPS is an XNAT-based data repository for capturing clinical, cognitive, and imaging data on NURIPS. NURIPS' features include: 1) a range of image upload/download methods, including DICOM, FTP, and web interface; 2) incorporation of new data types by automatically generating the necessary databasing components; 3) quality control modules and audit trails; 4) a secure web-based user interface; 5) a sophisticated search engine; 6) an online image viewer; and 7) automatic image processing pipeline support. In particular, data security and integrity features involve the following: data are transmitted over secure channels using SSL and stored on a RAID 5 device with backup; data access is restricted to authorized users with specific privileges (create, delete, read, edit) according to their role in the study. We will utilize the XNAT system's protocol-based data security features for data sharing with the research team members. At each time point, imaging information will be uploaded into the repository. The acquired image data will be sent directly from the scanner at CTI to the NURIPS DICOM receiver. The study team will be responsible for scan transfers and for entering information using built-in web-based forms.

Quality control features include: upon uploading to the system, imaging sequence details are validated against a study-specific protocol to ensure acquisition compliance; noncompliant scans are flagged for immediate follow-up; automated routines are executed to determine overall image quality; for fMRI, for example, signal to noise and movement histograms are generated. NURIPS also supports optional web-based radiological evaluation and manual quality assessments.

Study Documents

Study documents will all be stored securely in locked cabinets within a Northwestern University building in the Feinberg School of Medicine (Physical Therapy of Human Movement Sciences). Documents will be transported from CTI or the ABC lab to this Feinberg building by one of the research team after an MRI or intervention session. Only study team members will have access to the location where the documents are stored. The CRF will define each participant's study identifier (e.g. C01), which will be used in all MRI and physiological recording data to maintain anonymity in those files. Communication logs, screening and demographic information, and study data from the CRF and debriefing questionnaires will be input to REDCap for secure storage. Study documents (e.g. CRF, consent forms, payment forms, screening forms) may also

be scanned and uploaded to Northwestern Study Tracker, a secure, HIPAA-compliant research tool that can only be accessed by members of the study team.

Unidentified data from this study may be shared with the wider scientific community; participants will be specifically informed of this during the consenting process.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS

Overall framework for safety monitoring and what information will be monitored

Data and safety monitoring will be overseen by the project PI and physician Co-Investigator (Dr. Sorond), as well as a designated Independent Medical Monitor (as per NINDS instructions).

The independent medical monitor (IMM) will be a qualified physician appointed by the PI to oversee this clinical trial. They are independent of the study team and clinical trial, with no conflict of interest, although they may be employed by NU/NM. The IMM will be approved by the NINDS program official and operate in a manner similar to that of a SMC or DSMB.

The IMM will meet with the PI, Dr. Sorond, and other study team members at least every 6 months during this 2-year study. At each meeting, the IMM will review the research protocol and ongoing study activities with an emphasis on data integrity, protocol adherence, and study participant safety issues. The IMM will compile a review focused on any adverse events and participant drop-out, and raise any concerns or issues with the PI and NINDS program official, recommending either continuation, modification, or conclusion of the trial. The IMM will protect the confidentiality of the trial data and results of this monitoring.

In addition to these regular official IMM reviews, all reportable new safety information will be reported by the PI to the IRB via the online eIRB portal within 5 days per University and IRB policy, and to the NIH funding institute. (Note, all Serious Adverse Events will be reported within 24 hours.) This information includes, but is not limited to: new or increased risk, unexpected adverse events, serious adverse events, non-compliance with IRB policy, researcher error/protocol violation, and unresolved participant complaint.

Data related to participant safety will be recorded at all study visits. Continuous physiological monitoring data during MRI scanning (SpO_2, pulse, breathing) will be recorded in an anonymous manner. All MR imaging data will be anonymized and transferred to NURIPS for secure long-term storage, and also stored in a secure network drive operated by the Feinberg School of Medicine with access limited to the relevant study team members. Physiological and imaging data will be analyzed by the research team using computers (and associated peripheral devices) encrypted by Northwestern University.

