Colorado-Oregon Altitude Study Research and Statistical Analysis Plan
NCT: Not yet assigned
June 2, 2022

Research Plan

IMPORTANT: When completing this outline, please use the **Research Plan Guidance** for the content necessary to develop a comprehensive yet succinct Research Plan. Using the guidance to complete this outline will help facilitate timely IRB review.

Study Title: **C**olorado **O**regon **A**ltitude **ST**udy (COAST)
Protocol Number: TBD
Principal Investigator: Andrew T. Lovering, PhD

A. Introduction and Background

Rapid and prolonged exposure to high altitude presents a serious threat to the health of people. Unfortunately, no major advances have been made in promoting health and performance at high altitude in the last 25 years. To remedy this problem, we propose to test three new ideas to rapidly advance human performance at high altitude.

While a two-week period of acclimatization to high altitude substantially improves physical and cognitive function (2), this strategy is clearly impractical for many people who travel from low altitude to high altitude quickly. Pharmacological countermeasures to offset the negative effects of high altitude are thus attractive to ensure optimal health and performance at high altitude. Unfortunately, current pharmacological strategies only work moderately well, or are fraught with side effects and risks (1, 3). Our team has recently conducted a series of Department of Defense (DoD) funded studies aimed at understanding the molecular and genetic basis of human adaptation to high altitude as a means to identify novel ways to promote acclimatization. Based in part on our research and research from their labs, we have identified two compounds that we believe will improve human health and performance at high altitude: **iron and erythropoietin.** Moreover, we will study the molecular biomarkers of natural exposure and acclimatization in a placebo group and the two intervention groups to discover how these interventions aid performance, and to look for markers of use of each compound to aid detection of illicit use in the face of high altitude exposure. In addition, we propose to study the effect of these drugs on altitude acclimatization in those with and without a patent foramen ovale (PFO), a small hole in the atrial septum present in ~30% of the population. The PFO has been shown to alter ventilatory responses to hypoxia and increase susceptibility to acute mountain sickness, although the mechanism remains unclear.

Iron and erythropoietin are linked by each playing a role in the oxygen sensing pathway regulated by hypoxia inducible factor (HIF). We know that HIF plays a key role in sensing low oxygen, and thus plays a key role regulating human's response to low oxygen during acute and chronic exposure to high altitude. By using these compounds to modulate the HIF pathway we propose to discover the most important components of the pathway for overcoming hypoxia exposure in healthy, fit humans.

B. Specific Aims/Study Objectives

The overarching goal of the study is to determine the effect of iron and erythropoietin (EPO) supplementation on acute and chronic physiological responses to high altitude.

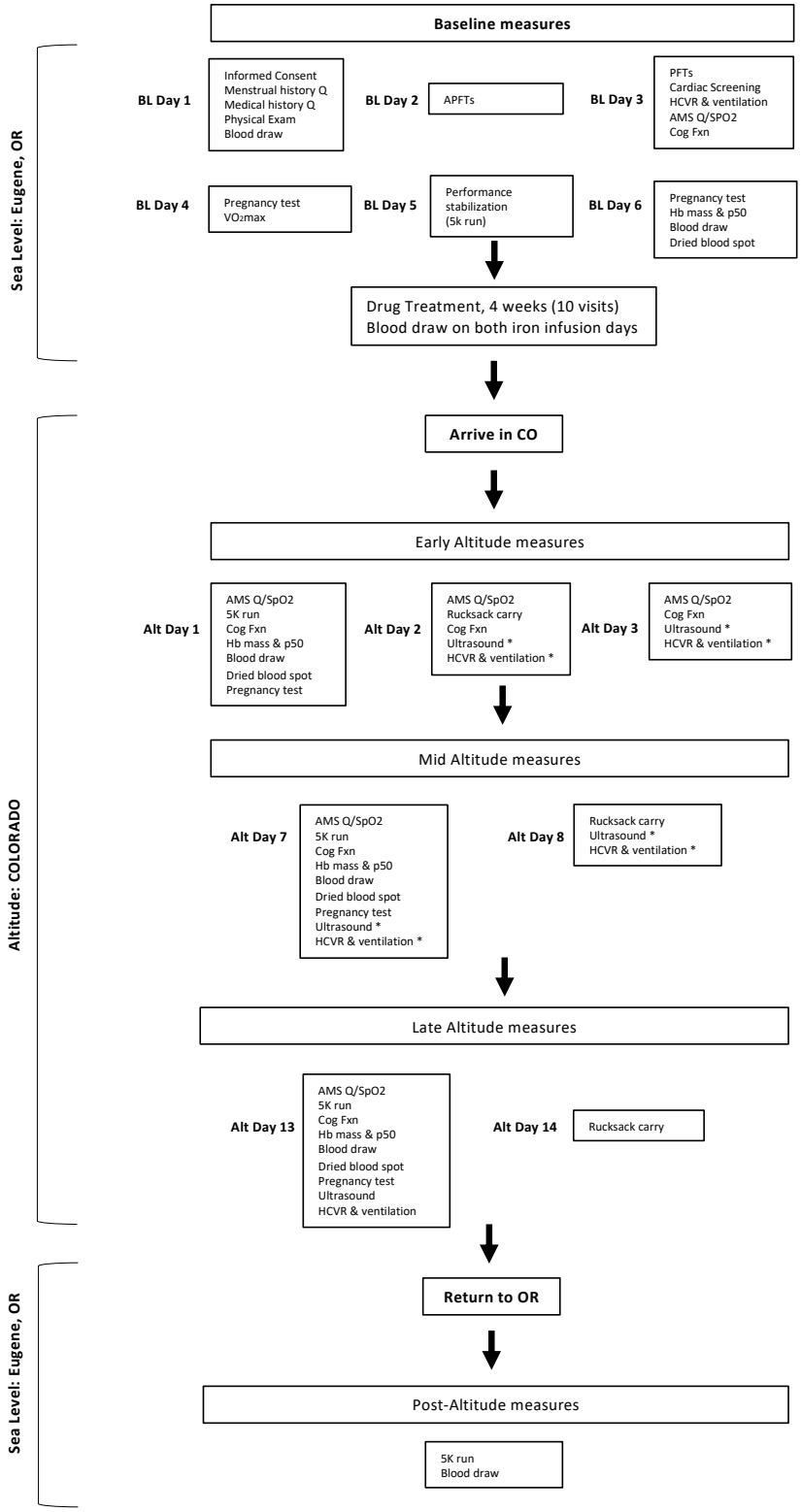
Specific aims

1. To determine the effects of iron and EPO on exercise performance acutely (first 3 days), during acclimatization (days 7-8), and chronically (days 13-14) at altitudes of 10,000 to 14,000 feet in PFO- and PFO+ subjects.
2. To determine the effects of iron and EPO on acute and chronic changes in hemoglobin (Hb) mass, cognitive performance, ventilation, and molecular markers of hypoxia exposure at altitudes of 10,000 to 14,000 feet in PFO- and PFO+ subjects.
3. To determine the effects of iron and EPO on development of acute mountain sickness (AMS) at altitudes of 10,000 to 14,000 feet in PFO- and PFO+ subjects.

Please note that all baseline studies will provide novel data on men and women with and without a patent foramen ovale (PFO) so that even if the altitude studies become unfeasible, new information will be gained from these studies.

C. Methods, Materials and Analysis

Overview: The initial components of the study will be performed over 6 days at baseline to 1) screen subjects for inclusion in the study and 2) obtain baseline measurements, with all studies conducted at the Cardiopulmonary Lab, Eugene, OR. These screening and baseline studies will be followed by 4 weeks of placebo or drug interventions (Cardiopulmonary Lab, Eugene, OR) and 14 days living at high altitude (Breckenridge or Leadville, CO). Within 1 week after returning to Eugene, OR, post-altitude measures will be made. Refer to Figure 1 below for a schematic representation of the timing and location of measurements. Each subject, regardless of intervention arm, will perform all protocols described below. All screening in Eugene, OR is scheduled for 6 days, but screening could take up to 10 days in the case of equipment malfunction or if the subject prefers to spread tests out over additional days.



* Indicates protocol that will be done on either day listed in early or mid altitude measures

What follows is a detailed description of each study day:

BASELINE: EUGENE, OR RESEARCH SITE

All studies done in Eugene will be done in either laboratory space within Pacific Hall and/or on running tracks and trails within the Eugene/Springfield metropolitan area or very close to this community.

Any subject with childbearing potential will be required to take a pregnancy test on VO2max days and Hemoglobin mass testing days (both at baseline and altitude) as the other testing days will not involve any activity that would otherwise affect the fetus.

Day 1 – Informed consent, medical History, blood draw: Cardiopulmonary Lab, Eugene, OR (~60 min)

Subjects who express interest in the study will have any initial questions answered on the phone or over email, depending on subject preference. Those interested in continuing with the study will arrive to the lab, and subjects will undergo informed consent and a complete a self-report health history questionnaire, and complete a menstrual cycle history questionnaire (biological women only) (~30 min). Subjects will have the option to undergo informed consent remotely prior to arriving to the lab (see 'Informed Consent Process' below) but will complete all other paperwork, including signing the informed consent, upon arrival to the lab. Menstrual cycle history will be documented for control purposes for blood biomarker analysis, although subjects will not be required to be in a certain phase for testing, nor will they be excluded for contraceptive use.

