Central blood volume shifts in trained vs. untrained: Effects on lung diffusing capacity

Authors:	Desmond A. Young, M.Sc. Desi P. Fuhr, M.Sc. Andrew R. Brotto, M.Sc. Sean van Diepen, M.D. Jason Weatherald, M.D. Michael K. Stickland, Ph.D.
Program:	Faculty of Medicine and Dentistry
Institutions:	University of Alberta
Corresponding Author:	Michael K. Stickland, Ph.D. Faculty of Medicine and Dentistry 3-135 Clinical Sciences Building University of Alberta Edmonton, Canada T6G 2J3 Tel: 780-492-3995 Fax: 780-492-4483 Michael.Stickland@ualberta.ca

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List of Abbreviations

CT	Computed Tomography
DLCO	Diffusion Capacity of Carbon Monoxide
Dm	Membrane Diffusing Capacity
FiO_2	Fraction of Inspired Oxygen
Hb	Hemoglobin
HDT	Head Down Tilt
HUT	Head Up Tilt
LBNP	Lower Body Negative Pressure
LBPP	Lower Body Positive Pressure
TAPSE	Tricuspid Annular Plane Systolic Excursion
TVI	Tissue Velocity Index
Vc	Pulmonary Capillary Blood Volume
$\dot{V}_E$	Ventilation
$\dot{V}CO_2$	Volume of Carbon Dioxide
$\dot{V}O_2$	Volume of Oxygen
$\dot{V}O_2max$	Maximal Oxygen Consumption

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1 Introduction/Significance

Pulmonary diffusing capacity (DLCO) is a pivotal component of gas exchange and acid-base balance. Indeed, increases in lung diffusing capacity are critical to meeting the elevated metabolic demands during exercise and subsequently preventing a diffusion limitation, excessive increases in alveolar-arterial oxygen difference and possibly exercise induced arterial hypoxemia (Stickland et al., 2013). Furthermore, reduced DLCO in those with respiratory diseases, such as chronic obstructive pulmonary disease, contributes to exercise intolerance and arterial hypoxemia (Ross et al., 2020). Examining DLCO in healthy individuals provides a safe and effective model to understand pulmonary-cardiac mechanics that can benefit those with pulmonary diseases in the future. To date, it is well established that interindividual factors such as aerobic fitness (Lalande et al., 2012; Tedjasaputra et al., 2016) and biological sex (Ehnes et al., 2025; Neas & Schwartz, 1996) may impact DLCO at rest and during exercise; however, the mechanisms underpinning these differences remain unknown.

Endurance-trained individuals have higher DLCO at rest and during exercise than untrained individuals. This difference may suggest a pulmonary adaptation to exercise training; however, the reasons for this difference are unknown. A greater blood volume in endurance-trained individuals may explain the higher lung diffusing capacity, measured as the diffusing capacity for carbon monoxide (DLCO). Endurance training increases blood volume, via expanding plasma volume (Convertino, 1991). Higher plasma volume could facilitate a greater DLCO via recruitment and distention of pulmonary capillaries, increasing pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm), which are the two primary determinants of DLCO (Hsia, 2002; Johnson et al., 1960; Mostyn et al., 1963). Indeed, translocating blood volume toward the pulmonary circulation by moving from the sitting to the supine position increases resting DLCO (Katz et al., 2018), with endurance-trained individuals demonstrating a greater increase in Vc than untrained individuals when moving to the supine position (Brotto et al., 2025). Additionally, aerobic capacity ($\dot{V}O_2max$) is associated with pulmonary vascular distensibility, indicating that individuals with higher aerobic fitness can facilitate a greater pulmonary capillary blood volume for a given increase in pulmonary artery pressure (Lalande et al., 2012). Higher distensibility alongside higher blood volume may explain bigger postural shifts in DLCO in endurance-trained individuals. However, the relationship between endurance training status, manipulations in central blood volume, and the resulting changes in DLCO, Vc, and Dm remains untested.

