Intermittent Hypoxia to Enhance Motor Function after Spinal Cord Injury

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1. Title: Intermittent hypoxia to enhance motor function after spinal cord injury: a pilot study.

2. Investigators:

Principal Investigator:

- Emily Fox, PT, DPT, PhD

Co-Investigators and study staff:

- Tommy Sutor, MS, CSCS
- Kathryn Doughty, DPT, NCS
- Alicia Vose, MS, CCC-SLP
- Joseph Welch, PhD
- Kelly Hawkins, DPT, NCS
- Christy Conroy, MSPT
- Arian Vistamehr, PhD
- Paul Freeborn, BS (Study Coordinator)

3. Abstract: Spinal cord injury (SCI) affects the trunk muscles that control respiration and posture. Decreased respiratory muscle function can lead to diseases of the respiratory system, which are the primary cause of death and significant cause of re-hospitalization after SCI. Deficits in postural muscle function affect one's ability to balance, safely maintain a seated position, or ambulation after SCI, severely impacting daily activities such as self-care and feeding skills. Most people have incomplete SCI, and therefore have spared motor pathways. Thus, the majority of people with SCI would potentially benefit from treatments that strengthen spared motor pathways. One such treatment that strengthens spared motor pathways after spinal cord injury is acute intermittent hypoxia.

Acute intermittent hypoxia induces an episodic release of serotonin near motor neurons, beginning an intracellular cascade of events which results in an increased affinity of the motor neuron for excitatory neurotransmitters. Through this mechanism, spared motor pathways after SCI can potentially be strengthened by acute intermittent hypoxia. A number of studies have demonstrated improved function in individuals with SCI with daily (5-10 days) mild episodes of acute intermittent hypoxia. Benefits have been demonstrated in ventilation, maximal ankle plantarflexion torque, walking speed and walking endurance. Based on the mechanisms of action and the demonstrated benefits of acute intermittent hypoxia, this intervention also may improve function of muscles that control respiration and posture.

This study will test the hypothesis that a single session of acute intermittent hypoxia will increase strength and activation of the trunk muscles that control respiration and posture, leading to improved scores on functional assessments in individuals with SCI. Our long term goal is to better understand the therapeutic potential of acute intermittent hypoxia combined with physical rehabilitation for individuals with SCI.

4. Background:

Most individuals, regardless of the severity of their SCI, maintain some intact spinal connections, creating an anatomical connection to the brain after injury (Flynn et al., 2011; Sherwood et al., 1992; McKay et al., 2004; Squair et al., 2016.) Different types of

interventions, such as physical therapy, occupational therapy, electrical stimulation, or a combination thereof seek to take advantage of these intact connections, including spared motor pathways, to enhance function after SCI. The abundance of research on functions such as walking or grip strength has led to many intervention options for rehabilitating these abilities. However, research and interventions to enhance strength of trunk muscles which control respiration and posture is lacking, and therefore many people with SCI experience respiratory and postural impairments (Wang, 2016). For example, forceful respiratory maneuvers such as clearing the airway of secretions depend highly on abdominal and trunk motor function (De Troyer et al., 1986; Yokoba et al., 2003). Due to weak trunk muscles which contribute to these respiratory maneuvers, respiratory complications remain the second-leading cause of re-hospitalization after SCI (Kirshblum et al., 2004.) A majority of people with SCI would benefit from improved strength and activation of trunk muscles that control respiration and posture. Acute intermittent hypoxia may be a way to strengthen and activate those muscles through spared motor pathways.