After completing paperwork, subjects will undergo physical examinations by either a Nurse Practitioner or Physician Assistant 25 mL blood draw for iron, ferritin, transferrin, basic metabolic panel, and complete blood count measurements to be analyzed by QUEST. Subjects will be asked to either give or deny permission to the Lovering Lab to re-contact them after the end of the study. Lastly, subjects will be asked to use an online Qualtrics survey, which will be emailed to every consenting subject, to log daily exercise activity for the duration of the study. (~30 min).

Day 2 – Army Physical Fitness Tests (APFTs): Cardiopulmonary Lab, Eugene, OR (~60 min)

Subjects will perform a modified version of the Army Physical Fitness Tests (APFTs) outdoors at the University of Oregon. The APFT will include 2 minutes of push-ups and sit-ups and a 5k run. Total time ~1 hour.

Day 3 – Lung function. cardiac screening, blood draw, Acute Mountain Sickness (AMS) questionnaire with finger saturation, and cognitive function testing: Cardiopulmonary Lab, Eugene, OR (~125 min)

Subjects will perform pulmonary function testing (~15 minutes) and cognitive function testing (~20 minutes).

Subjects will have an intravenous catheter (IV) placed. Subjects will then undergo comprehensive ultrasound screening. Amount of blood flow travelling through the PFO/ASD and IPAVA will be graded using saline contrast echocardiography while the subject is breathing room air. Pulmonary artery pressure, cardiac output, and TAPSE will also be measured using ultrasound (~60 minutes).

Subjects will complete the Lake Louise Questionnaire (LLQ) and Environmental Symptoms Questionnaire (ESQ) to determine acute mountain sickness (AMS). Subjects will be instrumented with a pulse oximeter to measure oxygen saturation (~15 minutes).
Lastly, subjects will have resting ventilation and HCVR measured (~15 minutes).

Day 4 – VO2max testing day: Cardiopulmonary Lab, Eugene, OR (~30 min)
Subjects will perform a VO_{2max} test (~30 minutes). Additionally, resting, exercise, and post-exercise heart rate and systemic blood pressure will be measured. Any subject with child bearing potential will be required to take a pregnancy test prior to the VO2max test.

All subjects will arrive at the Cardiopulmonary lab and will be given a telemetric core temperature pill to take 5-10 hours before arriving for **Day 5** testing. If subjects do not take the pill the night prior to exercise testing, they will have to take the temperature pill as a suppository the morning of testing.

To take the core temperature pills, subjects will be told to swallow with a glass of water or other beverage. There are no restrictions for fluid or food consumption after the pill has been ingested and subjects will be told not to chew the telemetric pill. In order to insert the pill as a suppository, subjects will be instructed to insert the pill past the first digit of your finger and given lubricant and a glove to assist with the insertion process. Research staff will be available to give additional information about the insertion process if subjects have more questions. These directions apply to every time subjects take the core temperature pills.

Day 5 – Performance Stabilization, Eugene, OR (occurs ~30 days before departure for Colorado) (~30 min)
To further ensure accurate baseline measurements of physical performance, the top 80 subjects (and 20 alternates) with the most similar physical characteristics will repeat the 5K. This strategy will minimize the influence of learning effects associated with the tests and facilitate matching between groups. (~30 min).

Day 6 – Hemoglobin mass, Eugene, OR (occurs ~30 days before departure for Colorado) (~75 min)
Subjects will perform hemoglobin mass (Hb mass) and p50 measures (~75 minutes). This measurement involves placing an IV and drawing an additional 10 mL of venous blood. Any subject with child bearing potential will be required to take a pregnancy test prior to Hb mass testing. In addition, subjects will have baseline venous blood drawn (60 mL of venous blood will be analyzed for various biomarkers and 5 mL for sickle cell screening to be analyzed by QUEST). Dried Blood Spot samples will be taken utilizing a commercially available dried blood spot sampling device (80 uL) (TASSO, Tasso Inc, Seattle, USA). Dried blood will be analyzed for HGB, CD71/Band3, ESAs, and reticulocytes. Total Blood drawn for this day of ~76 mL.

Drug intervention, Eugene, OR
For the 4 weeks immediately prior to the scheduled departure date, subjects will arrive at the Cardiopulmonary Lab as required by their intervention schedule (see 'Drug Intervention' below for details) to receive their assigned intervention via a sub-cutaneous injection and/or intravenous

injection. After the last dose, subjects will have venous blood drawn (~60 mL) prior to departure to Colorado for measurements of various biomarkers. A dried blood drop sample (80 uL) will also be taken.

COLORADO FIELD SITE:
All studies performed in Colorado will happen in laboratory space created by the investigators and/or on running tracks and trails in or very near Leadville or Breckenridge, CO.

Early high-altitude measures: Days 1-3
Subjects will fly from Eugene, OR to Denver, CO and be driven from Denver to the field site (~10,000 ft) where they will be housed and fed for 2 weeks. Upon arrival, subjects will fill out AMS questionnaires and have saturation measured. Prior to departure from Eugene, a research staff member will meet subjects at the airport and they will be given a telemetric core temperature pill to take 5-10 hours prior to the 5k time trial in Colorado. If subjects do not take the pill the night prior to exercise testing, they will have to take the temperature pill as a suppository before testing. After, subjects will run the 5k time trial, perform cognitive function tests, and then will be taken to preview the course for the rucksack carry to be performed on day 2. Dried Blood Spot samples will be taken utilizing a commercially available dried blood spot sampling device (80 uL) (TASSO, Tasso Inc, Seattle, USA). Dried blood will be analyzed for HGB, CD71/Band3, ESAs, and reticulocytes. In the evening, subjects will have an IV placed and venous blood drawn, and will perform Hb mass & p50 measurements (10 mL blood draw). Venous blood (60 mL) will be analyzed for various biomarkers. Total blood drawn for this day of ~71 mL. Subjects will fill out AMS questionnaires and have saturation measured before going to sleep. Any subject with child bearing potential will be required to take a pregnancy test on this day.

On day 2, AMS scores will be taken and saturation measured (morning and night), and subjects will participate in the rucksack carry. Cognitive function will also be measured.

On day 3, AMS scores will be taken and saturation measured (morning and night). Cognitive function will also be measured.

Subjects will also undergo ultrasound measurements and have resting ventilation and HCVR measured on either day 2 or 3.

Mid high altitude measures: Days 7-8
On day 7, AMS scores will be taken and saturation measured (morning and night), and subjects will run the 5k time trial, perform cognitive function tests, perform Hb mass & p50 measurements (10 mL blood draw), and have venous blood drawn (60mL). Venous blood will be analyzed for various biomarkers. Dried blood spot samples will also be taken (80 uL). Total blood drawn for this day of ~71 mL. This day will be a repeat of day 1 at altitude. Any subject with child bearing potential will be required to take a pregnancy test on this day. Subjects will be given a telemetric core temperature pill to take 5-10 hours prior to the 5k time trial on day 7/8. If subjects do not take the pill the night prior to exercise testing, they will have to take the temperature pill as a suppository before testing.

On day 8, subjects will participate in the rucksack carry.

Subjects will also undergo ultrasound measurements and have resting ventilation and HCVR measured on either day 7 or 8.

Late high altitude measures: Days 13-14

On day 13, AMS scores will be taken and saturation measured (morning and night), and subjects will undergo ultrasound measurements, run the 5k time trial, perform cognitive function tests, perform Hb mass & p50 measurements (10 mL blood draw), and have venous blood drawn (60mL). Dried blood spot samples will also be taken (80 uL). Venous blood will be analyzed for various biomarkers. Total blood drawn for this day of ~71 mL. This day will be a repeat of day 1 at altitude. Any subject with child bearing potential will be required to take a pregnancy test on this day. Subjects will be given a telemetric core temperature pill to take 5-10 hours prior to the 5k time trial on day 7/8. If subjects do not take the pill the night prior to exercise testing, they will have to take the temperature pill as a suppository before testing.

Subjects will also undergo ultrasound measurements and have resting ventilation and HCVR measured on day 13.

On the morning of day 14, subjects will participate in the rucksack carry. Subjects will be driven back to Denver, CO where they will fly back to Eugene, OR.

Post-altitude measures, Eugene, OR

Within 1 week of returning to Eugene, OR, subjects will return to the Cardiopulmonary Lab (Eugene, OR) to repeat the 5k run and blood draw only. Tests will be performed over post-Altitude days 3-5. The 5k run will be done either on the same day as the other tests, or on a separate day depending on subject availability. Total blood draw 60 mL.

Note: During the 14 days at high altitude, subjects will be involved in data collection on days 1, 2, 3, 7, 8, 13 and 14. On all other days, there will be group activities and the possibility for subjects to hike or bike ride in small groups. They will not participate in any research activities during these "off" days, and they will be reminded frequently to follow CDC guidelines for COVID-19 risk mitigation (see 'COVID-19 exposure scenario' below).

