Physiological and Biochemical Response to Prolonged Exposure to Hypoxic Breathing in Healthy Volunteers

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ClinicalTrials.gov Protocol Number: NCT02860975

Detailed Protocol Version 11.0
Date: June 4th, 2018
This document has been written following the PHRC instructions about Detailed Protocol writing.

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I. BACKGROUND AND SIGNIFICANCE

a. Background

Human cells require energy in the form of ATP to carry out countless, crucial cellular processes including thermogenesis, ion homeostasis, immune responses, production of reactive oxidation species, and programmed cell death. Mitochondria are the organelles responsible for making ~90% of cellular ATP and consume ~90% of inhaled oxygen (O_2) to fuel such processes (*1*). A wide range of human diseases including Leigh Syndrome, Leber's hereditary optic neuropathy (LHON), and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome (MELAS) (*2-4*) result directly from a faulty mitochondrial respiratory chain (RC) which generates ATP through oxidative phosphorylation. In fact, over 150 single genes have been associated with disorders of the mitochondrial RC, making them the largest class of inborn errors of metabolism. Though each individual mutation is rare, the overall disease prevalence is estimated to be 1:4300 live births (*5*). These patients can present in both childhood and adulthood with multisystemic involvement, including brain, muscle, eye, liver and kidney. In addition to rare mitochondrial diseases, dysfunctional mitochondria have been associated with more common conditions including Alzheimer's disease, Parkinson's disease, type 2 diabetes, obesity, and skeletal muscle atrophy (*2*). Furthermore, mitochondrial disease has dramatic effects on the morbidity and mortality of affected individuals. Despite ongoing research, treatment options for mitochondrial disease are severely limited and have poor efficacy (*2, 6*). To date, no studies in adults or children have shown improvement in survival.

b. Previous pre-clinical or clinical studies leading up to, and supporting the proposed research and rationale behind the proposed research, and potential benefits to patients and/or society

Recently, Drs. Warren Zapol and Dr. Vamsi Mootha's laboratories, both at Massachusetts General Hospital, demonstrated in a mouse model that hypoxia is a potential new therapy for a primary mitochondrial disease (*7*). Hypoxia activates an evolutionarily-conserved adaptive response that allows mammals to adapt to limited oxygen levels and so decreases their reliance on mitochondrial oxidative metabolism. This includes the hypoxia-inducible factor (HIF)-dependent transcriptional program which is known to activate key oxygen-independent pathways (e.g. increased glycolysis and decreased flux through the Krebs cycle). Consequently, oxygen consumption is reduced to meet limited oxygen availability. In many mitochondrial diseases, there is no hypoxic signal and so this

protective response is not triggered, which potentially contributes to reactive oxygen species (ROS) toxicity.

Using a genome-wide *Cas9*-mediated knockout screen in cells, Dr. Zapol's and Mootha's study demonstrated inhibition of Von Hippel-Lindau (VHL) as the most effective therapeutic target for mitochondrial dysfunction. VHL inhibition leads to normoxic activation of HIF and thereby the hypoxia response. Across multiple cell lines treated with RC inhibitors, activation of the hypoxia response through small molecules (FG-4592) or knock-out of VHL led to rescue of growth defects in a dose-dependent manner. These cells increased glycolysis while decreasing basal oxygen consumption—a mechanism which may be further protective against ROS-toxicity in the setting of mitochondrial disease. The benefits of hypoxia extended to zebrafish models: activation of the hypoxia response through VHL-KO or small molecule activators showed a significant improvement in survival when the RC was inhibited.

In a murine model of Leigh syndrome, chronic hypoxic exposure led to marked improvements in both morbidity and mortality (please see figure A, B and C below)[7]. WT mice and mice lacking *Ndufs4*, a gene implicated in one of the more severe Leigh disease forms, were allowed to live in normobaric chambers containing 11% O_2 or 21% O_2. Normoxia-exposed KO mice recapitulated many of the Leigh syndrome symptoms (ataxia, failure to thrive, etc.) and neuropathologic characteristics. They began to lose weight at 30d of age (Figure B and C), became hypothermic, and all died at a median age of ~60d (Figure A). Meanwhile, the growth rate kinetics and core temperature of hypoxia-treated KO mice resembled that of hypoxia-treated WT mice. Furthermore, no hypoxia-treated KO mice died and the oldest mouse at the time of manuscript submission was >170d old. As a measure of morbidity, locomotor activity was tested using the rotarod test which assesses grip strength, balance and time to fatigue by placing mice on an accelerating, rotating rod (*8*). Normoxia-treated KO mice were unable to perform for more than a few seconds by 50d of age due to a combination of muscular weakness, inability to balance, and a loss of visual activity. In contrast, hypoxia-treated KO mice performed similarly to WT mice, demonstrating a near complete rescue of the Leigh syndrome locomotor defect.