The mechanisms by which acute intermittent hypoxia strengthens and increases activation of muscles after SCI has been established by extensive animal research. Briefly, acute intermittent hypoxia activates the carotid body chemoreceptors, triggering intermittent serotonin release in or near spinal motor nuclei. Subsequent serotonin receptor activation triggers new brain-derived neurotrophic factor synthesis, enhancing synaptic input, motor output, and the strength of muscle contractions (Gonzalez-Rothi et al., 2015; Baker-Herman et al., 2004; Fuller et al., 2003). Regarding functional recovery, rodent studies demonstrate acute intermittent hypoxia improves breathing and forelimb function (Lovett-Barr et al., 2012), and complex locomotor tasks (Prosser-Loose et al., 2015) after SCI. In each of these aforementioned studies, no negative reactions to intermittent hypoxia were reported for any of the rodents.

Human clinical studies have yielded results similar to those of animal studies. Acute intermittent hypoxia can enhance ventilation (Tester et al., 2014; Sankari et al., 2015), ankle strength (Trumbower et al., 2012; Lynch et al., 2016), walking speed, and walking endurance (Hayes et al., 2014; Navarrete-Opazo et al., 2016b) after SCI. In these human studies, no adverse events were reported. Subjects tolerated the intervention and were unable to distinguish between a sham and experimental condition.

In one study, Trumbower et al. (2012) reported a mean increase in voluntary ankle torque of 82% in individuals with incomplete spinal cord injury, following 15, 60-90 second exposures of 9% oxygen alternated with 15, 60-second exposures to room air. In another study, Navarrete-Opazo et al. (2016b) found significant improvements in 10-meter walk times and 6 minute walk test differences for individuals with spinal cord injury. These results were accomplished after four weeks of repeated walking training and repeated bouts of 15, 90-second exposures to 9% oxygen alternated with 15, 90-second exposures to room air. In both studies, results were significantly improved over control subjects who received sham treatment, and no adverse events were reported for any subject.

Research examining the effects of acute intermittent hypoxia on trunk muscles that control respiration and posture has not been conducted. However, based on the mechanisms and prior studies demonstrating benefits of intermittent hypoxia, we expect acute intermittent hypoxia to improve the strength and activation of trunk muscles that control respiration and posture. The fundamental hypothesis guiding this proposal is a

single session of acute intermittent hypoxia improves trunk muscle strength and activation, thereby improving respiratory strength and postural function after SCI.

5. Specific Aims:

Specific Aim 1: test the hypothesis that a single session of acute intermittent hypoxia improves respiratory strength in individuals with SCI compared to a sham treatment. Respiratory strength will be quantified using standardized measures of maximal inspiratory pressure, maximal expiratory pressure, forced vital capacity, and mouth occlusion pressure (P0.1). Muscle activation during the respiratory tests will be quantified using surface electromyography (EMG).

Specific Aim 2: test the hypothesis that a single session of acute intermittent hypoxia will enhance postural strength and function in individuals with SCI compared to a sham treatment. Postural strength will be assessed in a seated position with standardized procedures, using a hand-held force dynamometer. Postural function will be quantified by assessing performance in 4 tests of sitting ability contained within the Neuromuscular Recovery Scale. Additionally, trunk and lower extremity muscle activation during postural tasks will be assessed using EMG. Additional non-invasive sensors may be worn to assess joint angles or forces applied during testing.

Aim 2a: Postural control and trunk strength contributes significantly to the ability to stand and walk. For subjects who are able to safely stand or walk short distances, a 10-meter walk test, 30-second chair stand test, or timed up-and-go test may be performed. Trunk and lower extremity muscle EMG will be recorded during testing. Sensors also may be worn to assess joint angles or forces generated during these tests.

6. Research Plan:

Inclusion criteria:

1) Male or female, ages 18-65; 2) medically stable; 3) >6 months post-SCI; 4) SCI affecting segments between C4-T12; 5) no other known neurological disorders; 6) no severe musculoskeletal impairments, open wounds, or skin lesions that would limit participation in functional assessments; 7) able to provide informed consent

Exclusion criteria:

1) Not meeting any of the above inclusion criteria; 2) presence of a self-reported uncontrolled medical condition including, but not limited to: cardiovascular disease; sleep apnea; obstructive lung disease; severe neuropathic or chronic pain; severe recurrent autonomic dysreflexia; 3) severe, untreated bladder or urinary tract infection; 4) women who report being pregnant or test positive on a pregnancy test.