In addition to a single postural shift, manipulating lower body pressure in the supine position can further modulate central blood volume to assess the graded effects of central blood volume shifts on DLCO and its components. First, lower body positive pressure (LBPP) in a box or head down tilt (HDT) on a tilt table increases central blood volume (Fu et al., 2000; Fu et al., 1998; Shi et al., 1993). However, to our knowledge no study has assessed the effect of LBPP on DLCO and its components. Second lower body negative pressure (LBNP) in a box or head up tilt (HUT) on a tilt table causes blood to shift to the legs with profound systematic adjustments to maintain cerebral

perfusion (Goswami et al., 2019). Notably, endurance-trained individuals show reduced tolerance to LBNP than their untrained counterparts, explained by greater reliance on Frank-Starling mechanics (i.e., higher end diastolic volume) causing a greater reduction in stroke volume during LBNP when preload is reduced (Goswami et al., 2019; Levine, Buckey, et al., 1991; Levine, Lane, et al., 1991). Therefore, it is essential to measure total blood volume as well as changes in central blood volume with LBPP/LBNP or HDT/HUT to understand the vascular mechanics that govern DLCO responses.

Accordingly, the purpose of the current study is to determine the impact of endurance training status and biological sex on DLCO and its components (Vc and Dm) during acute changes in central blood volume using varying levels of LBPP/LBNP and HDT/HUT. We hypothesize that for a given reduction (i.e., LBNP, HUT) or increase (i.e., LBPP, HDT) in central blood volume, endurance-trained individuals will demonstrate greater changes in DLCO compared to untrained individuals. As a second exploratory aim, we hypothesize that females will have larger changes in DLCO compared to males.

2 Study Objectives

2.1 2.1 Objectives

The *primary* objective of this study is to determine the impact of aerobic fitness and biological sex on acute central blood volume mobilization-mediated changes in DLCO and its components.

2.2 2.2 Hypotheses

We hypothesize that:

1) Endurance-trained individuals will demonstrate greater changes in DLCO compared to untrained individuals.
2) Females will have larger changes in DLCO compared to males.

3 Study Design

3.1 General Design

This study will be a cross-sectional design. Data will be collected over five experimental visits. **Visit 1:** Characterization of pulmonary function and exercise capacity by standard pulmonary function and cardiopulmonary exercise testing; **Visits 2–3:** Measurement of DLCO and its components via standardized breath hold techniques during LBNP/LBPP or HUT/HDT, and exercise. **Visit 4:** Ultrasound imaging of the heart during LBNP/LBPP or HUT/HDT, and exercise. **Visit 5:** Computed tomography to assess lung structure.

3.1.1 Primary Outcome Variable

The primary outcome variables are: DLCO and its components, Vc and Dm.

3.1.2 Participant Selection and Withdrawal

The study design includes a cohort of healthy individuals aged 18–40 years across a range of aerobic capacity.

3.1.3 Inclusion Criteria

Individuals between the ages of 18–40 years. This age range was selected to decrease the potential impact of ageing on the primary cardiopulmonary outcomes.

3.1.4 Exclusion Criteria

Participants will be screened to exclude:

1. Severe obesity (body mass index >35 kg/m^2).
2. Self-reported blood donation or transfusion in the past 60 days. Large artificial fluctuations in blood volume may impact the findings of the present study.
3. Pregnancy. To confirm this, women not using contraception will be given a pregnancy test. Pregnancy presents additional complications and may confound the results of the study; therefore, pregnant women will be excluded.
4. Known chronic disease (heart failure, hypertension, diabetes, asthma, chronic obstructive pulmonary disease, renal disease).
5. Participants with known postural orthostatic tachycardic syndrome or orthostatic hypotension.
6. Participants who currently smoke cigarettes (within the last month) or those who have >1 pack-year smoking history.
7. Participants who currently smoke cannabis products (within the last month) or those who have >1 joint-year cannabis history.

3.2 Experimental Visits:

Participants will report to the Clinical Physiology Laboratory in Clinical Sciences Building at the University of Alberta. There will be 5 experimental visits in this study. First, is a screening visit including pulmonary function testing and cardiopulmonary exercise testing which will take ~2 hours. On visits 2–3, we will measure lung diffusing capacity and its components using the diffusing capacity for carbon monoxide (DLCO; more details provided below) method. The DLCO technique will be performed at each condition of central blood volume mobilization (detailed below): visit 2 will be LBNP/LBPP or HUT/HDT, and visit 3 will be exercise. Visits 2–3 will each take ~2 hours. For visits 2–3, we will a blood sample for sex hormone analyses. Visit 4 will include ultrasound imaging of the heart during LBNP/LBPP or HUT/HDT, and exercise conditions. Participants will then undergo a blood volume measurement using a carbon monoxide rebreathe. Visit 4 will take ~2 hours. For visits 1–4, participants will report to the laboratory after refraining from alcohol for 24 hours, exercise for 12 hours, and food and caffeine for 2 hours prior to testing. On visit 5 participants will undergo a computed tomography scan to examine lung structure. Visit 5 will take ~1 hour.

