Title: An Exploration of Acute Intermittent Hypoxia as a tool to Enhance Neural Recovery in Stroke Survivors; a pilot safety study.

NCT: NCT04019522

STU: STU00208610

Date: 12/11/2023

Permission to Take Part in a Human Research Study

Do not sign this consent if today's date is later than the stated expiration date above.

Title of Research Study: An Exploration of Acute Intermittent Hypoxia as a tool to Enhance Neural Recovery in Stroke Survivors*; a pilot safety study.*

Investigator: William Z Rymer

Supported By: This research is supported by the American Heart Association

Key Information:
The first few pages of this document include a summary of this study to help you decide whether or not to participate. Detailed information is provided after the summary.

Why am I being asked to take part in this research study?
We are asking you to take part in this research study because you are over the age of 18, have experienced a first time unilateral, ischemic, hemispheric stroke, which was confirmed by an MRI over 6 months ago. Additionally you also speak and understand English with the ability to independently consent and have a reliable companion to attend each research visit.

What should I know about a research study?

- Someone will explain this research study to you.
- Whether or not you take part is up to you.
- You can choose not to take part.
- You can agree to take part and later change your mind.
- Your decision will not be held against you.
- You can ask all the questions you want before you decide.

Why is this research being done?

The aim of this study is to answer questions related to safety and preliminary efficacy of a new intervention, acute intermittent hypoxia (AIH) in stroke survivors.

First, we will be establish whether brief and moderate reductions of oxygen in breathing air can be safely tolerated in subjects. A clinician will closely monitor you for any untoward medical occurrences also known as an adverse event.

The second aim is to establish what effects AIH may have on arm, grip and pinch strength. You will be closely monitored for any adverse events during these experiments. Data will be analyzed to determine if there is an improvement in functional outcome at any of the oxygen dose levels.

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How long will the research last and what will I need to do?
We expect that you will be in this research study for two to three weeks.

You will be asked to come in for 6 visits total (one screening visit, four treatment visits and a follow up appointment). You will be giving a small blood sample of 20mls, which is approximately 1.5 tablespoons, at 5 of the visits in addition to performing some clinical assessments.

More detailed information about the study procedures can be found under the section **What happens if I say, "Yes, I want to be in this research"?**

Is there any way being in this study could be bad for me?
There are two major risk outcomes that could be experienced; in the brain (cerebral) or in the heart (cardiac). You will be screened before your therapies to make sure that these events are unlikely, but a small possibility remains that either cardiac or cerebral event might occur.

Cardiac adverse events could be a silent (non-symptomatic) or non-silent (symptomatic) event. In the case of a silent event, you will be given a referral to a cardiologist at Northwestern to undergo further comprehensive tests to confirm the severity of the adverse event. This will be indicated by your troponin levels (a cardiac protein marker) out of the normal clinical range. The troponin level is identified from the blood sample you provided at that visit. A non-silent event will require the resident on call to be called to the clinic room for immediate and urgent attention for any chest discomfort pain experienced. A cerebral adverse event is experienced through a change in your vision and from the dramatic changes in your ability to complete the neurological assessments such as changes in thinking, speaking etc. The Medical monitor will be informed of this outcome and provide advice accordingly with your safety as a priority.

More detailed information about the risks of this study can be found under **"Is there any way being in this study could be bad for me? (Detailed Risks)"**

Will being in this study help me anyway?
There are no direct benefits to you from taking part in this research. We cannot promise any benefits to others from taking part in this research. However, possible benefits to others include improvement in their stroke impairments.

Participation in the experiment may result in no improvement or, in the rare event, worsening of your condition, including debilitation and prolonged recovery time.

What happens if I do not want to be in this research?
Participation in research is voluntary. You decide whether to participate. If you choose to not participate, there will be no penalty to you or loss of benefit to which you are entitled.

Your alternative to participating in this research study is to not participate.

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Detailed Information:
The rest of this document includes detailed information about this study (in addition to the information listed above).

Whom can I talk to?
If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team contacts ; Alexander Barry, (312) 238-1435/ abarry@sralab.org or Milap Sandhu, (312) 238-6529/ msandhu@sralab.org for any informational or logistical questions. Otherwise, call the 24hr clinic hotline for medical questions/concerns at 1-312-238-1000 or 911 depending on the urgency.

This research has been reviewed and approved by an Institutional Review Board (IRB). You may talk to them at (312) 503-9338 or irb@northwestern.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research participant.
- You want to get information or provide input about this research.

How many people will be studied?
We expect about 12-16 people will participate in this research study.

What happens if I say "Yes, I want to be in this research"?
Each visit will include a series of outcome assessments, intermittent hypoxia delivery, monitoring assessments and a blood draw of 20mls (1.5 tablespoons). Each visit is broken down with the exact task that will occur. A detailed explanation of the assessments is listed at the end.

Before participation in the study we will also need to determine history of Covid-19. To participate in the screening session we must record proof of covid vaccination injection, OR we must have proof of a negative COVID PCR test within 72 hours prior to the initial visit.

Experimental Hypoxia Sessions:
Hypoxia means reducing the amount of oxygen in one's environment. It will be administered by a face mask while breathing into a gas mixing device, called Hypoxico.
The hypoxia administering unit will be manually adjusted to reach the targeted level (approximately 21%-normal oxygen, 17%, 13%, and 9%). These hypoxia sessions consist of breathing in normal air followed by 15 cycles of hypoxia for up to 60 seconds combined with 90 second room air breaths between hypoxic sessions. All sessions will be supervised by a nurse, with real time oversight from a physician who will be present during the hypoxia administration. Additionally the nurse or medical monitor will confirm your blood work (troponin results) to ensure levels are acceptable (within the normal ranges) from a safety

standard so that you can be safely dismissed from the research visit. In total, each session will last approximately 30mins.

PROCEDURES: research visits will all take place on the 14th floor of the Shirley Ryan AbilityLab in a procedure suite

Visit 1: Prescreening Length of visit: 4 hours

- Discuss the research project, complete the informed consent process and confirm eligibility
- Magnetic resonance imaging (MRI) This study uses functional magnetic resonance imaging (MRI) to look at the brain that will be examined. Magnetic resonance imaging is a type of scan that uses magnetic fields and radio waves to make a picture of the brain. The MRI will be a nonfunctional/resting state MRI looking at a change in size, volume and location of the stroke damage.
- Medical history
- Demographic data
- Current medications/comorbidities
- Pregnancy test for women of childbearing potential via a blood draw
- Outcome assessments
- Monitoring assessments (including a 12-lead ECG)Blood test (approx. 1.5 tablespoon of blood). The risks of taking blood include pain, a bruise at the point where the blood is taken, redness and swelling of the vein and infection, and a rare risk of fainting

Visit 2: 21% oxygen the same exposure as normal breathing air. Intervention Day 1, Length of visit: 4 hours