Description of Data Collection Procedures:

Pulmonary Function Tests: Subjects will perform standard non-invasive spirometry to measure a maximal inspiratory and expiratory flow-volume loop, forced vital capacity (FVC), slow vital capacity (SVC), forced expiratory volume in 1 sec (FEV1), and mid expiratory flow (FEF25-75%). These tests will require the subject to blow in and out of a mouthpiece connected to a computerized flowmeter (pneumotachometer). This is a routine clinical test.

VO_{2max} tests: The VO2max test will be an incremental test to volitional exhaustion on a cycle ergometer. Subjects will breathe through a mouthpiece and will begin cycling at a low resistance (50 W), with the resistance increasing every minute by 25 W. The test will continue until the subject reaches volitional exhaustion or a plateau in VO_2 as measured by our metabolic cart (Medgraphics Ultima CPX) is reached. During the entire protocol, subjects will breathe room air through a 2-way non-rebreathing small sampling flowmeter (pneumotachometer), which will allow us to measure

ventilation (V_E), VO_2, and breath-by-breath metabolic data. Subjects will be instrumented with a forehead oxygen sensor to measure heart rate and SpO2 at rest and during exercise.

Army physical fitness tests (APFTs): The Army Physical Fitness Test (APFT) is used by the military to assess the fitness of soldiers. The test consists of the maximum number of push ups in 2 minutes, then the maximum number of sit ups in 2 minutes, and finally the fastest time possible for a 2 mile run. The entire protocol must be completed within 2 hours. No strict cut offs for push ups or sit ups will be used as screening criteria, however, to meet the higher standards required of the Special Operations Forces (SOF), the run has been extended from 3.2 km (2 miles) to 5.0 km, and more stringent selection criteria will be applied. Specifically, all participants must be able to achieve at least 80% of the age adjusted minimum performance level on each of the three APFT criteria, but preference will be given to those who complete the criteria at 100% or more. For example, 100% passing for males (age 18-21), means they are able to complete 42 push ups in 2 minutes, 53 sit-ups in 2 minutes, and 5k run <24:42 (7:57 per mile pace). 100% passing for females (age 18-21), means they are able to complete 19 push ups in 2 minutes, 53 sit-ups in 2 minutes, and 5k run < 29:22 (9:27 per mile pace). The APFT will be performed once at baseline for all subjects and possibly twice for performance stabilization. Below is the table with the 100% passing criteria for all age groups included in the study.

	Male APFT Standards				Female APFT Standards			
Age Group	Push Up	Sit Up	Run (Pace)	Run Time (min)	Push Up	Sit Up	Run (Pace)	Run Time (min)
18-21	42	53	7:57	24.42	19	53	9:27	29.22
22-26	40	50	8:18	25.47	17	50	9:48	30.27
27-31	39	45	8:30	26.25	17	45	10:15	31.51
32-36	36	42	8:51	27.29	15	42	10:51	33.43
37-40	34	38	9:09	28.26	13	38	11:21	35.15

Measurements of Core Temperature: Core temperature (Tcore) will be measured using a Vital Sense Monitor and a telemetric pill (JonahTM ingestible temperature sensor, Mini Mitter Inc, Bend OR). The sterile telemetric pill will be activated by study staff then will be taken orally (with liquid) the 5-10 hours prior to testing or the volunteer will self-insert the pill into the rectum the morning of testing. Core temperature will be measured during the 5k runs (performance stabilization at baseline), and approximately days 1, 7, and 13 at high altitude).

Intravenous catheter and venous blood draw: We will place a 20-22 gauge (small diameter) IV into a vein in the subject's arm. This IV will be used to for various tests – blood draws for Hb mass & p50, blood draws to measure various biomarkers in the blood, iron/saline infusion during drug treatment, and for contrast bubble injections during ultrasound measures at sea level.

Venous blood draw: Blood for measuring biomarkers will be drawn from an IV (60 mL per blood draw) and centrifuged, and de-identified plasma and serum stored at -80°C until assayed. Additionally, blood will be drawn for baseline iron, transferrin, ferritin, complete blood count, and basic metabolic panel analysis (via QUEST diagnostics, 25 mL) on baseline day 1, and blood will be drawn for sickle cell screening on baseline day 6 (via QUEST diagnostics, 5 mL). Intravenous blood for biomarker analysis will be drawn on baseline 6, and on the second day of iron or saline infusion

to minimize the number of needle sticks. Blood will also be drawn days 1, 7, and 13 at high altitude, and at sea-level post altitude. 60 mL of blood will be drawn for biomarker analysis on each of these blood draw days.

Hypercapnic ventilatory response: We will use a published, well-established method (Duffin method) for assessing chemo sensitivity to carbon dioxide (MacKay et al., Adv Physiol Education, 2016). Subjects will be fitted with a nose-clip and breathe room air through a two-way rebreathing valve that can be directed between a filtered 6L non-diffusing bag filled with a mixture of CO_2 and O_2, with values dependent on whether studies are being conducted at baseline or altitude (CO_2 range: 3% to 7%, O_2 range: 60% to 97%) (medical grade), and room air. If O_2 and CO_2 do not equal 100%, the tank will be balanced with nitrogen. Ventilation, end tidal CO_2 ($PETCO_2$), end tidal O_2 ($PETO_2$) and saturation will be measured continuously throughout this test (Medgraphics). To establish a baseline before hypercapnic testing using the modified rebreathing protocol, subjects will breathe room air for 5 min. Subjects will then be coached to hyperventilate by increasing the lung volume and frequency of breathing for 1 minute to reach a target $PETCO_2$ of ~20-25 mm Hg (~40-45 mm Hg is normal); this part of the test is required to reduce CO_2 stores in the body. After 1 minute the subject will be asked to take a full breath in and out then they will be switched to the bag filled with the hyperoxic O_2 and hypercapnic CO_2 mixture and will be coached to take 3 large breaths then resume normal breathing until: 1) their breathing increases so much that it becomes in tolerable and they signal to quit; 2) the $PETCO_2$ = 55 mm Hg or 3) the rebreathing bag becomes deflated. Subjects will be informed of what to expect with this test prior to performing it, i.e they will be told that we will coach them through it and that when they start to breathe out of the bag they will initially have very little urge to breathe but as the rebreathing progresses they will have a very strong urge to breathe.

Comprehensive ultrasound screening: Ultrasound screening will be performed using a previously placed IV (see above). Subjects will sit in the left lateral decubitis position for ultrasound screening. An agitated saline contrast injection will be made while transthoracic saline contrast echocardiography (TTSCE) is performed on the subject to evaluate extent of blood flow through IPAVA and PFO/ASD (see below). Subjects will be asked to perform a Valsalva maneuver while breathing room air. This maneuver enhances blood flow across the PFO/ASD. Multiple saline contrast injections (up to 3) may be performed to verify bubble grades/presence of a PFO/ASD. Pulmonary artery systolic pressure (PASP), cardiac output, and TAPSE (tricuspid annular plane systolic excursion) will be measured with ultrasound. Comprehensive measurements will be done on baseline 3, and PASP, cardiac output, and TAPSE will be measured on days 1, 3, 7, and 13 at high altitude.

Transthoracic Saline Contrast Echocardiography (TTSCE): Echocardiography requires a medical sonographer to place a small probe against the subject's ribcage, which transmits and receives sound waves to produce images that are captured and stored on a computer. Saline contrast is made by manually agitating (mixing) 3-5 ml of sterile saline and 1 ml of air to create a suspension (mixture of liquid and gas) of very small bubbles called microbubbles. This suspension is injected through an IV, which allows us to detect the transpulmonary passage of microbubbles.

Cognitive Function: We will use the Defense Automated Neurobehavioral Assessment (DANA) to assess neurocognitive function across the study. We have recently documented decrements in several components of the DANA during a simulated rapid deployment to high altitude that improved with acclimatization (2) and thus believe the tests are sensitive and specific to the

cognitive challenges SOF face in this environmental extreme. Using a handheld computer, the following nine cognitive function tests will be administered:

1. Simple Reaction Time-1 (measured at the beginning of neurocognitive testing to gain an understanding of pure visual-motor response);
2. Simple Reaction Time-2, repeated at the end of neurocognitive testing to assess diminished reserve of cognitive effort on reaction time;
3. Procedural Reaction Time, a measure of choice reaction time and accuracy;
4. Go-No-Go, a measure of speed, accuracy and impulsivity;
5. Code Substitution—Simultaneous, a measure of visual scanning and attention, learning, and immediate recall of digit-symbol pairings;
6. Code Substitution—Delayed Recall, a measure of short-term memory for digit-symbol pairings;
7. Spatial Discrimination, a measure of visuospatial analytic ability;
8. Matching to Sample, an assessment of attention and memory for visuospatial discrimination;
9. Sternberg's Memory Search, a measure of working memory for letters.

The total time required to complete the battery of tests is ~20 minutes. DANA will be administered once at sea level (baseline day 3) and in the evening of days 1, 3, 7, and 13 at high altitude.

Acute Mountain Sickness (AMS) and oxygen saturation (SpO_2): The severity of AMS symptoms will be determined using a subset of the Environmental Symptoms Questionnaire (ESQ) and the Lake Louise AMS Questionnaire (LLQ), the two most common and accepted measures of AMS.

