Does Patent Foramen Ovale Size Matter in Men and Women

NCT #: Not Assigned

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Study Title: Does patent foramen ovale size matter in men and women?

Protocol Number: 04302018.049

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A. Introduction and Background

A patent foramen ovale (PFO) is present in ~30% of the general population. The PFO has historically been considered to be trivial. However, recent work by our group and others has identified that, compared to individuals without a PFO, those with a PFO have worse pulmonary gas exchange efficiency, have a higher core body temperature, blunted ventilatory responses to chronic hypoxia and acute carbon dioxide and increased susceptibility to altitude illnesses such as acute mountain sickness, and high altitude pulmonary edema (Lovering, Elliott & Davis J Appl Physiol 2016). Specific to this application, subjects with a PFO may have worse pulmonary gas exchange efficiency because a PFO is a potential source of right-to-left shunt that will make pulmonary gas exchange efficiency worse. If true, then this may negatively impact exercise capacity and/or exercise tolerance.

Further, in those with a PFO compared to those without, preliminary work from our lab indicates that there may be an effect of PFO size on pulmonary gas exchange efficiency. This is such that those with a large PFO (grade 3 or higher) display significantly worse gas exchange efficiency compared to those with a small (grade 2 or lower) or no PFO, even at low exercise workloads. Additionally, we were curious as to whether there would be a sex effect, but due to logistical constraints, we were unable to recruit an equal number of female and male subjects. Thus, in addition to the potential size effect on our outcome measures, we would like to build on this work by examining the potential effect of biological sex.

Although a PFO has been traditionally considered to have a minimal impact of physiology and pathophysiology, emerging evidence suggests this may not be the case. Our lab is focused on understanding how and why a relatively small hole in the heart (PFO) can have a relatively large impact on cardiopulmonary and respiratory physiology, and how these impacts may be based on the size of the PFO.

B. Specific Aims/Study Objectives

The overarching goal of this study is to examine how cardiopulmonary and respiratory physiology may be altered by the size of a PFO and/or biological sex.

To do this, we will:

1) Quantify pulmonary gas exchange efficiency (alveolar to arterial O2 difference) and arterial oxygenation at rest and during exercise.

2) Quantify aerobic exercise capacity and six-minute walk test.

3) Quantify Q_{IPAVA} at rest.

4) Quantify plasma inflammatory markers (TNFα, IL-1, 6 & CRP)

5) Quantify core body temperature

Hypotheses: We hypothesize that those individuals with a large PFO will display worse pulmonary gas exchange efficiency, compared to those with a small or no PFO; there will not be a sex effect. A large PFO, when compared to a small or no PFO, will be associated with increased plasma inflammatory mediators, and will result in a higher core body temperature.

C. Methods, Materials and Analysis

This study will be performed over the course of 3 days at the Cardiopulmonary and Respiratory Physiology Lab at the University of Oregon.

Screening at each study visit: Female subjects will be required to take a urine pregnancy test before each visit begins. A positive test at anytime will result in exclusion. A Modified Allen's test will be performed on Day 1 for all subjects and during the day when an arterial line is placed (Day 3). Failing the Modified Allen's test at anytime will result in exclusion.

Description of Study Days

Day 1 (~ 2 hours)

Subjects will undergo informed consent, fill out health history questionnaire, and perform a Modified Allen's Test (~45 min)

Subjects will be comprehensively screened for any cardiac abnormalities. Amount of blood flow travelling through the PFO and IPAVA will be graded using saline contrast echocardiography while the subject is breathing room air (PFO and IPAVA blood flow) and breathing 100% O2 (PFO blood flow only). Pulmonary artery pressure will also be measured using ultrasound. Intravenous blood draw, we will take 21mls (~2 Tablespoons) of iv blood (~1.25 hrs)

Subjects will perform pulmonary function, plethysmography and diffusing capacity (30min)

Day 2 (~ 1 hour)

Subjects will perform a 6 minute walk test (~15 min)

Subjects will perform a VO2max test (~30 min)

Day 3 (~ 3.5 hours)

Subjects will be instrumented with a radial artery catheter by Dr. Jerry Hawn, MD. An esophageal temperature probe and an iv for saline contrast injections will also be placed. (~1hr)

Arterial blood will be taken at rest while subject is breathing room air and 100% O2 for 10-20 minutes to calculate shunt fraction (~30 min).