3.2.1 Visit 1: Screening:

On the screening day, the participant will complete the informed consent procedure, fill out a medical history form, and be screened for exercise using the modified medical research council (mMRC) dyspnea scale, PAR-Q+ exercise readiness questionnaire and the Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q). We then will perform pulmonary function tests on each participant. Finally, the participant will complete a graded exercise test ($\dot{V}O_2$max, expired gas analyses, operating lung volumes) on an upright cycle ergometer to volitional exhaustion with measurement of heart rate and blood pressure. Evaluation of dyspnea including intensity and unpleasantness will be performed at each workload. Further, reasons for test termination, dyspnea, and qualitative descriptors of dyspnea will be evaluated at peak exercise (Simon et al., 1990). Hemoglobin concentration will be measured via finger prick prior to and following completion of exercise testing.

3.2.2 Visit 2: Central Blood Volume Manipulation and DLCO:

The participant will perform the DLCO test at various levels of LBNP/LBPP ranging from −80 mmHg to +40 mmHg, or HUT/HDT ranging between 90° and −12°. Head tilt and lower body pressure changes provide a similar intervention that can be used interchangeably based on equipment availability (Fu et al., 2000). Changes in central blood volume during LBNP/HUT are equivalent to standing upright (−80 mmHg LBNP or 90° HUT), and changes in central blood volume during LBPP/HDT are equivalent to lying down with your feet above your head (+40 mmHg LBPP or −12° HDT) (Diaz-Canestro et al., 2022; Goswami et al., 2019). For the LBNP/LBPP trials, participants will lie supine with their lower body (from the iliac crest to their toes) inside of the pressure chamber. The participant's lower body will be sealed within the differential pressure chamber using a neoprene skirt to prevent any air leakage from the insides of the chamber. Negative and positive pressures are induced by a vacuum pump that either reduces or increases atmospheric pressure inside the chamber in a controlled and precise manner. The vacuum pump is controlled by a trained member of the research team, and chamber pressure is continuously monitored via a digital manometer. For the HUT/HDT trials, the participant will lie supine on a tilt table. The table will be electronically adjusted to the desired angle at a rate of 1°/second.

Once participants are secured in the lower body pressure chamber or on the tilt table, a certified technician will insert an intravenous (IV) catheter into an antecubital vein which will allow peripheral venous pressure measurements. Participants will also be equipped with a continuous blood pressure monitor, a brachial blood pressure cuff, an electrocardiogram, and a thoracic impedance device. Each stage of LBPP/LBNP or HUT/HDT will begin with two minutes of stabilization followed by two DLCO breath-hold maneuvers. At each stage participants will also breathe on a mouthpiece for two minutes to collect metabolic data: ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$). Cardiovascular parameters (blood pressure, peripheral venous pressure, heart rate, thoracic impedance) will be measured

continuously. Hemoglobin will be measured at each stage via a small (< 1 mL) blood sample from the IV. At the end of the trial, a certified technician will obtain a ~5 mL blood sample from the IV for sex hormone analyses.

Participants will not complete more than 15 DLCO breath holds during each experimental visit. As per recommendations (Zavorsky et al., 2017), participants will perform standard DLCO breath-hold maneuvers for 6 seconds, and maneuvers will be performed twice at each stage. A washout period of at least 4 minutes between each breath hold will be completed to ensure adequate recovery and blood gas equilibration (Zavorsky et al., 2017).

