Cardiopulmonary complications of electronic cigarettes

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List of Abbreviations

BV5	Volume of Pulmonary Blood Vessels <5 mm^2 in Cross-Sectional Area
BV5-10	Volume of Pulmonary Blood Vessels Between 5-10 mm^2 in Cross-Sectional Area
CBV	Central Blood Volume
CT	Computed Tomography
DLCO	Diffusion Capacity of Carbon Monoxide
Dm	Membrane Diffusing Capacity
Hb	Hemoglobin
LBNP	Lower Body Negative Pressure
LBPP	Lower Body Positive Pressure
RVSP	Right Ventricular Systolic Pressure
TAPSE	Tricuspid Annular Plane Systolic Excursion
TVI	Tissue Velocity Index
TVV	Total Vessel Volume
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
$\dot{V}O_{2peak}$	Peak Oxygen Consumption

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1 Introduction/Significance

The invention of electronic cigarettes (E-CIGs) in 2003 upended the trajectory of a decades-long public health campaign against cigarette smoking. Promoted as a safer method of inhaling nicotine compared to cigarettes and as smoking cessation tools, E-CIGs have substantially grown in popularity with rates of uptake now exceeding those of cigarette smoking (Health Canada, 2023). Surveys estimate that 5.8% of Canadians 15 years and older have used E-CIGs in the last 30 days (Health Canada, 2023). Young Canadians appear particularly susceptible: 13.6% of youths aged 15-19 and 19.7% aged 20-24 report past 30 day E-CIG use (Health Canada, 2023). Despite this rapid growth, questions remain as to their long-term safety. In 2019, an outbreak in the United States of acute lung injury related to E-CIGs (Layden et al., 2020; Schier et al., 2019) (later termed E-cigarette or Vaping Use-Associated Lung Injury or EVALI) served as a warning that these devices were not benign. As Canadians increasingly reach for E-CIGs, especially at younger ages and not just for the purposes of smoking cessation, greater clarity into the pulmonary toxicities of E-CIG use is urgently needed.

E-CIG use has been linked to cardiopulmonary risks with potential long-term consequences for health (Marques et al., 2021). Even mild reductions in cardiopulmonary function during young adulthood are associated with increased risk of disease, morbidity, and all-cause mortality, underscoring the importance of protecting cardiopulmonary health (Barnes, 2015; Huang et al., 2013; Lang et al., 2024; Mandsager et al., 2018). Recent work has shown that E-CIG use is associated with reduced exercise capacity ($\dot{V}O_{2peak}$), increased exertional dyspnea and greater ventilatory inefficiency (Ammar et al., 2023; Simovic et al., 2024; Williams et al., 2026). Additionally, emerging evidence linking E-CIG use with greater ventilation/perfusion heterogeneity and blunted pulmonary diffusing capacity for carbon monoxide (DL_{CO}) recruitment during a volume-loading challenge suggests the presence of pulmonary vascular dysfunction (Kizhakke Puliyakote et al., 2021; Williams et al., 2026). However, the mechanisms behind these findings remain largely unknown.

DL_{CO} is a pivotal component of gas exchange and acid-base balance (Johnson et al., 1960). 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 DL_{CO} in those with respiratory diseases, such as chronic obstructive pulmonary disease, contributes to exercise intolerance and arterial hypoxemia (Ross et al., 2020). Physiological stressors such as exercise and controlled alterations in central blood volume (CBV) provide a means of challenging the cardiopulmonary system to reveal early dysfunction (Laveneziana et al., 2021; Stickland et al., 2022). Exercise increases cardiac output and pulmonary blood flow, requiring proportional increases in DL_{CO} secondary to its components, pulmonary capillary blood volume (V_C) and membrane diffusing capacity (D_M) to maintain efficient gas exchange (Hanson et al., 1989; Hsia, 2002; Reeves et al., 2005). Similarly, lower body positive

and negative pressure (LBPP/LBNP) transiently manipulate CBV, enabling assessment of pulmonary vascular responsiveness under controlled loading conditions. Evaluating DL_{CO} during these stressors may therefore uncover abnormalities that are not detectable at rest, providing novel insight into the early pulmonary consequences of chronic E-CIG use. Additionally, as many individuals also consume cannabis, we will also recruit a cannabis use only and a combined E-CIG and cannabis group.