Furthermore:

- Participant blood pressure will be monitored throughout the study, at each visit.
- During all hypoxic interventions, SpO_2 recordings will be monitored and recorded using the Nonin Model 7500 Pulse Oximeter,, with clear stopping criteria (Table 1 and Table 2).
- A questionnaire will be administered at every hypoxic intervention to assess any side effects experienced by the participant.
- Prescription medications and medical diagnoses will be collected at each study visit and changes will be reviewed by the clinical co-investigator.

- End-tidal O_2 and end-tidal CO_2 as well as pulse and breathing rate will be continuously recorded during all gas inhalation challenges during MRI, with clear stopping criteria (Table 1).
- Structural and diffusion weighted scans will be acquired at every MRI scan session.
- A debriefing questionnaire will be administered after every MRI session involving a gas inhalation challenge, to ascertain participant discomfort and any other experience of the gases and gas delivery protocols.

All research materials will be held in strictest confidence until the study is completed, after which all subject-sensitive information will be destroyed. Results of this study may be used for publications or presentations at scientific meetings. If individual results are discussed, their identity will be protected by using a study code rather than names or other identifying information

Frequency of monitoring, plans for interim analysis and stopping rules

Monitoring will occur in real-time through the discrete recording of blood pressure readings at each visit and continuous recording of physiologic data (SpO_2, end-tidal gas levels) during all gas inhalation challenges. If any of the following are observed during a gas challenge, the challenge will be immediately stopped and room air supplied to the participant:

- SpO_2 below 75% (Intervention visit) or below 85% (MRI visit)
- End-tidal O_2 below 52 mmHg
- End-tidal CO_2 above 54 mmHg

All of these factors are expected to resolve immediately after cessation of the gas challenge. The study team can work with the participant to resume the gas challenge with modifications, or they or the participant can choose to end the session. Due to the low risk of this healthy control study, we do not have stopping criteria for the overall study. However, increases in blood pressure more than 15 mmHg (systolic), or decreases below the level of 90 mmHg (systolic) will result in the withdrawal of an individual from interventions. Furthermore, if the participant has an SpO2 below 75% during an intervention visit (requiring early termination of that intervention visit) more than 2 consecutive or more than 3 non-consecutive instances, they will be removed from the study.

Participant debriefing questionnaires administered during all scan and intervention sessions will be reviewed immediately by the study team and entered into the REDCap database. Paper Case Report Forms, including description of any protocol deviations, pauses or early termination of scan sessions, and any adverse events, will be scanned and uploaded to a secure encrypted network cloud storage maintained by the Northwestern University Feinberg School of Medicine. The study team will discuss debriefing questionnaires and Case Report Form data at a monthly meeting to determine if any problematic patterns in participant discomfort or monitoring data are apparent, and discuss their mitigation. The PI will regularly review individual participant records with the Clinical Research Coordinator to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records.

We will follow the standard procedures adopted by the Center for Translational Imaging for dealing with any incidental findings in the MRI scans as described earlier.

Management and reporting of adverse events and serious adverse events

Upon first knowledge of an adverse event or serious adverse event (including any reportable new information), the study personnel will report to the PI and physician Co-Investigator Dr. Sorond. After rapid review by the study team, the PI will generate and submit the IRB report via the Northwestern University eIRB portal within 5 days (24 hours for Serious Adverse Events) per University and IRB policy. The PI will report all unanticipated adverse and serious adverse events to the appropriate NIH funding institute within the same time frame. The entire study team will meet monthly to discuss study progress, and every 6 months with the IMM, who will review and report on all study safety data.

Individual responsible for trial monitoring

Overall trial monitoring will be the responsibility of the PI, who will share all data and findings with the IMM and NIH officials as appropriate. The PI will utilize the research assistant and research coordinator on the study team to generate the safety and data monitoring reports.