3.2.3 Visit 3: DLCO with Exercise:

Exercise will be used as a physiological stressor to increase central blood volume beyond that of LBPP or HDT. The participant will perform the DLCO maneuvers during exercise at varying exercise intensities (~20%–100% of an individual's $\dot{V}O_2max$). Participants will not complete more than 15 breath holds during the experimental visit. As per recommendations (Zavorsky et al., 2017), participants will perform standard DLCO breath-hold maneuvers for 6 seconds, and maneuvers will be performed twice at each stage. At each exercise intensity, participants will breathe on a mouthpiece for two minutes to collect metabolic data; heart rate and peripheral oxygen saturation will be measured continuously for the same two minutes at each intensity. Blood pressure will be measured once per stage with an automatic sphygmomanometer. Hemoglobin via finger prick will also be measured at each intensity. A washout period of at least 2 minutes between each breath hold during exercise will be completed to ensure adequate recovery and blood gas equilibration (Zavorsky et al., 2017). At the end of the trial, a certified technician will obtain a ~5 mL blood sample from an antecubital vein for sex hormone analyses.

3.2.4 Visit 4: Ultrasound with LBNP/HUT, LBPP/ HDT, and Exercise:

Participants will complete the same interventions as visit 2 (LBNP/LBPP −80 to +40 mmHg, or HUT/HDT 90° to −12°) and visit 3 (exercise at ~20%–100% of an individual's $\dot{V}O_2max$). At each stage of LBNP/LBPP or HUT/HDT, and exercise, a trained sonographer will obtain images of the heart via echocardiography. Participants will then undergo a carbon monoxide rebreathe protocol to estimate blood volume (Burge & Skinner, 1995; Schmidt & Prommer, 2005; Siebenmann et al., 2017). A certified technician will insert an IV catheter into an antecubital vein to collect serial blood samples. While lying supine, participants will inhale a bolus of carbon monoxide (0.7–1.0 mL/kg bodyweight) mixed with 100% oxygen on a rebreathing circuit for two minutes and will then continue to rest for eight additional minutes. Venous blood samples measuring carboxyhemoglobin before the rebreathe and eight minutes after the rebreathe will allow the calculation of blood volume.

3.2.5 Visit 5: Computed Tomography:

Participants will report to a Medical Imaging Centre for the computed tomography (CT) scan. Following a briefing by the Medical Imaging Centre staff, participants will lay supine on the CT

scanner and the region of interest in the chest will be obtained. The participant will remain as still as possible and inhale to total lung capacity and hold their breath. During the breath hold, images will be acquired. The participant will then empty their lungs as far as they can and imaging will be repeated.

3.3 Health Canada License Numbers

The devices discussed in Section 3.4 below are approved by Health Canada. Their Health Canada License Numbers are provided below.

- Encore 229, Vmax: 22536
- Radical 7, Masimo: 15928
- Tango M2, Suntech: 92724
- Physioflow PF-07, Manatec: 75857
- VAMP Plus, Edwards Lifesciences: 100235
- TruWave Transducer, Edwards Lifesciences: 97516
- Hb 201+, Hemocue: 77132
- ABL90 FLEX PLUS, Radiometer: 85942
- Hypair PFS: 105182

3.4 Measures:

Questionnaires: Participants will be screened for exercise using the modified medical research council (mMRC) dyspnea scale, the Canadian Society for Exercise Physiology PAR-Q+ exercise readiness questionnaire, and the Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q).

Pulmonary Function: Spirometry, plethysmography, forced oscillometry, and diffusing capacity for carbon monoxide (DLCO) will be completed as per guidelines (Graham et al., 2017; Miller et al., 2005; Wanger et al., 2005). Measurements will be expressed as percent predicted normal values when applicable (Quanjer et al., 2012; Stanojevic et al., 2017). As these are clinical tests specifically for respiratory conditions, the risk of these procedures for the populations being recruited for the study is very low.

Metabolic Monitoring: Measurements of expired gases through a metabolic cart (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA) will provide values for $\dot{V}_E$, $\dot{V}O_2$, and $\dot{V}CO_2$. Participants will rate their perceived breathing and leg discomfort using the modified Borg scale (Borg, 1982) as well as descriptors of dyspnea (Simon et al., 1990) at rest, within the last 30 seconds of every two minute exercise interval, and at the end of exercise followed by an inspiratory capacity maneuver. All previously mentioned expired gas measures will be collected continuously during the exercise test on visit 1, and a 30 second average from 1:00–1:30 of each stage will be analyzed to avoid contamination of expired gas data from the inspiratory capacity maneuvers. At symptom limitation, participants will complete maximal Borg ratings of breathing and leg discomfort (Simon et al., 1990). Participants will also be asked to report their primary reason for

termination of exercise and to select qualitative phrases that best describe their breathing during exercise and at peak exercise (Simon et al., 1990). Oxygen saturation (Radical-7 Pulse CO-Oximeter and LNCS SpO_2 sensor, Masimo, CA, USA) will be measured during cardiopulmonary exercise testing and during the experimental session. The same metabolic cart will be utilized for metabolic measurements on visits 2 and 3.