We aim to screen 40 individuals with SCI and enroll 30 subjects in this study.

Recruitment and enrollment

The target population will be individuals with SCI. Study procedures will take place at Brooks Rehabilitation in Jacksonville, FL, and the University of Florida's Health Science Center Department of Physical Therapy Research Laboratories. Subjects will be recruited in four ways.

1) The study team will notify Brooks clinicians about the study via e-mail. The study team may also notify other clinicians who work with individuals with SCI through e-mail. Those clinicians may then notify their patients about the study, who may then contact Dr. Emily Fox, the principal investigator, or other study staff for more information.
2) Potential subjects also may be informed of the study via IRB approved flyers, which will be hung in the Brooks Health System, the Health Science Center at UF, and in the community. Contact information will be provided on the flyer so that interested potential subjects may contact Dr. Emily Fox for more information.
3) Information regarding the study also may be provided through word of mouth or general presentations such as at support groups and community meetings (i.e. such as meetings for those with SCIs).
4) All individuals admitted to Brooks Health System sign a Brooks Notice of Privacy Practices upon admission which indicates their information may be used for research. This is a standard practice for the Brooks Health System facilities. For individuals who sign this form, Brooks Research Recruitment personnel have permission to access their medical records for research-related purposes. Therefore, Brooks Research Recruitment personnel would be aware of our study (as it is being conducted at a Brooks facility) and would include this as one of several potential research study opportunities when contacting Brooks patients. This procedure is independent of our research study procedures and is carried out by Brooks Research Recruitment personnel; potential participants will only be contacted for screening for this specific study if they indicate to Brooks Research Recruitment personnel that they want to learn more about our study.

Individuals who are potentially interested in enrollment in the study will have ample opportunity to ask questions, talk with study staff and review the informed consent document. If the individual indicates that he/she would like to determine if they qualify to participate in the study, they will be asked to complete a basic screening questionnaire to determine if they are eligible to participate. The screening may occur over the phone or in-person depending on the preference of the potential participant. The IRB-approved screening form, designed to avoid collection of protected health information, (see attached) will ask subjects general information about their eligibility. This information may include health status, physical functioning, history of neurologic impairments, the date of their SCI, and other information pertaining to inclusion/exclusion criteria. If an individual completes the screening process, is eligible to participate, and would like to enroll, a study staff member will meet with them to complete the informed consent process. The informed consent form (ICF) will be used to guide the process. The potential participant will have the opportunity to review the ICF ahead of time. Potential participants will be informed there may be no direct benefit of participation. Study staff will be available to answer questions. The informed consent process will occur prior to study procedures but may occur on the same visit as the anticipated study procedures. The informed consent process also may be scheduled on a day prior to study procedures based on the preference of the potential participant. We anticipate that some potential participants may want to meet with study staff ahead of time and others will prefer to complete the informed consent form and procedures on a single visit.

Participants may be asked to participate in the following activities during an assessment. They will be informed that they may choose not to perform any aspect of the study.

Overview

This pilot study will utilize a single blind, placebo-controlled, randomized, repeated measures experimental design. Participants will be asked to come in for two visits at least 7 days apart. If study procedures are not completed in the two sessions (such as due to equipment malfunction or unexpected time restrictions/events), an additional session may be required to complete procedures. This will be scheduled at the participant's convenience. Participants will be asked to wear comfortable, casual clothing for participation ahead of time, during their phone screening. In the first visit, participants will be asked to complete a demographic health and function questionnaire. Questions may address their injury level, medical history, impact of their condition on physical functioning, breathing function, and their walking or other functional abilities. Participants may then be asked to undergo standardized assessments of muscle strength and sensory function.
Following collection of this baseline information, the remainder of the first visit will be similar to all subsequent visits. Visits will include:

- Pre-assessments of respiratory strength, postural strength and function, EMG of trunk muscles, and trunk positioning using non-invasive sensors. For subjects who can stand or walking with assistance, ambulation assessments may be conducted while also recording trunk and lower extremity EMGs, and joint angles or forces using non-invasive sensors.
- 15 short exposures to hypoxic air, or a sham exposure, in a randomized order (details below)
- Post-assessments of respiratory strength, postural function, ambulation, EMG of trunk muscles, and trunk and joint positioning using non-invasive sensors

Each session will last about 3 hours and no longer than 6 hours. Rest breaks will be provided at regular intervals and at any time the participant requests. A study clinician will oversee the study procedures and monitor the participants, including a baseline assessment of vital signs (blood pressure, heart rate, and blood oxygen saturation) for safety.

For participants who provided consent for photography and/or video, recordings or photographs may be obtained during their session. Recordings or photographs will only occur based on the consent provided by each participant. The participant will be informed that they may refuse the use of photography or video recordings at any time during the procedures.

Study Procedures

Participants will be asked not to ingest any caffeine within four hours of each visit, as caffeine can affect the response to acute intermittent hypoxia. After consenting, female subjects of potential childbearing age will be asked to take a pregnancy test. A pregnancy test will not be required if a medical condition that prohibits pregnancy, such as hysterectomy, is reported. Participants will be able to sit or lie on a mat table comfortably during set-up. Instrumentation will generally include EMG sensors over the trunk,

respiratory, and lower extremity muscles, which may be secured with hypoallergenic tape. Electrogoniometers to measure joint angles may be attached at the hips, knees, or along the vertebral column with double-sided medical tape. Pressure sensors also may be placed under the heel or ball of the subject's feet, and worn in the shoes. All sensors are non-invasive and will be removed at the conclusion of testing.

1) Baseline and demographic assessments:

Participants may undergo the ISNCSCI (International Standards for the Neurological Classification of Spinal Cord Injury) examination. The ISNCSCI is a standardized assessment used to determine the motor and sensory impairment and severity of a spinal cord injury (Kirshblum et al., 2011; previously known as the ASIA examination based on the American Spinal Injury Association). A study clinician will complete this assessment using standardized procedures. Subjects may also be asked general demographic information via and IRB-approved demographics form about their injury, rehabilitation history, current health status, and current activity and exercise levels.

2) Standardized assessments of respiratory function

These clinical tests of respiratory function will be administered according to standardized guidelines (American Thoracic Society, 2002; Miller et al., 2005). Clinical outcomes from these assessments will allow us to assess the level of respiratory function of subjects vs. predicted values derived from able-bodied individuals based on the subject's age. Participants will complete up to, but no more than 10 trials of each test in order to ensure a maximal effort was obtained. Rest breaks will be allowed at the subject's request as often and as long as they are needed. Oxygen saturation and breathlessness will be monitored for safety. The assessments may include:

a) Maximum inspiratory pressure – an evaluation of inspiratory respiratory muscle strength, assessed using a digital respiratory pressure meter.

b) Maximum expiratory pressure – an evaluation of expiratory respiratory muscle strength, assessed using a digital respiratory pressure meter.

c) Forced vital capacity – The forced vital capacity is an evaluation of how much air a person can forcefully exhale after a maximal inspiratory effort. Tests are conducted using a digital spirometer or respiratory monitor.

d) Mouth occlusion pressure (P0.1) – P0.1 is an index of the neuromuscular drive to breathe, independent of lung mechanics. Tests are performed by having participants breathe naturally through a mouthpiece with their eyes closed. Upon completion of one breath, at random, the tester manually occludes the mouthpiece for less than half of one second. The pressure generated in the first 0.1 seconds of the participant's initiation of inhalation will be recorded as the participant's P0.1.