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS

If any participant expresses desire to not participate or learn about the study, we will respect their rights and cease the information or consent process immediately after thanking them for their time. Throughout the consent process and study participation, the participants will be encouraged to ask questions, and we will clearly inform them that participation can be ended at any time and for any reason. Participants will have one primary contact person on the study team, and they will also have the ability to speak with the Principal Investigator if at any time they are uncomfortable or need further information about the study. Unless necessary (as deemed by the PI), only the one main contact person on the study team will have the relevant contact information for the participant.

COMPENSATION FOR RESEARCH-RELATED INJURY

If subjects become ill or are injured as a result of this study (medications, devices or procedures), they will be told to seek medical treatment through their doctor or treatment center of choice. We will ask all subjects to promptly tell the study doctor about any illness or injury during their enrollment.

We will not pay for medical care required because of a bad outcome resulting from participation in this research study. However, this does not keep participants from seeking to be paid back for care required because of a bad outcome

ECONOMIC BURDEN TO PARTICIPANTS

There is no direct cost to the participant for participating in this study. The participant may incur indirect costs related to visiting Northwestern Campus. However, we minimize these costs by offering compensation for participating in the study that should exceed reasonable transportation costs.

CONSENT PROCESS

Consent will take place at the beginning of the first study visit (practice session) at the Center for Translational Imaging, or at a precursor in-person meeting at the (PI's laboratory in the Department of Physical Therapy and Human Movement Sciences (645 N. Michigan Ave., Chicago, IL 60611). The English language will be used by those obtaining consent and understood by the prospective participant. Participants may be informed of the research study on an occasion prior to signing the consent form. If more than a day lapses between first explaining the study to the potential participant and signing the consent document, all elements of the study consent form will again be reviewed with the patient just prior to signing the consent. The informed consent process will always be carried out in person, including for those patients who are initially contacted about this study via mailed recruitment letter and consent form.

The procedures and policies of "SOP: Informed Consent Process for Research (HRP 090)" will be followed during the consent process. Accordingly, the most recently approved version of the consent form will be used, a copy of the consent form will be provided to the potential participant, and the potential participant will be given as much time as they need to review the form independently. The individual obtaining consent will then review all contents of the consent form with the potential participant and if the participant prefers, any family or friends that they would like to be involved. Time will be given to the participant (and if applicable, friends/family) to ask any questions and clarification will be given by the individual obtaining consent.

Consent will be documented per the procedures of "SOP: Written Documentation of Consent (HRP 091)." As stated in this SOP, once all study information has been reviewed with the participant and all questions have been answered, the participant will print and sign their name, as well as date the consent form. The individual performing consent will then do the same. A copy of the signed consent form will be provided to the participant for their records.

Throughout the scan session, communication between the participant and the researcher will be maintained to ensure ongoing consent.

NON-ENGLISH SPEAKING PARTICIPANTS

As part of our inclusion criteria, this study will only recruit participants who are conversant in English.

QUALIFICATIONS TO CONDUCT RESEARCH AND RESOURCES AVAILABLE

Data collection, MR scanning, and analysis will be performed on-site at Northwestern University. All MRI scans will be performed on a 3.0 T scanner (Siemens Medical Systems, Erlangen, Germany) located in the Center for Translational Imaging (CTI) in the Olson Pavilion at Northwestern Memorial Hospital on the Chicago campus. Technologists at CTI are available to assist with operating the MRI scanner if needed. Intervention visits will occur at the "ABC" lab of Dr. Farzaneh Sorond located within NMH (7-240). The Hyp-123 and RAMR™ devices to deliver the AIH and Sham intervention and the sustained gas challenges during MRI scanning are already owned and used by the Bright lab. The study team includes Max Wang, who is a

licensed Emergency Medical Technician hired to help administer the AIH intervention and further support the MRI scan visits.