Accordingly, the purpose of the current study is to:

1. Describe the relationship between pulmonary vascular structural and functional parameters with exercise tolerance in E-CIG users, and
2. Examine if blunted resting DL_{CO} recruitment in E-CIG users can be normalized by increasing pulmonary blood volume through LBPP.

We hypothesize that:

1. E-CIG users will show a blunted DL_{CO} response to exercise, particularly in females, and impaired pulmonary vascular structure/function is associated with exercise intolerance in E-CIG users, and
2. Increasing central blood volume via LBPP will normalize resting DL_{CO} in E-CIG users

2 Project Aims

2.1 Aim #1: To describe the relationship between pulmonary vascular structural (CT scan) and functional (rest/exercise DL_{CO}) parameters and exercise tolerance (i.e., $\dot{V}O_{2peak}$) in E-CIG users. Hypotheses: 1) The stress of exercise will fail to appropriately recruit and distend pulmonary capillaries (as evaluated by DL_{CO}, V_C, and D_M) in E-CIG users, with the magnitude of DL_{CO} impairment being larger in females. 2) Impaired pulmonary vascular structure/function will be associated with exercise intolerance in E-CIG users. Significance: This study will provide insight into whether impaired pulmonary vascular structure/function is associated with the reduced exercise tolerance observed in E-CIG users, and whether E-CIG impairments are greater in females.

2.2 Aim #2: To examine if the impairment in resting DL_{CO} recruitment in E-CIG users can be reversed by increasing pulmonary blood volume through LBPP. Hypothesis: Increasing central blood volume via LBPP will normalize resting DL_{CO} in E-CIG users. Significance: Should LBPP normalize DL_{CO}, this will provide evidence of a vascular dysfunction that may be reversible with increased pulmonary blood volume. In contrast, if DL_{CO} does not normalize, this will indicate a fixed vascular impairment (potentially remodeling/destruction) that is not easily reversible.

3 Overview of Study Outcomes by Aim

Aim	Intervention/Exposure	Outcomes	Strata/Covariate
1. Exercise	Exercise Intensity,	$\dot{V}O_{2peak}$, Exercise	Age group, Sex,

	E-CIG Group	DL_{CO}, V_C, D_M	Physical Activity, DL_{CO}, RVSP, TVV, BV5, BV5-10, and $\dot{V}_E/\dot{V}CO_2$ nadir
2. LBPP	LBPP Condition, E-CIG Group	DL_{CO}, V_C, D_M	Age group, Sex

4 Study Design

4.1 General Design

This study will be a cross-sectional design. Participants will report to the Clinical Physiology Laboratory in Clinical Sciences Building at the University of Alberta. Data will be collected over six experimental visits. **Visit 1:** Characterization of pulmonary function and exercise capacity by standard pulmonary function and cardiopulmonary exercise testing. A venous blood draw will be taken to quantify plasma cotinine and tetrahydrocannabinol (THC) (~4 hours); **Visit 2:** Measurement of DL_{CO} and its components via standardized breath hold techniques during exercise (~2 hours). **Visit 3:** Measurement of DL_{CO} and its components via standardized breath hold techniques during LBPP/LBNP (~2 hours). **Visit 4:** Ultrasound imaging of the heart during exercise and LBPP/LBNP (~2 hours). **Visit 5:** Estimation of blood volume via carbon monoxide rebreathe protocol (~1 hour; adult cohort only). **Visit 6:** Computed tomography to assess lung structure (~1 hour; adult cohort only). Visits 2-6 may occur in any order, depending on the availability of the participant and the investigator. For all visits, 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. E-CIG users will refrain from E-CIG use for at least 6 hours before visits 2-6, in line with previous work (Kizhakke Puliyakote et al., 2021).

4.1.1 Primary Outcome Variable

The primary outcome variables are DL_{CO} and its components, V_C and D_M.

4.1.2 Participant Selection

The study design includes a cohort of individuals aged 12-40 years who have consented to be contacted from previous studies.