- Hypoxia session with 21% oxygen: 30mins
- Outcome assessments
- Monitoring assessments
- Blood drawn: troponin (approx. 1.5 tablespoons will be taken)

Visit 3: 17% oxygen Intervention Day 4, Length of visit: 4 hours

- Hypoxia session with 17% oxygen: 30mins
- Outcome assessments
- Monitoring assessments
- Blood drawn: troponin (approx. 1.5 tablespoons will be taken)

Visit 4: 13% oxygen Intervention Day 8, Length of visit: 4 hours

- Hypoxia session with 13% oxygen: 30mins
- Outcome assessments
- Monitoring assessments
- Blood drawn: troponin (approx. 1.5 tablespoons will be taken)

Visit 5: 9% oxygen Intervention Day 12, Length of visit: 4 hours

- Hypoxia session with 9% oxygen: 30mins

- Outcome assessments
- Monitoring assessments
- Blood drawn: troponin (approx. 1.5 tablespoons will be taken)

Visit 6: Final Visit Post-intervention Day 16 Length of visit: 3/3.5hours

- MRI- resting state
- Outcome assessments
- 12-lead ECG

Outcome assessments:
You may experience slight pain with passive limb movement (or attempted active) – this mostly occurs if post-stroke patients have a lot of tightness

1. Fugl Meyer motor assessment: This test measures recovery after stroke. You will be asked to perform a variety of tasks measuring your motor functioning.
2. Manual Muscle testing (MMT): Test to measure muscle strength
3. Grip strength: You will be asked to grasp a A device repeatedly in order to measures your strength. We will ask you to perform this with one or both hands.
4. Elbow strength: a device measures strength at the elbow joint. A total of three trials will be taken on each side, with a rest break between trials.
5. Neurological test: This test will be used to asses any neurological changes in coordination, movement, reflexes and cranial nerves
6. NIH stroke scale: is a tool used by healthcare providers to measure the injury caused by the stroke.
7. Stroop test: A neuropsychological measure that seeks to evaluate attention and inhibition. You will be asked to read words or name ink colors as quickly as possible within a given time limit.
8. Modified Ashworth Scale: This scale assess your muscle tone. which be assessed for both of your elbows.
9. Pinch Strength: A device measures muscle strength or weakness. You will be asked to pinch the device three times using each hand.

Monitoring assessments: (monitored throughout the hypoxia administration)

10. Vitals: We will measure your blood pressure, blood oxygen saturation (measured from the finger and from a wearable sensor), and pulse rate.
11. ECG: is a test that gives us a measure of the heart's electrical activity. Several small electrode pads (like stickers) will be placed on the body. 30min ECG to be done during the hypoxia administration, with a 12 lead ECG on V1 and V6.
12. Symptom check list: A simple yes/no subjective checklist of physical symtpoms, such as chest pain, will be asked and repeated to the subject at each time point: 2, 6, 14, 24 and 30 mins from the beginning of the hypoxia session.

You may be photographed or videotaped during the procedures, which is optional. You will be given the option to accept or decline later in this consent form. All attempts will be made to minimize any identifiable information.

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What are my responsibilities if I take part in this research?
If you take part in this research, you will be responsible to arrive at the Shirley Ryan AbilityLab for all the scheduled visits and complete the research procedures with the staff.

What happens if I say "Yes", but I change my mind later?
You can leave the research at any time; it will not be held against you. If you decide to leave the research, contact the investigator.

Choosing not to be in this study or to stop being in this study will not result in any penalty to you or loss of benefit to which you are entitled. Specifically, your choice not to be in this study will not negatively affect your right to any present or future medical treatment,

Detailed Risks: Is there any way being in this study could be bad for me?
Cardiac adverse events could be silent (non-symptomatic) or non-silent (symptomatic).
In the event of a silent event subjects will be given a referral to a cardiologist at Northwestern Memorial Hospital to undergo more comprehensive assessments to confirm the severity of the adverse event. This will be indicated by the troponin levels out of the normal clinical range. The troponin level is identified from the blood sample provided at that visit.
A non-silent event will require the ROC (resident on call) to be called to the clinic room for immediate and urgent attention for the chest pain experienced.
The medical professional will decide the best care plan for you, which could include a trip to the emergency room.

A cerebral adverse event is experienced through a change in vision and/or from changes in the neurological assessment, such as blurred vision or slurred speech. The Medical monitor will be informed of this outcome and will provide advice accordingly, which may result in being withdrawn from this research study for your own safety.

Breathing low levels of oxygen causes low levels of oxygen in the blood. Some of the side effects could include lightheaded sensation, dizziness, reduced vision, feeling short of breath, abnormally elevated heart rate, euphoria, and fainting. We will monitor for these side effects, as well as monitor different vital signs to assure your safety during the session. In the event of an emergency, you will be withdrawn from the breathing apparatus, and the resident on call will be notified for further management. There may also be other side effects that we cannot predict, or there may be risks that may hurt you in ways that are unknown. These may be a minor inconvenience or so severe as to cause death.

Some people cannot have an MRI because they have some type of metal in their body. For instance, if you have a heart pacemaker, artificial heart valves, metal implants such as metal ear implants, bullet pieces, chemotherapy or insulin pumps or any other metal such as metal clips or rings, they cannot have an MRI. During this test, you will lie in a small closed area inside a large magnetic tube. Some people are scared or anxious in small places (claustrophobic). The

MRI scanner makes loud banging noises while taking a measurement, so either ear plugs or specially designed headphones will be used to reduce the noise.

For any additional concerns, the number of the researcher is listed in the section **"If I have questions or concerns about this research study, whom can I call?"**

The safety of breathing low oxygen during pregnancy is unknown. There may be some unknown or unanticipated risks or discomforts, because some of the procedures are relatively new and are attempts to advance medical knowledge. Every known precaution will be taken to ensure your personal safety and to minimize discomfort.

This study involves the use of your identifiable, personal information and there is a chance that a loss of confidentiality could occur. The researchers have procedures in place to lessen the possibility of this happening. See the section below titled: **"What happens to the information collected for the research?".**

What do I need to know about reproductive health and/or sexual activity if I am in this study?
The effect of this study's breathing intervention on human sperm and eggs has not been studied. The effects on the developing fetus during breathing low oxygen levels during pregnancy and the risk of birth defects are also unknown. Therefore, both men and women should not attempt pregnancy and women should not be pregnant or breast-feeding while taking part in this study.

If you are sexually active, both men and women should use at least one effective means of birth control while participating in this research study. According to the World Health Organization and the United States Center for Disease Control and Prevention, the most effective forms of birth control include complete abstinence, surgical sterilization (both male and female), intrauterine devices (IUDs), and the contraceptive implant. The next most effective forms of birth control include injectable, oral contraceptive pills, the contraceptive ring, or the contraceptive patch. Acceptable but least effective methods of birth control include male condoms (with or without spermicide) and female condoms.

