Does patent foramen ovale closure improve exercise capacity & prevent blood flow through intrapulmonary shunt?

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Study Title: Does patent foramen ovale closure improve exercise capacity & prevent blood flow through intrapulmonary shunt?

Protocol Number: #12132016.027

Principal Investigator: Andrew T Lovering, PhD

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 a higher core body temperature, significantly worse pulmonary gas exchange efficiency, 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 maybe 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.

Our lab group has demonstrated that hypoxemia increases blood flow through intrapulmonary arteriovenous anastomoses (IPAVA) in healthy and subjects with COPD (Lovering, Duke & Elliott J Physiol, 2015; Norris et al Exp Physiol, 2016). When these subjects breathe 100% O2 it prevents or reduces blood flow through IPAVA. This suggests that hypoxemia per se induces blood flow through IPAVA. The blood flow through IPAVA and presence of a PFO is also associated with increased risk of stroke and/or transient ischemic attack (Duke, Elliott, Lovering Echocardiography 2015). In addition, an atrial septal defect (ASD) is a hole within the interatrial septum, and is considered a congenital heart defect. An ASD is typically larger than a PFO, and thus, the symptoms may be worse in those with an ASD, compared to those with a PFO. Thus, some hypoxemic patients who have had a stroke or transient ischemic attack, who also have a PFO/ASD may undergo surgical closure of their PFO/ASD to prevent subsequent neurological sequelae. This surgical closure may also prevent the hypoxemia thereby reducing or preventing blood flow through IPAVA. Of note, blood flow through IPAVA has been demonstrated to be strongly correlated with TIA and/or stroke and has not previously been taken into consideration in randomized clinical trials mentioned below (Abushora et al *JASE*, 2013).

Three randomized clinical trials have determined that PFO closure is not superior to regular medical management, for the prevention of subsequent stroke and/or TIA. Nevertheless, the American Heart Association still recommends that “in patients with cryptogenic [unexplained] TIA or stroke, a PFO, and DVT, guidelines from the American College of Chest Physicians currently recommend vitamin K antagonist therapy for 3 months and consideration of PFO closure rather than no vitamin K antagonist therapy or aspirin therapy.” (Whitlock et al., *CHEST* 2012; 141(2)(Suppl):e576S–e600S, Kernan et al., *Stroke*. 2014;45:2160-2236). Additionally, in the largest single center retrospective study performed to date, PFO closure for the purpose of preventing hypoxemia was found to result in “improvement in echocardiographic evidence of right to left shunt, New York Heat Association functional class, and oxygen requirement.” (Fenster et al *Am J Cardiol* 2013).

Thus, PFO/ASD closure remains a potentially beneficial option for both hypoxemic and stroke/TIA patients.

Lastly, preliminary data also suggest greater levels of plasma inflammatory mediators in subjects with a PFO and systemic inflammation is associated with increased risk of cardiovascular diseases. Importantly, exercise is known to reduce so of these systemic inflammatory mediator levels. Thus, PFO/ASD closure may allow for greater exercise capacity and a subsequent reduction in inflammation.

Thus, 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/ASD) can have a relatively large impact on cardiopulmonary and respiratory physiology.

B. Specific Aims/Study Objectives

The overarching goal of this study is to examine cardiopulmonary and respiratory physiology pre and post PFO/ASD closure in patients who are undergoing surgical closure of their PFO/ASD.

Pre and post PFO/ASD closure, 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, daily activity, six-minute walk test.

3) Quantify Q_{IPAVA} at rest and assess recurrence of stroke or TIA at 3 months.

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

5) Measure hypercapnic ventilatory response

6) Quantify core body temperature

Hypotheses: We hypothesize that closure of PFO/ASD will improve pulmonary gas exchange efficiency, improve exercise capacity, and reduce blood flow through PFO/ASD and IPAVA thereby preventing subsequent stroke. PFO/ASD closure will also reduce plasma inflammatory mediators, increase ventilation in response to a hypercapnic challenge, and will result in a lower core body temperature.

C. Methods, Materials and Analysis

This study will be performed over the course of 7 days at the Cardiopulmonary and Respiratory Physiology Lab at the University of Oregon. Days 1-3 are Pre-PFO/ASD closure procedure and Days 4-7 repeat all research activities beginning at 3 months post PFO/ASD closure procedure. Those *cleared for exercise* by their cardiologists (see recruitment below)

will participate in all aspects of the study, Days 1-7. Those *not cleared for exercise* by their cardiologists will participate in all non-exercise aspects of the study Days 1-4 **[gray highlight for non-exercise subject information]**.