4.1.3 Inclusion Criteria

Adolescent Cohort

1. Participants aged ≥12 and <18 years
2. Non-smoking and Non-E-CIG Use: <100 lifetime cigarettes and < 200 E-CIG puffs
3. E-CIG Use Only: currently using E-CIGs every day or some days
4. Cannabis Use: cannabis smoking history of > 1 joint-year
5. Combined E-CIG and Cannabis Use: adolescents who meet both #3 and #4

Adult Cohort

1. Participants aged 18-40 years

2. Non-smoking and Non-E-CIG Use: <100 lifetime cigarettes and < 200 E-CIG puffs
3. E-CIG Use Only: currently using E-CIGs every day or some days
4. Cannabis Use: cannabis smoking history of > 1 joint-year
5. Combined E-CIG and Cannabis Use: adults who meet both #3 and #4

4.1.4 Exclusion Criteria

1. Contraindication to pulmonary function testing (i.e. recent thoracic or abdominal surgery, aneurysms, cardiovascular instability)
2. Unable to provide informed consent
3. Currently undergoing treatment for lung cancer
4. Cigarette smoking in the past 6 months
5. Physician-diagnosed chronic lung disease (e.g. cystic fibrosis, asthma, primary ciliary dyskinesia, bronchopulmonary dysplasia) (adolescent arm only)
6. Self-reported premature infancy, referring to individuals who were born premature (born before the 37th week of pregnancy). Previous literature and work in our lab have shown that lung development is blunted in these individuals, and we are therefore excluding those born premature so that underdeveloped lungs do not impact the findings of the present study (PMID: 37734855, 37707478, 27259338).
7. Pregnancy. To confirm this, women not using contraception will be given a pregnancy test prior starting the study. Pregnancy presents additional complications and may confound the results of the study; therefore, pregnant women will be excluded.

4.1.5 Screen Failure Criteria

Screen failures will be defined as an individual who meets one or more exclusion criteria, cannot be assigned to one of the study cohorts based on the eligibility criteria, is unable to provide informed consent, or withdraws during the screening process.

Individuals identified as screen failures will be informed that they are not eligible for the study and will not proceed with study enrollment or subsequent study visits.

4.1.6 Rationale for Study Population

This study aims to characterize the early effects of E-CIG and cannabis use on pulmonary vascular structure and function before clinically apparent disease develops. Therefore, the inclusion of otherwise healthy participants is essential to address the study objectives. Adolescents are included because this is a period of continued lung growth and maturation during adolescence which exposure may have unique physiological effects. Emerging evidence suggests that even acute or occasional e-cigarette exposure may have measurable effects on the pulmonary and cardiovascular systems, such as increased airway inflammation, blood pressure, and endothelial dysfunction (Tsai et al., 2020). These effects may occur before the development of clinically apparent disease and may therefore be detectable in otherwise healthy individuals with relatively low levels of exposure.

Therefore, participants with occasional e-cigarette use will be eligible for inclusion in the study. The study procedures involve physiological testing that is considered low risk when performed in appropriately screened, healthy individuals under close supervision. Recruiting participants with underlying cardiopulmonary disease would likely increase the risks associated with procedures and would also introduce important confounders. In addition, adolescent participants will not undergo the carbon monoxide rebreathe protocol (which requires an IV) and the quantitative CT scan. Risks have been minimized through careful eligibility screening, predefined exclusion criteria, continuous monitoring by trained personnel, established stopping criteria, and emergency procedures, as described in the Risk Mitigation section below.

The findings from this study are not intended to directly determine therapeutic options but are critical to a better understanding of the physiological effects of e-cigarette use on pulmonary and cardiovascular function. This information is critical to help identify potential physiological targets for future research and may ultimately contribute to the development or evaluation of strategies to prevent or mitigate adverse health effects associated with e-cigarette use.

4.1.7 Informed Consent and Withdrawal

The informed consent form used must be approved by the reviewing Research Ethics Board and should be in accordance with the current revision of the Declaration of Helsinki. Consent forms describing in detail the study procedures and risks will be given to each participant and written documentation of informed consent is required prior to starting the study. Each participant should have sufficient opportunity to discuss the study, have all their questions addressed and consider the information in the consent process prior to agreeing to participate. Participants may withdraw consent at any time during the course of the study without prejudice. An investigator will be available to answer questions about the study if required. The informed consent form will be signed and dated by the participant and the investigator or delegate. The original signed informed consent form will be retained in the participant's study files and a copy will be provided to the participant.