If you or your partner become pregnant while participating in this research study or for a week after you complete the study, it is important that you tell the study doctor or other research team member immediately. You may be required to stop participation in this study; however, other treatment options will be discussed with you at that time if necessary.

If you or your partner [are/is] considered to be postmenopausal, you are not required to use contraception while participating in this research study. Postmenopausal women rarely become pregnant. If you or your partner become pregnant while participating in this research study or for a week after you complete the study, it is important that you tell the study doctor or other research team member immediately. You may be required to stop participation in this study; however, other treatment options will be discussed with you at that time if necessary.

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Will it cost me anything to participate in this research study?
Taking part in this research study will be of no cost to you. All visits are entirely research related and will not result in a cost to you or your insurance.

What happens to the information collected for the research?
We limit the use and disclosure of your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this institution and the American Heart Association.

The sponsor, monitors, auditors, the IRB, the Northwestern University Office for Research Integrity, the US Office of Research Integrity (ORI), the US Office for the Protection of Human Research Protections (OHRP), and the US Food and Drug Administration (FDA) may be granted direct access to your medical records to conduct and oversee the research. By signing this document, you are authorizing this access. We may publish the results of this research. However, we will keep your name and other identifying information confidential.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Data Sharing
De-identified data from this study may be shared with the research community at large to advance science and health. We will remove or code any personal information that could identify you before files are shared with other researchers to ensure that by current scientific standards and known methods, no one will be able to identify you from the information we share. Despite these measures, we cannot guarantee anonymity of your personal data.

Can I be removed from the research without my OK?
The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include inability to comply with study requirement and/or appointments.

We will tell you about any new information that may affect your health, welfare, or choice to stay in the research.

What else do I need to know?
If you become ill or are injured as a result of this study (medications, devices or procedures), you should seek medical treatment through your doctor or treatment center of choice. You should promptly tell the study doctor about any illness or injury.

The hospital [university, researchers] will not pay for medical care required because of a bad outcome resulting from your participation in this research study. This does not keep you from seeking to be paid back for care required because of a bad outcome.

If you agree to take part in this research study, we will pay you a total of $500 for your time and effort, after all 6 visits have been completed.

Payment will be provided through ClinCard, a form of debit card used for research studies (see below). You will be compensated:

- $50 for the screening visit (lasting up to four hours).
- $100 total for each of the four intervention visits (lasting up to four hours).
- $50 for the final follow up visit post- intervention (lasting up to three and a half hours)
- Total subject compensation= $500

For all visits over the allocated time, $20 extra will be paid every hour beyond the expected. For any session attended but is not completed you will be compensated $30.

- During Covid-19 pandemic, we will reimburse round-trip private transportation cost including driver tip (e.g. accessible Uber, Lyft, Taxi, parking fees etc.) for visits to the Shirley Ryan AbilityLab. A receipt is required for full reimbursement of transportation cost.

The Shirley Ryan AbilityLab will issue you a ClinCard, which is a specially designed debit card for clinical research. Once a visit that qualifies for compensation is completed, funds will be approved and loaded onto your card. The funds will be available within 1 day after being loaded and can be used at your discretion.

You will be issued one card for the duration of your participation. If your card is lost or stolen, please call (866) 952-3795 or ask a coordinator for a replacement ClinCard.

Fees are incurred if used at an ATM (fees vary by location). However, if the card is used for in-store or online purchases via credit or debit, there are no associated fees and no expiration date.

Please be advised: Inactivity on the card for more than 3 months will incur a monthly fee. However, as long as there is activity on the card within 3 months (funds are added or a transaction is completed), the month period will reset and no monthly fee will be assessed.

If you do incur a monthly fee, please contact Greenphire Support at the number on the back of your card and they will reverse the fee. See "Tips for Using the Attached ClinCard" for more information.

The Finance Department at the Shirley Ryan AbilityLab will be provided with your information, including your Social Security Number, in order to issue payment for your study participation.

Study payments are considered taxable income and reportable to the Internal Revenue Service (IRS). An IRS Form 1099 will be sent to you if your total payments are $600 or more in a calendar year.

You may be given access to new inventions that are being developed by the investigator, the study sponsor, or other people involved in the study. Certain laws can make it harder to obtain legal protection for a new invention shared with a study participant, unless the study participant agrees to keep information about the invention confidential. You agree to keep confidential information you may receive about new inventions, such as new drugs, new devices, or new methods.

HIPAA Authorization

We are committed to respect your privacy and to keep your personal information confidential. When choosing to take part in this study, you are giving us the permission to use your personal health information that includes health information in your medical records and information that can identify you. For example, personal health information may include your name, address, phone number or social security number. Your health information we may collect and use for this research includes:

- Results of physical examinations
- Medical history
- Lab tests, or certain health information indicating or relating to a particular condition
- Records about current study medication or drugs

During this study, you may be coming to a Northwestern Memorial Healthcare Corporation entity (for example, Northwestern Memorial Hospital, Prentice Women's Hospital) for research appointments or to get clinical services, such as lab tests, needed for the study. When that happens, you will be scheduled for these services through the NMHC computer system. When a clinical exam or lab is done by NMHC or one of its employees for the purpose of this research study, that information will be kept in both NMHC's clinical records and in the study records.

The following clinical providers may give the researchers information about you: all current and previous health care providers, including but not limited to the Shirley Ryan AbilityLab (SRALAB), Northwestern Medical Group (NMG), Northwestern Memorial Hospital (NMH), and Northwestern Lake Forest Hospital (NLFH).

Once we have the health information listed above, we may share some of this information with the following offices or entities outside of Northwestern University and its clinical partners (or affiliates): the Northwestern University Institutional Review Board Office and Office for Research Integrity; the US Office of Research Integrity; the US Office for Human Research Protections; the US Food and Drug Administration.

Any research information shared with outside entities will not contain your name, address, telephone or social security number or any other personal identifier unless disclosure of the

identifier is necessary for review by such parties or is required by law or University policy [except that such information may be viewed by the Study sponsor and its partners or contractors at the Principal Investigator's office].

The following entities may receive your health information:

- Authorized members of the Northwestern University and the Shirley Ryan AbilityLab) workforce, who may need to see your information, such as administrative staff members from the Office for Research, Office for Research Integrity and members of the Institutional Review Board.
- Clinical affiliates, including but not limited to the Shirley Ryan AbilityLab (SRALAB), Northwestern Medical Group (NMG), Northwestern Memorial Hospital (NMH), Northwestern Lake Forest Hospital (NLFH), and the Ann & Robert H. Lurie Children's Hospital of Chicago (Lurie Children's). Your participation in this clinical trial may be tracked in an electronic database and may be seen by investigators running other trials that you are enrolled in and by your healthcare providers.
- Clinical affiliates, including but not limited to Northwestern Medical Group (NMG), Northwestern Memorial Hospital (NMH), and Northwestern Lake Forest Hospital (NLFH), for purposes including, but not limited to, the affiliate's provision of care to you and/or the affiliate's scheduling of appointments and/or billing activities.
- Study monitors and auditors who make sure that the study is being done properly,
- American Heart Association who is sponsoring the study, and that company's contractors and partners.
- Government agencies and public health authorities, such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (DHHS).