Special note on post PFO/ASD closure procedures: The timing of post PFO/ASD closure procedure visits were carefully selected (i.e., 3 and 6 months) based on recommendations from the referring physicians (Dr. Richard Padgett and Dr. Samuel Lau). It is their collective recommendation that 6 months (and in some cases 3 months) is sufficient to allow for physical closure/endothelialization of the PFO/ASD. However, if is it determined at 6 months post PFO/ASD closure procedure that the PFO/ASD has been deemed NOT PHYSICALLY CLOSED/ENDOTHELIALIZED by the referring physician, the subject will not partake in the post-closure visit(s) scheduled at 6 months, **AND** they will be excluded from the study.

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 each day when an arterial line is placed. Failing the Modified Allen's test at anytime will result in exclusion.

Protected Health Information (PHI): Participating Physicians Dr. Padgett and Lau will provide PHI to the investigators. The PHI provided will include: the reason(s) for PFO/ASD closure procedure (e.g., TIA, Stroke, dyspnea upon exertion, etc.), when the PFO/ASD has physically closed/endothelialized per referring physician (e.g., at 3 or 6 months), current and past medication information, and results from clinical exercise studies as they pertain to determining the reason(s) for closure (e.g., arterial oxygen saturation at rest and during exercise, rating of perceived exertion, etc). Subjects will be asked to sign a HIPAA authorization form on Day 1. The PHI will be used for research purposes only. Of note, this information is only solicited via self-report from those who have reported a prior history at the time of enrollment.

The following Figures (i.e., 1 & 2) are meant to assist in determining the path through the study days, and should be used in conjunction with the detailed study-day descriptions.