Subjects will exercise on a cycle ergometer at standardized workloads of 70, 100, 130 and 160 Watts (W) for 3 minutes each, followed by 15 W/min increments until VO2Max is reached. This will be done with subjects breathing room air (~1.5hr)

After exercise breathing room air, subjects will take a 30-minute break. (~30min)

Subject will repeat the exercise protocol above while breathing 55% O2 (~1.5hrs); note 60% O2 is the maximal level of oxygen that can be breathed and still get metabolic data required for calculating the AaDO2.

Description of Study Day Procedures

Modified Allen's Test:
This test examines collateral blood flow in the hand, and is performed on the day there is an arterial line placed. The subject clenches their hand for ~30 seconds while the radial and ulnar arteries are occluded by the investigator. The ulnar artery is released and the return of circulation (color to the hand) is examined. If the color doesn't return, then this is considered a negative test (fail). Both hands will be tested, two failures (right and left hand) is exclusionary.

Comprehensive Ultrasound Screening:
An intravenous catheter will be placed in the subject. 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. This will be repeated while breathing 100% O2 for 10 minutes. Subjects will be asked to perform a Valsalva maneuver while breathing room air and 100% O2. A Valsalva maneuver is performed by completing a moderately forceful exhalation against a closed glottis aka 'bearing down.' This maneuver enhances blood flow across the PFO. Multiple saline contrast injections (up to 3) may be performed to verify bubble grades/presence of a PFO.

Pulmonary Function Tests:

Subjects will perform standard non-invasive spirometry to measure a maximal inspiratory and expiratory flow-volume loop, forced vital capacity (FVC), 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 performed in pulmonary function labs in the United States.

Diffusion Capacity (DLCO):
This is a standard non-invasive test for diffusion capacity for carbon monoxide (CO) using the single breath, breath-hold technique. It requires the subject to breathe in a single breath of a commercially available (MedGraphics/Airgas) diffusion gas containing 0.3% CO. The subject will breathe in this breath through a mouthpiece connected to a flowmeter and hold it for approximately 10 seconds before exhaling the gas. This may be repeated a maximum of 3 to 5 times. This is a routine clinical test performed in pulmonary function labs in the United States.

Whole-body Plethysmography:
Subjects perform a standard non-invasive procedure to measure functional residual capacity (FRC), which is the amount of air within the lungs at the end of a passive expiration. Subjects also perform a standard non-invasive slow vital capacity (SVC) maneuver to determine the vital capacity (usable capacity) of the lungs. These procedures will require the subject to sit inside a clear plexiglass box called a whole body plethysmograph (MedGraphics Elite) and breathe room air through a mouthpiece (pneumotachometer) connected to a flowmeter. Using the value obtained for FRC and the value obtained for SVC total lung capacity (TLC) can be determined. This is a routine clinical test performed in pulmonary function labs in the United States.

Nitrogen Washout:
Subjects may perform a standard non-invasive procedure to measure residual volume for calculation of total lung capacity. This procedure will require subjects to breathe 100% oxygen through a mouthpiece for 7-10 minutes while we monitor nitrogen washout of the lungs. This will be done following ATS/ERS standard criteria. This is a routine clinical test performed in pulmonary function labs in the United States. <u>This will only be performed if the plethysmograph is not functioning properly, i.e. it is a contingent test. Of note we have never needed to perform this test.</u>

Transthoracic Saline Contrast Echocardiography (TTSCE):
Echocardiography requires a medical sonographer from PeaceHealth 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.

Intravenous Catheter (IV):
We will place a 20-22 gauge (small diameter) IV into a vein in the subject’s arm that will be used for the rapid injection of the agitated sterile saline for TTSCE. This

catheter will also be used to measure plasma inflammatory mediators. Blood will be centrifuged and de-identified plasma stored at -80°C until assayed by BioLegend's LEGENDplex immunoassay. Total IV blood draw is approximately 21 mL.

VO_{2MAX} test and 6 minute walk test:
The six minute walk test will be performed on the second floor of the Center for Medical Education and Research. The subjects begins walking laps around a continuous hallway for six minutes. One lab of the hallway is measured using a roller-wheel measuring tape. The number of laps completed by the subject is recorded, and the total distance is then calculated.

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 very low resistance (50 Watts), 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 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 12 lead EKG.