Those persons who get your health information may not be required by Federal privacy laws (such as the Privacy Rule) to protect it. Some of those persons may be able to share your information with others without your separate permission.

The results of this study may also be used for teaching, publications, or for presentation at scientific meetings.

Unless you revoke your consent it will expire at the end of the research study.

Although you may revoke consent to participation in this research at any time and in any format, you must revoke authorization for use or disclosure of your health information in writing. To revoke your authorization, write to:

William Z. Rymer, Principal Investigator
Shirley Ryan AbilityLab Arms and Hands Lab
355 E Erie St. Chicago, IL 60611

You do not have to authorize the use or disclosure of your health information; however, you will not be allowed to take part in this research study. If you do not authorize the use or

disclosure of your health information, it will not affect your treatment by health care providers, or the payment or enrollment in any health plans, or affect your eligibility for benefits.

A copy of this signed consent document, information about this study, and the results of any test or procedure done may be included in your medical records and may be seen by your insurance company.

Optional Elements:
The following research activities are optional, meaning that you do not have to agree to them in order to participate in the research study. Please indicate your willingness to participate in these optional activities by placing your initials next to each activity.

I agree	**I disagree**	
_____	_____	The researcher may audio or video record me to aid with data analysis. The researcher will not share these recordings with anyone outside of the immediate study team.
_____	_____	The researcher may audio or video record me for use in scholarly presentations or publications. My identity may be shared as part of this activity and could be permanently available on the internet if used for publication, however the researchers will make all attempts to limit or minimize such identification. I understand the risks associated with such identification.
_____	_____	The researcher may contact me in the future to see whether I am interested in participating in other research studies by the Principal Investigator of this study.

Signature Block for Capable Adult:

Your signature documents your permission to take part in this research. You will be provided a copy of this signed document.

_____ _____
Signature of Participant Date

_____
Printed Name of Participant

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

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PROTOCOL TITLE:
An Exploration of Acute Intermittent Hypoxia as a tool to Enhance Neural Recovery in Stroke Survivors; a pilot safety study.

PRINCIPAL INVESTIGATOR:
Name: William Z Rymer
Department: Arms and Hands Laboratory, Shirley Ryan AbilityLab
Telephone Number: 312-238-6529
*Email Address: z*rymer@sralab.org

VERSION NUMBER:
12

VERSION DATE:
1st December 2023

STUDY SUMMARY:

Investigational Agent(s) (Drugs or Devices)	N/A
IND / IDE / HDE #	N/A
/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	12-16 subjects
Funding Source	American Heart Association
Indicate the type of consent to be obtained	X 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 x No
DSMB / DMC / IDMC	x Yes ☐No

OBJECTIVES:

Aim 1: To establish safety boundaries for administering acute intermittent hypoxia therapy in subjects with chronic thrombotic stroke, in order to provide guidance for future therapeutic hypoxia interventions.

This is a Phase I safety study. Our plan consists of dose-escalation exposures with continual assessment of hypoxic conditioning impact in individuals with chronic stroke.

Aim 2: To collect preliminary data about the potential efficacy of this approach in improving voluntary muscle strength, as a prelude to a more extensive clinical trial.

We plan to measure changes in arm strength and hand grip strength before AIH, and following each AIH exposure sequence. We will use a three-second maximum contraction, and calculate the mean of three grip strength estimates at each time epoch, with at least a 60 second rest periods between trials to prevent fatigue. We will test elbow flexion using a commercial dynamometer.

We hypothesize that similar to our SCI studies, we will see increases in voluntary strength in the impaired arm within 30 minutes; peaking at about 90 minutes after the AIH sequence is completed.

BACKGROUND:

Of the 795,000 people who experience a stroke every year in the US, only a small percentage will achieve full recovery. While current therapies promote strength and endurance, none directly address the unique potential of the brain to reorganize following injury. The goal of this project is to explore the effects of a novel therapy, acute intermittent hypoxia (AIH). During this therapy, individuals receive brief bouts of reduced oxygen levels by inhalation through a face mask. (This is akin to being on top of a tall mountain). In brief exposures, AIH is known to trigger the release of specific proteins that help the brain adapt to oxygen reductions. Published results in people with incomplete spinal cord injury have shown that AIH enhances muscle strength and coordination rather quickly. We now aim to study the effects of AIH in stroke survivors.

Stroke is the second leading cause of death and a leading cause of long-term disability worldwide. Despite the spontaneous recovery that occurs following a hemispheric stroke, more than half of stroke patients show substantial residual impairments, imposing a significant human and economic burden. This burden is likely to increase in coming decades, due to a rapidly aging population, and the associated progression of cardiovascular risk factors. Accordingly, new interventions to alleviate impairment in stroke survivors are urgently needed. ***The development and testing of one such novel intervention, termed Acute Intermittent Hypoxia (AIH), is the primary focus of this AHA Innovative Project Award.***

The aim is to answer questions related to safety and preliminary efficacy of AIH in stroke survivors.

First, we will establish whether brief reductions in inhaled oxygen concentration can be safely tolerated in stroke survivors. A clinician will closely monitor subjects for any adverse events.

The second aim is to establish the effects of AIH on elbow flexion on hand grip and pinch strength.

Subjects will be monitored closely for any adverse events during these experiments. Data will be analyzed to determine if there is an improvement in key outcomes at any dose level.

STUDY ENDPOINTS:

Primary study endpoint: Safety will be assessed through the continuing clinical review of the clinical/neurological status over the duration of the study.

All adverse events will be reviewed for safety and study continuation by the medical monitor. It will be determined if the subject should continue the experimental hypoxia or if the study should be terminated.

Each subject will serve as his/her own control group; comparisons will be taken at baseline via MRI scans and radiological assessments then compared against the post hypoxia sessions via MRI scans and radiological assessments.

Secondary outcomes: Clinical outcomes: NIH Stroke Scale, Fugl-Meyer Assessment scale, Modified Ashworth, manual muscle testing, Grip strength, Pinch strength, Elbow strength, Blood tests (troponin), D-KEFS Color-Word Interference Test and a 5-minute neurological assessment of clinical status.

Imaging outcomes: The 3T-MRI protocol duration will be up to one hour, to be done prior to initiating our tests at baseline, and at the follow up visit. The MRI will be a nonfunctional/resting state MRI looking at a change in size, volume and location of the stroke damage. This will be conducted as a standard of care MRI sequencing; just performed and purchased by the grant. The sequence of the MRI will be the type typically used in the standard of care for stroke evaluation.