The informed consent process must be conducted, and form signed before the participant undergoes any screening procedures that are performed solely for the purpose of determining eligibility for the study. All research team members obtaining informed consent must be qualified through training and knowledgeable about the study. The delegate who obtains consent will not have a personal relationship with the potential participant and will not be part of the participant's circle of care.

4.2 Study Procedures

4.2.1 Visit 1: Screening

The participant will undergo a venous blood draw to measure cotinine and THC, and complete detailed questionnaires regarding demographic, medical, E-CIG, other substance use, and respiratory symptoms. We then will perform pulmonary function tests on each participant. Finally, the participant will complete a graded exercise test ($\dot{V}O_{2max}$, 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 (Hb) concentration will be measured via finger prick prior to and following completion of exercise testing.

At the end of the visit, participants will be provided with an activity monitor to quantify physical activity. They will be instructed to wear the device 24-hours per day for seven consecutive days and return it to the research coordinator at the end of the monitoring period.

4.2.2 Visit 2: DL_{CO} with Rest and Exercise

Exercise will be used as a physiological stressor to increase central blood volume. The participant will perform DL_{CO} maneuvers at rest and during exercise at varying exercise intensities between ~20%-100% of their $\dot{V}O_{2max}$. Participants will also be equipped with a continuous blood pressure monitor, a brachial blood pressure cuff, an electrocardiogram, and a thoracic impedance device. 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. Hb via finger prick will also be measured at each intensity.

As per recommendations (Zavorsky et al., 2017), participants will perform standard DL_{CO} breath-hold maneuvers for 6 seconds, and maneuvers will be performed twice at each intensity. A washout period of at least 4 minutes at rest and 2 minutes between each breath hold during exercise will be completed to ensure adequate recovery and blood gas equilibration (Zavorsky et al., 2017). Participants will not complete more than 15 DL_{CO} breath holds during each experimental visit.

4.2.3 Visit 3: Central Blood Volume Manipulation and DL_{CO}

The participant will perform the DL_{CO} test at various levels of LBNP/LBPP ranging from −80 mmHg to +40 mmHg. Changes in central blood volume during LBNP are equivalent to standing upright (−80 mmHg LBNP), and changes in central blood volume during LBPP are equivalent to lying down with your feet above your head (+40 mmHg LBPP) (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 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 inside 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.

Participants will 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 will begin with two minutes of stabilization followed by two DL_{CO} breath-hold maneuvers. At each stage, participants will also breathe on a mouthpiece for two minutes to collect metabolic data, and oscillometry measurements will be completed twice per stage. Cardiovascular parameters will be measured continuously. Hb via finger prick will be measured prior to the first pressure stage and again at the transition between LBNP and LBPP conditions.

Participants will not complete more than 15 breath holds during the experimental visit (Zavorsky et al., 2017). As per recommendations (Zavorsky et al., 2017), participants will perform standard DL_{CO} 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).

4.2.4 Visit 4: Ultrasound with Exercise and LBPP/LBNP

Participants will complete the same interventions as visit 3 (exercise at ~20%–100% of their $\dot{V}O_{2max}$) and visit 4 (LBNP/LBPP −80 to +40 mmHg). At each stage of exercise and LBNP/LBPP, a trained sonographer will obtain images of the heart via echocardiography.

4.2.5 Visit 5: Blood Volume Measurement

Participants from the adult cohort only will 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 at eight and 10 minutes after the rebreathe will allow the calculation of blood volume.

4.2.6 Visit 6: Computed Tomography

Participants from the adult cohort only will report to a Medical Imaging Centre for the CT scan. Following a briefing by the Medical Imaging Centre staff, participants will lie 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.

4.3 Measures

Questionnaires: Participants will be screened for exercise using the Canadian Society for Exercise Physiology PAR-Q+ exercise readiness questionnaire, and the Physical Activity and

Sedentary Behaviour Questionnaire (PASB-Q). Participants with a positive PAR-Q+ screen will require further medical evaluation and/or physician clearance before participation.