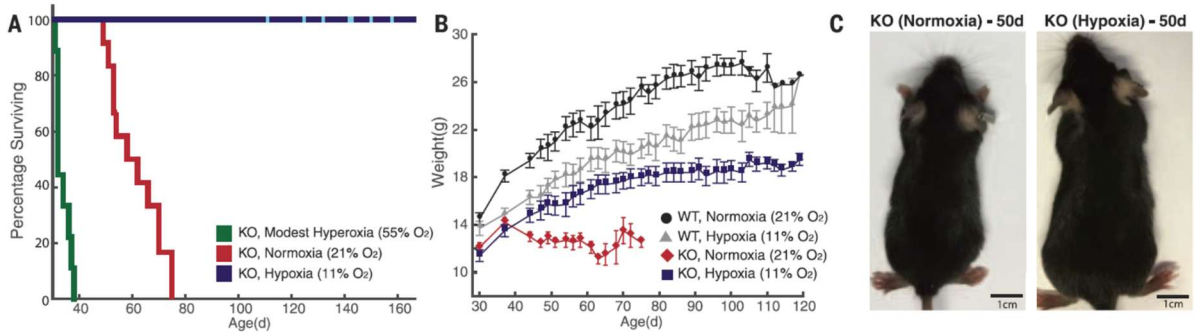


***Figure A, B and C**. Chronic hypoxia extends life span and alleviates disease in a mouse model of Leigh syndrome, whereas chronic hyperoxia exacerbates disease [7].*
***Figure A.** Ndufs4 KO mice of both genders were chronically exposed to hypoxia (11% O2), normoxia (21% O2), or hyperoxia (55% O2), at 30 days of age and survival was recorded (n = 12, n = 12, n = 9 mice, respectively). Cyan bars represent the current age of hypoxic KO mice. **Figure A.** Body weights were measured in WT and KO mice exposed to normoxia or hypoxia, three times a week upon enrollment in the study. Weights are shown as mean ± SE. **Figure C.** Representative image of 50-day-old KO mice exposed to normoxia or hypoxia.* From Jain, I.H., et al., Hypoxia as a therapy for mitochondrial disease. *Science*, 2016. 352(6281): p. 54-61.

Based on these results, hypoxia offers a potential treatment option for mitochondrial disease -- for which little or no treatment can be offered at the present time. Here we propose a safety trial to study physiological and biochemical effects of prolonged hypoxia in healthy subjects. This would be the first step for future studies in determining whether the marked clinical benefits seen with chronic hypoxia in an animal model can be translated into a safe, effective therapy for patients with mitochondrial dysfunction. Intermittent hypoxic therapy is well-researched in the field of sports medicine in an effort to find ideal training conditions for high-level athletes (*9-13*). However, findings from these studies do not have broad applicability to the medical field for multiple reasons: (a) the study population often consists of highly-trained athletes, (b) methodology and data collection prioritizes finding increases in exercise capacity, and (c) data points are not gathered systematically outside of exercise-stress trials. In contrast, many patients with mitochondrial disease are known to have exercise intolerance which makes interpretation and application of the available data to these patients difficult. Existing studies also generally look for benefits with short, intermittent bursts of hypoxia on the order of minutes to several hours. In contrast, we propose to study healthy individuals (and not competitive athletes) exposed to chronic hypoxic conditions with systematic monitoring.

Members of our research team have a long history of safe, successful studies on healthy human subjects exposed to both hypobaric hypoxia (high altitude) and normobaric

hypoxia (sea level testing with a decreased fraction of inspired oxygen – FiO_2). These human studies have occurred both locally (with our colleagues at the U.S. Army Research for Environmental Medicine in Natick and Pikes Peak Summit Labs) and with our colleagues in Nepal (Everest Base Camp 17,500 ft), Mt. Kilimanjaro (19,340 ft), and Denali (20,320 ft) (*14-18*). In each of these settings over the past 17 years, even more severe hypoxia challenges have been administered and safely managed under demanding environmental conditions (e.g., extreme cold, wind, distance from easy evacuation, linguistic and cultural barriers, lethal environmental hazards (e.g. avalanche, glacial crevasse fall, structure collapse in earthquake). These studies have been closely monitored by the MGH IRB and have shown excellent results and safety based on thoughtful trial design. We propose a similarly demanding, safe, and thoughtful protocol here. This will allow rigorous study of safety, biochemical and physiological changes, while also allowing us to develop an efficacious and safe in-hospital delivery system of hypoxic gas mixture.

II. SPECIFIC AIMS

The ultimate goal of our studies is to determine whether the morbidity and mortality benefits of hypoxic exposure observed in a pre-clinical mouse model of Leigh syndrome can safely be translated to humans with mitochondrial disease [7]. The goal of the present study in healthy adult volunteers is to define the safety and biochemical-physiological response of prolonged exposure to a hypoxic atmosphere. This study has been published as a clinical trial on clinicaltrials.gov (NCT02860975). Our specific aims are as follows:

AIM 1: To provide a safe, controlled setting for prolonged exposure of monitored, healthy adults to a normobaric, low-oxygen environment.

Along with the MGH Respiratory Therapy service, we will deliver a nitrogen-enriched, humidified mixture of gas by mask, nasal cannulae or by providing a small room-sized tent with the same humidified gas. To provide the correct gas mixture, we will utilize medical nitrogen tanks or nitrogen generators. Humidified, high flow, hypoxic gas by nasal cannula or by mask will allow the participants to walk short distances, eat, execute basic personal needs (e.g., use of toilette, washing, bathing etc.), or take breaks from wearing the tight mask. A small room-sized tent, that contains a hospital bed, will be available for resting and at all time. We will gradually decrease the FiO_2 to 11% over a period of five days to obtain a peripheral capillary O_2 saturation (SpO_2) between 80%-85% (corresponding to 40-55 mmHg of PaO_2).