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

Hypoxic Experimental Therapy: The hypoxic stimulus will be implemented through a facemask. Hypoxia sessions will consist of a baseline breathing normoxic, ambient air, followed by 15 cycles hypoxia for up to 60 seconds, interspersed with 90 second normoxic breathing between hypoxic sessions. All conditioning sessions will be supervised by a nurse, who will be:

1) assisting the lab techs to complete a pregnancy test prior to the hypoxia session. Pregnancy test will be determined via blood serum test for WOCBP due to potential incontinence of this patient population.

2) monitoring the 2-lead EKG, SPO2, blood pressure and pulse rate throughout the hypoxia session. EKG's will be reviewed and signed off by the nurse confirming that no adverse events (dysrhythmias) occurred. If there are concerns, the medical monitor will review.

Additionally, the nurse or medical monitor will confirm troponin results, to ensure levels are acceptable (within the normal ranges) from a safety standard in order to allow the subject to leave the research facility.

In the past five years, the main findings relevant to the use of AIH in persons with incomplete SCI are:

1. The neuroplasticity mechanisms linked to AIH described initially in animal models appear to be present and active in humans.
2. In persons with incomplete SCI, the use of AIH alone (15, 60-second episodes of 9% oxygen) can augment muscle strength and joint torque at the ankle. The effects of AIH are often dramatic, increasing voluntary strength in ankle muscles by 30-40% within 90-120 minutes.(Trumbower, Jayaraman et al. 2012)
3. Repeated (daily Acute Intermittent hypoxia) day exposure combined with conventional rehabilitation therapy such as over ground walking can greatly improve walking speed and endurance(Hayes, Jayaraman et al. 2014) beyond that achieved with either intervention applied separately.

Recent work has also demonstrated the effectiveness of dAIH to enhance spinal motor plasticity related to respiration. (Tester, Fuller et al. 2011, Yokhana, Gerst et al. 2012) A randomized, triple-blind, two-arm parallel clinical trial was recently done in Chile.(Navarrete-Opazo, Alcayaga et al. 2016)

This study compared the effects of a 4-week protocol of dAIH combined with body weight-supported treadmill training on walking in persons with incomplete SCI. Subjects received dAIH or sham dAIH combined with 45 minutes of training for 5 consecutive days, followed by dAIH or sham dAIH 3 times per week for 3 additional weeks. They found that walking recovery was significantly better after dAIH + training, compared to training alone. Moreover, additional 3 times per week dAIH prolonged or enhanced daily IH-induced walking speed and endurance improvements up to 5 weeks post dAIH. In addition, this protocol of dAIH does not elicit any visual or verbal memory impairment.(Navarrete-Opazo, Alcayaga et al. 2016)

DATA AND SPECIMEN BANKINGS

Data will be stored in password-protected database; Redcap which is managed by Northwestern University. The data points collected will be specifically designed around the structure of the study visits dates and assessments being carried out.
If needed an excel file will be managed with password protection; located on the secure network for the Shirley Ryan AbilityLab.

Upon enrollment, each subject will be assigned an identifier number such that the data does not contain identifiable private information; the data will only be coded with the identifier number. Thus, there is no identifiable data. The data will be uploaded into Redcap. Only authorized personnel of this study (IRB-approved) will have direct access.

Once all patient data has been collected and uploaded into Redcap; the statisticians will analyze the data and provide reports, which will be used to publish relevant findings. Names of participants will not be used in study reports.

We will retain study documentation including all case report forms, data correction forms or queries, source documents, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms) for at least three years after the completion and final study report of this investigational study.

SHARING RESULTS WITH PARTICIPANTS

This is not a blinded study; all subjects will each be given the same treatment.
Subjects will be provided with a phone number to call if they have any questions about the study treatment they received or the results of the clinical trial. It is not anticipated that any information, unless an adverse event occurs, will need to be shared with primary care or other physicians.

STUDY TIMELINES

Participants will be initially screened over the phone or in person to ensure eligibility based on the established inclusion and exclusion criteria. We anticipate that each participant will take about 2-3 weeks to complete the study procedures/testing (depending on scheduling and study staff availability). We anticipate a total of 2-3 years to enroll, complete data collection and complete the primary analyses.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria:

- Age ≥18 years;
- A first time, unilateral, ischemic, hemispheric stroke, confirmed by magnetic resonance imaging (MRI);
- Chedoke assessment ≥ 3
- Ability to open and close affected hand
- Able to understand and communicate in English
- Be able to consent independently
- ≥ 6 months post stroke
- Must have a hemoglobin level above 10g/dl (to be confirmed using handheld noninvasive lab equipment)
- Must have ability to leave research visit with a companion/group transportation
- WOCBP must be comfortable confirming negative pregnancy prior to hypoxia experimental therapy.
- Must not be involved in any other research intervention study testing upper extremities
- Verification of Covid vaccination status or must show a negative COVID PCR test within 72 hours prior to the screening visit

Exclusion criteria:

- Brain stem or cerebellar stroke; mean Fazekas score rated on initial fluid-attenuated inversion recovery MRI≥3;
- Severe aphasia, preventing subject from understanding the protocol and giving written consent;
- History of prior neurological disorder;
- Pre-existing hypoxic pulmonary disease,
    - Includes positive COVID-pneumonia diagnosis within 1 year of screening visit
- Severe hypertension (>160/100)
- Ischemic cardiac disease.

PROCEDURES: LOCATION: 14TH FLOOR SUITE

Hypoxia sessions consist of a baseline breathing normoxic, ambient air, followed by 15 cycles of hypoxia for up to 60 seconds, interspersed with 90 second normoxic breathing between hypoxic sessions.
Outcome and Monitoring Assessments are defined below.

Visit 1: Prescreening Length of visit: 4 hours

- MRI – resting state
- Medical history
- Demographic data
- Current medications/comorbidities
- Pregnancy test for WOCBP
- Outcome assessments
- Monitoring assessments as baseline values (including a 12-lead ECG)
- Blood test: troponin (approx. 1.5 tablespoon of blood).

Visit 2: 21% “normal oxygen” Intervention Day 1 Length of visit: 4 hours

- Must be within 1-month of prescreening visit
- Hypoxia session: 30mins
- Outcome assessments
- Monitoring assessments
- Blood drawn: troponin

Visit 3: 17% oxygen Intervention Day 4 Length of visit: 4 hours

- Hypoxia session: 30mins
- Outcome assessments
- Monitoring assessments
- Blood drawn: troponin

Visit 4: 13% oxygen Intervention Day 8 Length of visit: 4 hours

- Hypoxia session: 30mins

- Outcome assessments
- Monitoring assessments
- Blood drawn: troponin

Visit 5: 9% oxygen Intervention Day 12 Length of visit: 4 hours

- Hypoxia session: 30mins
- Outcome assessments
- Monitoring assessments
- Blood drawn: troponin

Visit 6: Final Visit Post-intervention Day 16 Length of visit: 3/3.5 hours

- MRI-resting state
- Outcome assessments
- 12-lead ECG

Intervention methods:
During each session, study participants will receive a single sequence of AIH, consisting of 15 x periods of hypoxia, lasting up to 60 seconds, alternating with 90-seconds of normoxia (21% O_2), for a total of 35 minutes, whilst in a seated upright position. AIH will be applied by directing gas flow to a reservoir bag connected via plastic tubing to a non re-breathing facemask/respiratory valve system while the participants are in a seated position. Defined gas mixtures will be delivered by manual adjustment of one-way valves attached to a hypoxia generator (HYP123, Hypoxico Inc.). An oxygen monitor will continuously measure and record the fraction of inspired oxygen delivered to the subject (MAX-250E, Maxtec Inc.).