Personnel

Molly Bright, SB, DPhil, Principal Investigator, is an Assistant Professor of Biomedical Engineering and Physical Therapy and Human Movement Science. She has extensive experience in using MRI to measure human cerebrovascular function, and specific expertise in administering gas challenges during scanning to quantify cerebrovascular reactivity. She is also currently collaborating (as study PI) with researchers at the Shirley Ryan AbilityLab (SRAL) on a project funded by the Craig H. Neilsen Foundation, applying neurovascular MRI to understand how Acute Intermittent Hypoxia induces motor plasticity in spinal cord injury. The intervention protocol used in the proposed project mimics the protocol she is using in this spinal cord injury study and also being used in several ongoing clinical trials at SRAL. She has >10 years of experience working with gas inhalation challenges during MRI, including using the RAMR™ system. Dr. Bright will be responsible for study design, training and managing the research assistant, monitoring data acquisition and analysis, overseeing safety monitoring, and leading the interpretation and dissemination of study results.

Farzaneh Sorond, MD PhD, Co-Investigator, will guide the safe execution of this clinical trial, overseeing screening criteria and safety monitoring data. Dr. Sorond is the Chief of Stroke and Neurocritical Care in the Feinberg School of Medicine Department of Neurology at Northwestern, and is an established vascular neurologist with expertise and research experience in vascular aging, small vessel disease, and cerebrovascular imaging. Study intervention visits will be held in Dr. Sorond's laboratory in Northwestern Memorial Hospital, and she will be on call to rapidly respond to any adverse events.

Todd Parrish, PhD, Co-Investigator, will contribute his 30+ years of experience in MRI neuroimaging to the design, optimization, quality control, analysis, and interpretation of the imaging data in this study. As the Director of Neuroimaging research at Northwestern University, Dr. Parrish further specializes in characterizing brain physiology, including the use of respiratory challenges and quantitative blood flow mapping as included in this study.

Yufen Chen, PhD, Co-Investigator, will provide her technical expertise in implementing and analyzing arterial spin labeling (ASL) MRI sequences to facilitate the quantitative perfusion mapping central to this study. Dr. Chen is a Research Assistant Professor in Radiology and also the research manager of the Neuroimaging facility at the Center for Translational Imaging at Northwestern University, and will be responsible for maximizing perfusion MRI data quality and contribute to perfusion modeling.

Additional personnel will include a research assistant, clinical research coordinator, and/or student and postdoctoral trainees in the Bright lab, as well as the Independent Medical Monitor to be appointed and approved by NINDS to oversee and report on this clinical trial. All study team members will have completed CITI training in human subjects research. Monthly meetings for the study team and more regular meetings of imaging and intervention sub-teams will ensure regular communication and coherence of study execution.

REFERENCES

1. Quaegebeur A, Lange C, Carmeliet P. The neurovascular link in health and disease: molecular mechanisms and therapeutic implications. Neuron. 2011 Aug 11;71(3):406–24.

2. Sirevaag AM, Black JE, Shafron D, Greenough WT. Direct evidence that complex experience increases capillary branching and surface area in visual cortex of young rats. Brain Res. 1988 Oct 1;471(2):299–304.

3. Argandoña EG, Lafuente JV. Influence of visual experience deprivation on the postnatal development of the microvascular bed in layer IV of the rat visual cortex. Brain Res. 2000 Feb 7;855(1):137–42.

4. Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. Proceedings of the National Academy of Sciences. 1990 Jul;87(14):5568–72. PMCID: PMC54366

5. Swain RA, Harris AB, Wiener EC, Dutka MV, Morris HD, Theien BE, et al. Prolonged exercise induces angiogenesis and increases cerebral blood volume in primary motor cortex of the rat. Neuroscience. 2003;117(4):1037–46.

6. Ding Y, Li J, Luan X, Ding YH, Lai Q, Rafols JA, et al. Exercise pre-conditioning reduces brain damage in ischemic rats that may be associated with regional angiogenesis and cellular overexpression of neurotrophin. Neuroscience. 2004;124(3):583–91.

7. Morgan R, Kreipke CW, Roberts G, Bagchi M, Rafols JA. Neovascularization following traumatic brain injury: possible evidence for both angiogenesis and vasculogenesis. Neurol. Res. 2007 Jun;29(4):375–81.

8. Park E, Bell JD, Siddiq IP, Baker AJ. An analysis of regional microvascular loss and recovery following two grades of fluid percussion trauma: a role for hypoxia-inducible factors in traumatic brain injury. J Cereb Blood Flow Metab. 2009 Mar;29(3):575–84.