AIM 2: To carry out a seven-day long pilot safety-study in six healthy young subjects by inducing hypoxia with a humidified normobaric gas mixture containing O_2 content as low as 11% to reach a SpO_2 between 80%-85%.

We aim to enroll 12 healthy adults between 18 and 40 years old who are non-smoking, without chronic disease or organ dysfunction, and not taking any regular medications. We will continuously monitor SpO_2 and heart rate. We will perform daily transthoracic echocardiography to non-invasively measure pulmonary arterial pressures. We will obtain daily urine and venous blood samples from volunteers to measure effects of induced hypoxia on a series of markers listed in chapter V. a. (Study visits and parameters to be measured).

III. SUBJECT SELECTION

a. Inclusion/Exclusion criteria

Inclusion criteria

- Have a photo ID
- Male or female individuals age between 18 and 40 years old
- BMI between 19 and 29.9 kg/m^2
- Having capacity to consent to the study
- Have health insurance

Exclusion criteria

- Evidence of any physical, mental, and/or medical conditions that would make the proposed studies relatively more hazardous
- Prior high altitude pulmonary edema (HAPE) or high-altitude cerebral edema (HACE) diagnosis
- Born at altitudes greater than 2,100 m (~7,000 ft)
- Systemic disease with or without any functional limitation; including
    - controlled hypertension
    - controlled diabetes without systemic effects
    - any cardiac conditions with or without functional limitation, such as, coronary artery disease or valve disease
- Pregnancy determined by serum pregnancy test, detecting presence of human chorionic gonadotropin (hCG), less than six weeks postpartum, or planning to conceive during the study period.
- Women who are not willing to receive serum pregnancy tests
- Active smoking and tobacco chewers. Volunteers may be enrolled if they quit smoking for more than 1 year.
- Excess alcohol use: more than ½ L/day of wine consumption or equivalent
- Any current medication use except oral contraceptives.
- Living in areas that are more than 1,200 m (~4,000 feet), or have traveled to areas that are more than 1,200 m for more than four days within the last 2 months
- Anemia, as defined by hemoglobin < 10g/dL
- Abnormal hemoglobin (e.g. presence of hemoglobin S)
- Evidence of apnea or other sleeping disorders
- Evidence of asthma
- Peripheral vascular disease
- Raynaud's syndrome
- Lower respiratory infection within the last 30 days

- If applicable, unwilling to refrain from using energy drinks or other caffeinated beverages for 7 days prior to and during the study
- If applicable, unwilling to refrain from use of all over-the-counter oral medications, herbal remedies, and nutritional supplements for 7 days prior to and during the study
- Not willing to have blood drawn from an arm vein each test day of the study
- Claustrophobia (inability to wear a facemask) or other active psychiatric conditions or not willingness to cooperate with the investigators and the other medical team
- Currently enrolled in another research study
- Facial abnormalities that would preclude proper use of a face mask
- COPD
- History of ARDS or severe pneumonia (residual shunt)
- Taking any sulfa-containing medications, dapsone (metHb)
- Sickle Cell Disease or Thalassemia or variant
- History of TIA or CVA

Pregnancy Prevention/Testing: Women using oral, subdermal or injectable contraceptives, and those using other means of birth control may participate. A serum pregnancy test will be conducted as part of the screening process for study participation no more than 7 days before starting the study and it will be repeated on the day of enrollment. The test result will be read by a female staff member who will keep the result confidential. If a woman declines to have a pregnancy test, she will not be able to participate.

b. Description of the Informed Consent Process:

- Drs. Harris and Berra will be responsible for explaining the study, answering questions, and obtaining informed consent for this portion of the study. As in multiple prior DoD-funded studies at MGH, USARIEM and international field studies, our subjects' questions will be addressed during the consent process and throughout the trial.
- Subjects will be provided privacy and up to 48 hours to make a decision. They will be made aware that they may withdraw from this protocol at any point. The potential human subject will be allowed to discuss the study with anyone before making a decision.

Consented volunteers will be given a study-specific medical clearance by Drs. Berra and Harris as employed in multiple prior Department of Defense funded protocols. Screening will consist of a physical examination, 12-lead electrocardiogram, cardiac echocardiography, sickle cell screening test, and current (within 12 weeks of clearance exam with no intervening medical treatment) routine blood and urine analyses. If the echocardiographic study reveals any unexpected cardiac disease the subjects will be informed and also their