Arterial Blood Gas Measurement:
Prior to catheter placement the physician will perform a modified Allen's Test to confirm adequacy of collateral circulation of the hand. A physician (Jerold Hawn, MD) will perform catheterization of the radial artery. Using a standard, sterile procedure Dr. Hawn will place a small catheter into the radial artery (arterial catheterization). Dr. Hawn will initially numb the wrist with lidocaine/nitroglycerin solution. Then Dr. Hawn will place a needle into the radial artery. Dr. Hawn will then place a small guide wire through the needle in to the vessel. Then he will place a flexible catheter into the vessel using the guide wire for direction. This catheter will be used to collect blood totaling <100 mL (< 7 Tablespoons). Blood draws will be accompanied by a heparinized saline flush (1unit heparin/ml) to reduce chances of catheter clotting. 12 lead ECG, heart rate, metabolic, ventilation data will be continuously collected and arterial blood oxygen saturation will be continuously measured via a non-invasive transcutaneous forehead sensor.

*Note: TOTAL blood draw for Subjects (i.e., IV + Arterial Line) will be less than 250 mL.

Exercise with arterial line:
After arterial line placement (see above) the subjects will perform two bouts of graded exercise tests on the cycle ergometer at standardized workload stages of 70, 100, 130 and 160 W with each stage lasting 3 minutes, followed by 15W/min increments until VO2Max is reached. During one bout, subjects will breathe normal oxygen levels (21% oxygen), during the other bout, subjects will breathe high oxygen levels (55% oxygen). Bouts will be randomized and separated by 30 minutes of rest breathing room air.

Intrapulmonary & intracardiac Shunting with arterial line placement:
Saline contrast echocardiography will be performed during the third minute of each exercise stage to assess intrapulmonary and intracardiac shunting. Saline contrast echocardiography will also be performed in NON-exercise subjects at rest.

Measurement of core body temperature:
An esophageal temperature probe (Nelcor used with Mon-a-Therm System) will be placed. We will be using esophageal temperature monitoring because core temperature is a primary outcome variable of this study and esophageal temperature is considered to be the gold standard technique for measuring core temperature during short duration exercise testing. We also use this temperature to correct our blood gas values. In order to place the esophageal probe, subjects will be given lidocaine Jelly (2% lidocaine, 1 ml intranasal administration). After administration of the lidocaine Jelly, researchers will place the esophageal probe through one of the nostrils, and advance the probe until it is visible in the back of the throat. Once the probe is visible, the subject will then begin to sip water through a straw while the researcher advances the probe to the appropriate depth. The swallowing of the water helps to insure that the probe goes down the esophagus and not the trachea. Researchers will make no more than 2 attempts per nostril to place the esophageal probe. In the event subjects cannot tolerate the esophageal temperature probe, subjects will swallow an ingestible pill, about the size of a multi-vitamin, that is designed for human use (FDA approved) and is accurate to 0.1ºC (CoreTemp, HQI Technologies, Inc.). This pill will be used to measure core body temperature at rest and during cycle ergometer exercise. Prior to ingestion the temperature pill will be calibrated with an external wireless recording device. This will allow us to wirelessly acquire the subjects core temperature when necessary as the temperature pill transmits a signal to the external device that will then display the appropriate temperature. This pill will harmlessly pass through the subject's intestinal tract. The pill is not recovered, is disposable, and every subject receives a new pill for each study day.

D. Research Population & Recruitment Methods & Compensation

Sample size, Population and Recruitment Methods:

<u>Sample Size and Data Analyses:</u>

Necessary sample size was determined with our primary outcome variables ($AaDO_2$, VO_{2MAX}, saline contrast bubble scores, TNFα levels) using our preliminary data, previously published work and *a priori* power analysis. Using G-Power and information about a physiologically-meaningful effect from published studies, we determined that n = 15 individuals would be sufficient to test our hypotheses. Thus, we are asking to recruit 45 subjects (sex of these subjects may be chosen specifically to ensure even distribution of subjects in order to appropriately analyze the effect of sex on our outcome measures) in the event our actual pre-to-post changes are less than expected and/or there is greater variability than our preliminary data suggest.

Mean differences in $AaDO_2$ and core body temperature between males and females, and between those with a small or no PFO compared to a large PFO will be analyzed

using a mixed analysis of covariance (MANCOVA). Mean differences in $VO_{2MAX,}$ 6 minute walk test, inflammatory markers between males and females, and between those with a small or no PFO compared to a large PFO will be analyzed using an unpaired t-test.