9. Hayward NMEA, Tuunanen PI, Immonen R, Ndode-Ekane XE, Pitkänen A, Gröhn O. Magnetic resonance imaging of regional hemodynamic and cerebrovascular recovery after lateral fluid-percussion brain injury in rats. J Cereb Blood Flow Metab. 2011 Jan;31(1):166–77. PMCID: PMC3049481

10. Salehi A, Zhang JH, Obenaus A. Response of the cerebral vasculature following traumatic brain injury. J Cereb Blood Flow Metab. 2017 Jul;37(7):2320–39. PMCID: PMC5531360

11. Edvinsson LIH, Povlsen GK. Vascular plasticity in cerebrovascular disorders. J Cereb Blood Flow Metab. 2011 Jul;31(7):1554–71. PMCID: PMC3137480

12. Nishijima T, Torres-Aleman I, Soya H. Exercise and cerebrovascular plasticity. 1st ed. New Horizons in Neurovascular Coupling: A Bridge Between Brain Circulation and Neural Plasticity. Elsevier B.V; 2016. p. 243–68.

13. Pozehl BJ, Duncan K, Hertzog M, McGuire R, Norman JF, Artinian NT, et al. Study of adherence to exercise in heart failure: the HEART camp trial protocol. BMC Cardiovasc Disord. 2nd ed. 2014 Nov 29;14(1):172. PMCID: PMC4280683

14. Lamanna JC, Kuo N-T, Lust WD. Hypoxia-Induced Brain Angiogenesis. Oxygen Transport to Tissue XX. Springer, Boston, MA; 1998. p. 287–93.

15. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. J Neuroimmune Pharmacol. 2014 Mar;9(2):142–60. PMCID: PMC4048289

16. Navarrete-Opazo A, Mitchell GS. Therapeutic potential of intermittent hypoxia: a matter of dose. AJP: Regulatory, Integrative and Comparative Physiology. 2014 Nov 15;307(10):R1181–97. PMCID: PMC4315448

17. Beaudin AE, Hartmann SE, Pun M, Poulin MJ. Human cerebral blood flow control during hypoxia: focus on chronic pulmonary obstructive disease and obstructive sleep apnea. J. Appl. Physiol. 2017 Nov 1;123(5):1350–61.

18. Mallet RT, Manukhina EB, Ruelas SS, Caffrey JL, Downey HF. Cardioprotection by intermittent hypoxia conditioning: evidence, mechanisms, and therapeutic potential. Am. J. Physiol. Heart Circ. Physiol. 2018 Aug 1;315(2):H216–32. PMCID: PMC6139623

19. Burtscher M, Pachinger O, Ehrenbourg I, Mitterbauer G, Faulhaber M, Pühringer R, et al. Intermittent hypoxia increases exercise tolerance in elderly men with and without coronary artery disease. Int. J. Cardiol. 2004 Aug;96(2):247–54.

20. Burtscher M, Haider T, Domej W, Linser T, Gatterer H, Faulhaber M, et al. Intermittent hypoxia increases exercise tolerance in patients at risk for or with mild COPD. Respiratory Physiology & Neurobiology. 2009 Jan 1;165(1):97–103.

21. Lyamina NP, Lyamina SV, Senchiknin VN, Mallet RT, Downey HF, Manukhina EB. Normobaric hypoxia conditioning reduces blood pressure and normalizes nitric oxide synthesis in patients with arterial hypertension. J. Hypertens. 2011 Nov;29(11):2265–72.

22. Sandhu MS, Gray E, Kocherginsky M, Jayaraman A, Mitchell GS, Rymer WZ. Prednisolone Pretreatment Enhances Intermittent Hypoxia-Induced Plasticity in Persons With Chronic Incomplete Spinal Cord Injury. Neurorehabil Neural Repair. 2019 Sep 15;33(11):911–21.