Inspired fraction of oxygen (FiO2) of the gas mixture will be individually adjusted using the valve settings to reach the targeted SpO_2. The gas mixtures administered during the four sessions will be 21% O_2 (target SpO_2 = 95%), 17% O_2 (target SpO_2 = 92%), 13% O_2 (target SpO_2 = 87%) and 9% O_2 (target SpO_2 = 82%).

We have extensive experience with this protocol and have administered it to both healthy and spinal cord injury individuals. As the individuals get hypoxic gas for only a brief period, i.e., 60 seconds, the risks associated are minimal. To put into perspective, SpO_2 of 82% is akin to levels at the top of a tall mountain. SpO_2 as low as 75% will be accepted, if the SpO_2 drops below 75%, we will terminate the hypoxia exposure immediately. Similarly, if the participant's SpO_2 is unable to reach within 5% of the specific target SpO_2, hypoxia exposure will be terminated.
All conditioning sessions will be supervised by a nurse, who will be: 1) administering the pregnancy test prior to the first hypoxia session (if applicable), 2) monitoring the 2-lead EKG, SPO_2, blood pressure and pulse rate throughout the session. EKG's will be reviewed and signed off by the nurse confirming no adverse events occurred. If there are concerns the medical monitor will review. Additionally, the nurse or medical monitor will confirm troponin results, to ensure levels are acceptable (within the normal ranges) from a safety standard in order to dismiss the subject from the research visit. Levels of troponin will be monitored directly after

each AIH session; performed a total of five times (before the screening through to visit 5). 20mls of blood will be collected to test.

If there is an unforeseen alteration in the cardiopulmonary parameters being assessed the experiment will be stopped and appropriate care will be provided by the overseeing physician and the emergency medical team at the Shirley Ryan Abilitylab will be promptly notified.

Radiologic imaging (MRI) will be used to determine whether there are alterations in cerebral perfusion. Subjects will have a total of two MRIs in our Shirley Ryan Lab Siemens 3T machine: the first before the first AIH exposure and the second after the last hypoxia session. The MRI will be read by a physician to confirm:
1) location of stroke
2) size/dimensions of damage, to certify the results were collected and reviewed correctly with the appropriate next steps.

Any and all adverse events will be documented and appropriately reported, as required by the IRB review board.

Outcome assessments:

1. Fugl Meyer motor assessment: Each test element will be graded on a 3-point ordinal scale and summed up to provide a maximum upper limb score of 66. Reliability and validity have been demonstrated. The FMA will be administered while the subject is seated.
2. Grip strength: A dynamometer measures maximum gross grasp (lb.) averaged over three attempts with each hand. The minimum possible value of zero lb. will be assigned when the participant cannot actively flex the fingers or grasp the dynamometer. Completed bilaterally, if possible.
3. Elbow strength: monitoring changes in isometric elbow flexion force using a dynamometer. A total of three trials will be taken on each side, with a rest break between trials. The average of the three trials will be recorded.
4. Neurological test: Tests to be done; cranial nerves, motor, reflexes, sensory, coordination
5. NIH stroke scale: is a tool used by healthcare providers to objectively quantify the impairment caused by a stroke. The NIHSS is composed of 11 items, each of which scores a specific ability between a 0 and 4. For each item, a score of 0 typically indicates normal function in that specific ability, while a higher score is indicative of some level of impairment. The individual scores from each item are summed in order to calculate a patient's total NIHSS score ranging from 0-42.
6. D-KEFS Color-Word Interference Test: A neuropsychological measure that seeks to evaluate attention and inhibition. Subjects will be asked to read words or name ink colors as quickly as possible within a given time limit. The test supplies the examiner with three separate scores, including an ability to calculate an interference score. This final score allows for interpretation of cognitive flexibility, creativity and cognitive stress. This measure will be utilized to monitor subjects throughout their participation at

specific time-points. Audio recording may be taken to ensure accurate recording of responses.

7. Modified Ashworth Scale: This scale allows for characterization of increases in muscle tone, from low or normal tone to complete limb rigidity. Specifically, we will evaluate the elbow flexors, bilaterally.
8. Pinch Strength (Strength Gauge Dynamometry): Hydraulic Handheld Dynamometer): A dynamometer measures maximum gross grasp (lb.) averaged over 3 attempts with each hand. The minimum possible value of zero lb. will be assigned when the participant cannot actively flex the fingers or grasp the dynamometer.

Monitoring assessments: (monitored throughout the hypoxia administration)

9. Vitals: Blood pressure guidelines for participation include: systolic blood pressure at/between 85-160mmHg, diastolic blood pressure at/between 55-110mmHg, and participant is asymptomatic before beginning each hypoxia session; Oxygen saturation levels measured from finger or wearable sensor, and pulse rate
10. EKG: Standard bedside monitoring that is a 2 lead tracing to identify dysrhythmias. 30min ECG to be performed during hypoxia administration. 15min 12 lead ECG to be performed at V1 and V6 at resting state with no other ongoing activities.
11. Symptom check list: A simple yes/no subjective checklist will be asked and repeated to the subject at each time point: 2, 6, 14, 24 and 30mins from the beginning of the hypoxia session.

Subjective Symptom checklist:

Symptom	Yes	No
Chest pain		
Shortness of breath		
Light headedness		
Neck pain		
Dizziness		
Arm pain (left side) for cardiac symptoms		
Sweaty/feeling warm		
Sensory changes: new signs of numbness		
Increased weakness		

VULNERABLE POPULATIONS

Not applicable

PARTICIPANT POPULATION(S)

The local recruitment goal is between 12-14 subjects

RECRUITMENT METHODS

Potential participants will be identified through a variety of means, including: medical records, physician contacts, recruitment flyers within the Shirley Ryan AbilityLab and surrounding community hospitals, the Clinical Neuroscience Research Registry, and community outreach presentations.

Shirley Ryan AbilityLab Research Registries: Subjects will be recruited through the Clinical Neuroscience Research Registry. The database is maintained through combined efforts of the Shirley Ryan AbilityLab and Northwestern University Department of Physical Therapy. It includes up to 1000 individuals with various neurologic conditions, including spinal cord injury and stroke. Researchers are able to modify their search criteria based on level of injury, severity of injury, and primary means of mobility. The letter of support is attached. Patients will also be recruited through the Shirley Ryan Ability Lab's electronic medical records and through the PM&R clinic physicians.