Blood Pressure: Upper arm blood pressure measurements will be taken via auscultation with a sphygmomanometer throughout exercise (Tango, Suntech, Morrisville, NC). During visits 2 and 3, beat-by-beat blood pressure data will be collected using finger photoplethysmography (ADInstruments NIBP). Upper arm blood pressure measurements will serve as a calibration value to which finger data can be adjusted.

Electrocardiogram: A standard 12-lead electrocardiogram will be collected via surface electrodes placed on the chest to determine heart rate during the cardiopulmonary exercise testing. A standard 3-lead electrocardiogram alongside a thoracic impedance device (PhysioFlow PF-07 Enduro, Manatec Biomedical, France) will be used during experimental sessions.

Peripheral Venous Pressure: A certified technician will administer an IV in an antecubital vein during visits 2 and 4. During visit 2, the IV will be attached to a pressure transducer (VAMP Plus with TruWave Transducer, Edwards Lifesciences, Irvine, CA, USA) placed at the height of the phlebostatic axis. The VAMP Plus has a continuous saline line that attaches to the IV, so the pressure transducer is never exposed to the participant directly. Peripheral venous pressure will be measured during LBNP/LBPP or HUT/HDT during visit 2. During visit 4, the IV will be used for blood collection during the carbon monoxide rebreathe.

Hemoglobin and Carboxyhemoglobin: Blood collected from a finger prick will be analyzed for hemoglobin concentration using a handheld Hb measurement device (Hemocue, HB 201+, Hemocue America, Brea, CA). Blood collected from an IV inserted by a certified technician in an antecubital vein will be analyzed for carboxyhemoglobin concentration using a blood gas analyzer (ABL90 FLEX PLUS, Radiometer Medical Aps, Denmark). Real-time carboxyhemoglobin saturation will be measured using a pulse oximeter (Radical-7 Pulse CO-Oximeter and LNCS-II SpCO sensor, Masimo, CA, USA).

Carbon Monoxide Rebreathe: The participant will lay supine for the entire rebreathe protocol. The participant will breathe through a mouthpiece connected to a 3-way valve with one port open to room air and the second port connected to a 6 L bag containing 100% oxygen. The segment between the 3-way valve and the 6 L bag will have a small port through which a researcher can administer a small bolus of carbon monoxide. While resting supine, the participant will breathe normally with the 3-way valve open to room air and closed to 100% oxygen. A certified technician will collect a 1 mL venous blood sample from an IV for carboxyhemoglobin analysis. In a non-

smoking healthy individual this number should be between 0.0%–3.0%. A trained researcher will prepare a bolus of 99.5% pure carbon monoxide (0.7–1.0 mL/kg bodyweight) in a 100 mL syringe and attach it to the port on the rebreathing apparatus (Schmidt & Prommer, 2005; Siebenmann et al., 2017). Once instructed, the participant will empty their lungs completely, at which time the valves on the device will be switched. The participant will then take a full, deep breath, mixing the carbon monoxide from the syringe and the 100% oxygen from the 6 L bag. The participant will hold their breath for 10 seconds and then breathe normally on the closed rebreathing circuit for an additional 1 minute and 50 seconds, for a total of two minutes on the closed system. The 100% oxygen in the 6 L bag will provide adequate oxygen throughout the rebreathe. Following the two-minute rebreathe, the mouthpiece will be removed, and the participant will rest supine for an additional eight minutes. A certified technician will collect a 1 mL venous blood sample immediately following the eight minutes of supine rest. The carbon monoxide rebreathe will increase carboxyhemoglobin to ~6% (Schmidt & Prommer, 2005; Siebenmann et al., 2017) which is approximately the same as a habitual cigarette smoker.