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Figure 1. Exercise

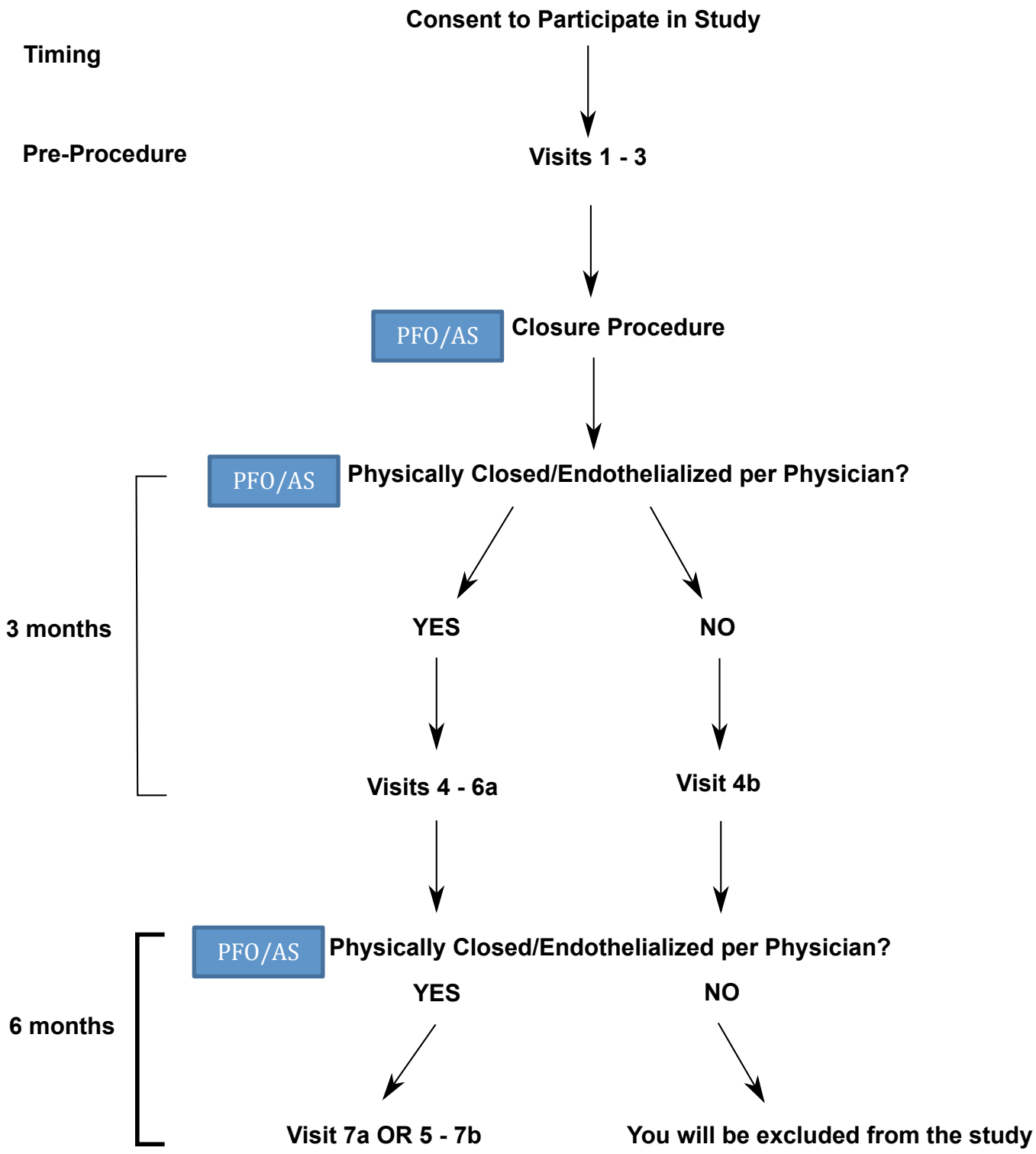
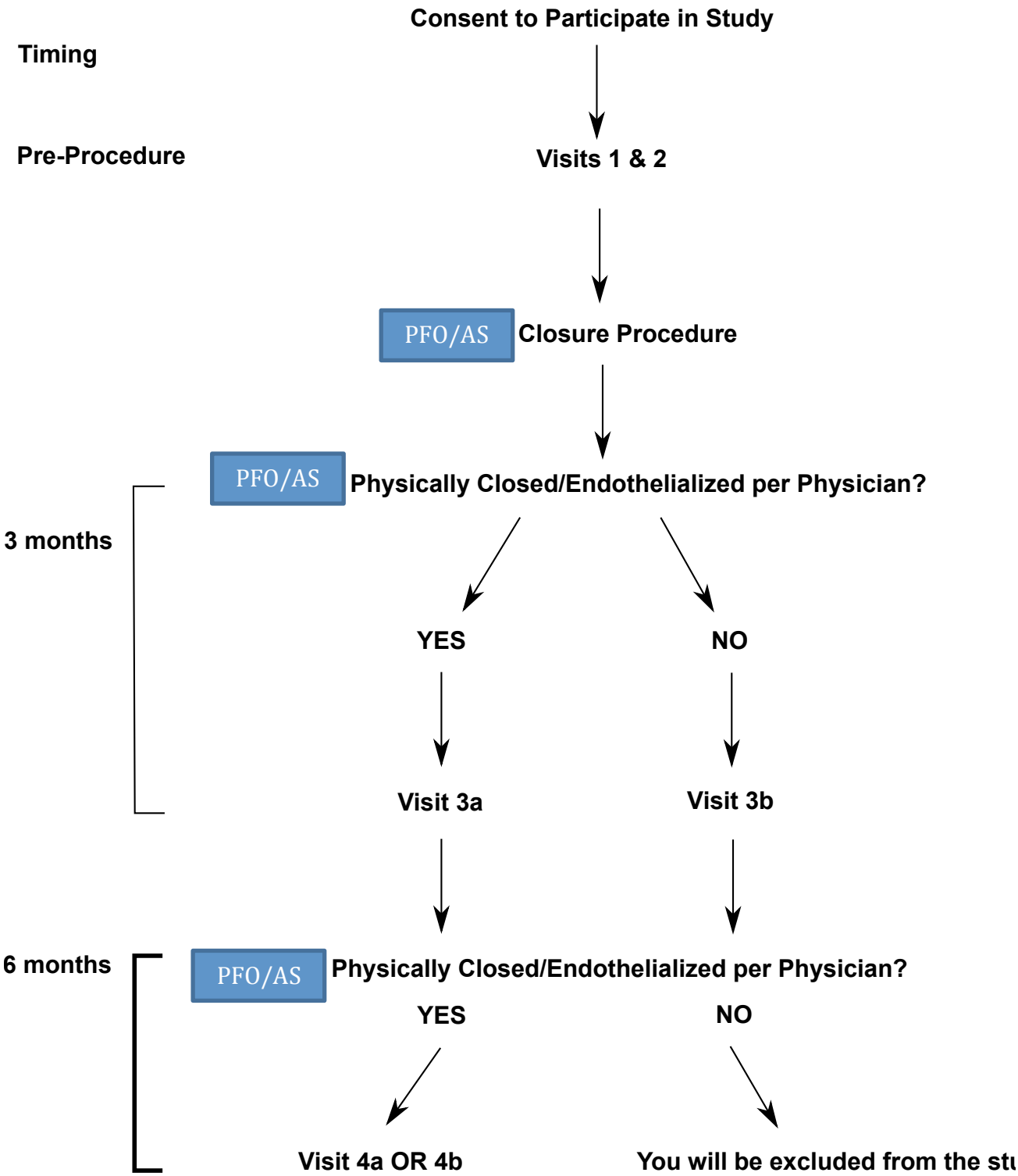

Timing
Consent to Participate in Study
Pre-Procedure
Visits 1 - 3
PFO/AS
Closure Procedure
PFO/AS
Physically Closed/Endothelialized per Physician?
3 months
YES
NO
Visits 4 - 6a
Visit 4b
PFO/AS
Physically Closed/Endothelialized per Physician?
6 months
YES
NO
Visit 7a OR 5 - 7b
You will be excluded from the study

Figure 2. Non-Exercise



EXERCISE SUBJECTS

What follows is a detailed description of the study days. For convenience, please refer to Figure 1. Exercise (page 4) to assist in determining the timing of study days.