For the adult cohort, respiratory symptoms will be assessed by the St. George's Respiratory Questionnaire (SGRQ), COPD Assessment Test (CAT), Modified Medical Research Council Dyspnea Scale (mmRC), and McMaster Cough Severity Questionnaire (MCSQ). E-CIG history will obtain information including use frequency, device type, e-liquid flavourings, and nicotine content. E-CIG dependence will be assessed using a modified Fagerström test.

Adolescent participants will complete the same questionnaires as adult participants. However, the CAT, mmRC, and MCSQ will be replaced with the American Thoracic Society Questionnaire and the Visual Analog Scale.

Pulmonary Function: Spirometry, plethysmography, forced oscillometry, and DL_{CO} 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 (Oostveen et al., 2013; 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 [HC#22536] or equivalent HC approved device) 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 [HC#15928]) will be measured during cardiopulmonary exercise testing and during the experimental session. The same metabolic cart will be utilized for metabolic measurements on visits 3 and 4.

Blood Pressure: Upper arm blood pressure measurements will be taken via auscultation with a sphygmomanometer throughout exercise (Tango M2, Suntech, Morrisville, NC, USA [HC#92724]). During visits 3 and 4, 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 will be used during visits 3, 4, and 5, and a thoracic impedance device (PhysioFlow PF-07 Enduro, Manatec Biomedical, France [HC#75857]) will be used during visits 1, 3, 4, and 5.

Venous Blood Draw: From a vein in the antecubital fossa, up to 30 mL of blood will be collected prior to the pulmonary function testing on visit 1 using standard venipuncture techniques. Half of the 30 mL sample will be allowed to coagulate for a minimum of 30 minutes at room temperature before centrifuged at 12,000 rpm for 10 minutes. Serum will be collected and aliquoted into samples of 200 μL and stored in a -80 degrees Celsius freezer. The other half of the 30 mL sample will not be allowed to coagulate; the sample will be centrifuged at 12,000 rpm for 10 minutes before plasma is collected and aliquoted into samples of 200 μL and stored in a -80 degrees Celsius freezer and used later for analysis of cotinine, THC, and biomarkers of stress, inflammation (i.e., of IL-6, TNFα, CRP, nitrates, and nitrites) and organ/tissue damage.

Intravenous Blood Draw: A certified technician will administer an IV in an antecubital vein during visit 6. 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, USA [HC#77132]). 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 [HC#85942]). 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 before the rebreathe and at eight and 10 minutes after the rebreathe. 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.

DL_{CO}: Measurements of capillary blood volume will be derived from the lung diffusing capacity of carbon monoxide using the Hypair® pulmonary function system (Ultima™ Cardio2, Novus Medical, Ontario, Canada [HC#105182]) (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 (E95, GE Health, GE Healthcare, Chicago, IL, USA [HC#95856]) 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; Quiñones 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: 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. The range of pressures used in the current study will be equivalent to the physiological stress of standing upright (−80 mmHg) and lying down with your feet above your head (+40 mmHg) (Diaz-Canestro et al., 2022; Goswami et al., 2019).

Physical Activity: Minute-by-minute data from an activity monitor (Fitbit Versa 4, Google LLC, Mountain View, CA, USA or equivalent HC approved device) will be exported to quantify steps

per day, heart rate, caloric expenditure, and time spent at moderate to vigorous physical activity (i.e. > 3 times resting values) (Burchartz et al., 2020; Matthews et al., 2012).

Computed Tomography (CT) Scan: Participants will lie in a CT scanner where a series of X-ray images are taken (from different angles around the lungs), and computer processing is used to examine cross-sectional images (slices) of the blood vessels in the lungs.

4.4 Participant Safety and Emergency Procedures

Before exercise testing, all participants will complete the Canadian Society for Exercise Physiology Physical Activity Readiness Questionnaire for Everyone (Akben-Marchand, 2021). This standardized questionnaire consists of several general health questions to determine if it is necessary to seek further medical advice prior to becoming more physically active. As per the guidelines of the questionnaire, if the participant answers 'no' to all of the questions, the participant is cleared to participate in the study. If the participant answers 'yes' to any of the questions, the questionnaire will be reviewed by either: the study physicians (Dr. Sean van Diepen, cardiologist; Dr. Joanna MacLean, pediatric pulmonologist; Dr. Jason Weatherald, pulmonologist) or a certified exercise physiologist who will determine whether the participant is appropriate to participate in the study. If deemed required by the study staff, n physician may be present to oversee the exercise test. Importantly, the present study aims to recruit adolescents and adults without known cardiopulmonary disease or other contraindications to exercise testing. Moreover, all adult participants will be < 40 years of age, further minimizing risk.