The ESQ is a self-reported, 68-question inventory used to document symptoms induced by high altitude and other stressful environments. A weighted average of scores from 9 symptoms (headache, lightheaded, dizzy, etc.) designated AMS-C will be calculated. AMS-C scores greater than 0.7 are considered positive for AMS (77).

The LLQ consists of a six question self-reported assessment of AMS symptoms, with a score of ≥ 3, including headache, defined as AMS. The high altitude-illness assessment questionnaires will be administered on paper forms and tabulated in digital format at the end of each day (78).

After completing the AMS assessment forms, arterial saturation will be monitored by pulse oximetry. Each AMS assessment and pulse oximetry measurement will take ~10 minutes to complete. These measurements will be made once at baseline, immediately upon arrival at high altitude, and every night and morning of days 1, 2, 3, 7, and 13 at high altitude. Based on our previous study of 164 subjects, this ascent profile induced symptoms of AMS in ~50% of the subjects on the first evening at high altitude.

Hemoglobin mass (Hb mass) and p50: Determination of blood volume is done by measuring hemoglobin mass using the carbon monoxide (CO) rebreathing method and doing some calculations (see below). Prior to the test the rebreathing circuit is flushed and prefilled with 100% oxygen and sealed. Upon arriving to the laboratory subjects will have an IV placed into their arm by an investigator as described above. Prior to any measurements, subjects rest in the supine position, quietly for ~20 minutes. Then, we obtain 1 blood sample (2 mL) for p50 measurements. After, using a noseclip and mouthpiece, subjects will breathe 100% oxygen for 4 minutes. At the end of this 4 minute period, another 2 mL blood sample will be taken to measure hemoglobin concentration,

hematocrit, baseline proportion of hemoglobin bound with CO (HbCO), and baseline proportion of hemoglobin bound with oxygen (HbO2). HbCO is typically 1.5 to 2.0% in nonsmoking city dwellers. Once the baseline blood sample has been taken, subjects will be instructed to breathe out completely. Then they take a slow breath from the rebreathe circuit prefilled with 100% oxygen to completely fill their lungs while a bolus of 99.9% pure CO is injected into the circuit. The bolus volume of CO is calculated from body mass (1.0 mL/kg body mass for men; 0.8 mL/kg body mass for women). This dose of CO is expected to raise HbCO by ~6% to ~8%, which is below the levels associated with side effects (>15%; see below). After CO administration the subjects begin breathing normally for 10 minutes. At the end of this 10-minute period, subjects expire all of their air back into the spirometer and the spirometer is sealed and a post-rebreathe blood sample of 2mL is taken to measure the above-mentioned blood parameters (with the exception of p50, which is measured once prior to Hb mass testing). Subjects then goes back to resting while breathing ambient air. Finally, we obtain the total volume of gas in the rebreathe circuit and determine the concentration of CO left in the rebreathe circuit after the test. Using established formulas, we calculate hemoglobin mass and then use the following equations to obtain red cell volume (Red Cell Volume = hemoglobin mass x hematocrit x hemoglobin-1 x 100-1) and then blood volume (Blood Volume = red cell volume x 100 x hematocrit-1 x 0.91-1). Subjects will then repeat the above procedures within 10 minutes after completion. Procedures, including volume of CO injected and blood withdrawn, are identical, with the exception of p50 measurements, which will only be done once prior to CO inhalation. The second test is performed to establish a low test-retest variable as it minimizes biological variation that would occur between days. If these two tests yield results within 3% then the test is complete. If greater than 3% we will repeat the 2 tests on a different day once CO levels in the blood have returned to baseline (~48 hours). In total, 10 mL of blood will be taken (8mL for Hb mass - 4mL for each test, and 2mL for p50). CO level in the blood is not expected to exceed 20% following the second test. If it does, subjects will breathe 100% oxygen for 10 minutes. p50, the partial pressure of oxygen when Hb is 50% saturated with oxygen, is calculated using pre-rebreathe values for pH, PvO2, PvCO2, Hb concentration, HbO2, and HbCO. Hb mass will be measured in this manner, i.e., duplicate tests, at baseline day 6 and on days 1, 7, and 13 at high altitude. p50 will be measured on every day Hb mass is measured, as well as on baseline day 3 and day 3 at high altitude when Hb mass is not measured. On p50 only days, one 2mL blood sample will be taken to measure p50, but no Hb mass measurement will be made.

Blood measures: Blood samples will be used to measure biomarkers such as soluble transferrin receptor, ferritin, hepcidin, erythropoietin, C-reactive protein, erythropoiesis agents (ESAs), hemoglobin, hematocrit, red blood cell count, reticulocyte number, reticulocyte percentage, mean corpuscular volume, mean corpuscular hemoglobin, CD71/Band3, cytokines including interleukins (IL) -1β, 6, 8, 10, 12p (70), 17A, 18, 23, and 33, interferons -α2 and -γ, tumor necrosis factor-α, monocyte chemoattractant protein-1, nitric oxide synthase, endothelin-1, myoglobin, matrix metalloproteinases 2 and 9, cystatin C, myeloid-related protein 8/14, osteopontin, neutrophil gelatinase-associated lipocalin, myeloperoxidase, serum amyloid A, insulin like growth factor binding protein 4, intracellular adhesion molecule 1, and vascular cell adhesion protein 1. Serum will also be collected for Luminex analysis of genetics and metabolism regarding inflammation which will include many of the previously mentioned markers. Additionally, serum will be saved for cell culture experiments in which endothelial and vascular smooth muscle cells will be exposed to serum, these cells will be analyzed for mechanistic changes involving inflammation and health status change of the cells.

Plasma, buffy coat, and serum samples will all be stored for later use; however, these samples will be unidentifiable, only stored with an ID number that will indicate which treatment the sample had been exposed to.

Abnormal blood test results will be shared with participants with a recommendation to see their physician.

Dried Blood Spot Sampling: We will use a commercially available and FDA-approved for patient use blood spot single-use disposable sampling device (TASSO, TASSO Inc, Seattle, USA). The device adheres to skin which has been cleaned with isopropyl alcohol with a light adhesive. Upon activation, a vacuum forms and a lancet pricks the surface of the skin. The vacuum draws blood out of the capillaries and into the sample pod attached to the bottom of the device, collecting 4 samples of 20 microliters (0.020 mL) each over the course of 5 minutes and storing the samples on a dried blood spot card contained within the device. After collection, the device is removed from the skin, the sample card separates from the device and is submitted for analysis. Samples will be taken every time a venous blood draw is taken at sea level (baseline 6, and post dosing) and altitude (day 1, 7, and 13).

5k run: The 5-kilometer run will be performed as part of the APFTs at baseline (see above) and on its own on days 1, 7 and 13 in Colorado. The run will be performed on a 400 m track or local running trail (Pre's trail) in Eugene, OR and on the high school track or local paved trails near the research site in Colorado. Subjects will be asked to complete the run as fast as possible while being timed.

Drug intervention: In a single-blind, randomized, placebo-controlled design, subjects will receive a placebo (saline placebo), iron, or erythropoietin.

Iron. Volunteers will receive an intravenous injection of Fe(III)-hydroxide sucrose (200 mg) or placebo twice before high altitude exposure, once 4 weeks and once within several days before traveling to high altitude. All subjects will report to the lab as if they are receiving iron, but only those randomly assigned to the iron arm will receive iron. The remaining subjects will receive intravenous saline. This is infused over the course of approximately 2-5 minutes. This is the same dose shown to prevent AMS, and to block hypoxia-induced pulmonary hypertension[28]. All subjects, regardless of whether they receive iron or placebo, will remain in the lab for an additional 15 minutes after administration to ensure they do not have an allergic reaction (see risks below) and to ensure placebo subjects are blinded to the treatment.

EPO. Volunteers will receive a sub-cutaneous injection of epoetin alpha 150 IU/kg injections given weekly (50 IU/kg 3 x week) for three consecutive weeks prior to high altitude exposure. This dose and timing match the AMS prevention study protocol of Heo et al.[11]

To ensure subjects are blinded to the intervention, all subjects will have the same intervention schedule. For those in the iron group, they will come to the lab 3x per week as required by the EPO intervention schedule, for example, and receive saline interventions. For those in the EPO group, they will come to the lab one time 4 weeks prior to departing to Colorado to receive a placebo injection while the iron group receives their iron injection. The placebo group will receive saline at all visits.

The table below provides the intervention schedule for each group. Blue indicates the placebo group, red indicates the iron group, and green indicates the EPO group.

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
4 weeks until departure				Saline Iron Saline			
3 weeks until departure		Saline Saline EPO		Saline Saline EPO		Saline Saline EPO	
2 weeks until departure		Saline Saline EPO		Saline Saline EPO		Saline Saline EPO	
1 week until departure		Saline Saline EPO		Saline Iron EPO		Saline Saline EPO	**Departure**

Rucksack carry: Subjects will be instructed to complete a 3.1-mile uphill hike as fast as possible while carrying a 35-pound backpack. We previously developed this test to simulate military style field operations for another DoD funded study. The course follows a rugged hiking/jeep trail that begins in a wooded area at ~10,500 feet and ends on a ridge above tree line at ~12,500 feet. Subjects will hike the course as a group after the 5k run first day at high altitude to familiarize themselves with the terrain. The following morning (2^{nd} day) they will be asked to complete the course as fast as possible while being timed. Subjects will also run the course on days 8 and 14. Fit subjects, free of AMS, can finish the course in ~60 minutes.