Visit 1 Pre PFO/ASD Closure Procedure (~2.5hrs) AND:

Visit 4a (PFO/ASD Physically Closed/Endothelialized) (~2 hours) OR,

Visit 4b (PFO/ASD Not Physically Closed/Endothelialized) (~30 min)

Visit 4a (If PFO/ASD is **physically closed/endothelialized** at 3 months per referring physician)

Subjects will undergo informed consent, fill out health history questionnaire, sign a HIPAA authorization form 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/ASD and IPAVA will be graded using saline contrast echocardiography while the subject is breathing room air (PFO/ASD and IPAVA blood flow) and breathing 100% O2 (PFO/ASD 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)

Visit 4b (If PFO/ASD is **not physically closed/endothelialized** at 3 months per referring physician)

Subjects will receive and intravenous blood draw, and we will take 21mls (~2 Tablespoons) of IV blood (30 min).

Visit 2 Pre PFO/ASD Closure Procedure (~2.5 hrs) AND:

Visit 5a (PFO/ASD Physically Closed/Endothelialized) (~2.5 hrs) OR,

Visit 5b (PFO/ASD Not Physically Closed/Endothelialized) (~2 hrs):

Visit 5a (If PFO/ASD **physically closed/endothelialized**) at 3 months per referring physician)

Subjects will undergo a hypercapnic breathing challenge (~1hr)

Subjects will be given an activity monitor and given instructions on how and when to use it (~30 min) **(Detailed Instructions on Visit 2 ONLY. On Visit 5, they will be reminded of the instructions, with details as required)**

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

Subjects will perform a VO2max test (~30 min)

Visit 5b (If PFO/ASD **not physically closed/endothelialized** at 3 months per referring physician)

Subjects will undergo informed consent, fill out health history questionnaire, sign a HIPAA authorization form 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/ASD and IPAVA will be graded using saline contrast echocardiography while the subject is breathing room air (PFO/ASD and IPAVA blood flow) and breathing 100% O2 (PFO/ASD 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)

Visit 3 Pre PFO/ASD Closure Procedure (~5 hrs) AND:

Visit 6a (PFO/ASD Physically Closed/Endothelialized) (~5 hrs) OR,

Visit 6b (PFO/ASD Not Physically Closed/Endothelialized) (~2.5 hrs)

Visit 6a (If PFO/ASD **physically closed/endothelialized** at 3 months per referring physician)

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 for 4 minutes per stage at 25, 50, 75 and 90% of VO2 max breathing room air. Subjects will take up to 15 minute breaks between exercise stages (~1.5hr)

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

Subject will repeat the exercise protocol above while breathing 40% 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.

Visit 6b (If PFO/ASD **not physically closed/endothelialized** at 3 months per referring physician)

Subjects will undergo a hypercapnic breathing challenge (~1hr)

Subjects will be given an activity monitor and given instructions on how and when to use it (~30 min) **(Detailed Instructions on Visit 2 ONLY. On Visit 6b, they will be reminded of the instructions, with details as required)**

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

Subjects will perform a VO2max test (~30 min)

Visit 7a (PFO/ASD Physically Closed/Endothelialized) (~ 30 mins) OR,

Visit 7b (PFO/ASD Not Physically Closed/Endothelialized) (~ 5 hrs)

Visit 7a (If PFO/ASD **physically closed/endothelialized** at 3 months per referring physician)

Intravenous blood draw. We will take roughly 21 mls (~2 Tablespoons) of IV blood.

Visit 7b (If PFO/ASD **not physically closed/endothelialized** at 3 months per referring physician)

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 for 4 minutes per stage at 25, 50, 75 and 90% of VO2 max breathing room air. Subjects will take up to 15 minute breaks between exercise stages (~1.5hr)

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

Subject will repeat the exercise protocol above while breathing 40% 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.

NON-EXERCISE

What follows is a detailed description of the study days. For your convenience, please refer to Figure 2. Non- Exercise (page 5) to assist in determining the timing of study days.