23. Trumbower RD, Jayaraman A, Mitchell GS, Rymer WZ. Exposure to acute intermittent hypoxia augments somatic motor function in humans with incomplete spinal cord injury. Neurorehabil Neural Repair. 2012 Feb;26(2):163–72.

24. Hayes HB, Jayaraman A, Herrmann M, Mitchell GS, Rymer WZ, Trumbower RD. Daily intermittent hypoxia enhances walking after chronic spinal cord injury: A randomized trial. Neurology. American Academy of Neurology; 2014 Jan 14;82(2):104–13. PMCID: PMC3897437

25. Li Y, Lucas-Osma AM, Black S, Bandet MV, Stephens MJ, Vavrek R, et al. Pericytes impair capillary blood flow and motor function after chronic spinal cord injury. Nat Med. 2017 Jun;23(6):733–41. PMCID: PMC5716958

26. Shi Y, Thrippleton MJ, Makin SD, Marshall I, Geerlings MI, de Craen AJM, et al. Cerebral blood flow in small vessel disease: A systematic review and meta-analysis. J Cereb Blood Flow Metab. 2016 Oct;36(10):1653–67. PMCID: PMC5076792

27. Sam K, Crawley AP, Conklin J, Poublanc J, Sobczyk O, Mandell DM, et al. Development of White Matter Hyperintensity Is Preceded by Reduced Cerebrovascular Reactivity. Ann Neurol. John Wiley & Sons, Ltd; 2016 Aug 1;80(2):277–85.

28. Inglese M, Park S-J, Johnson G, Babb JS, Miles L, Jaggi H, et al. Deep gray matter perfusion in multiple sclerosis: dynamic susceptibility contrast perfusion magnetic resonance imaging at 3 T. Arch. Neurol. 2007 Feb;64(2):196–202.

29. De Keyser J, Steen C, Mostert JP, Koch MW. Hypoperfusion of the cerebral white matter in multiple sclerosis: possible mechanisms and pathophysiological significance. J Cereb Blood Flow Metab. 2008 Oct;28(10):1645–51.

30. Debernard L, Melzer TR, Van Stockum S, Graham C, Wheeler-Kingshott CA, Dalrymple-Alford JC, et al. Reduced grey matter perfusion without volume loss in early relapsing-remitting multiple sclerosis. J. Neurol. Neurosurg. Psychiatr. 2014 May;85(5):544–51.

31. Marshall O, Lu H, Brisset J-C, Xu F, Liu P, Herbert J, et al. Impaired Cerebrovascular Reactivity in Multiple Sclerosis. JAMA Neurol. 2014 Aug 18;71(10):1275–81. PMCID: PMC4376108

32. Pelizzari L, Laganà MM, Rossetto F, Bergsland N, Galli M, Baselli G, et al. Cerebral blood flow and cerebrovascular reactivity correlate with severity of motor symptoms in Parkinson's disease. Ther Adv Neurol Disord. 2019;12:1756286419838354. PMCID: PMC6431769

33. Glodzik L, Randall C, Rusinek H, de Leon MJ. Cerebrovascular reactivity to carbon dioxide in Alzheimer's disease. J. Alzheimers Dis. 2013;35(3):427–40. PMCID: PMC3776495

34. Suri S, Mackay CE, Kelly ME, Germuska M, Tunbridge EM, Frisoni GB, et al. Reduced cerebrovascular reactivity in young adults carrying the APOE ε4 allele. Alzheimers Dement. 2015 Jun;11(6):648–57.e1.

35. Manukhina EB, Downey HF, Shi X, Mallet RT. Intermittent hypoxia training protects cerebrovascular function in Alzheimer's disease. Exp. Biol. Med. (Maywood). 2016 Jun;241(12):1351–63. PMCID: PMC4950272

36. Wang H, Shi X, Schenck H, Hall JR, Ross SE, Kline GP, et al. Intermittent Hypoxia Training for Treating Mild Cognitive Impairment: A Pilot Study. Am J Alzheimers Dis Other Demen. 2020 Jan;35(17):1533317519896725.