Cardiopulmonary exercise testing is a well-established clinical and research procedure that is generally well tolerated when performed with appropriate screening and supervision. Although published data quantifying the risk of serious adverse events in healthy adolescents are limited, CPET is routinely and safely performed in pediatric clinical populations, including adolescents with cardiopulmonary disease. In healthy adults, the overall risk of a serious cardiac event is very small (less than 9 in 10,000 hours) (Kasiak, 2026).

All study team members are certified in CPR and use of an automated external defibrillator (AED). All study team members are trained to recognize and respond to adverse events according to our laboratory SOP (please see attached to application). Research staff will be present at all times during study visits and will have immediate access to emergency equipment, including an AED to respond to any adverse medical situation that may arise during testing. Immediate management of an adverse event will be started as soon as it is identified and completed as soon as feasible. Research staff will ensure that the needs of the participant are being attended to, immediate first aid and/or medical aid is offered, immediate practical support (e.g., access to a telephone, snacks, quiet space), and will maintain or create a safe environment for the participant and staff. In the event of a serious adverse event requiring emergency medical care, research staff will activate emergency medical services (EMS) by calling 911 and will remain with the participant, providing appropriate first aid and/or CPR until EMS assumes care.

Additionally, the carbon monoxide rebreathe procedure is conducted by trained research personnel according to an established laboratory protocol with predefined safety monitoring and stopping criteria (12% carboxyhemoglobin). As outlined in section 3.1.4.0 of the ARISE protocol, participants will wear a carboxyhemoglobin saturation monitor and the test will be terminated if carboxyhemoglobin saturation exceeds 12%. Importantly, COHb of 20% has been shown to be well tolerated and generally safe (Hanada et al., 2003; Schmidt & Prommer, 2005; Siebenmann et al., 2017; Zavorsky et al., 2014). We will also have 100% oxygen available for participants to breathe if needed, which will improve oxygen delivery immediately and speed up the removal of carbon monoxide from the blood. 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 carbon monoxide is delivered at the start of the test, any symptoms should occur at the onset of the test and improve thereafter.

4.5 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: Some participants may feel uncomfortable answering some of the questions. They do not have to answer any questions that they do not want to answer.

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 discomfort 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. Participants will be screened prior to participation using the Canadian Society for Exercise Physiology (CSEP) PAR-Q+ exercise readiness questionnaire and the Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q) to identify any contraindications or conditions requiring further medical evaluation before exercise testing.

Pulmonary Function Tests (Including DL_{CO}): 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.

Venipuncture and Intravenous: There is a minimal risk of infection. Aseptic technique will be used, and only a certified technician will insert the IV and/or draw blood samples. Some participants may experience mild, brief discomfort during blood collection. 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, 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.

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: There is a small risk of brief light-headedness and possibly fainting during LBNP. However, blood pressure and heart rate are monitored continuously and 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.

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. Participants will be asked to perform CT no more than once per year (or longer). The dose of radiation that participants will receive from the research CT scan is estimated at below 3.0 mSv, which is the approximate annual background radiation everyone experiences due to naturally occurring radioactive materials. These radiation doses are also lower than typical clinical chest CT protocols (5-6 mSv).

However, the CT scan may not be safe during pregnancy. Females of childbearing potential not using contraception must have a negative urine pregnancy test before the start of the study (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.

Lastly, some participants may experience a sense of uneasiness in small spaces during the CT scan. They will be able to communicate this to the researcher and scanning will be paused or stopped if there is any discomfort.

Post-Visit Monitoring: If participants develop symptoms during or after the study visits, they will remain under observation until symptoms resolve or appropriate medical care has been arranged. Participants will be given the study team's contact information for reporting adverse events during business hours. Those who develop severe or concerning symptoms after leaving the study site will be instructed to seek immediate medical attention by calling 911 or presenting to the nearest emergency department. Participants will also be asked to notify the research team as soon as reasonably possible following any adverse events related to study participation.