Traveling and accommodations:
Subjects will be transported to Denver, Colorado in groups of 10-20 by commercial airlines or bus and then immediately driven to Leadville or Breckenridge, Colorado by charter buses. The total travel time from baseline to high altitude will be ~8 hours. Subjects will follow a strict regimen of tasks designed to simulate a high level of physical activity at high altitude (10,000 to 14,000 feet) over three days and two nights (acute) followed by 11 additional days of residence at high altitude (chronic exposure) before returning to Oregon on the 14^{th} day.

Subjects will be housed and fed in condominiums in the Leadville or Breckenridge area. All meals will be cooked and served communally. Omnivorous and vegetarian options will be provided. Subjects will sleep in individual beds, but will share quarters and bathrooms (see note below about COVID management in 'COVID-19 exposure scenario'). Caffeine intake will be limited to the equivalent of two cups of coffee per day. Subjects will refrain from taking any non-study related supplements (over-the-counter, prescription, and recreational) or other non-study drugs or alcohol for the duration of the study beginning with baseline studies through the completion of the study. Violation of these policies will yield a subject's data invalid for the whole study, and thus will result in expulsion from the study and prorated payment.

D. Research Population & Recruitment Methods

Participant population

We will study civilians who are recreational athletes who meet the basic health and physical fitness eligibility standards as evaluated using the modified Army Physical Fitness Test (APFT). We use the APFT because of its standard of use across all US Armed Forces, and our experience with it successfully selecting broadly fit students, i.e. a specialized runner might struggle, but a male or female with overall high aerobic fitness and strength will do well. Subjects will be matched and assigned to either the placebo or the two intervention groups to achieve the necessary statistical power to detect meaningful changes in health and performance at high altitude. Our team has extensive experience in recruiting and screening subjects on college campuses to achieve this goal (4-64).

Up to 300 volunteers will be screened in Eugene, Oregon to identify 100 subjects meeting the inclusion and exclusion criteria (see below).

Inclusion criteria	**Exclusion criteria**
Ages 18 to 40	Smokers
Recreational athletes able to pass the APFTs	Previous severe COVID infection or contraction of any COVID infection occurring during the study. Previous mild to moderate COVID infections will be considered for the study on a case-by-case basis as determined safe by clinical research staff (see inclusion criteria).
Men and women of any ethnic background	
Medical and dental insurance	
Able to read and speak English	Carboxyhemoglobin values (HbCO) 3% or greater at baseline
Fully vaccinated against COVID-19	Diseases or disorders known to be affected by hypoxia or the drugs used in this study, including but not limited to hypotension, anemia, sickle cell trait or disease, and diabetes.
*If a subject is taking medication that is deemed safe and will not interfere with the main outcome measurements of the study as determined by clinical research staff, they will be included.	Anyone unable to receive the investigational drugs used in this protocol (EPO or iron).
*If a subject had a previous mild to moderate COVID-19 infection but is deemed safe for all research activities by clinical research staff, then they will be included in the study.	Those with a history of significant head injury, migraines or seizures.
	Anyone that is pregnant or trying to become pregnant.

	Any medication determined by the clinical research staff to be unsafe or to interfere with the outcome measurements of the study. Those with inability to be headache-free when consuming the amount of caffeine in two six-ounce cups of coffee or less per day. Extended exposure (>6 hours) to high altitude above 1000m in the month leading up to departure to Colorado. Those who have been on an airline flight over six hours (the lowered cabin pressure for an extended period of time approximates exposure to high altitude) within the month leading up to departure to Colorado. Those who are unable to achieve the minimum physical criteria as outlined above. Anyone with lung function below the lower limit of normal per GLI standards. Previous diagnosis of high altitude pulmonary edema or high altitude cerebral edema upon altitude exposure. Failure to get fully vaccinated against COVID-19. Choosing not to be vaccinated will result in exclusion. Family history of clotting disorders, anemia or venous thrombosis. Plans for professional competition during or within 1 week after participation in this study as participation may enhance your aerobic performance Presence or absence of a PFO once we have enrolled a sufficient number of each group representation in the general population (e.g., ~40% of population has a PFO and ~60% does not). However, all subjects will be allowed to

	complete all baseline screening to assure that we will have enough subjects to go to Colorado Biological sex once we have enrolled a sufficient number of males and/or females as we are aiming to enroll ~50% of each sex. However, all subjects will be allowed to complete all baseline screening to assure that we will have enough subjects to go to Colorado. Subjects will be excluded from telemetric pill ONLY if they have a history of obstructive diseases of the gastrointestinal tract including diverticulosis, diverticulitis, inflammatory bowel disease, peptic ulcer disease, Crohn's disease, ulcerative colitis, or previous GI surgery.

Detailed recruitment methods

Recruitment of participants will begin immediately following IRB approval. Participants will be solicited through flyers posted locally (posted in Eugene and on University of Oregon's campus), online, via word of mouth, through directly contacting UO students, faculty, and staff using the list maintained by the lab of potentially participants who have expressed interest in future participation, and through newspaper and radio ads. Screening will be conducted at University of Oregon, under the direction of Dr. Andy Lovering, and University of Oregon personnel will seek informed consent.

The PI (Andy Lovering) and graduate students will be responsible for actively recruiting participants and seeking informed consent.

Potential subjects responding to advertisements will be given a basic description of the study over either phone or email (depending on personal preference) and given a chance to ask any initial questions. Questions in the phone and email script will be used to ensure those most likely to qualify present for the comprehensive screening days. If the subjects wish to participate in the study, we will schedule their first visit.

During subject recruitment, we will give examples of exclusion criteria to enable participant self-identification for exclusion. For example, "have you ever had a blood clot, or been told that you are anemic?"

Compensation

In return for the time and effort, subjects will be compensated for the study visits completed as follows:

- Baseline Screening: $75
- Performance Stabilization: $25

- Colorado Week 1: $400
- Colorado Week 2: $400
- Post-study visits: $100

All expenses (airfare, lodging and food) will be paid for the subject's trip to Colorado. Subjects will receive a check for a total of $1000, if they complete the entire study. Subjects will be mailed a check for $600 by Research Logistics LLC, the study sponsor, approximately 2 weeks after completion of the study and they will receive a separate check for $400 from the University of Oregon's Cardiopulmonary and Respiratory Physiology Laboratory. If subjects do not complete all study procedures or violate the study rules (such as drinking alcohol or excess caffeine or violating CDC guidelines for lessening COVID-19 exposure risks), payment will be prorated based on the above compensation schedule, e.g., if subjects complete baseline screening and performance stabilization before dropping out they will be paid $100. Subjects who do not comply with the study rules will be housed until they are able to be sent home on the next available flight without excessive costs to the investigators and will be paid only for the portions of the study they have completed before they were removed from the study.

E. Informed Consent Process

Informed consent will be administered to each subject by the PI and colleagues. The PI is well versed in the process of informed consent and has trained his co-investigators thoroughly on how to best perform this procedure. The PI will ensure that all investigators obtaining consent have experience in the informed consent procedure and are capable of adequately discussing the related physiology, study procedure and potential risks.

The researcher will first verbally explain the study in its entirety and in doing so walk through the informed consent in person or via an online video conferencing service. Subjects will then be given a sufficient length of time to read through the informed consent form privately and instructed/encouraged to write down or remember any questions/concerns they may have. Afterward, the researcher will rejoin the subject and address any question or concern they may have while subsequently going back through the informed consent form with the subject and obtaining any needed initials and signature at the end of the document. Furthermore, the investigator will verbally address any questions the subject may have regarding the seriousness and/or likelihood for the occurrence of the risks described in the Informed Consent Form. The research team will address probability and severity of any adverse reactions with the subject by carefully explaining the statements regarding probability and severity contained within the Informed Consent. The investigator will also provide appropriate statistics (where available) regarding the probability of adverse reactions. The investigator also advises the subject of what he/she can expect to feel during a particular procedure, for example, during contrast injection. Investigator further explains any questions regarding physiology or reason for a particular procedure in plain language.

This process will be done either in person when they arrive for their physical exam and informed consent visit or done via an online video conferencing service prior to coming to the lab for their physical exam. Remote informed consent via an online video conferencing service will be conducted in groups sized 1-20, and we will allow individual subjects to ask questions privately in breakout rooms. Subjects will be asked to sign the informed consent once they arrive for their first visit to the lab.