Visit 1 Pre PFO/ASD Closure Procedure (~2.5hrs):

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/ASD and IPAVA will be graded using saline contrast echocardiography while the subject is breathing room air (PFO/ASD and IPAVA blood flow) and breathing 100% O2 (PFO/ASD 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)

Visit 2 Pre PFO/ASD Closure Procedure (~3 hrs):

Subjects will undergo a hypercapnic breathing challenge (~1hr)

Subjects will be given an activity monitor and given instructions on how to use it (~30 min)**(Detailed Instructions on Visit 2 ONLY. On Visit 3, they will be reminded of the instructions, with details as required)**

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 (~30 min).

Visit 3a (PFO/ASD Physically Closed/Endothelialized) (~4.5 hrs) OR,

Visit 3b (PFO/ASD Not Physically Closed/Endothelialized) (~30 min)

Visit 3a (If PFO/ASD **physically closed/endothelialized** at 3 months per referring physician)

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 be comprehensively screened for any cardiac abnormalities. 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 (PFO/ASD and IPAVA blood flow) and breathing 100% O2 (PFO/ASD 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)

Subjects will undergo a hypercapnic breathing challenge (~1hr)

Visit 3b (If PFO/ASD **not physically closed/endothelialized** at 3 months per referring physician)

Subjects will undergo an intravenous blood draw, and we will take 21mls (~2 Tablespoons) of IV blood (30 min).

Visit 4a (PFO/ASD Physically Closed/Endothelialized) (~30 min) OR,

Visit 4b (PFO/ASD Not Physically Closed/Endothelialized) (~4.5 hrs)

Visit 4a (If PFO/ASD **physically closed/endothelialized** at 3 months per referring physician)

Subjects will undergo an intravenous blood draw, and we will take 21mls (~2 Tablespoons) of IV blood (30 min).

Visit 4b (If PFO/ASD **not physically closed/endothelialized** at 3 months per referring physician)

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 be comprehensively screened for any cardiac abnormalities. 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 (PFO/ASD and IPAVA blood flow) and breathing 100% O2 (PFO/ASD 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)

Subjects will undergo a hypercapnic breathing challenge (~1hr)

Description of Data Collection Procedures (timing of these procedures can be found in Table 1 and 2 below:

- Modified Allen's Test (Days 1, 3 & 6a OR 7b Exercise; Days 1, 2 & 3a OR 4b NON-Exercise):

This test examines collateral blood flow in the hand, and is performed on the first day and each day there is an arterial line placed. The subject clenches their fist 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 (Days 1 & 4a OR 5b Exercise; Days 1 & 3a OR 4b NON-Exercise):

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/ASD. This will be repeated while breathing 100% O2 for 10 minutes. Subject will be asked to perform a Valsalva maneuver while breathing room air and 100% O2. 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 Function Tests (Days 1 & 4a OR 5b Exercise; Days 1 & 3a OR 4b NON-Exercise):

- 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.

- Diffusing Capacity (DLCO) (Days 1 & 4a OR 5b Exercise; Days 1 & 3a OR 4b NON-Exercise):

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 (Days 1 & 4a OR 5b Exercise; Days 1 & 3a OR 4b NON-Exercise):

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 (Days 1 & 4a OR 5b Exercise; Days 1 & 3a OR 4b NON-Exercise):

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) (Days 1, 3, 4a OR 5b & 6a OR 7b Exercise; Days 1-3a OR 4b NON-Exercise):

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) (Days 1,3,4a OR 4b, 5b, 6a, 7a OR 7b Exercise; Days 1-3a, 4a OR 4b NON-Exercise):

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 during Days 1,3,4a OR 4b, 5b, 6a, and 7a OR 7b Exercise; Days 1-4 NON-Exercise. 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 ELISA kit. Total IV blood draw is approximately 63 mL for exercise or non-exercise participants.

- Hypercapnic ventilatory response (Days 2 & 4a OR 5b Exercise; Days 2-3a OR 4b NON-Exercise):

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 7% CO2 and 93% O2 (medical grade) and room air. Ventilation, end tidal CO2 (PETCO2), end tidal O2 (PETO2) and saturation will be measured continuously throughout this testing (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 5 minutes to reach a target PETCO2 of ~20-25 mm Hg (~40-45 mm Hg is normal); this part of the test is required to reduce CO2 stores in the body. At 5 minutes the subject will be asked to take a full breath in and out then they will be switched to the bag filled with 7% CO2 and 93% O2 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 PETCO2 = 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.

- Actigraphy (Days 2 & 4a OR 5b Exercise; Days 2-3a OR 4b NON-Exercise):

All subjects will wear an ActiGraph (Pensacola, FL) GT3X accelerometer daily for approximately 1 week Pre PFO/ASD closure (baseline) and 1 week Post PFO/ASD closure. Subjects will be instructed to wear the device at all times around their waist except when showering, bathing, swimming, or sleeping. Accelerometry data will be downloaded to a computer using ActiLife v.6 software. Total daily energy expenditure (TDEE; kcal/day) will be estimated, and physical activity levels (PALs; TDEE/basal metabolic rate [BMR]) calculated to adjust for body size. Activity energy expenditure (AEE; kcal/day), calculated as TDEE-BMR, will be used to describe the caloric costs of physical activity. In addition, time spent in standard categories of activity (sedentary, moderate, vigorous) will be assessed. Members of the Lovering lab are responsible for uploading and analyzing de-identified data from the ActiGraphs every week.