Disclosure Risks: There is the possible harm from accidental disclosure of participants' health information, however, study investigators have taken precautions to minimize this risk, such as minimizing the amount of personal information collected and keeping it in a separate locked cabinet from the other coded study data.

Incidental Findings: All CT scans will be reviewed by a radiologist and there is a chance that the scan will discover a finding that the study team was not looking for. If the radiologist thinks that there may be an abnormality in the CT scan, the study team will contact the participant and with their permission, contact their family physician to assist with follow-up. No information generated in this study will become part of participants' health record. However, if the study detects an abnormality in a participant's CT scan and further follow-up is required, then this information may become part of their health record.

4.5 Statistical Plan

4.5.1 Sample Size Determination

Our pilot work shows a blunted DL_{CO} response in E-CIG users compared to controls during 60 watts of exercise (mean group difference 7.3±10.4 ml/min/mmHg; Aim #1) and LBPP (mean group difference 12.9±10.5 ml/min/mmHg; Aim #2). Power analyses indicated that with 80% power and an α-level of 0.05, 18 participants per group are required to detect between-group differences for Aim #1, while 8 participants per group are required for Aim #2. To conduct sex- (male/female; sample size x2) and age-group-stratified (adolescents, young adults (18-25 years), adults (26-40 years); x3) analyses, and account for 10% drop-out, we will recruit 480 individuals (120 adolescents, 120 young adults, 120 adults, 25% E-CIG users, 25% cannabis users, 25% combined E-CIG and cannabis users, 50% female).

4.5.2 Statistical Methods

4.5.2.1 Aim #1

Baseline pulmonary vascular structure/function differences will be assessed using linear mixed effects modelling where pulmonary vascular parameters (rest/exercise DL_{CO}, V_C, D_M, CT parameters) are considered the outcome (one outcome per analysis) and exposure (control, E-CIG, cannabis, combined E-CIG and cannabis) is the primary exposure. Each model will be adjusted for age, sex, and exercise workload (where appropriate) as covariates. The differences in each

outcome between exposure groups will be reported for the adjusted models. We will also test for exposure group-by-biological sex and exposure group-by-age group interactions to see if the association is modified by sex or age group. The relationship between pulmonary vascular parameters and exercise tolerance (i.e., $\dot{V}O_{2peak}$) will be evaluated using linear regression analyses to estimate the relationship between each of the parameters: DL_{CO}, V_C, D_M, RVSP, TVV, BV5, BV5-10, and $\dot{V}_E/\dot{V}CO_2$ nadir with $\dot{V}O_{2peak}$ (outcome) in each group, adjusting for sex and physical activity. This analysis will help determine if underlying pulmonary vascular structure/function is correlated with $\dot{V}O_{2peak}$ in E-CIG and cannabis users or if the lower $\dot{V}O_{2peak}$ in E-CIG and cannabis users is confounded by low physical activity.

4.5.2.2 Aim #2

A mixed effects model will be used to estimate the effect of positive pressure (control vs. LBPP) on $DL_{CO.}$ Random intercepts (individual) and slopes (group) models with sex, randomization order, pressure condition will be included. An interaction between positive pressure (LBPP) and group (control, E-CIG, cannabis, combined E-CIG and cannabis) will be used as the primary effect. Models will be stratified by age category (adolescent, young adult, adult) to compare the effect of lung maturation/age group.

5 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. De-identified data will also be stored with the Scientific and Health Data Assets in Respiratory Research database.

5.1 Confidentiality

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

5.2 Records Retention

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

6 Study Auditing and Inspecting

Not applicable.

7 Funding

Funding for this study is provided by Dr. Stickland's Canadian Institutes of Health Research (CIHR) Project Grant.

8 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.

9 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)	
Born prematurely?	
Time since last ENDS/cannabis use	

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:	
Are you post-menopausal?	No Yes
Age at last menstrual cycle?	
Pregnant?	No Yes
Contraception?	No Yes
	If yes, brand/type:
Day of cycle	
Medications • _____ **Dose**_____ • _____ **Dose**_____ • _____ **Dose**_____ • _____ **Dose**_____	

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