3) Standardized assessments of functional ability

3a) Functional ability of the trunk muscles

The degree to which subjects can independently perform certain tasks that require trunk muscles will be assessed with the Neuromuscular Recovery Scale. Four components of this standard, reliable and responsive clinical test of functional ability for people with SCI

will be used (Tester et al., 2016). Standardized instructions will be provided to the subjects for each component. A study staff member trained in administering the Neuromuscular Recovery Scale will supervise testing to ensure proper execution and subject safety. These components include:

a) Sit – testing the subject's ability to sit tall with proper posture when the feet are flat on the floor.

b) Reverse Sit-up – subjects will be asked to lower themselves from sitting up to lying supine on a mat as slowly and controlled as possible.

c) Sit-up – the subject's ability to sit up from lying supine on a table while the feet are on the ground will be assessed.

d) Trunk extension in sitting – while seated on a table with feet on the floor, subjects will be asked to lean forward with their arms hanging down, and then return to a seated position without using their hands.

3b) Assessment of postural muscle strength in sitting

A standardized and reliable protocol using a hand-held force dynamometer will be used to assess postural muscle strength for maintaining upright sitting (Larson et al., 2010). A study staff member will apply force to the participant's chest (anterior strength), upper back (posterior strength), or either shoulder (lateral strength) while the participant is asked to resist the force to remain seated, unsupported, on a mat table. The force applied at the point that the participant loses balance or can no longer sit unsupported will be recorded as strength in that direction. This test of seated strength will be carried out by a study staff member experienced in administering the test, and the participant will also be spotted by an additional therapist or research assistant in case the participant is unable to balance themselves during the test.

3c) Assessments of ambulation

These are standard, clinical tests of functional ability that may be used to assess mobility in subjects who can safely attempt these tests. Outcomes from these assessments will allow us to quantify the level of functional mobility of our subjects, and if acute intermittent hypoxia affects levels of functional mobility. These assessments may include:

a) Timed Up and Go Test – Assessment of walking balance, and fall risk based on the participant's ability to stand up form a seated position, walking 10 meters, turn around and return to the chair and sit down.
b) 10 meter walk test – Assessment of walking speed over a distance of 10 meters
c) 30 second chair stand test – Assessment of lower body strength and power in mobility impaired populations, by assessing the maximum amount of times that a participant can safely stand up from a seated position in 30 seconds.

4) EMG activation and activation patterns of respiratory, postural, and lower extremity muscles

Muscle activity will be collected and measured by surface EMG. By analyzing the electrical activity generated during a muscle contraction, EMG analyses allow us to understand timing and amplitude of muscle activation as well as the frequency content of the EMG signal. Thus, we will be able to assess how the nervous system's control of

muscles is disrupted following SCI, and whether this control will be affected by acute intermittent hypoxia.

Using standard surface preparation (cleaning the skin with water or alcohol pad, shaving of excess hair) and electrode placement, EMGs will be collected from postural muscles of the trunk, accessory respiratory muscles, and lower extremity muscles using surface EMG electrodes. EMG sensors will be attached to the skin with hypoallergenic medical tape. Before data collection, tests of each EMG channel may be performed to ensure clear signals and to check for crosstalk. EMG information will be used to assess when different postural, respiratory, or lower extremity muscles fire during the assessments, as well as when certain muscles fire in relation to one another.

5) Trunk positioning and function using non-invasive sensors.
Electrogoniometers may be placed on the hips, knees, or spinous process on the back of subjects to allow for quantification of the angle of the spine or hips achieved during each task. These sensors will be attached to the skin using double-sided, medical grade, hypoallergenic tape. Information gained from these movement sensors will allow for quantification of how a subject performs respiratory tests and postural muscle functional tasks.