- VO_{2MAX} test and 6 minute walk test (Days 2 & 4a OR 5b; Exercise subjects Only):

The six minute walk test will be performed on the second floor of the Center for Medical Education and Research. This is the same space used to perform these tests in Dr. Dreyer's subjects.

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 (25 to 75 W depending on fitness level), 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 (Days 3, 6a OR 7b Exercise subjects; Days 2-3a OR 4b NON-Exercise subjects):

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) in the exercising subjects and < 25 ml (<2 Tablespoons) in resting subjects. 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 Exercise subjects (i.e., IV + Arterial Line) will be less than 250 mL.
TOTAL blood draw for Non-Exercise subjects (i.e., IV + Arterial Line) will be less than 125 mL.

However, if it is deemed at 6 months by the referring physician that the PFO/ASD has not been physically closed/endothelialized, subjects will not partake in the scheduled 6-month post PFO/ASD closure procedure visits. Thus, the amount of blood drawn from these subjects would be significantly reduced. Specifically:

Exercise subjects without 6 month visits (i.e., total blood drawn): < 125 mL
Non-exercise subjects without 6 month visits (i.e., total blood drawn): < 75 mL

Risks and interventions to mitigate such are included in Section G.

- Exercise with arterial line (Days 3, 6a OR 7b Exercise subjects only):

After arterial line placement (see above) the subjects will perform two bouts of graded submaximal exercise tests on the cycle ergometer at 25%, 50%, 75% and 90% of their VO_{2max} with each stage lasting 4 minutes with up to 15 minute breaks between stages. During one bout, subjects will breathe normal oxygen levels (21% oxygen), during the other bout, subjects will breathe high oxygen levels (40% oxygen). Bouts will be randomized and separated by 30 minutes of rest breathing room air.

- Intrapulmonary & intracardiac Shunting with arterial line placement (Days 3, 6a OR 7b Exercise subjects; Days 2-3a OR 4b NON-Exercise subjects):

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 (Days 3, 6a OR 7b Exercise subjects; Days 2-3a OR 4b NON-Exercise subjects):

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.

Table 1 – Exercise

Group - Exercise											
Visits:	1	2	3	4a	4b	5a	5b	6a	6b	7a	7b
Pre- PFO/ASD Close	x	x	x								
Post-PFO/ASD Close				x	x	x	x	x	x	x	x
Study Procedures:											
IC & Health Hx, HIPAA form	x										
Modified Allen Test	x		x					x			x
Comprehensive Screening	x			x			x				
Pulmonary Function	x			x			x				
Diffusing Capacity	x			x			x				
Plethysmography	x			x			x				
Nitrogen Washout*	x			x			x				
Transthoracic Saline Contrast Echo (TTSCE)	x		x	x			x	x			x
Intravenous catheter (IV)	x		x	x	x		x	x		x	x
Hypercapnic Ventilatory Response		x				x	x				

Actigraphy**		x		x			x				
VO2Max & 6 min walk		x		x			x				
Arterial Blood Gas Measurement			x					x			x
Exercise w/arterial line			x					x			x
Intrapulmonary/intracardiac shunt w/art line			x					x			x
Core Body Temp			x					x			x
Compensation:											
IC & Health Hx	5										
Echo screen/blood draw	10			15			15				
Blood Draw					10					10	
PFT, diff capacity, plethysmography	10			15			15				
Hypercapnic breathing challenge		10				15			15		
activity monitor training		5									
six min walk		5				5			5		
vo2max		15				15			15		
arterial line			100					130			130
exercise tests			75					100			100
blood draws/breathing room air											
Total for completed study visit	25	35	175	30	10	35	30	230	35	10	230
Total for all aspects of the study	540										

Table 2 – Non-Exercise

Group – Non-Exercise						
Visits:	1	2	3a	3b	4a	4b
Pre- PFO/ASD Close	x	x				
Post-PFO/ASD Close			x	x	x	x
Study Procedures:						
IC & Health Hx, HIPAA form	x					
Modified Allen Test	x	x	x			x
Comprehensive Screening	x		x			x
Pulmonary Function	x		x			x
Diffusing Capacity	x		x			x