Before all tests, the subject will be telephoned or e-mailed (depending on preference) or told in person (while in Colorado) to confirm participation, remind them of the activities that will take place, checking for questions, and confirming continued interest and as a reminder to: 1) stay hydrated by drinking at least 2 liters of water a day, and 500 ml the morning of the test day; 2) to limit caffeine intake to the equivalent of two 8 oz cups of coffee a day; 3) limit exercise to that prescribed in the study design; with no exercise the day before the VO2Max, APFT and 5K tests; and 4) for exercise tests to time their eating so 1-2 hrs elapse between last meal and heavy exercise (VO2Max, 5K run or ruck hike). In addition, subjects will be emailed (while in Oregon) or reminded in person (while in Colorado) to complete their COVID-19 compliance survey prior to arriving to the lab.

A copy of the consent will be posted after the trial is closed to recruitment and no later than 60 days after the last visit by any subject in this study.

F. Provisions for Participant Privacy and Data Confidentiality

Each subject folder will be stored in locking file cabinets inside the primary investigators' locking offices (Dr. Andrew Lovering, Dr. Robert Roach, Dr. Andrew Subudhi, or Dr. Joseph Duke). All other computer files associated with the subject will be identified only through their unique subject ID and stored on password protected lab computers. The de-identified data will be kept for at least 7 years after publication, per NIH guidelines. In the unlikely event the data are not published, they will be kept for at least 10 years after collection. This will ensure sufficient time for publication after data have been collected considering some trainees take up to 6 years to graduate, and often publication does not occur until many years after graduation.

Each subject will be assigned an ID using a random number code system consisting of three to five letters describing the study (e.g., COAST) and a random, non-repeating number (1-1000). This ID will be associated with their data folder, which will contain all study documents and data collected including all associated forms (i.e. informed consent document).

The PI will maintain a subject ID key capable of identifying subject IDs to subject names and contact information to provide us with the ability to identify subjects as additional questions or research findings arise. This ID key will be kept in a locked filing cabinet also within the office of the primary investigator. No contact information will be stored with subject data.

De-identified data may potentially be shared with other investigators for research purposes.

Specimens collected for research will be de-identified using the subject's unique ID and stored at the University of Oregon until future analysis. Blood collected to be analyzed by a third party (QUEST diagnostics) will be labeled with the subject's ID and birthdate, as required by QUEST, and results from those tests will be available only to members of the Lovering lab who have access to the password protected QUEST Quanum online platform. No subject names are included online or on their specimens, and all specimens get destroyed after analysis.

G. Potential Research Risks or Discomforts to Participants

Confidentiality:

If data is lost or stolen, subjects could experience invasion of privacy. To minimize the potential invasion of privacy, we are not collecting social security numbers so that the potential economic impact is greatly minimized. All of our files will be kept in a locked filing cabinet to prevent theft and

data will be de-identified. Data acquired on computers will be password protected. As such, the ***probability*** of the adverse outcomes discussed above is low, and the ***severity*** is minimal.

Psychological:
For the echocardiography tests, female subjects will need to have no sports bra on to allow for imaging of the heart and the placement of small electrodes on the chest to record heart rate. To avoid any discomfort, subjects will be given a large, loose-fitting shirt (provided by researchers) to cover up. Male subjects will either go shirtless or be given a loose-fitting shirt. As such, the ***probability*** of the adverse outcomes discussed above is low, and the ***severity*** is minimal.

Physiological:
Travel: All subjects will be transported between Eugene and Colorado by automobiles and airplanes. Travel by road or air, carries the risk of injury or death. According to the National Transportation Safety Board, there are 0.0003 fatal accidents for every 1,000,000 miles flown by US carriers. The National Highway Traffic Safety Administration reports a fatality rate of 1.13 per 100,000,000 miles driven on US roads. To minimize this risk, only commercial, US carriers will be used for air travel and all traffic laws will be strictly obeyed.

Pulmonary Function Tests: Risks associated with pulmonary function testing include shortness of breath, cough, dizziness, and possible loss of consciousness. To minimize risks, the co-investigators will administer all pulmonary function tests and allow subjects to rest between measurements. Lung function testing performed in our lab is a routine assessment performed in pulmonary function labs all over the world according to American Thoracic Society and European Respiratory Society standards. Subjects can stop the test at any time if they feel any of the above symptoms. The probability and severity of these risks is very low.

VO_{2MAX} testing*:* Subjects will perform a VO2max test where they exercise to volitional exhaustion. Criteria for terminating a VO2max include achieving: 1) heart rate >85% of age-predicted max (220-age), 2) a plateau in oxygen consumption and/or a respiratory exchange ratio >1.15; all of these criteria are continuously monitored on our metabolic system and Nellcor pulse oximeter. This carries the rare risk of dizziness, confusion, nausea, fatigue, difficulty breathing, abnormal heart rhythms, stroke, heart attack, and sudden death. In subjects less than 35 years old, the risk of sudden death of all causes is estimated to be 1 in 133,000 for men (Van Camp et al MSSE 1995). In this study only 100 of 136 deaths with identifiable causes were caused by cardiac events, so this estimate of risk may overestimate incidence of cardiac events. In the event of a cardiac event, research personnel will respond in a manner appropriate to their level of training. All graduate students, study coordinators, and PIs are minimum CPR/AED/first aid certified and will therefore immediately call emergency services and perform CPR if appropriate. The overall risk of sudden death caused by a heart problem for all ages and sexes is estimated to be 1 in 15,000 to 18,000. (*Source:* American College of Sports Medicine - ACSM). As such, the ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is very low (e.g. dizziness) to very high (e.g. sudden death).

Additional exercise tests (APFTs, 5k run, and rucksack carry): Hard exercise has inherent risks associated with it, including stroke and heart attack. These risks are very low in young, healthy individuals (less than 0.00006%). Subjects may also feel dizzy, lightheaded, or unusually winded. These symptoms can be worse during exercise at high altitude. If subjects feel faint or feel like they

can't get enough oxygen, subjects will be instructed to tell study personnel as soon as possible. For field exercise tests, like the rucksack carry, research personnel will be stationed at various points along the course, including the start point, turn-around point, and various mid-way points. All research personnel will be equipped with subject lists, and each subject will wear an identifiable running number on their clothing. Therefore, if a subject feels feint during this test, they will be close to the aid of research personnel and easily identifiable to the research team. Subjects may experience muscle injury and stiffness following exercise. Proper warm-up and cool-down procedures will minimize the chances of this occurring.

Risks associated with core temperature pill: The risks of using the temperature pills include mechanical injury to the mucus membranes if adequate care is not used. Risks of the temperature pill will be minimized by explaining the procedures to the volunteers. Additionally, ample lubricant (suppository) or water (oral) will be provided to the volunteer. Volunteers with history of obstructive diseases of the gastrointestinal tract including diverticulosis, diverticulitis, inflammatory bowel disease, peptic ulcer disease, Crohn's disease, ulcerative colitis, or previous GI surgery will not use a telemetric pill.

Hypercapnia during rebreathing tests: Risks associated with breathing hypercapnia (high carbon dioxide) at rest include feeling light-headed, headache, fatigue, dizziness, shortness of breath. Subjects will be monitored with a peripheral estimate of arterial oxygen saturation using a forehead monitor to ensure you are well oxygenated and we will continuously monitor inspired and expired oxygen and carbon dioxide levels. As such, the ***probability*** of the adverse reactions discussed above is moderate, and the ***severity*** is minimal.

Intravenous catheter: Risks associated with placement of an IV include pain and/or bleeding during placement, vasovagal syncope, hematoma (pooling of blood under the skin), infection, and vessel blockage. The placement of the IV may cause some discomfort with rare bleeding or bruising at the puncture site. It also carries the risk of infection. To mitigate risks associated with vasovagal syncope, the subject will be safely positioned upright and sitting in an IV chair, and the subject will be continually monitored by Dr. Lovering and colleagues, all of whom are trained to place IVs. As such, the ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is minimal.

Blood Removal: Risks associated with the removal of blood include an aversion to seeing blood that could result in nausea, vasovagal syncope, increased stress, and/or feeling faint. To mitigate these potential risks, the subject is continually monitored by Andrew Lovering PhD and colleagues. In addition, the subject is safely and comfortably positioned on a gurney or cot. In this way, the potential risk of vasovagal syncope (i.e., fainting) is mitigated.

A total of ~435-465 mL of blood will be drawn throughout the study. The range up to 465ml is included in case extra samples are required for screening metabolic panels due to abnormalities in findings or compromised samples. Of that volume, 25mL will be drawn baseline day 1 as part of the physical examination. In total, ~162 mL will be drawn prior to departure to Colorado for metabolic panels, biomarker analysis, dried blood spots, Hb mass, and p50. An additional ~213 mL will be drawn during the 2 weeks at high altitude (max volume of ~71mL per day), and ~60 mL will be drawn as part of post-altitude testing. 1 pint of blood, the normal volume of blood donated on a given day, is ~473mL. Throughout the entire study, we will be taking less than that, and the maximum volume for a given day does not exceed ~76 mL. As such, the ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is minimal.