Very thin, light pressure sensors made of flexible plastic may be worn in the subjects' shoes, under their heels and/or the ball of their feet. As the subjects will be seated with their feet on the ground during the tests of postural muscle function, the non-invasive pressure sensors may help give an idea of how much force the subject is imparting on the ground during different tasks. Since the seated tasks of postural muscle function are dynamic in nature, the amount of pressure subjects will have to apply to the ground to accomplish the tasks must change. Quantifying the amount of force the subject applies to the ground may better inform us how well they are doing a particular task.

<u>Delivering an acute intermittent hypoxia or a sham procedure</u>
Acute intermittent hypoxia is a safe, non-invasive technique to improve muscle strength and activation after SCI. All sessions will be monitored by a study clinician with experience in both delivering acute intermittent hypoxia, and treating individuals with SCI. Hypoxic intervals will be delivered with a commercially available sports performance hypoxicator. A unit capable of delivering a fraction of inspired oxygen ranging from 21%-9% will be used. Air will be delivered into a mask that will be secured comfortably over the subject's nose and mouth. An SpO2 sensor that monitors oxygen saturation of the blood will be used to monitor the subject's response to hypoxic intervals; if the user's SpO2 falls below 80% during a round of hypoxia, the fraction of inspired oxygen delivered by the hypoxicator will be rapidly increased in order to bring the user's SpO2 back over the safety threshold. 80% SpO2 is adequate to induce the effects of hypoxia without causing adverse safety events (Trumbower et al., 2012; Hayes et al., 2014; Lynch et al., 2016; Navarrete-Opazo et al., 2016a, 2016b).

Acute intermittent hypoxia will be provided to the subject by delivering 15 brief exposures (60 to 120 seconds) of hypoxic air alternated with 15 brief exposures (60 to 120 seconds) of room air. The amount of oxygen delivered during hypoxic exposures may range from 15%-9% fraction of inspired oxygen, compared to 21% oxygen in normal atmospheric air. Various protocols have previously used intervals and oxygen concentrations within this range (Trumbower et al., 2012; Tester et al., 2014; Navarrete-Opazo et al., 2016b). To switch from hypoxic to room air, a four-way valve will be attached to the subject's mask which can allow air from the hypoxicator, or ambient air from the room to flow into the mask. The amount of oxygen delivered and the length of the hypoxic and room air exposures will be adjusted during the protocol to maintain safety, tolerability, and a consistent hypoxic response of the subject. The total time of all episodes of alternating room and hypoxic air will be between 30-45 minutes. Alternatively, a sham protocol will be administered in which 21% fraction of inspired oxygen will be delivered by the hypoxicator during hypoxic intervals, and room air will be delivered through the four-way valve during room air intervals.
Subjects will rest and be asked not to ingest any food or drink other than water for 30 minutes after the delivery of acute intermittent hypoxia, because fullness of the stomach may impede full depression of the diaphragm and compromise maximal respiratory measures. Following this, maximal inspiratory pressure, maximal expiratory pressure, forced vital capacity, mouth occlusion pressure, postural strength assessments, Neuromuscular Recovery Scale components, and assessments of ambulation will be re-assessed as outlined above. EMG electrodes, electrogoniometers, and pressure sensors will be worn during the acute intermittent hypoxia and rest period, and will be removed following the conclusion of all testing.

Following the procedures for each visit, a $75.00 gift card or check will be provided to participants. Thus, upon full completion of two visits, subjects will have been provided with $150.00 following their participation in the study. If participants are asked to come in for a third session, an additional $75.00 will be provided.

No standard therapies for people with SCI currently include acute intermittent hypoxia. Thus, this research does not intend to directly deliver therapeutic benefits to subjects. Rather, this research seeks to determine the therapeutic potential of acute intermittent hypoxia on respiratory strength and postural muscle function for people with SCI. As an alternative to participating in this study, subjects may not enroll or choose to withdraw at any time.