The population of subjects will include males and females between the ages of 18-40 and will be recruited via subject fliers. In addition, subjects known to have or not have a PFO based on previous participation in studies in the Cardiopulmonary and Respiratory Physiology Lab at the University of Oregon may be contacted to evaluate whether they would be interested in participating in this study.

Subject fliers, initial phone script and email script are attached.

Detailed Recruitment Information

A) Once a subject contacts us (via flier) or is contacted by us (via previous study participation) we will use the phone script (attached) to determine their eligibility. The phone script is the first instance where the subject will be informed about restrictions regarding caffeine/alcohol/food and medications.

B) **Screening at each study visit:** Female subjects will be required to take a urine pregnancy test before each visit begins. A positive test at anytime will result in exclusion. A Modified Allen's test will be performed on Day 1 for all subjects and during day when an arterial line is placed (Day 3). Failing the Modified Allen's test at anytime will result in exclusion.

C) Two days before the first visit, the subject will be telephoned or e-mailed (depending on preference) to confirm participation and as a reminder to 1) not drink caffeine for 12 hours before each study day, 2) not exercise or drink alcohol for 24 hours before the study day and 3) not eat for 2 hours before arriving for the study day and 4) to stop taking medications or herbal supplements for 48 hours before the study visit (as determined by their referring physician). Male subjects will be required to go shirtless for echocardiography. Female subjects will need to wear a sports bra for echocardiography. To minimize any risk of embarrassment both male and female subjects will be allowed to wear a loose-fitting shirt (provided by the researchers) that allows the upper body to be covered but also allows for imaging of the heart and the placement of small electrodes that record heart rate.

D) Subjects may be either referred to us by a flier, OR, we may contact subjects who have previously enrolled in studies in our lab. Thus, to verify that it is safe for subjects to refrain from taking medications 48 hours prior to the study day, we will contact the associated physician to determine whether or not this is appropriate.

Table 1

Inclusion	**Exclusion**

Inclusion	Exclusion
Men and women aged 18-40 recruited from subjects in the surrounding community, as well as individuals who have previously participated in research studies in the Cardiopulmonary and Respiratory Physiology Lab at the University of Oregon who are known to have/not have a PFO. Specifically, we are looking to recruit 6 male and 9 female subjects, have them fully participate in the study, and then we will be able to appropriately analyze the effect of biological sex on our outcome variables. It may be the case where we end up recruiting more subjects, if for example other exclusionary criteria are met.	Previous history of coronary artery disease (ischemic heart disease such as angina, heart attack, myocardial infarction). Failure of Modified Allen's Test in both hands. Currently taking medications or herbal supplements for any heart or respiratory disease that they cannot stop taking for 48hrs prior to testing (seasonal allergy medication not included in exclusion medications). Lidocaine, nitroglycerine or heparin allergy. Women who are pregnant or trying to become pregnant. Previous history of any condition that would prevent the subject from performing cycle ergometer exercise (for exercise study only). Because we are looking to recruit enough male (6) and female (9) subjects to fill in data from our preliminary work, if (for example) we reach the point where we have recruited and studied 9 female subjects, the next female who may be interested in participating may end up being excluded. This is to ensure we have an even number of male/female subjects to appropriately test the potential effect of biological sex on our outcome measures. Recreational drug use.

Compensation

Subject Compensation (maximum of $220:

Visit #1: $25: Completing the informed consent and health history questionnaire ($5.00) and echocardiographic screening and blood draw ($10.00) will pay $15.00 total. The pulmonary function tests, the diffusion capacity test, and the whole body plethysmography or nitrogen washout will pay $10.00. Subjects who choose not to participate upon review of the informed consent or are excluded based on any findings during this visit will be paid for their degree of visit completion.

Visit #2: $20: Completing the six minute walk test ($5) completing the VO2max test ($15).

Visit #3: $175: Completing the arterial blood line ($100) and exercise tests ($75).

Any subject that withdraws (or is withdrawn) before the end of the study will receive prorated payment up to the point of completion.

The following table is meant to assist as a screenshot of what tests are conducted on each of the three study days, as well as shown the compensation for each test.