primary care physician (PCP) will be informed. If the subject does not have a PCP, we will provide information about choosing a PCP. Subjects must be between the ages of 18 and 40, and if female must not be pregnant (as noted above). Subjects will be excluded if they were born at altitudes over 2100 m (7000 feet), are currently living above 1200 m (4000 feet), have traveled to altitudes greater than 4000 feet for more than four days in the past two months, currently smoke or chew tobacco, or take medications that interfere with oxygen delivery and transport (includes sedatives, sleeping aids, diuretics, alpha and beta blockers, tranquilizers, and any medication that depresses ventilation). Subjects will be excluded if they have a prior HAPE or HACE diagnosis, asthma, sleep apnea or evidence of any physical, mental, and/or medical conditions that would make the proposed studies relatively more hazardous. In addition, subjects must have normal hemoglobin and hematocrit levels and absence of hemoglobin S. Subjects will also be excluded if they do not want to be exposed to hypoxia and remain inside the hospital for consecutive days, do not agree to the screening medical clearance exam or laboratory work, or are unwilling to participate in the experimental imaging (echocardiography) and testing in a hospital setting. If a subject has a BMI between 25 and 29.9 kg/m^2, he or she will have bioelectrical impedance and skinfold measurements taken to assess body fat percentage per the protocol used extensively by the White 12 TCRC nutritionists. Subjects in this BMI range will be allowed to participate in the study if their body fat percentages lie within the healthy range: 20-35% for women and 8-25% for men.
Lastly during the screening visit, Resting Energy Expenditure (REE) will be assessed by indirect calorimetry using the VMAX Encore Metabolic Cart (Care Fusion, Yorba Linda, CA). This system uses a dilution canopy to measure oxygen consumption (VO2) and carbon dioxide production (VCO2). The calculation of REE is facilitated by the Weir equation (*19*). This information will be used to estimate each subject's caloric need during the 7-day study. All subjects will receive a similar composition of macronutrients during their stay at MGH to standardize diet for study purposes.

c. Source of subjects and recruitment methods

Recruitment will be done in three ways;

1. Email via Broadcast MGH: Research Studies In Need of Volunteers
2. Enrollment in the Research Study Volunteer Program (RSVP) for Health of MGH and BWH website: http://www.rsvpforhealth.org
3. Advertising at the Partners clinical studies web site http://clinicaltrials.partners.org

Potential study subjects will be able to contact study investigators by phone or email to verify eligibility according to the inclusion/exclusion criteria and to set up the first visit, at which point the study will be explained in detail and they will be asked to provide informed consent.

Subjects may contact the study investigators at any time to withdraw from the study. Confidentiality will be preserved to the fullest extent.

IV. SUBJECT ENROLLMENT

a. Methods of enrollment, including procedures for patient registration

We aim to study 12 healthy subjects for seven days. A de-identified code will be assigned to the patient and registered on a dedicated enrollment log.

b. Procedures for obtaining informed consent (including timing of consent process)

The study will be explained to each volunteer in detail. Signing of an Informed Consent Form will be requested for participation in the study. Consent will be signed by the volunteer at the beginning of the study. The PI and/or Co-PIs of the study will be available to answer any questions the volunteer may have. Consent can be withdrawn at any time. Personal Medical Information (PMI) will be accessed by the investigators for study purposes.

c. Treatment assignment and randomization (if applicable)

There is no randomization in this interventional physiological study. All patients will receive the same procedures in the same order.

V. STUDY PROCEDURES

a. Study visits and parameters to be measured

Subject will be asked to come to the Clinical Research Center on White 13 (or starting in September 2016, White 12) at the Massachusetts General Hospital. The duration of the study protocol is seven days and subjects will be admitted for the entire 7-day period on White 13/ White 12. Though no additional procedures will be performed after this 7-day period, subjects will be asked to return for a 3-month follow-up to evaluate for any changes in health from baseline related to the study.

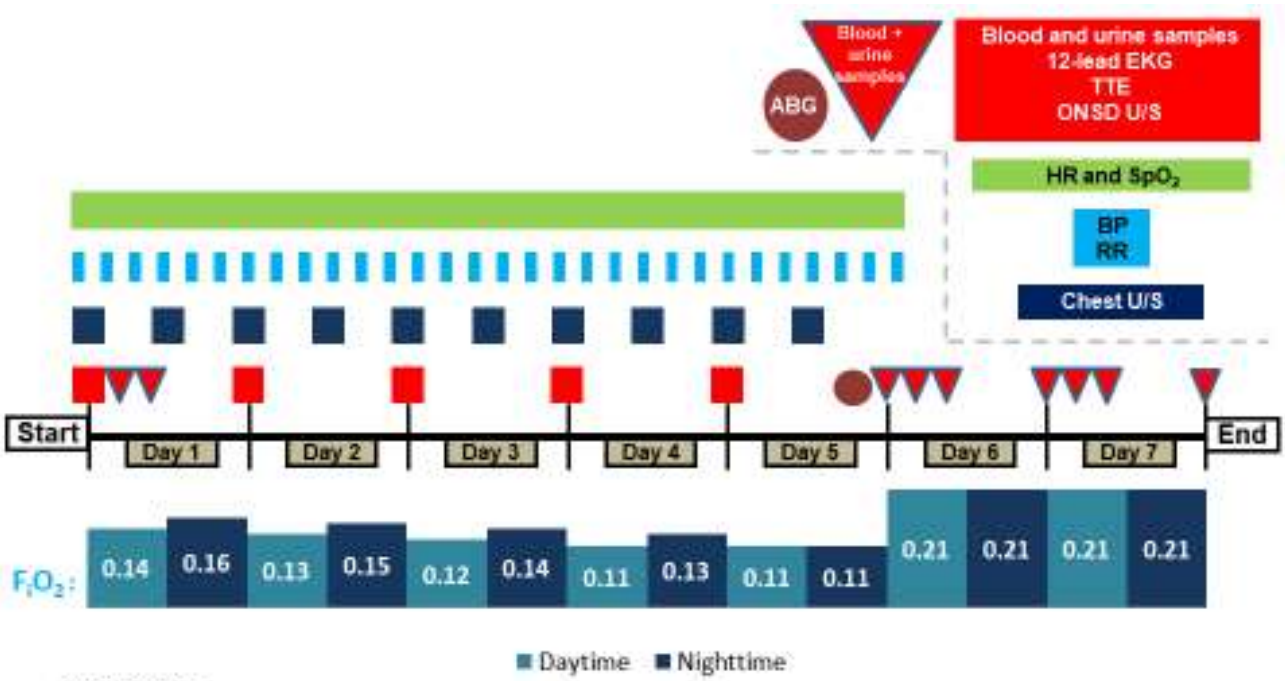
Along with the MGH Respiratory Therapy service, we will deliver a humidified mixture of gas by mask, nasal cannula, or a small room-sized tent. We will gradually decrease the FiO_2 to 11% over a period or five days to obtain a SpO_2 between 80%-85%, as described in Appendix 1 (FiO_2 protocol) and shown in figure below.