The following table is meant to breakdown the volume of venous blood that will be drawn on a given day, as well as what it will be used for:

	Informed consent	Baseline 6	Post Treatment	Altitude 1	Altitude 7	Altitude 13	Post-altitude
Screening panels	25 mL	5mL	None	None	None	None	None
Biomarkers	None	60 mL	60 mL	60 mL	60 mL	60 mL	60 mL
Hb Mass & p50	None	10 mL	None	10 mL	10 mL	10 mL	None
Dried blood spot	None	0.08 mL	0.08 mL	0.08 mL	0.08 mL	0.08 mL	None

Saline Contrast Echocardiography: The PI has been using saline contrast echocardiography since 2003 to detect blood flow through intracardiac and intrapulmonary shunts. Risks: transient dizziness associated with agitated sterile saline injection in patients with cardiac shunting. With respect to exercise, the Principal Investigator has >10 years of experience using TTSCE in a research setting. In 4 years (2003-2007) at the University of Wisconsin Madison, approximately 60 human (male and female) subjects (including 8 subjects with a patent foramen ovale) were tested without a single adverse event related to TTSCE. Additionally, research done at the University or Oregon between 2008 and 2016 has involved >100 subjects using TTSCE at rest and during exercise without incident related to the TTSCE. We will use agitated sterile saline without preservatives. Furthermore, we will use a minimal volume (3-5 mL) of sterile saline. Dr. Lovering and colleagues will perform saline contrast injections, while a trained ultrasonographer will perform echocardiography. Mixed saline (saltwater), either alone or with 5% sugar in water **has been used to help see the ultrasound pictures (echocardiogram) for over thirty years**. Saline contrast bubble injections are routinely used to screen for the presence of a patent foramen ovale in the clinic. The American Society of Echocardiography Guidelines (2014) state that "...life threatening reactions are rare (<1 in 10,000)" when using contrast injections (including bubbles with protein shells) and The European Association of Echocardiography (2009) has stated that "... the evidence shows that contrast echocardiography is very safe in clinical practice." And this includes using stabilized bubbles with protein shells and we only use non-stabilized saline contrast bubbles in our lab. We only use a small amount of air mixed with saline, thus the ***probability*** of any ***severe*** adverse reaction is very low. Given the evidence presented above, the ***probability*** of the adverse reactions discussed above is low. Although the ***severity*** of arterial gas emboli is high, given the amount of air used and the short life span of intravascular bubbles of this size, the likelihood of the constellation of unfortunate events required for a serious adverse reaction to occur is very small.

Hb mass measurement: Risks associated with CO determination of blood volume include exposure to carbon monoxide. Most non-smoking city dwellers have 1.5-2.0% CO in their blood and the duplicate tests will increase it to ~15%. The level most commonly associated with symptoms is >15%, with no symptoms reported at 10% or lower. Minor symptoms are present when CO levels rise to 30%. These symptoms may include: confusion, headache, fatigue, nausea, shortness of breath, dizziness, cough, and cherry-colored lips. To protect against an excessive increase in CO we

will only test individuals whose baseline CO level is 3% or less. Further, if an individual's post-CO rises to 20% or higher following the duplicate test we will initiate oxygen therapy. This will have subjects breathe 100% oxygen for 10 min, which reduces the CO half-life from 5 hours to 80 minutes and is the approved therapy used in a clinical setting to treat CO poisoning. In the unlikely event that a subject's CO bound to hemoglobin is excessive and does not appear to be responding to O_2 therapy we will call appropriate police dispatch, depending on the location of the incident (Eugene, OR or Colorado). This method has been in use in research in a wide variety of populations to measure Hb mass including elite athletes (male and female), untrained individuals (male and female), adolescents (11-15 year old boys and girls), elderly subjects, and pregnant women with no ill effects reported. The use of CO as a marker for measuring blood volume has been in use for over 100 years without notable complications. Subjects will be verbally reminded of the symptoms associated with increased carbon monoxide levels prior to their participation in the CO uptake test and will be asked to notify researchers if they are experiencing any of the side effects of carbon monoxide during or after the test.

Altitude associated risks: Staying at high altitude causes systemic hypoxia. This decreased oxygenation can lead to several things depending on how high one goes, how fast one goes there, and how well adjusted to high altitude one is. The consequences can range from mild to severe.

The most common symptoms of high altitude exposure are feeling lightheaded, dizzy, or short of breath during exercise. We expect up to 40% of people to feel one of these things while at high altitude. A smaller number of people will develop acute mountain sickness (AMS). AMS is a name for the combination of headache and lack of appetite, difficulty sleeping, upset stomach, throwing up, or feeling weak. These symptoms are usually temporary and manageable. They typically do not threaten peoples' health. Participants will not be treated for AMS symptoms and AMS should be gone by day 3 at the altitudes studied and therefore very unlikely to overlap with COVID symptoms and COVID testing will occur 4-5 days after arrival to Colorado to confirm the presence or absence of COVID infection. In addition, while PFO+ subjects have shown to have increased susceptibility to AMS, this illness is non-life threatening and does not increase risks for those with a PFO traveling to altitude. More severe altitude illness will be identified and treated as outlined below.

Traveling from sea level to high altitude can, in rare occasions, lead to more serious conditions. Two of these include high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE). Both of these conditions are potentially fatal if left untreated. HAPE is a serious disorder that sometimes occurs with a fast change in altitude (going up). It occurs in less than 1% of people going from baseline to altitudes used in this study. The APFT will be at 9,075 feet above baseline. The uphill hike will start at 10,627 feet and go to 12,595 feet. Subjects will sleep at 10,570 feet. This sleeping altitude is not expected to produce HAPE. HAPE symptoms are treatable if found early. They usually show up 2-3 days after ascent to high altitude. HAPE may be preceded by AMS. The inability to breathe while resting is the hallmark of HAPE. Subjects may also have a bad cough, blue lips, fever, or fast heart beat. Subjects will be instructed to immediately notify study personnel if they feel out of breath at rest. Suspected HAPE cases will be treated by a doctor familiar with the condition.

The other serious complication of high altitude is HACE. HACE is very rare. The possibility of seeing HACE is extremely low. In our previous studies involving >150 volunteers at these altitudes in Colorado, HAPE and HACE have never been seen. As with HAPE, it is treatable if recognized early.

Loss of coordination and mental confusion are the major symptoms of HACE. HACE victims typically show AMS or HAPE first. If a subject starts to behave irrationally or appear very uncoordinated, they will be treated by a doctor immediately.

To protect subjects during their stay in Colorado, we will monitor them in several ways. Their oxygen levels will be monitored with a standard finger oximetry (Nellcor N-200). Subjects will also fill out a questionnaire designed to quantify the level of AMS they are experiencing, if any. Furthermore, subjects will be monitored for other signs that they are proceeding down a path that may threaten their health. These include severe AMS symptoms: a severe headache that does not go away, uncontrolled vomiting, extreme dizziness, or excessive weakness. Other signs include: blue fingernails, trouble breathing while resting, unsteady walk, extreme paleness, or incoherent or bizarre behavior. In the event that the research team notices these signs and symptoms, they will call emergency services immediately so that appropriate medical personnel can treat these symptoms. The research being done in Colorado, whether in Leadville or Breckenridge, will occur in and around town and will never be far from hospital care or quick emergency transport. Oxygen and transportation back to Denver will be available at all times. In the event of a life-threatening emergency, we will call 911. Although we do not expect most of these symptoms to develop, we cannot rule out the possibility. It is important to note that the combined experience of the research team amounts to over a century of doing this type of study with no severe adverse events in that time.

During the rucksack carry, there is cell phone service throughout the hikes. Subjects are accompanied with research teams ahead of and behind the subjects, with research assistants setup along the trail with radios (walkie talkies) to contact the teams ahead of and behind the runners with updates on participant status. The Colorado group has completed over 300 runs with research subjects in these exact same conditions with no medical adverse events to date. Any of the vehicles we will be driving can transport a subject to the Frisco medical center within 15-20 minutes. In Leadville, St. Vincent Health Hospital is 1.4 miles from College Mountain College (where subject's will be staying) and subjects can be transported within 5-10 minutes. A physician will be on the course with a vehicle, with supplemental medical oxygen, and a full field first aid kit at all times subjects are on the course.

There are also risks of being in the mountains. The most significant environmental risks include lightning strikes, traumatic injury from falls in rough mountain terrain, and sunburn from the higher UV-light exposure. These risks will be minimized by requiring subjects to remain in the vehicle or indoors during electrical storms; by prohibiting subjects from any rock climbing activity, and by making subjects aware of the sunburn risk and supplying them with sunscreen. Finally, the investigators have more than three decades of experience conducting field research and dealing with these challenges,[4-64] including recent experience with an identical protocol in identical mountain setting with nearly identical testing procedures and with no serious unanticipated outcomes.

Risks associated with drug interventions: Some of the known risks associated with EPO are joint/ muscle/bone pain, fever, cough, rash, nausea, vomitting, soreness of mouth, itching, headache, redness and pain in the skin where EPO shots are given, myocardial infarction, stroke, venous thromboembolism, thrombosis of vascular access, and tumor progression or recurrence. Some of the known risks associated with iron are pain and swelling at the site of injection. In order to

minimize these risks, all precautions and warning labels will be followed, and dosing volumes will be strictly adhered to. Serious allergic reactions to this drug are unlikely, but may include: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing. Subjects will be excluded from the study for any health problems that may negatively effect their reaction to these drugs (see table of inclusion and exclusion criteria and drug appendices for specific exclusion criteria). FDA recall lists for drugs being administered to participants will be checked weekly beginning with the initiation of drug administration.