Table 2

Visits:	1	2	3
Days	x	x	x
Study Procedures:			
IC & Health Hx, HIPAA form	x		
Modified Allen Test	x		x
Comprehensive Screening	x		
Pulmonary Function	x		
Diffusing Capacity	x		
Plethysmography	x		
Nitrogen Washout*	x		
Transthoracic Saline Contrast Echo (TTSCE)	x		x
Intravenous catheter (IV)	x		x
VO2Max & 6 min walk		x	
Arterial Blood Gas Measurement			x
Exercise w/arterial line			x
Intrapulmonary/intracardiac shunt w/art line			x
Core Body Temp			x
Compensation:			
IC & Health Hx	5		
Echo screen/blood draw	10		
PFT, diff capacity, plethysmography	10		
six min walk		5	
vo2max		15	
arterial line			100
exercise tests			75
blood draws/breathing room air			
Total for completed study visit	25	20	175
Total for all aspects of the study	220		

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E. Informed Consent Process

Once a subject contacts our lab, OR we contact an individual who has previously participated in our studies, we will use the phone script to determine whether or not that subject would be eligible for the study (see phone script document for the five exclusionary questions). That being said, no participant identifiable data will be obtained. It is merely used as a pre-screening tool to determine if the subject would fit the inclusion/exclusion criteria.

Informed consent will be administered to each subject by the primary investigator and colleagues. During the initial meeting where we will attain informed consent, the subject will be provided with the document entitled 'PFO Fill HIPAA.' The primary investigator and colleagues will verbally explain this document, and answer any questions the subject has. Detailed inclusion/exclusion criteria will be discussed with the subject.

The primary investigator 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. 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.

F. Provisions for Participant Privacy and Data Confidentiality

Each subject folder will be stored in a locking file cabinet inside the primary investigators locking office located in the University of Oregon Cardiopulmonary and Respiratory Physiology Lab. This lab is located on the 2nd floor of the Center for Medical Education and Research Building at 722 E. 11th St., Eugene, OR 97403. All other computer files associated with the subject will be identified only through their unique subject ID and stored on a 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., FILL) and a random, non-repeating number (1-1000). This ID will be associated with their unique folder, which will contain all study documents and data collected including all associated forms (i.e. informed consent document). This ID will also be used to label subject blood samples, which will be centrifuged, and the de-identified plasma will be stored at -80 degrees C until assayed. The remaining sample (not plasma) will be discarded into a biohazard waste container and will be collected and destroyed by the Environmental Hazard and Safety Department at the University of Oregon.

The primary investigator 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.

If the subject, upon their first visit does not qualify for the study (see Table 1 above), any subject information will be immediately destroyed. Thus, if the subject does not consent to participate AND/OR they fail the screening procedures, the individually identifiable PHI obtained for recruitment will be destroyed.

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:

Male subjects will be required to go shirtless for echocardiography and the VO_{2Max} test. Female subjects will need to wear a sports bra for echocardiography and the VO_{2Max} test. Female subjects will be allowed to wear a loose-fitting scrub top (provided by the researchers) over their sports bra that allows for echocardiographic imaging and EKG electrode placement. Accordingly, both male and female subjects could potentially feel embarrassed or have modesty issues by being shirtless (males) or when only wearing a sports bra (females). To minimize the risk of embarrassment or modesty issues, both male and female subjects will be allowed to wear a loose-fitting scrub top (provided by the researchers) that allows for concealment of upper body but also allows for echocardiographic imaging and EKG electrode placement. Female subjects will still be allowed to wear the sports bra with the scrub top which will allow for required instrumentation, but will also allow the subject to cover up as much as possible for the study. Both males and females may wear sweat pants or shorts, i.e. whatever makes the subject comfortable. As such, the ***probability*** of the adverse outcomes discussed above is low, and the ***severity*** is minimal.

Physiological:

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. You can stop the test at any time if you feel any of the above symptoms. The probability and severity of these risks is very low.

Whole Body Plethysmography: Risks associated with whole body plethysmography include shortness of breath, dizziness, and cough. To minimize risks, the co-investigators will administer all whole-body plethysmography tests and allow subjects to rest between measurements. As with pulmonary function testing, the ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is minimal.

Nitrogen Washout: There are no risks associated with breathing 100% oxygen for 7-10 minutes.