The following parameters and information will be recorded at the following times:

- After informed consent signature: demographic and medical data regarding current and past history will be recorded.
- Continuous monitoring: heart rate, SpO_2
- Every four hours for the first five days of the study, the following parameters will be recorded: blood pressure, respiratory rate, and heart rate.
- Cardiac monitoring: every morning while the subject is under hypoxia (first five days), transthoracic echocardiography will be performed to noninvasively measure pulmonary arterial pressure and a 12-lead EKG will be obtained and reviewed by a physician on this trial with an active medical license.
- Neurologic and pulmonary monitoring: Twice daily while the subject is under hypoxia (first five days), we will perform bedside chest ultrasound to assess for pulmonary edema and bedside ultrasound evaluating optic nerve sheath diameter as a surrogate for intracranial pressure.
- Blood samples: before the study and every morning (at fasting prior to breakfast) blood samples will be drawn for hemoglobin, hematocrit, reticulocyte count, erythropoietin levels, and serum electrolytes. In addition, blood will be drawn at the following times: i) 1hr and 3hr after initiation of hypoxia on Day 1, ii) 1hr and 3hr after cessation of hypoxia on Day 6, iii) 1hr and 3hr after morning blood draw on Day 7 (corresponding to the additional draws on Days 1 and 6), and iv) three times on Day 0 (i.e. the day before the subject enters the hypoxia chamber) at times corresponding to the estimated blood draw times to take place on Day 1/6/7. During all blood draws, we will save a portion of the plasma in EDTA tubes for metabolomics measurements that will provide levels of over 400 metabolites in each. These measurements will allow us to focus on known metabolic markers

of mitochondrial activity and oxidative stress such as lactate, β-hydroxybutyrate, α-hydroxybutrate, and methionine sulfoxide while also searching broadly for other metabolic consequences of hypoxia. We will also subject plasma samples to proteomic and genomic measurements that will yield levels for over 1000 proteins and genes including factors involved in energy metabolism, hypoxia response and inflammation. (Total blood draw for the entire study will be ~280mL, as shown in the figure below). We will store plasma and buffy coats samples at -80° C for future analyses.

- Urine samples: every morning a 20mL urine sample will be collected (at fasting prior to breakfast) for urinary electrolytes, urinary chemistry, F2-isoprostane, and potential metabolomics follow-up. Additionally, we will collect urine samples at the following times (only if the subject is able to provide any urine): i) 1hr and 3hr after initiation of hypoxia on Day 1, ii) 1hr and 3hr after cessation of hypoxia on Day 6, iii) 1hr and 3hr after morning urine collection on Day 7 (corresponding to the additional collections on Days 1 and 6), and iv) three times on Day 0 (i.e. the day before the subject enters the hypoxia chamber) at times corresponding to the estimated urine collection times to take place on Day 1/6/7. If during these additional times, a subject is unable to urinate, we will skip that collection and attempt again during the subsequent collection time. We will perform metabolomics measurements on urine samples to similarly focus on known markers of oxidative stress while casting a broad net for other metabolic changes. Urine samples will also be stored at -80° C for future analyses.
- Arterial blood gas: In the hour before cessation of hypoxia, an arterial puncture will be performed by an anesthesiologist to obtain a one-time measurement of arterial blood gases.
- Daily review of system to assess for any side effects or new symptom onset
- Twice daily mental status exams
- A daily questionnaire will be given to the subjects to assess symptoms of mountain sickness (Lake Louis score, Questionnaire 1)



Study Schema
Daytime refers to the time period between 10:00AM and 10:00PM. Nighttime refers to the time period between 10:00PM and 10:00AM. The fraction of inspired oxygen (FiO_2) will be varied during the daytime and nighttime as depicted. All FiO_2 values are target values, and may vary +/- 0.02. At 10:00AM on each day of the study, blood and urine samples will be collected. Blood will additionally be drawn at both 1hr and 3hr after initiation of hypoxia, 1hr and 3hr after cessation of hypoxia, and again at the corresponding times on Day 7. An arterial puncture to measure blood gases will be done in the hour before cessation of hypoxia. At 10:00AM on the first five days of the study, 12-lead electrocardiogram (EKG), trans-thoracic echocardiography (TTE), and optic nerve sheath diameter (ONSD U/S) will be performed. Every four hours for the first five days of the study, blood pressure (BP) and respiratory rate (RR) will be measured. Twice daily for the first five days of the study, chest ultrasound (chest U/S) will be performed to assess for pulmonary edema. Lastly, during the first five days of the study, subjects will be continuously monitored via pulse oximetry.