Statistical Plan

Percent changes in maximal inspiratory pressure, maximal expiratory pressure, forced vital capacity, mouth occlusion pressure, EMG parameters, spine and hip angles, postural strength measures, absolute Neuromuscular Recovery Scale scores, and assessments of ambulation will be compared pre- and post-intervention for both experimental conditions. Changes for each subject will be averaged, and the group mean change ± standard deviation will be reported and compared across experimental conditions. Statistical differences between the group means for each variable in each experimental

condition will be compared using t-tests. Multiple comparisons will be accounted for. Statistical analyses will be carried out by the study investigators.

Acute Intermittent Hypoxia Safety Profile

Acute intermittent hypoxia is a safe, non-invasive, relatively inexpensive way to strengthen spared motor pathways after SCI. With the mild to moderate acute intermittent hypoxia protocols used in neurorehabilitation research (Trumbower et al., 2012; Hayes et al., 2014; Navarrete-Opazo, 2016b), the severity of hypoxia, number of episodes, and exposure duration are low (e.g. 10-15, 1-2 minute episodes per day). The acute intermittent hypoxia protocols for this study are not similar to these more severe hypoxia exposures, as may be experienced during sleep apnea (Gozal & Kheirandish-Gozal, 2008).Chronic intermittent hypoxia experienced in obstructive sleep apnea is severe and can consist of 80-400 episodes per night for many years, and is known to have detrimental systemic effects. While too high a dosage of intermittent hypoxia can be dangerous, lower doses, such as those previously published in acute intermittent hypoxia studies, can be both safe and beneficial after SCI (Navarrete-Opazo & Mitchell, 2014). All studies to date have demonstrated acute intermittent hypoxia-induced functional benefits in humans with SCI without adverse events or unwanted side effects (Trumbower et al., 2012; Hayes et al., 2014; Tester et al., 2014; Sankari et al., 2015; Lynch et al., 2016; Navarrete-Opazo et al., 2016a, 2016b). In these studies, changes in SpO2, blood pressure and heart rate were closely monitored, and no adverse events were reported. All study participants tolerated procedures without discomfort, and most were unable to distinguish acute intermittent hypoxia versus sham normoxia. In rodent models, repetitive acute intermittent hypoxia does not cause hypertension or weight loss (Wilkerson et al., 2009), hippocampal gliosis or neuronal cell death (Lovett-Barr et al., 2012; Satriotomo et al., 2012). Accumulating data indicate that acute intermittent hypoxia is safe and will induce meaningful gains in respiratory function in adults with chronic incomplete SCI.

Safety monitoring

The testing procedures are not particularly strenuous, but could be tiring for some individuals. All participants will be given rest breaks and informed that they may rest or terminate their participation at any time. During brief hypoxic episodes, subjects may become sleepy. If subjects appear to be falling asleep they will be asked to stay awake and report any concerns or feeling of discomfort. The criteria for termination include subject complaints of confusion, dyspnea, onset of angina, and/or atypical bradycardia (drop in heart rate greater than 10 beats per minute). In addition, should the subject's heart rate exceed 70% of their age-predicted maximum (220 – age) then the assessment will halt. In this case, the subject will be asked to rest while blood pressure and heart rate are monitored and will resume only when they return to acceptable values. If any of these conditions persist after rest, the individual's primary physician may be called and referred for evaluation. If the individual complains of angina at rest, loss of consciousness occurs, or cardiac arrest, emergency medical services through 911 will be called immediately.

SpO2 will be continuously monitored throughout the study. If SpO2 drops below a safety threshold of 80%, the fraction of inspired oxygen delivered by the hypoxicator will be rapidly increased until the subject is back over 80% SpO2. This threshold is chosen based

on 80% being the average number the SpO2 has dropped to in previous studies without any adverse events occurring (Trumbower et al., 2012; Hayes et al., 2014; Lynch et al., 2016; Navarrete-Opazo et al., 2016a). If the subject's SpO2 remains under 80% or continues to decline after the percent oxygen delivered has been increased, the mask will be removed and the procedure will be aborted. In the event that a subject reports discomfort and does not wish to continue, the mask will be removed and the procedure will be aborted. None of these adverse events are anticipated, however if they occur, each will be handled using the aforementioned methods.