37. Serebrovska ZO, Serebrovska TV, Kholin VA, Tumanovska LV, Shysh AM, Pashevin DA, et al. Intermittent Hypoxia-Hyperoxia Training Improves Cognitive Function and

Decreases Circulating Biomarkers of Alzheimer's Disease in Patients with Mild Cognitive Impairment: A Pilot Study. Int J Mol Sci. 2019 Oct 30;20(21):5405. PMCID: PMC6862463

38. Iwamoto E, Hanson BE, Bock JM, Casey DP. Intermittent hypoxia enhances shear-mediated dilation of the internal carotid artery in young adults. Journal of Applied Physiology. American Physiological Society Bethesda, MD; 2020 Sep 10.

39. Fan J-L, Burgess KR, Basnyat R, Thomas KN, Peebles KC, Lucas SJE, et al. Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO 2. The Journal of Physiology. Wiley/Blackwell (10.1111); 2010 Jan 29;588(3):539–49.

40. Bickler PE, Feiner JR, Lipnick MS, Batchelder P, MacLeod DB, Severinghaus JW. Effects of Acute, Profound Hypoxia on Healthy Humans: Implications for Safety of Tests Evaluating Pulse Oximetry or Tissue Oximetry Performance. Anesth. Analg. Anesthesia & Analgesia; 2017 Jan;124(1):146–53.

41. Bright MG, Murphy K. Reliable quantification of BOLD fMRI cerebrovascular reactivity despite poor breath-hold performance. Neuroimage. 2013 Dec;83:559–68. PMCID: PMC3899001

42. Dunworth SA, Natoli MJ, Cooter M, Cherry AD, Peacher DF, Potter JF, et al. Hypercapnia in diving: a review of CO_2 retention in submersed exercise at depth. Undersea Hyperb Med. 2017 May;44(3):191–209.

43. Spano VR, Mandell DM, Poublanc J, Sam K, Battisti-Charbonney A, Pucci O, et al. CO2 blood oxygen level-dependent MR mapping of cerebrovascular reserve in a clinical population: safety, tolerability, and technical feasibility. Radiology. 2013 Feb;266(2):592–8.

44. Liu Y, Zhu X, Feinberg D, Guenther M, Gregori J, Weiner MW, et al. Arterial spin labeling MRI study of age and gender effects on brain perfusion hemodynamics. Magn. Reson. Med. John Wiley & Sons, Ltd; 2012 Sep;68(3):912–22.

45. Murrell CJ, Cotter JD, Thomas KN, Lucas SJE, Williams MJA, Ainslie PN. Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: Effect of age and 12-week exercise training. AGE. 2012 Jun 6;35(3):905–20. PMCID: PMC3636405

46. Robertson AD, Marzolini S, Middleton LE, Basile VS, Oh PI, MacIntosh BJ. Exercise Training Increases Parietal Lobe Cerebral Blood Flow in Chronic Stroke: An Observational Study. Front Aging Neurosci. Frontiers; 2017 Sep 29;9.

47. Ismaili ARA, Vestergaard MB, Hansen AE, Larsson HBW, Johannesen HH, Law I, et al. Components of day-to-day variability of cerebral perfusion measurements - Analysis of phase contrast mapping magnetic resonance imaging measurements in healthy volunteers. Bachschmid MM, editor. PLoS ONE. Public Library of Science; 2018;13(6):e0197807. PMCID: PMC5991708

48. Spencer MD, Tyndall AV, Davenport MH, Argourd L, Anderson TJ, Eskes GA, et al. Cerebrovascular Responsiveness to Hypercapnia Is Stable over Six Months in Older Adults. Zang Y-F, editor. PLoS ONE. Public Library of Science; 2015;10(11):e0143059. PMCID: PMC4658173

49. Leung J, Behpour A, Sokol N, Mohanta A, Kassner A. Assessment of intracranial blood flow velocities using a computer controlled vasoactive stimulus: a comparison between phase contrast magnetic resonance angiography and transcranial Doppler ultrasonography. J. Magn. Reson. Imaging. John Wiley & Sons, Ltd; 2013 Sep;38(3):733–8.