b. Drugs to be used

Hypoxic inspiratory gas mixture: Hypoxic inspiratory gas will be obtained by adding nitrogen (N_2) to normobaric, normoxic air with medical N_2 tanks or membrane technology nitrogen generators. The latter technology exploits the property by which different gases move through a selectively-permeable membrane at different rates based on partial pressures gradients. In the setup, a hollow-fiber membrane fills a cylindrical cartridge functioning as a spool with specifically reeled polymer fibers.

c. Devices to be used

At night, the gas mixture of N_2 and O_2 will be delivered in a tent that is implemented in the MGH burn ICU. During the day, the gas mixture will be delivered by either standard tight facemask or by high flow nasal cannula. Humidified, high flow, hypoxic gas by nasal cannula will allow the participant to walk short distances, eat, execute basic personal needs (i.e., use of toilette, washing, bathing etc...).

Payment: Subjects can expect to spend up to 7 days (168 hours) total time and will receive a stipend of $15/hour. A bonus of $480 will be given at completion on day 7. Additionally, $100 will be given for the screening visit and $80 for the 4-hour visit which occurs on the day prior to the 7-day in-house study in the hypoxia chamber. The total payment for participation on this study is of $3,180 at the study completion. If a subject exits the study earlier, he/she will be paid for the hours of participation (number of hours in the study multiplied by $15) and will not receive the bonus.

VI. BIOSTATISTICAL ANALYSIS

a. Specific data variables being collected for the study (e.g., data collection sheets)

The study data (vital signs and biochemical measurements) will be collected as per the attached workflow organization sheet in specific data sheet (Appendix 2).

b. Study endpoints

The primary endpoint of our study is to prove safety in healthy subjects breathing humidified hypoxic inspiratory gas mixture for 5 days.
The secondary endpoint of our study is to describe the physiological and biochemical changes during the 5-day hypoxic period and the 2 days after return to normoxia.

c. Statistical methods

This is an interventional prospective safety study. Data will be expressed as means ± SD or median/interquartile range as appropriate.

d. Power analysis (e.g., sample size. evaluable subjects. etc.)

Sample size: We aim to study 12 healthy subjects for the entire 7-day study period.
Sample size calculation: This is an intra-hospital safety trial with the purpose of testing methods to re-create a safe hypoxic environment for possible future studies in patients with mitochondrial dysfunction. While 2 or 3 subjects would likely be sufficient to test our

methods for reliable delivery of humidified N_2 and O_2 in predetermined concentrations, we will test our methods on 12 healthy subjects to reproduce our methods and obtain feedback from the volunteers.

VII. RISKS AND DISCOMFORTS

a. Complications of surgical and non-surgical procedures, etc.

Participants in the study will undergo 3 procedures during the study: hypoxic gas administration, phlebotomy, and echocardiography.

- Facemask.
  1. Claustrophobia is one of the exclusion criteria. If a subject develops claustrophobia, he/she may leave the trial at any point.
  2. The most common side-effects of facemask are pressure related, such as redness, irritation over the mask's borders. To decrease possible skin irritation and abrasions, subjects will be using a tent during the night and high flow nasal cannula during the day for breakfast, lunch and dinner. Additionally they will be able to choose whether to use high-flow nasal cannula or the facemask during the day at any time as an alternative to the facemask.
- Tent. Risks associated with use of tents include unintended hypoxia, hypercarbia, and claustrophobia. These tents are made at the MGH shop and routinely used on the burn unit to maintain elevated temperature (80-90 °F) and humidification in critically ill patients. Our subjects will be continuously monitored for hypoxia and clinical condition to limit risk of unintended hypoxia or hypercarbia. Claustrophobia will be handled as described previously.
- Nasal cannula and non-invasive high flow humidified gas. All participants will wear non-invasive high flow humidified gas for part of study period. Unlike masks, high flow nasal cannula is well tolerated in hospitalized patients and warm humidified air alleviates discomfort of the high flow. In order to avoid abrasions, we will inspect subjects' nasal mucosa in the morning and evening.
- IV/arterial punctures and phlebotomy. Risks related to venipuncture include bruising, hematoma formation, cellulitis, superficial thrombosis, bleeding and phlebitis. Risks of arterial puncture (done only once during study by an anesthesiologist) are similar and also include arteriospasm, nerve damage, and a vasovagal response. An IV nurse or an anesthesiologist will perform phlebotomy to minimize subject discomfort. A peripheral line will be used and changed every 3 days in the clinical research center at MGH. If the subject prefers, single venipuncture will be performed for each blood draw in place of a continuous IV line; this will allow for maximal patient comfort.
- Trans-thoracic echocardiography. Echocardiography will be performed on a daily basis. Echocardiographic assessment of pulmonary artery pressure and cardiac output is a noninvasive and painless maneuver (*20, 21*). It is commonly

used in clinical practice, has been used in the field under hypoxic conditions, and is safe for the study subjects.