Plethysmography	x		x			x
Nitrogen Washout*	x		x			x
Transthoracic Saline Contrast Echo (TTSCE)	x	x	x			x
Intravenous catheter (IV)	x	x	x		x	x
Hypercapnic Ventilatory Response		x	x			x
Actigraphy**		x	x			x
VO2Max & 6 min walk						
Arterial Blood Gas Measurement		x	x			x
Exercise w/arterial line						
Intrapulmonary/intracardiac shunt w/art line		x	x			x
Core Body Temp		x	x			x
Compensation:						
IC & Health Hx	5					
Echo screen/blood draw	1					
Blood draw				10	10	
PFT, diff capacity, plethysmography	1					
Hypercapnic breathing challenge		10	15			15
activity monitor training		5				
six min walk						
vo2max						
arterial line		100	130			130
exercise tests						
blood draws/breathing room air		15	20			20
Total for completed study visit	25	130	165	10	10	165
Total for all aspects of the study	330					

D. Research Population & Recruitment Methods & Compensation

1-2. Sample size, Population and Recruitment Methods:

Sample Size and Data Analyses:

Necessary sample size was determined with our primary outcome variables ($AaDO_2$, VO_{2MAX}, saline contrast bubble scores, TNFa 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 (i.e. reduction of $AaDO_2$ by 50%, VO_{2MAX} increasing by 10% and 6 min walk test increasing by 50 meters (15, 34, 41)(24, 40)(24, 40), TNF-a levels decreasing by 50%, a desired power of 0.80, and alpha (0.05), we determined that n = 8-10 individuals would be sufficient to test our hypotheses, using a repeated measures design. Thus, we are asking to recruit 20 subjects (any combination of male and female) 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 pre- and post-PFO/ASD closure will be analyzed using a repeated measures ANOVA with Tukey posttest. Mean differences in $VO_{2MAX,}$ 6 minute walk test, activity, inflammatory markers and hypercapnic ventilator response, between pre and post PFO/ASD closure will be analyzed using a paired t-test. Differences in bubble scores pre- and post-closure will be analyzed using a Mann-Whitney U test.

The population of subjects will include patients who are undergoing PFO/ASD closure at either PeaceHealth or McKenzie-Willamette Hospitals. Subjects will be identified by Rick Padgett, MD (PeaceHealth/Oregon Heart & Vascular Institute) and Sam Lau, MD (McKenzie-Willamette). These two cardiologists will only refer subjects to contact the Lovering Lab for informed consent and enrollment into the study. Drs. Lau and Padgett will identify potential subjects as "cleared for exercise" or "not cleared for exercise." (See statement of PHI above). Drs. Lau and Padgett will not be formally engaged in the research (i.e. they will not consent subjects, have access to identifiable data, etc). No research activities will take place at either hospital. Subject fliers, initial phone script and email script are attached.

3. Detailed Recruitment information:

A) Patients undergoing PFO/ASD closure will be identified by Dr. Lau and Padgett. The PI has verbally discussed the details of this study over the phone with Drs. Lau and Padgett. The reality is that the three of us have been discussing the potential to do these studies for years and we only recently received the funding to do them. The PI will send the cardiologist the criteria listed below in Table 3 so that they can refer to them before making the decision to recommend them for the study. Because Drs. Lau and Padgett understand that this study has an exercise arm and a non-exercise arm, they will use their best judgment along with available medical records to determine whether or not a subject **would be** cleared for exercise or not cleared for exercise. For example, a subject with a history of chest pain upon exertion and a clinical exercise study revealing an elevated ST segment (EKG abnormality) would not qualify for the exercise study whereas a subjects with history of running marathons would qualify for the exercise study.

B) If the cardiologist believes that their patient meets the qualifications for this study, they will simply hand them a flier (either the exercise or non-exercise flier based on cardiologist recommendation) and direct them to contact us (Lovering and colleagues) for questions.

C) Once a subject contacts us and agrees to come in for consent (after receiving a flier from their physician), we will contact the cardiologists via email/phone to VERIFY which group they qualify for (exercise/non-exercise).

D) **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 each day when an arterial line is placed. Failing the Modified Allen's test at anytime will result in exclusion.

Table 3

Inclusion	Exclusion
Men and women aged 18-80 recruited from patients in the surrounding community undergoing PFO/ASD closure. Subject's physician will determine inclusion in either exercise or non-exercise group, based on available medical information.	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). Physician determination. PFO/ASD deemed by referring physician as not fully closed/endothelialized at 6 months post-PFO/ASD closure procedure.

4. Compensation:

Subject Compensation (maximum of $540 for exercise subjects and $330 for NON-exercise subjects; paid via check at the end of the study):

Visit #1 (Exercise Subjects): $25.00: Completing the informed consent and health history questionnaire ($5.00) and echocardiographic screening and blood draw ($10.00) will pay $15.00. 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, that is $5.00 if the subject decides not to participate after reviewing the informed consent; $ 10.00 for participation in the screening procedures; total compensation for reviewing the informed consent and participating in screening is $15.00.