DLCO: Measurements of capillary blood volume will be derived from the lung diffusing capacity of carbon monoxide using the multiple FiO_2 DLCO technique (Roughton & Forster, 1957; Zavorsky et al., 2017), and the Hypair® pulmonary function system (Zavorsky et al., 2017). We will utilize a 6-second breath hold as employed by similar studies (Tedjasaputra et al., 2016; Tedjasaputra et al., 2018) and as per current recommendations (Hsia, 2002; Zavorsky et al., 2017).

Cardiac and Vascular Structure and Function: Doppler ultrasound of the heart will be performed by a trained sonographer to estimate pulmonary arterial pressures using tricuspid valve regurgitant jet velocity and inferior vena cava diameter (Gargani et al., 2023; Quinones et al., 2002). B-Mode and M-Mode images will be used to measure cardiac volumes and tricuspid annular plane systolic excursion (TAPSE). Pulse wave and continuous wave Doppler will be used to measure blood velocities (A-V valve inflow, pulmonic/aortic outflow), and tissue velocity index (TVI) will be used to measure tissue velocity. The E/e' ratio will be used to estimate left ventricular filling pressure in conjunction with left ventricular propagation velocity (Geske et al., 2007). Stroke volume will be derived from doppler images of end systolic and end diastolic volumes.

Lower-body Negative and Positive Pressure or Head Up and Head Down Tilt: The participant will be lying supine in a differential pressure chamber that will be sealed at the level of the waist via a neoprene skirt (i.e., compression-style waistband). Chamber pressure is controlled by a trained member of the experimental team via an industrial vacuum with a pressure range of −100 to +60 mmHg and will be monitored via a digital manometer. For the head up and head down tilt, participants will be lying on a tilt table that will be electronically adjusted at a rate of 1°/second by a trained member of the study team to achieve the target angle. The range of pressures and postural stress used in the current study will be equivalent to the physiological stress of standing

upright (−80 mmHg or 90° HUT) and lying down with your feet above your head (+40 mmHg or −12° HDT) (Diaz-Canestro et al., 2022; Goswami et al., 2019).

Sex Hormone Analysis: Blood samples collected on visits 2 and 3 will be analyzed for sex hormones (progesterone, estrogen, and testosterone).

3.5 Risk Mitigation

Participation in this study may involve some added risks or discomforts. Precautions and screenings will be in place to minimize these risks. These risks include:

Questionnaires: There are no foreseeable risks associated with the questionnaires.

Exercise Tests: There is risk associated with exercise, whether it is conducted within our laboratory, or in day-to-day life. This can include some minor discomforts such as temporary fatigue, shortness of breath or muscle soreness afterward, all of which typically stop within minutes of finishing exercise. There is a very small risk of a serious cardiac event, such as an arrhythmia, heart attack or death, associated with heavy exercise in healthy people (less than 9 in 10,000). If a participant experiences chest pain, light headedness, or clammy skin, we will stop the test to ensure participant safety. All study team members are certified in CPR and are trained to handle adverse events. As per our laboratory SOP, study team members are responsible for knowing and following the current Canadian Society for Exercise Physiology (CSEP) and American College of Sports Medicine guidelines for (ACSM; ACSM's guidelines for Exercise Testing and Prescription, Eleventh Edition) exercise testing, which includes contraindications for termination. An emergency protocol has been established to respond to any adverse medical situation that may arise during testing.

Pulmonary Function Tests (Including DLCO): There are no foreseeable risks associated with breathing room-air or increased oxygen gas mixtures. Some gas mixtures will contain trace amounts of carbon monoxide and methane gas. The small quantities of these gases are not harmful and leave the body quickly (via breathing). These gas mixtures are used routinely in clinical tests of lung function.

Metabolic Monitoring: There are no foreseeable risks associated with metabolic monitoring.

Blood Pressure: There are no foreseeable risks associated with the use of finger or arm cuffs to examine blood pressure. Some individuals may feel acute discomfort and numbness in the fingertip during prolonged testing sessions, but this is resolved immediately upon cuff removal. Standard sphygmomanometer (arm cuff) measures of arterial blood pressure will also be obtained periodically – there are no known risks beyond discomfort during inflation. Discomfort associated with blood pressure measurements will be mitigated by immediately deflating the cuff, after which the discomfort will dissipate.

Electrocardiogram & Peripheral Oxygen/Carbon Monoxide Saturation: There are no foreseeable risks associated with electrocardiogram and peripheral oxygen and carbon monoxide saturation monitoring.