Diffusion Capacity (DLco): Risks associated with the diffusion capacity testing include exposure to carbon monoxide (CO), shortness of breath, dizziness, and cough. The percentage of CO in the gas being breathed will be 0.3%. Cigarette smoke contains 3% CO or ten times the amount of CO that will be breathed. Performing the test 5 times will increase the level of CO bound to hemoglobin in the subject's blood by 3.5%. Normal resting levels of CO bound to hemoglobin are less than 3% as a non-smoker. This means that if a subject performs 5 tests, their blood levels of CO will increase from 3 to 3.1 %, maximally. The half-life of CO in blood is between 5 and 6 hr, meaning that blood with 3.1 % CO content to start with, would have less than 1% carbon monoxide 15 to 18 hours later. As such, the ***probability*** of the

adverse reactions discussed above is low, and the ***severity*** is minimal. The diffusing capacity test 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. As with the lung function testing, the probability and severity of these risks is low. To minimize risks associated with testing diffusion capacity of the lung using CO, tests will be performed using a commercially available diffusion gas mixture with 0.3% CO and each subject will be limited to 3 to 5 single breath tests separated by a minimum of 4 min according to ATS/ERS recommendations to minimize exposure to CO. The co-investigators will administer all diffusion capacity tests.

Esophageal temperature probe: Risks associated with placing the esophageal temperature probe include nose and throat irritation, gagging sensation and an extremely remote theoretical risk of esophageal puncture. As such, the ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is minimal. In the event subjects cannot tolerate the esophageal temperature probe, subjects will swallow an ingestible pill, about the size of a multi-vitamin, that is designed for human use (FDA approved) and is accurate to 0.1ºC (CoreTemp, HQI Technologies, Inc.). This pill will be used to measure core body temperature at rest and during cycle ergometer exercise. Prior to ingestion the temperature pill will be calibrated with an external wireless recording device. This will allow us to wirelessly acquire the subjects core temperature when necessary as the temperature pill transmits a signal to the external device that will then display the appropriate temperature. This pill will harmlessly pass through the subject's intestinal tract. The pill is not recovered, is disposable, and every subject receives a new pill for each study day. The ***probability*** and ***severity*** of risks associated with the temperature pill is very minimal.

VO_{2MAX} and exercise testing*:* Subjects will perform a VO2max test where they exercise to volitional exhaustion. Criteria for terminating a VO2 max 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 12 lead EKG. 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. 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).

Arterial catheterization: Risks associated with the radial artery catheter include pain and/or bleeding during insertion, vasovagal syncope, and hematoma (collection of blood under the skin at the puncture site). Extremely rare risks usually associated with long-term insertion (many days) of an arterial catheter include infection and blood vessel blockage. If a blood vessel blockage were to occur, immediate surgical

treatment may be required. Arterial catheterization will be performed by a board-certified cardiologist (Dr. Jerold Hawn) while the subject is comfortably and safely positioned on a gurney to mitigate the potential risks associated with vasovagal syncope. As such, the ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is moderate. The Principal Investigator has extensive experience using this technique in a research setting.

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/or a graduate student. As such, the ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is minimal. Dr. Lovering or a graduate student will place IVs.

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 (IV placement) and colleagues. In addition, the subject is safely and comfortably positioned on either a gurney or IV chair. In this way, the potential risk of vasovagal syncope (i.e., fainting) is mitigated. As such, the ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is minimal.

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 or a graduate student will perform saline contrast injections, while Randy Goodman or Eben Futral from PeaceHealth 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.

Heparin and lidocaine allergy: Both carry the potential risk of eliciting an allergic reaction. The risk of developing an allergic reaction to each of these individually or cumulatively is very small. Some symptoms of an allergic reaction include itching, tingling, chest tightness, difficulty breathing, nausea, or vomiting. Subjects will be continuously monitored for each of these symptoms. The ***probability*** of this risk is very low and ***severity*** of these risks is moderate.

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.

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.

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 all exercise and recovery procedures, all subjects will be non-invasively and continuously monitored for vital signs using: 1) a 12 lead EKG to monitor electrical activity of the heart, and 2) a forehead probe 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.

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 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.

The PI trains all graduate and undergraduate personnel on all laboratory procedures and protocols. Co-investigator Jerry Hawn, MD is a board certified cardiologist who has worked with Dr. Lovering's group for almost 10 years placing radial artery

catheters, interpreting echoes and providing significant intellectual input to the laboratory group.

Ultrasound technicians, Randy Goodman, RDCS and Eben Futral, RDCS have worked with Dr. Lovering's group for years as well (Goodman ~10 years; Futral ~5 years). These two registered diagnostic cardiac sonographers (RDCS) have performed thousands of resting and stress echoes in patients and research subjects.