In presence of an abnormal physical exam and/or additional abnormal laboratory results (including a positive pregnancy test), subjects will be informed.

b. Drug side effects and toxicities

We will test a gas mixture enriched with nitrogen to produce an O_2 concentration lower than 21%. It is expected that subjects may suffer to a certain degree with symptoms consist with the syndrome acute mountain sickness. Symptoms related to hypoxia may include: headache, dizziness, poor sleep, poor appetite and fatigue. These symptoms are well described in adults and children during family travel to commonly visited high altitude locations in the continental U.S. (e.g. hiking in Rocky Mountain National Park or on family skiing vacations (e.g., Vail/ Breckenridge in Colorado, Mammoth in California)) and will not require interruption of the study, unless the subject requests to exit the study. Furthermore, in our proposed model of normobaric hypoxia, the risk of acute mountain sickness symptoms has been reported to be lower than hypobaric hypoxia (simulated altitude) (*22*). The following risks might also occur:

<u>Risks Associated with Hypoxia:</u>

- On acute exposure to hypoxia, severe hypoxemia ($SpO_2 \leq 70\%$) can ensue. Acute exposure to even modest environmental hypoxia (e.g. altitudes greater than 8,000 ft which are experienced by an estimated 35 million Americans voluntarily every year) will routinely lead to measured pulse oximetry values that at sea level would inevitably be associated with profound illness and an alarming clinical course requiring immediate intervention. In contrast, at high altitude (or with normobaric hypoxia as in our study) lower oxygen saturations (as measured easily by pulse oximetry) are both physiologically normal and to be expected. At 14,000 ft, routine pulse oximetry values for a healthy, physically active and well-acclimatized individual may be in the mid 80s, with still lower values commonly experienced in asymptomatic, healthy low-landers who have recently ascended – especially during sleep. Our species has been exposed to hypoxic environments safely for over 10,000 years. Population studies find that high altitude is well-tolerated by millions of citizens each year as they voluntarily seek outdoor experiences during vacations. Our research team has 2 decades of experience with this normal physiologic response in both healthy adults and children voluntarily ascending to even higher elevations, both for work and vacation (*14-18*). We are markedly limiting the risk of these hypoxemic episodes by gradually increasing each subjects exposure to hypoxia in a graded fashion, allowing the powerful moderating influence of normal

acclimatization to limit hypoxemia. Over the 5 days of our study, normal physiologic responses (of lung, brain, kidney, and heart) will allow for increased minute ventilation at rest and vigorous bicarbonate diuresis to stabilize serum pH. Hypoxia is a stress our class and species has evolved to adjust to. In addition, without the added stress of hypobaria, we can immediately increase the fraction of inspired of oxygen, and so remove the risk of developing prolonged hypoxia events. If $SpO_2 \leq$ 70% decrease for more than one continuous minute, we will stop nitrogen and we will increase fraction of inspired of oxygen to 50%. Symptoms of hypoxia (light headedness, dizziness) typically resolve immediately upon return to a normal range of SpO_2. If hypoxia symptoms are present, subjects will exit the study only after all symptoms of light-headedness or dizziness are resolved, and headache or nausea is absent.

- Acute mountain sickness can very rarely progress to high altitude pulmonary edema (HAPE; (*23*)) or high altitude cerebral edema (HACE; (*14*)) and would be case reportable were either to occur in normobaria at the limited hypoxic challenge we will be employing. Though HAPE is very unlikely to occur, we will provide daily monitoring by echocardiography, lung ultrasounds (number of B lines in the anterior and lateral fields of the lungs), clinical exam (auscultation for rales and unusual dyspnea at rest solicited), and pulse oximetry. If HAPE does occur, the study will stop and the subject treated with oxygen until resolution of symptoms.
- HACE is also a very unlikely potential risk in this study. If a subject develops any evidence of ataxia or altered mental status, they will be immediately withdrawn from the study. FiO_2 will be increased to 50%, dexamethasone 8 mg IV will be administered, and head CT or MRI scan will be performed if clinically indicated. A brain MRI would allow assessment of characteristic T2 weighted signal change in the corpus collosum consistent with HACE.
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Any study subject who experiences side effects sufficient to prompt premature termination of the study will not be allowed to continue in the protocol. He or she will be monitored until vital signs normalize and transthoracic echocardiography shows normal right heart function.

As further background regarding the treatment of HACE and HAPE, consider the following: even in patients treated for severe HAPE, provision of oxygen and descent results in a rapid return to normality (*15, 23, 24*). Of note, some of studies worked with sick patients treated in a stone hut while remaining at 4,400 meters—far worse conditions compared to the controlled hospital setting we propose herein. It is more the rule than the exception to have a patient present profoundly ill with HAPE, with oxygen saturations in the 50s, who rapidly responds to oxygen and diltiazem. These patients become clinically unrecognizable as they briskly continue their ascent to higher

elevations within 3-4 days without complaint. Similarly, HACE when treated early has an excellent prognosis. Permanent neurologic dysfunction of any sort from acute altitude illness event at extreme elevations (e.g., < or = 25,000 ft) is extraordinarily rare when it is treated in a timely fashion. Typically, patients who have any lingering sequelae are ones who come from remote areas without access to medical care and who have not had adequate treatment over 2-6 days despite obvious preceding symptoms.
In addition, simply to help make clear the divergence between appalling oxygen saturations and excellent patient outcomes between sea level and high altitude, we have included an article by colleague Mike Grocott *et al.* have reported an average SaO2 of 54% at a Pa02 of 24.6 mmHg in a team of climbers on the summit of Everest (*25*). Despite low oxygen saturations, these healthy individuals did not suffer poor medical outcomes.
It should further be noted that our protocol calls for normobaric hypoxia, exposing subjects to an inspired oxygen partial pressure (PiO2) of ~80mmHg. The studies mentioned above support the assertion that hypoxia is safe and well-tolerated in healthy individuals despite healthy subjects receiving much lower PiO2 levels of 40s to 60s mmHg.