Confidentiality and protecting subject privacy

The risks related to collecting and sharing protected health information and study-related data will be addressed in several ways. First, upon enrollment in the study, a study ID will be assigned to the participant so that all study-related information pertaining to the subject uses the study ID. The document that links study-IDs to participant information will be stored in a separate location, not with the study-related data. The study procedures will be conducted in private locations at Brooks Rehabilitation or University of Florida Department of Physical Therapy laboratories, so that participant privacy may be maintained when conducting procedures such as clinical testing, administering questionnaires, or answering individual questions. All study procedures will be conducted in limited access areas with minimal traffic and personnel.

Protected health information collected as part of this study will be limited to the minimal necessary information to carry out procedures in this approved protocol. All hard-copy study related documents will be stored in locked file cabinets in locked offices. All electronic study related information and data will be stored on password protected computers and on password-protected institutional servers or encrypted electronic storage devices. Only approved study personnel will have access to research records.

7. Possible Discomforts and Risks:

Discomforts and risks from acute intermittent hypoxia are minimal. Many studies in animals (Gonzalez-Rothi, 2015; Baker-Herman, 2004; Fuller, 2003; Lovett-Barr, 2012; Prosser-Loose, 2015; Satriotomo, 2016) and humans (Trumbower et al., 2012; Tester et al., 2014; Hayes et al., 2014; Sankari et al., 2015; Navarrete-Opazo, 2016a, 2016b; Lynch et al., 2016) have established that acute intermittent hypoxia can be delivered safely with minimal risk of adverse events. In a human study with a sham control, subjects were not able to distinguish whether they were receiving acute intermittent hypoxia or the sham procedure (Hayes et al., 2014). All sessions will be monitored by a study clinician who has experience working with individuals with SCI and in delivering acute intermittent hypoxia.

Some participants may find the mask used during acute intermittent hypoxia uncomfortable, so the mask will be adjusted as necessary to ensure optimal comfort of the subject. Subjects may also feel sleepy during the brief episodes of hypoxia. However, this feeling typically lasts only for the duration of the hypoxic episode, as the episodes of hypoxia are alternated with breathing room air. Additionally, the percent of oxygen delivered will be increased, if necessary, to keep subjects above the safety threshold of 80% SpO2. All subjects will undergo the acute intermittent hypoxia protocol in a

comfortable chair with armrests and a headrest to ensure both comfort and safety if the subject does become sleepy.

Discomfort and risks of injury from functional assessments are minimal. All procedures are non-invasive and will be supervised by a study clinician with experience in testing breathing, postural muscle strength and function, and ambulation with individuals with SCI. Participants may rest at any time and may opt not to perform any of the study procedures. Participant responses will be closely monitored. During seated tasks of postural muscle function or ambulation, subjects may be assisted as necessary to safely complete the tasks.

The non-invasive sensors affixed with tape or wrapping may cause some mild discomfort or redness. Removal of the tape may be uncomfortable for some participants and participants will be made aware prior to application and removal. Some participants may not be accustomed to the study activities and may become fatigued during the testing. Rest breaks will be provided as needed.

8. Possible Benefits:

Participants may experience short-term improvements in total volume of air breathed in the hours following the procedure. Participants may also experience increased strength in postural muscles in the hours following the procedure. Any responses are not expected to last more than four hours. As these effects have not been tested formally in humans with SCI, responses will likely be variable across participants.

Future populations may benefit from knowledge of how acute intermittent hypoxia affects the outcomes of rehabilitation for people with SCI. Since all risks to the subjects are minimal, the risks are reasonable in relation to the knowledge that could potentially be gained from this pilot study.

9. Conflict of Interest:

There are no conflicts of interests relating to any of the investigators and this protocol beyond the professional benefit from academic publication or presentation of the results.

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