Visit #2 (Exercise Subjects): $35.00: Completing the hypercapnic breathing challenge ($10), completing the activity monitor training ($5), completing the six minute walk test ($5) completing the VO2max test ($15).

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

Visit #4a (Exercise Subjects): $30.00: Completing the echocardiographic screening and blood draw ($15.00) will pay $15.00. The pulmonary function tests, the diffusion capacity test, and the whole body plethysmography or nitrogen washout will pay $15.00. Reimbursement for Day 4 is more than Day 1 despite fewer procedures. This is to encourage subjects to return 3 months later.

Visit #4b (Exercise Subjects): **$10.00:** Completing the IV blood draw.

Visit #5a (Exercise Subjects): $35.00: Completing the hypercapnic breathing challenge ($15) and completing the six minute walk test ($5) completing the VO2max test ($15).

Visit #5b (Exercise Subjects): $30.00: Completing the echocardiographic screening and blood draw ($15.00) will pay $15.00. The pulmonary function tests, the diffusion capacity test, and the whole-body plethysmography or nitrogen washout will pay $15.00. Reimbursement for Visit 4 is more than Visit 1 despite fewer procedures. This is to encourage subjects to return 6 months later.

Visit #6a (Exercise Subjects): $230: Completing the arterial blood line ($130) and exercise tests ($100)

Visit #6b (Exercise Subjects): $35.00: Completing the hypercapnic breathing challenge ($15) and completing the six-minute walk test ($5) completing the VO2max test ($15).

Visit #7a (Exercise Subjects): $10.00: Completing the IV blood draw.

Visit #7b (Exercise Subjects): $230.00: Completing the arterial blood line ($130) and exercise tests ($100)

NOTE: Reimbursement for Visits 4-7 is more than Visits 1-3 despite fewer procedures. This is to encourage subjects to return 3 or 6 months later for the study.

Visit #1 (NON-Exercise Subjects): $25.00: Completing the informed consent and health history questionnaire ($5.00) and echocardiographic screening and blood draw ($10.00) will pay $15.00. 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, that is $5.00 if the subject decides not to participate after reviewing the informed consent; $ 10.00 for participation in the screening procedures; total compensation for reviewing the informed consent and participating in screening is $15.00.

Visit #2 (NON-Exercise Subjects): $130.00: Completing the hypercapnic breathing challenge ($10), completing the activity monitor training ($5), completing arterial line ($100) and arterial blood draws breathing room air and 100% O2 ($15).

Visit #3a (NON-Exercise Subjects): $165.00: Completing the hypercapnic breathing challenge ($15), completing arterial line ($130) and arterial blood draws breathing room air and 100% O2 ($20).

Visit #3b (NON-Exercise Subjects): $10.00: Completing the IV blood draw.

Visit #4a (NON-Exercise Subjects): $10.00: Completing the IV blood draw.

Visit #4b (NON-Exercise Subjects): $165.00: Completing the hypercapnic breathing challenge ($15), completing arterial line ($130) and arterial blood draws breathing room air and 100% O2 ($20).

NOTE: Reimbursement for Day 3/4 is more than Day 2 despite fewer procedures. This is to encourage subjects to return 3 months later.

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

E. Informed Consent Process

Informed consent will be administered to each subject by the primary investigator and colleagues.

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.

On the day before ALL visits, 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 each study day and 3) not eat for 2 hours before arriving for each study day. Male subjects will be required to go shirtless for all studies involving echocardiography. Female subjects will need to wear a sports bra for all studies involving 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.

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., CLOSE) 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).

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.

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 all echocardiography and exercise studies. Female subjects will need to wear a sports bra for all echocardiography and exercise studies. 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.

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).

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.

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.

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 Dr. Jerry Hawn (arterial line placement) and 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.

Risks for subjects undergoing PFO/ASD closure: Potential risks for subjects undergoing PFO/ASD closure are at least equal to the general population as outlined above. Because

these subjects have a hole in their heart, which allows for blood to flow across the heart without being filtered by pulmonary capillaries, there is an elevated risk for stroke, TIA and/or arterial hypoxemia (these are likely the reasons the subjects is getting the PFO/ASD closed). Indeed, the subjects who have been selected for PFO/ASD closure in this study will include those with previous history of stroke, TIA and/or exercise-induced arterial hypoxemia. The goal of the PFO/ASD closure is to reduce subsequent cerebrovascular accidents (stroke & TIA) and/or prevent/minimize exercise-induced arterial hypoxemia. Thus, post PFO/ASD closure subjects would have a reduced risk profile that would likely resemble those without a PFO/ASD.

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.

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.