Risks for subjects who are pregnant: Potential risks to subjects who are pregnant (beyond the risks outlined above for the general population) include exercising beyond than what is recommended by their doctor (see 'VO_{2max} testing' and 'additional exercise testing' above) and potential unknown risks to the child bearer or fetus from carbon monoxide inhalation (see 'Hb mass measurement' above). None of the additional risks described above are greater in those who are pregnant. Due to the increased risk of exercise testing and Hb mass measurements, any subject with childbearing potential will be required to take a pregnancy test on a day that involves maximal exercise testing (VO2max) and Hb mass testing while at baseline and in Colorado, and if such a test comes back positive, they will be immediately excluded from the study. Therefore, there is no added risk to the child bearer or the fetus from any of the above-mentioned protocols, as they will not undergo those procedures. As such, the probability of an adverse reaction is zero, and the severity of an adverse reaction is zero.

COVID-19 related risks: Due to the SARS-Cov2 pandemic, there is the added risk of COVID-19 infection for subjects and staff in this study. To mitigate this risk, **all research personnel will be fully vaccinated before the initiation of any study (see below).** Additionally, per standard protocol, all mouthpieces will be disinfected with Cidex and all surfaces wiped with disinfecting wipes between participants. Researchers will wear appropriate PPE, including masks, lab coats or scrubs, safety glasses, and gloves. Additionally, proper social distancing can be done with the most protocols described above, i.e. maintaining 6 feet between participants and researchers. When possible, testing will be performed outdoors to maximize social distancing (i.e. during 5km and uphill rucksack tests). While the severity of COVID-19 infection has the potential to be severe even in our young subject population, the measures described to mitigate this risk make the likelihood of an event low. Subjects will also be reminded during the consent process and throughout the study that failure to comply with these precautions will result in their removal from the study. Subjects will be cohabitating in Colorado so to minimize COVID outbreaks subjects will be assigned to research groups of 4-5 so that if one subject develops COVID, we can minimize transmission within our other groups. Subjects will wear masks, wash hands frequently and will have hand sanitizer in each room while cohabitating. In addition, we have created a COVID-19 plan outlined below, including additional precautions during travel, exposure scenarios, vaccination requirements, etc.

COVID-19 plan

All research team members and subjects will be required to be fully vaccinated prior to beginning the study. Subjects' vaccination cards will be scanned, and identifiable data (name, birthday, etc.) will be blacked out prior to photocopied vaccine card being added to subject folders. Subjects will be considered fully vaccinated to the extent that is recommended by the CDC and University of Oregon (for example, if a booster shot is recommended and available, subjects and researchers must receive it).

Prior to departure to Colorado, subjects will self-monitor symptoms and be told to not come in to the lab if they experience any symptoms of COVID and get tested if recommended by current CDC and University of Oregon guidelines. While in Colorado, research team members will screen all participants daily for symptoms and signs of COVID. If subjects report symptoms that suggest they have COVID, transportation will be provided to the closest COVID testing center. The subject or the subject's insurance company will be responsible for costs related to the COVID test, if there are costs incurred. If the subject's COVID test is positive prior to or while in Colorado, they will be withdrawn from the study and they will be treated by local medical personnel according to CDC guidelines. The subject or the subject's insurance company will be responsible for any costs related to medical care for COVID. Research team members will check on subjects closely and will work to arrange safe transportation back to Oregon when the medical personnel tell us it is safe for them to travel.

Compensation for an injury or assistance with medical bills resulting from the subject's participation, including contracting COVID, in the course of this research is not available from the University of Oregon or Research Logistics LLC. If subjects get sick or injured as part of your participation in this study, the subject or the subject's insurance provider will be charged for the cost of the care.

COVID Compliance protocol

Compliance to CDC guidelines for minimizing risk of exposure to COVID-19 must be adhered to by all participants for the duration of the study. In order to hold participants accountable, subjects will be emailed a Qualtrics survey to check 'yes, I have been following appropriate COVID protocols', or 'no, I have not been following appropriate COVID protocols' in their reminder email prior to every lab visit in Oregon. Subjects will also be required to fill this survey out starting weekly 30 days prior to departure to Colorado. Participants will be required to fill out this survey daily while in Colorado reporting that they are following CDC guidelines and will have the opportunity to report pod mates who are no following guidelines. Additionally, each week the participants will be updated on any changes to CDC guidelines which will be included as part of the survey.

COVID transportation protocol

Participants will be given a "covid-19 travel bag" which will include one 4 oz bottle of hand sanitizer (minimum 60% alcohol), 3 KN95 masks, and several alcohol wipes. We will ask the participants to do the following while traveling:

- Before walking into the airport: put KN95 mask on, this will stay on the entire duration of airplane travel
- When seated on airplane use alcohol wipes to disinfect all touch surfaces by the seat including the lap tray, buckle, arm rests, overhead light/air controls, and if in window seat, the window screen pull. Then, use hand sanitizer to clean hands.
- After exiting the airport in Colorado trash the first KN95 mask and replace with a fresh one, this mask will be used for the duration of the bus ride to Leadville or Breckenridge. Use hand sanitizer.
- When seated in the bus, use alcohol wipes to disinfect all touch surfaces by the seat. Use hand sanitizer.
- Upon arrival to Leadville or Breckenridge, trash the bus KN95 and replace with a fresh mask. Use hand sanitizer.
- Participants will be given a new "covid-19 travel bag" when they depart from Colorado so that their travel back may be as safe as possible. They will be expected to follow the same protocol for traveling to Eugene as they did while traveling to Colorado.

COVID testing protocol
If at any time during a subject's stay in Colorado they experience symptoms of COVID, they will be given a COVID test and their pod will be quarantined.

Safety Equipment available in the Cardiopulmonary and Respiratory Physiology Laboratory (where all testing and screening will be performed): Phillips FRx AED, a spare AED battery, and spare AED pads, bottled oxygen and face masks, all necessary equipment to run ACLS algorithms (suction, masks, breathing bag, etc.) and ACLS drugs such as chewable aspirin, and oxygen will be administered as required by AHA and ACLS guidelines. Additionally, a standard first aid kit is also available.

Emergency Procedures: According to American Heart guidelines, in the event of an adverse cardiopulmonary event, we will begin CPR, call 911 (as directed by U of O EH&S), and will continue CPR as required until emergency medical personnel arrive. Epinephrine auto-injectors (Epi Pens) will be available if the PI and colleagues judgement and/or 911 operator supported a decision to use an Epi Pen. In addition, a separate emergency procedure document is included.

Safety Monitoring: *Subjects will be given clear instructions that they should notify the investigators immediately if they experience any of the above-mentioned risk symptoms.* During the VO2max test and recovery procedures, all subjects will be non-invasively and continuously monitored for vital signs using a forehead monitor to monitor arterial oxygen saturation and heart rate. During all other procedures subjects will be visually monitored for any signs of discomfort, distress or problems. During both exercise and resting procedures, the investigators will continuously ask the subjects how they are feeling and how they are doing. If subjects have ingested a core temperature pill they will be given an orange safety bracelet (no MRI) to wear until the study staff can ensure the pill has been passed.

Medical Monitor: Dr. Bill Cornwell, MD, University of Colorado, Colorado Springs (UCCS) will serve as the medical monitor for this study. Dr. Cornwell will receive regular reports on volunteer status. He will also evaluate adverse event reports and monitor study progress. He will discuss research progress with the PI on a weekly basis. He will promptly report discrepancies or problems to the IRB of record and the HRPO. He shall have the authority to stop this research study in progress, remove individual subjects from a study, and take whatever steps are necessary to protect the safety and wellbeing of research subjects until the IRB can assess their report. The medical monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor will comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship of the event to participation in the study. The medical monitor will also indicate whether he concurs with the details of the report provided by the PI. Reports for events determined by either the PI or medical monitor to be possibly or definitely related to participation and reports of events resulting in death will be promptly forwarded to the USAMRMC ORP HRPO.

H. Potential Benefits of the Research

This study will not improve the health of subjects and is only being done to gather information. This study will not improve the health of the general subject population. Completing the aims of this study will have the benefit of contributing to generalizable knowledge.

I. Investigator Experience

The PI and Co-Investigators colleagues have been performing cardiopulmonary and respiratory physiology investigations at rest and during exercise in healthy and diseased populations at the University of Oregon for ~10 years. Dr. Lovering's CV is on file. Notably, Drs. Lovering, Subudhi and Roach successfully completed the 2012 AltitudeOmics research project which was also based in Oregon and Colorado, and also involved the safe transport and study of 21 research subjects to very high altitude in Bolivia. That study resulted in no serious adverse events in any research subject, and in more than 20 research publications.

The PI trains all graduate and undergraduate personnel on all laboratory procedures and protocols. Ultrasound technician Eben Futral, RDCS has worked with Dr. Lovering's group for years as well (Futral ~5 years). Futral, a registered diagnostic cardiac sonographers (RDCS), has performed thousands of resting and stress echoes in patients and research subjects.

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