Flyers: These will be primarily distributed through the Shirley Ryan AbilityLab, surrounding community hospitals, and at the surrounding Chicagoland support groups for individuals having suffered a stroke.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES

Payment will be provided through ClinCard. Each participant will be compensated:

- $50 for each screening visit (lasting up to four hours).
- $100 total at for each of the four intervention visits (lasting up to four hours).
- $50 for each final visit post- intervention (lasting up to three and a half hours)
- Total subject compensation= $500

Additional $20 per hour will be paid if the visit time exceeds the expected length.
For any session attended but is not completed, $30 will be paid.

- During Covid-19 pandemic, we will reimburse round-trip private transportation cost including driver tip (e.g., accessible Uber, Lyft, Taxi, parking fees etc.) for visits to the Shirley Ryan AbilityLab. A receipt is required for full reimbursement of transportation cost.

Data Safety Monitoring Board:

The independent Data Safety Monitoring Board will oversee the study, consisting of:

1. Dr. Richard Harvey, MD; Medical Director at SRALab. Chair of the DSMB
2. Dr Khushboo Doshi, MD; Attending Physician at SRALab
3. Biostatistician: Mary Kwasny; Statistician at Northwestern University

There are no conflicts of interest or specific investments in this study.
The DSMB will be responsible for oversight of the following activities:

1. **Initial visit:** The DSMB Chair will be notified when the first patient is enrolled in the study.

2. **First Visit**: To be conducted within the first week the first subject has completed the intervention. This board will confirm for protocol execution, satisfactory site facilities, clarify the applicable regulations and requirements of the protocol, and carefully review the process of implementing the protocol with patient's safety as priority. If major flags are discussed the board will 1) pause the study until corrections are made 2) terminate the study due to high risk/harm towards the subjects safety and overall research conduct.
3. **Ad hoc or for-cause**: Monitoring as needed or requested

WITHDRAWAL OF PARTICIPANTS

Subjects maybe withdraw from participation in the study at any time. Any data collected up to the time of withdrawal will be retained for study purposes.

Participants may withdraw from the study at any time at their own request or they may be removed at the discretion of the investigator for safety, behavioral or administrative reasons.

The reason(s) for discontinuation will be documented and may include:

- Participant voluntarily withdraws from treatment;
- Participant withdraws consent;
- Participant is unable to comply with protocol requirements;
- Researcher staff decides that continuation with the study would not be in the best interest of the participant.

Subjects will be considered as not evaluable if they choose to drop out voluntarily and will therefore be replaced. However, if an adverse event is experienced and it is deemed the subject must be removed from the research study for safety; that subject with not be replaced. This determination will be made by the medical monitor and will be reflected in subjects research record in Redcap.

Subject accrual will stop at any point in the study if two or more subjects (out of the 12-14) experience an AE at any of the dose levels following baseline. This AE will be defined as subjects experiencing the first sign of troponin leakage (detection in the blood), and will be immediately terminated from the study having been confirmed by Dr Richard Harvey. With troponin levels being drawn post each hypoxia session this will be easy to identify. As mentioned, subjects cannot leave the clinic until their results have been verified.

RISKS TO PARTICIPANTS

Potential risks related to acute intermittent hypoxia, although mild, may include headaches, lightheadedness, dizziness, altered vision, respiratory distress, cyanosis, increase in breathing rate, sweating, muscle spasms and fainting. There can be alterations in the field of vision, a sensation of numbness or tingling and feelings of euphoria.

In case of a sudden emergency ROC (Resident on Call) will be summoned via the hospitals loud speaker to attend to the participant.

There are two major risk groups that could be determined; cerebral or cardiac.

Cardiac adverse events could be silent (non-symptomatic) or non-silent (symptomatic).

In the event of a silent event, such as a significant change in EKG or in cardiac enzymes subjects will be given a referral to a cardiologist at Northwestern to undergo more comprehensive assessments to confirm the severity of the adverse event. This will be indicated by the troponin levels out of the normal clinical range. The troponin level is identified from the blood sample provided at that visit.
A non-silent event will require the ROC (resident on call) to be call to the clinic room for immediate and urgent attention for the chest pain experienced.

In both cases the medical monitor will determine the next steps for the safety of the subject, which may include a visit to the emergency room located at Northwestern Memorial Hospital.

A cerebral adverse event is experienced through a change in vision and/or from changes in the neurological assessment. The Medical monitor will be informed of this outcome and will provide advice accordingly.

POTENTIAL BENEFITS TO PARTICIPANTS
There may be no direct benefit for participants enrolled in this study.

DATA MANAGEMENT AND CONFIDENTIALITY
All case report forms will be kept in study binders for each subject. Each subject will only be identified by subject number (subject ID). Study binders will be kept in a locked cabinet on the 26th floor of the Shirley Ryan Abilitylab. This is not a public access floor.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS
The PI has the ultimate responsibility for this study. Dr Rymer will oversee the entire project with respect to study design, recruitment processes, study progress, participant safety, data interpretation and data reporting. The Data and Safety Monitoring Board (DSMB) will consist of at least three individuals. An independent Medical Safety Monitor will review all adverse events and make recommendations to the Data and Safety Monitoring Board and the PI.

The DSMB Chair will be notified when the first patient is enrolled in the study. The initial review of data by the full DSMB will take place within the first week the first subject has completed the intervention. DSMB Chair and the full DSMB will determine additional reviews of the data. If necessary, the DSMB Chair can request more frequent reports. Whenever the Chair thinks it is necessary, a full DSMB conference call will be held. The DSMB Chair will be notified if any

unanticipated adverse events, serious adverse events and/or any deviation from the investigational plan that affects the safety, rights and welfare of the patient occur.

Medical Monitor:
The medical monitor on this study is Dr. Roth, MD (Physical Medicine and Rehabilitation, Shirley Ryan AbilityLab). Responsibilities will include:

1. Observing recruitment and enrollment procedures and the consent process for individuals.
2. Overseeing study interventions and interactions.
3. Reviewing monitoring plans.
4. Overseeing data matching, data collection, and analysis
5. Placing the MRI order confirm the presence of a stroke before the subject can be scheduled for intervention visits
    A detailed analysis of the size and location of the stroke will be done by a radiologist during data collection/analysis after all subject data has been collected.
6. Reviewing the ECG reading to confirm clinical significance/non clinically significant

Additionally:
1. May discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research;
2. Shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report;
3. Shall have the responsibility to promptly report their observations and findings to the IRB

The medical monitor will be placing the order for the research MRI and reviewing the EKG. He will acknowledge receipt of the EKG strip and sign off by stating "not clinically significant" or "clinically significant". If readings are clinically significant it will be passed over to a cardiologist who take a deeper look into the adverse event.
Once results of the MRI have been generated medical monitor will review and sign off on the images to confirm stroke occurrence for safety of the subject receiving hypoxia. If results generate safety concerns, then a radiologist on subscription with SRALab will be contacted for assistance.

