The Impact of Carbon Monoxide on Endothelial Function

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

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Abstract

Endothelial function measured via flow-mediated dilation following mild, acute carbon monoxide (CO) exposure was quantified in 19 healthy young non-smoking adult participants (n=10 females). The Schmidt-Prommer rebreathe method for the measurement of blood volume was used for the CO exposure, and standardized flow-mediated dilation techniques were utilized. It was hypothesized that CO would increase endothelial-dependent vasodilation in vascular smooth muscle, potentially by modulation of endothelial nitric oxide synthase, intracellular NO release, or the dilatory cGMP pathway. While carboxyhemoglobin (COHb%) was elevated following the rebreathe (5.64±1.24%), mean arterial pressure, heart rate, blood flow, brachial artery diameter, and forearm vascular conduction were not significantly different following the CO rebreathe protocol. FMD and FMD%, as well as shear corrected FMD:ssAUC and FMD%:ssAUC were also not significantly different following CO rebreathe. These results suggest mildly elevated COHb% does not impact resting vascular and hemodynamic parameters, or impact endothelial-dependent vasodilation. Further research exploring CO release from hemoglobin to tissue could provide insight into endothelial cell exposure of CO following mild, acute exposure.

Preface

This thesis is an original work by Nicholas Cheung. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name “The Impact of Carbon Monoxide and Altitude on Vascular Function”, Pro00096251, approved 2/5/2021.

Acknowledgements

Thank you to the University of Alberta for funding received throughout my degree. I would like to thank CS, EV, AS and MT for their ongoing support and guidance through the protocol development, data collection, analysis and writing stages of this project. Thanks to ST for their work through this project in all its stages and for always being the most positive person in the room. Finally, I'd like to thank AC for their constant love and support throughout my degree and this project, I truly wouldn't have gotten here without you.

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List of Abbreviations

ATP – Adenosine 5’ triphosphate

NO – Nitric Oxide

eNOS – Endothelial nitric oxide synthase

SS_{AUC} – Shear stress area under curve

CO – Carbon Monoxide

COHb - Carboxyhemoglobin

HO-1 – Heme-oxygenase 1

D_A – Arterial Diameter

Q_A – Arterial Flow

Introduction

The human vasculature is an essential organ carrying nutrients, metabolites, and signalling factors to and from all cells in the body. Lining these vessels is a single layer of cells known as the endothelium, which contributes to vascular function via dilation and constriction (Tremblay et al., 2018). Endothelial function is often quantified using flow mediated dilation (FMD), or a measure of the ability of the endothelium lining an artery to respond to an increase in shear stress, often induced post ischemia (Bruce & Bruce, 2006). Increased shear stress can be induced through occlusion-release, pharmaceutically, or commonly experienced during exercise. (Thijssen et al., 2019). FMD analysis is used as a quantification of the functional outcomes of nitric-oxide mediated vasodilation (Green, Dawson, Groenewoud, Jones, & Thijssen, 2014). Nitric oxide is a highly vasoactive signalling molecule released from the endothelium into nearby vascular smooth muscle to trigger a vasodilatory cascade.

Inhaled carbon monoxide, is typically thought of as a fatal, silent killer (Barinaga, 1993). However, carbon monoxide (CO) is also produced endogenously within the vasculature (Stec, Drummond, & Vera, 2008) and influences vascular tone, blood flow and has been shown to modulate blood pressure *in vitro* (Zhao & Wang, 2001) and *in vivo* (Lee et al., 2017). Thus, alterations in CO (via exogenous sources) may also influence vascular regulation. Present literature supports carbon monoxide as a potent vasodilatory molecule with multiple pathways of potential action(Ndisang, Tabien, & Wang, 2004; Thorup, Jones, Gross, Moore, & Goligorsky, 1999; Wang, Wang, & Wu, 1997). This has broader implications including potential avenues for understanding vascular health and treatment of various cardiopulmonary blood pressure disorders including preeclampsia (McRae, Pudwell, Peterson, & Smith, 2019) and

atherosclerosis (Kim et al., 2011). Despite this, there is little known of the effect of acute carbon monoxide exposure on endothelium-dependent vasodilation.