IV & Peripheral Venous Pressure: There is a minimal risk of infection. Aseptic technique will be used and only a certified technician will insert the IV, draw blood samples, and set up the peripheral venous pressure transducer. The IV may result in some discomfort, minimal pain upon insertion, swelling, redness, or bruising. Once the IV is in place, the participant should have no discomfort. The IV will only be used for pulling blood samples or for a saline line to the venous pressure transducer, and no foreign materials will be inserted through the IV. The IV can also be removed at any point if the participant wishes to have it removed.

Phlebotomy: There is a minimal risk of infection. Aseptic technique will be used and only a certified technician will draw blood samples. Phlebotomy may result in some discomfort, minimal pain upon insertion, swelling, redness, or bruising. Once the needle is removed from the vein, the participant should have no discomfort.

Finger Prick Blood Sample: Minor discomfort may be felt while the finger is being pricked.

Carbon Monoxide Rebreathe: Carbon monoxide has a high affinity for hemoglobin in the blood and can therefore be toxic in high doses. As such, we have employed several risk mitigation strategies to maximize participant and researcher safety. First, though the room is well ventilated, we have a carbon monoxide sensor right beside the carbon monoxide cylinder that would detect any buildup of carbon monoxide in the testing area. Furthermore, only trained research staff will measure and administer a known bolus (0.7–1.0 mL carbon monoxide per kg bodyweight) of 99.5% pure carbon monoxide with extreme care to ensure the correct dose is given. Furthermore, the use of a 100 mL syringe ensures that a toxic dose cannot accidentally be administered. The above bolus is expected to increase carboxyhemoglobin saturation ~6% (Schmidt & Prommer, 2005; Siebenmann et al., 2017) which is similar to levels seen in habitual cigarette smokers. Previous work has shown that at 6% carboxyhemoglobin saturation participants have no symptoms, and that no cardiovascular effects would be seen up to 20% (3× higher than our expected values) (Hanada et al., 2003; Schmidt & Prommer, 2005; Siebenmann et al., 2017; Zavorsky et al., 2014). Even so, participants will wear a carboxyhemoglobin saturation monitor (Radical-7 Pulse CO-Oximeter and LNCS-II SpCO sensor, Masimo, CA, USA) and the test will be terminated if carboxyhemoglobin saturation exceeds 12%. Carboxyhemoglobin saturation up to 12% should cause no symptoms. Exceeding 12% could lead to a headache or nausea, and in extreme cases, signs of cognitive impairment. We will also have 100% oxygen available if needed, which will improve oxygen delivery immediately and speed up the removal of carbon monoxide from the blood. Furthermore, the 100% oxygen in the 6 L rebreathing bag will ensure sufficient oxygenation and dampen the affinity between the carbon monoxide and hemoglobin compared to

room air. Should the participant experience any symptoms during or following their carbon monoxide rebreathe, a trained researcher will stay with the participant until they feel better. Since the entire bolus is delivered at once, any symptoms should occur during the rebreathe or 8-minute rest and improve thereafter. The half-life of carbon monoxide in human blood is ~2 hours (Siebenmann et al., 2017), so any symptoms experienced by the participant will be resolved by then, returning completely to baseline levels within 12–14 hours.

Ultrasound Imaging: There are no known risks to ultrasound imaging of the heart or blood vessels. However, some minor discomfort may be felt during the heart imaging due to pressure from the ultrasound probe pressing against the chest.

LBNP/LBPP & HUT/HDT: There is a small risk of brief lightheadedness and possibly fainting during LBNP and HUT. However, blood pressure and heart rate are monitored continuously so that the test will be stopped if a rapid fall in blood pressure (25 mmHg in systolic blood pressure and 15 mmHg in diastolic blood pressure) occurs. We ask that participants tell the investigators if they feel light-headed, sweaty, or have "tunnel" vision so that the test can be stopped, after which participants will feel better almost instantly. There are no foreseeable risks with LBPP and HDT.