c. Device complications/malfunctions

Nitrogen generator: If the nitrogen generator is malfunctioning, we will enrich air with medical nitrogen from medical nitrogen-tanks. We will contact the manufacturer (Higher Peak LLC, Winchester, MA 01890) who will deliver a new nitrogen generator.

d. Psychosocial (non-medical) risks

Psychosocial risks include prolonged indoor exposure and perceived isolation from a subject's daily routine, family and friends. Subjects will have ready access to their personal communication devices (e.g. smart phone for voice/ social media use) during times when they are not actively participating in testing procedures. Subjects may be visited in person on the study floor. We do not anticipate any other psychosocial risks to the study subjects from participation in this protocol. Strict confidentiality will be maintained by the research team at all times, including keeping all data in a secure, locked cabinet with limited access. All specimens will be coded after they are obtained and the code key kept in a locked cabinet. Plasma samples and buffy coats will be stored for future genetic, proteomic, and metabolomic analyses. All electronic data will be stored in a Partners encrypted laptop. Samples given to parties outside of MGH for analysis will be coded to maintain confidentiality.

e. Radiation risks (statement provided by radiation Safety Committee)

Not applicable.

VIII. POTENTIAL BENEFITS

a. Potential benefits to participating individuals

There are no direct benefits to the healthy volunteers in this study.

b. Potential benefits to society (e.g., increased understanding of the disease process, etc.)

This is a safety trial to study physiological and biochemical effects of prolonged normobaric hypoxia in healthy subjects. This is the first step for future studies aimed to determine whether chronic hypoxia breathing can be safely translated into a therapy for patients with mitochondrial dysfunction. Approximately 1 in 4,000 children in the United States will develop a monogenic mitochondrial disease by the age of 10 years. Up to 4,000 children per year in the US are born with a type of mitochondrial disease. Because mitochondrial disorders contain many variations and subsets, some are very rare. At the present there is little, if any proven benefit from available treatments of mitochondrial disease.

IX. MONITORING AND QUALITY ASSURANCE

a. Independent monitoring of source data
The PI and co-Is will be responsible for the monitoring of the study. Additionally, a safety report will be submitted to the MGH IRB after study completion by three subjects as described in point b.

b. Safety monitoring (e.g., Data Safety Monitoring Board: etc.)
Trained personnel will be responsible for monitoring the physiological data in real time. This includes the nursing staff in the MGH Clinical Research Center and co-investigators who are part of this IRB application. Furthermore, Dr. Lorenzo Berra or Dr. Stuart Harris will be available on-call at all times for the duration of the study.

After completion of the study by three subjects, the three members of the DSMB and the Principal Investigator (Dr. Lorenzo Berra) will review the results and assess for safety, and a safety report will be submitted to the MGH IRB. The study will only be stopped if the stopping rules are met (please see point d below).

Members of the Data Safety management Board (DSMB) consist of an anesthesiologist, a pediatrician and a trauma surgeon with expertise in high altitude studies. Names and contacts of the DSMB members are:

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c. Outcomes monitoring
Subjects will be monitored until the end of the study protocol. Since the proposed study is going to investigate volunteers, no outcome data are going to be collected.

d. Adverse event reporting guidelines

The principal investigator will review any adverse events immediately following their occurrence and report them to the IRB in accordance with Partners Investigator Guidelines.

Stopping rules

The principal investigator and co-investigators will perform the review and decision regarding altering or stopping the protocol. Mild or moderate adverse events will be presented in progress reports at continuing reviews. Protocol exit criteria will be:

- Desaturation: SpO_2<70% for more than 1 minute not immediately responsive to oxygen therapy.
- Chest pain with evidence of ischemia on EKG (e.g., T-wave inversions, ST segment changes).
- Signs and symptoms of acute pulmonary edema:
  - Rapidly progressive, severe shortness of breath at rest..
  - Severe dyspnea, or a feeling of suffocating or drowning despite return to normoxia.
  - Wheezing or gasping for breath accompanied with anxiety, restlessness or a sense of apprehension.
  - A cough that produces frothy sputum that may be tinged with blood
- Signs and symptoms of acute cerebral edema:
  - Acute ataxia or altered mental status
  - Any focal neurologic deficit.
- Change in mental status as determined by clinical mental status exam
- Increase in systolic pulmonary artery pressure of 15 mmHg from baseline (first trans-echocardiography in ambient air)
- Systemic blood pressure increase to 150/100 or increase by 40% over baseline, whichever is lower.
- Syncope.
- Febrile illness (> 100.4 on two contiguous assays 1 hour apart)
- Subject may voluntarily withdraw from the study at any time.

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