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS
An ongoing dialogue will take place during the subject's introduction to the study and the informed consent process to ensure that the candidate fully understands the study, what is required, the risks and the benefits, and their rights as a participant. Only participants that are able to independently consent in English will be approached.

All information provided by participants will be de-identified. Documentation will be stored within a locked cabinet within a specified specialty lab within the Shirley Ryan AbilityLab. Furthermore, all data stored electronically will be password protected. Only authorized individuals will have access to files and data, as entered within the Northwestern IRB site.

COMPENSATION FOR RESEARCH-RELATED INJURY
N/A

ECONOMIC BURDEN TO PARTICIPANTS
There will be no costs to the subject for participating in this study.

CONSENT PROCESS
After identifying potential subjects, they will be invited to return to the Shirley Ryan Abilitylab for a research appointment to complete screening and consent. This appointment will be arranged and coordinated by the designated research coordinator for the study. The informed consent documentation will be reviewed with the participant and authorized research personnel prior to any testing or interventions. The principal investigator/medical monitor will be available for any questions regarding the consent and the participant.
This process will be completed in a private space within the Shirley Ryan AbilityLab. Participants will be encouraged to ask questions related to study purpose or procedures, or have family present. They will be provided a copy of the signed informed consent.

Individuals who are not yet adults, are not cognitively intact, unable to understand English or unable to consent will not be recruited for this study.

WAIVER OR ALTERATION OF CONSENT PROCESS
Not applicable

PROTECTED HEALTH INFORMATION (PHI AND HIPAA)
Protected health information will remain for the most part in the Cerner Research Medical Record which is the same system used for clinical medical records and is accessible only by authorized personnel with electronic ID and password. Any other PHI will be in paper records (e.g., CRF's) which will include only the subject's study ID and not their names. Paper records will be stored on the 26th floor of the Shirley Ryan Abilitylab in the research offices which are locked when unoccupied. These records are kept in locked cabinets as well.
All paper records will be kept for three years after the end of the study and then will be destroyed in accordance with the Shirley Ryan Abilitylab policies

SETTING:
This single site study will have all research procedures and visits take place at the Shirley Ryan AbilityLab, located on 355 E. Erie St, Chicago, IL 60611.

QUALIFICATIONS TO CONDUCT RESEARCH AND RESOURCES AVAILABLE
W. Zev Rymer, MD, PhD is the Primary Investigator and will oversee all aspects of the overall development, administration, and direction of the project. Dr. Rymer serves as Director of the single motor unit lab, a position he has held since 2016, and Director of Research Planning at the Shirley Ryan Abilitylab.

Alexander Barry, MS, CCRC is a clinical research coordinator and engineer at the Shirley Ryan AbilityLab. He oversees the protocol, logistics, and interventions of the study. He has extensive research experience with clinical trials taking place in the hospital.

Milap Sandhu, PT, PhD is a research scientist at the Shirley Ryan AbilityLab. He will oversee the administration of hypoxia and provide overall guidance in regard to the scientific approach.

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evaluate whether there are subclinical signs of cardiac damage, including increased levels of troponin, creatine kinase-MB (CK-MB), inflammatory biomarkers and other muscle enzymes. ***Subject accrual will stop at any point in the study if two or more subjects (out of the 12-14) experience an AE at any of the dose levels following baseline***.

Statistical Considerations for AE detection

Sample Size Considerations: We used simulation studies to determine operating characteristics for detecting excessive AEs for this trial. Under the null hypothesis, we assume that the AE rate is extremely low, $H_0: p_1 = p_2 = p_3 = 0.01$, where p_j corresponds to the probability of AE at dose level d_j. We further assume that p_j are uncorrelated under H_0. Under the alternative hypothesis, we consider three types of dose-toxicity curves: (I) $p_2 = p_1 + 0.025, p_3 = p_1 + 0.025$, (II) $p_2 = p_1 + 0.025, p_3 = p_1 + 0.050$, and (III) $p_2 = p_1 + 0.050, p_3 = p_1 + 0.050$. We considered p_1 ranging between 0.05 and 0.125. Under the alternative hypothesis, subjects' outcomes (AE, yes or no) at the three dose levels were simulated as correlated binary data, assuming within-subject correlation between AE occurrence at the various dose levels is $\rho = 0.3$. R=10,000 trials were simulated under each scenario using the "bindata" package in R. For each simulated trial, we calculated the number of AEs occurring among all patients at any dose level, and hypoxia treatment was declared unsafe if ≥ 2 subjects in a given trial experienced an AE at any dose level. Type I error rate (α-level) was calculated as the proportion of all R=10,000 trials simulated under H_0 that were declared unsafe. Power was calculated as the proportion of all trials under a particular alternative hypothesis scenario that were declared unsafe. We considered sample size n=12 or 14. Under H_0, the probability of declaring hypoxia unsafe (Type I error) was 0.048 when n=12 and 0.062 when n=14. For example, for a dose-toxicity curve of type III, we will have 80.8% power with n=12 when $\{p_1 = 0.08, p_2 = 0.13, p_3 = 0.13\}$. More generally, when n=12, we will have >80% power under all dose-toxicity scenarios with $p_1 \geq 0.10$. When n=14, we will have >80% power to under all dose-toxicity scenarios when $p_1 \geq 0.08$.

Aim 2: To collect preliminary data about the potential efficacy of this approach in improving voluntary muscle strength, as a prelude to a more extensive clinical trial. We plan to use a standard grip strength meter (Jamar Plus Digital dynamometer) to record maximum grip strength and pinch strength before AIH, and following each AIH exposure sequence. We will use a three second maximum contraction, and record the mean of three grip strength estimates at each time epoch, with at least a 60 second rest periods between trials to prevent fatigue. We will also test elbow flexion and extension strength, using a commercial dynamometer in a similar fashion. We hypothesize that similar to our SCI studies, we will start to see increases in voluntary strength in the impaired hand within 30 minutes, peaking at about 90 minutes after the AIH sequence is completed.

Statistical Analysis of Efficacy: AIH efficacy will be assessed at each O2 dose level for all subjects, as continuous outcomes (e.g., strength). Data will be analyzed using generalized linear mixed models with repeated measures, with the outcome measure as the response variable, and dose level as a categorical predictor to allow for a nonlinear dose-response relationship. Baseline measurements will be adjusted for in the model. Within-subject correlation between multiple doses will be accounted for with an appropriate variance-covariance structure (e.g., compound symmetry or autoregressive of order 1, AR(1)). Model fit and assumptions will be checked using model diagnostic techniques for mixed models, and the outcome variable may be transformed to satisfy the normality assumption. Post-hoc tests will be used to determine whether there is an improvement in outcome relative to baseline at any dose level, and whether there is a difference between dose levels.