Computed Tomography: The CT scan includes exposure to a small amount of radiation from X-rays, but the risk from radiation exposure in this research study is very low. However, the CT scan may not be safe during pregnancy. Therefore, pregnant or breastfeeding individuals will not undergo a CT scan. Females of childbearing potential not using contraception must have a negative urine pregnancy test (provided by the research team). Acceptable methods of contraception include tubal ligation, oral contraceptive, barrier methods (intra-uterine device, diaphragm, female condom, male condom). Abstinence is an acceptable form of contraception insofar as participants agree to use another acceptable method of birth control, preferably a barrier method, if they become sexually active during the study.

3.6 Statistical Plan

3.6.1 Sample Size Determination

Based on previous work which examined the impact of aerobic fitness in lung diffusing capacity (Tedjasaputra et al., 2016) (untrained: 84.9 ± 14.9 mL vs. trained: 100.7 ± 12.7 mL), at an α-level of 0.05 and 80% power, a total sample size of 28 is calculated to detect a between group difference. Additionally, based on prior evaluations of the impact of body posture (and therefore central blood volume changes) on capillary blood volume (Ross et al., 2020), (upright: 61 ± 6 mL vs. supine: 65 ± 7 mL), at an α-level of 0.05 and 80% power, a total sample size of 24 is calculated to observe an effect of central blood volume shifts on capillary blood volume. However, our main outcome is the interaction of fitness group × central blood volume shift (e.g., LBPP condition). Therefore, assuming the interaction of the above effects accounts for 10% of the variability in DLCO ($r^2 = 0.10$, therefore $f \approx 0.10$), $\alpha = 0.05$, 80% power, 2 groups (high aerobic capacity vs. low aerobic

capacity), and 4 measurements per group, the required sample size is 138 (69 per group). To account for participant dropouts, screening failures, and the experimental nature of this work, we propose recruiting up to 200 participants for this study.

3.6.2 Statistical Methods

All primary and secondary variables will be analyzed using two-way mixed analyses of variance (ANOVA) with the non-repeated factor of group and repeated factor of condition.

4 Data Handling and Record Keeping

We will collect and store data in a confidential manner. Only researchers will have access to the data, which will be stored in a secured location in a locked file cabinet. Electronic data will be kept on a password-protected computer.

4.1 Confidentiality

After participants are recruited, they will be assigned a participant code, and moving forward, that will be their only identifier.

4.2 Records Retention

Physical records will be kept for 5 years after which time they will be destroyed.

5 Study Auditing and Inspecting

Not applicable.

6 Funding

Funding for this study is provided by Dr. Stickland's Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant.

7 Publication Plan

We plan to publish our findings in a high impact physiology journal such as Chest, The Journal of Physiology, or The Journal of Applied Physiology.

8 Supplements

Preliminary Screening Sheet

Participant Information	
Initials	
Sex (M/ F)	
Height (cm)	
Weight (kg)	
BMI (kg/m^2)	
BSA (m^2)	

Date of Birth (dd/mm/yyyy)	
Age (y)	

Smoking History	
Average number of packs/day (1 pack = 25 cigarettes)	
Number of years spent smoking	
Pack years (# pack/day x years smoked)	
Year since quit	

Past History	
Coronary Artery	No Yes
Respiratory Disease	No Yes
Cerebrovascular	No Yes
Hypertension	No Yes
CHF	No Yes
Valvular Heart	No Yes
Per Vasc Disease	No Yes
Diabetes	No Yes
Dyslipidemia	No Yes
Liver Disease	No Yes
Mental Illness	No Yes
MSK	No Yes
Neuromuscular	No Yes
Renal Disease	No Yes
GERD *GI reflux*	No Yes
Cancer	No Yes
Surgery	No Yes
Other	
FEMALES ONLY: Menopausal history	
Age at last menstrual cycle	
Years since last menstrual cycle	
FEMALES ONLY: Menstrual cycle information	
Current menstrual phase & day of cycle	

Medications

- _____ **Dose**_____
- _____ **Dose**_____
- _____ **Dose**_____
- _____ **Dose**_____

Participant ID:

Height:

Date:

Weight:

TRIAL: CPET

Time	PO	HR	Q	SPO2	BP	RPE
Baseline 0-1						
1-2						
START 0-1						
1-2						
2-3						
3-4						
4-5						
5-6						
6-7						
7-8						
8-9						
9-10						
10-11						
11-12						
12-13						
13-14						
14-15						
15-16						
16-17						
17-18						

Total Test Duration (mm:ss):

Pre Hb Count:

Post Hb Count:

Notes:

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