This study aimed to quantify the effects of acute, mild carbon monoxide exposure on endothelial function using a standardized flow-mediated dilation technique in a healthy young non-smoking adult population. I hypothesized that the vasodilatory properties of carbon monoxide will improve endothelial function following a mild, acute carbon monoxide exposure – expressed as an augmented reactive hyperaemia and FMD.

Understanding the relationship of exogenous CO and endothelial function has important implications for the potential of inhaled CO as a therapeutic target in anti-inflammatory and vascular disease, as well as for better understanding of physiological consequences of acute environmental exposure from sources including but not limited to air pollution, second-hand smoke, or acute carbon monoxide poisoning.

Literature Review

The Vascular Tree

The human vascular system is a large network of vessels that play an integral role in the maintenance of respiratory, metabolic, and nutrient delivery needs of cells. This large network is comprised of arteries, veins, and capillaries. Arteries and veins are composed of three layers, the *tunica intima, tunica media, and tunica adventitia* (Kirsch & Mohr, 2014). Lining these vessels is a single-cell layer known as the endothelium. Arteries have the main function of supplying blood from the heart to tissue and sustain much higher pulse pressures than other blood vessels.

As a result, arteries have thicker walls with more elastic tissue and smooth muscle (Sandoo, Veldhuijzen van Zanten, Metsios, Carroll, & Kitas, 2015). Arteries can be further classified by size and composition. Conducting arteries are the largest arteries in the body and have a significant elastic tissue component to dampen oscillatory changes in blood pressure. Examples of conducting arteries include the aorta and the carotid artery (McEniery, Wilkinson, & Avolio, 2007). Branching from conducting arteries are conduit arteries whose main function is to deliver blood to specific regions of the body (Kirsch & Mohr, 2014). Examples of conduit arteries include the brachial and femoral arteries. Further division from the conduit arteries are resistance arteries, responsible for blood perfusion to organ tissue, and the microcirculation. Conduit and resistance arteries are highly innervated by the central nervous system (Alberts et al., 2002), allowing for central nervous system control of peripheral arterial function, diameter, and consequently flow.

Contraction and Relaxation of Vascular Smooth Muscle

Vascular smooth muscle cells are responsible for the modulation of artery diameter, allowing for the artery to respond to changes in hemodynamic conditions (Basatemur, Jørgensen, Clarke, Bennett, & Mallat, 2019). Several pathways and mechanisms within the vascular smooth muscle are responsible for signalling a change in artery diameter via contraction (constriction) or relaxation (dilation).

Contraction of the vascular smooth muscle is triggered by an elevation of intracellular calcium concentration initiated by either a membrane depolarization opening voltage-dependent calcium channels or via a pharmacomechanical activation of membrane receptors by a binding agonist such as norepinephrine, beginning a messaging cascade to release calcium from the sarcoplasmic reticulum. Intracellular calcium is then bound to calmodulin to form a calcium-

calmodulin complex, activating myosin light chain kinase. myosin light chain kinase is responsible for the phosphorylation of myosin heads, enabling the interaction of myosin and actin, cleaving the high-energy bonds in Adenosine 5' Triphosphate (ATP) and energizing the actin-myosin cross-bridge cycling, shortening the muscle cell (Consigny, 1991).

Relaxation of the vascular smooth muscle is triggered by a lowering of intracellular calcium levels, reversing the calcium-calmodulin binding responsible for contraction. Lowering of intracellular calcium can be achieved by the removal of a contractile stimulus, the removal of intracellular calcium, or by the elevation of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (Consigny, 1991). Cyclic guanosine monophosphate is elevated in vascular smooth muscle cells following the reaction of guanosine triphosphate and soluble guanylyl cyclase and reduces intracellular calcium levels by reducing calcium release from the sarcoplasmic reticulum, while stimulating the uptake of calcium back into the sarcoplasmic reticulum, promoting relaxation (Figure 1) (Sandoo et al., 2015).

Vascular Tone and the Endothelium

The endothelium acts as a barrier between blood and tissue and plays important roles in vascular remodelling and in the regulation of blood flow (Triggle et al., 2012). The location of endothelial cells allows for a sensitivity to hemodynamic changes both molecular and mechanical, as well as rapid diffusion of vasoactive compounds from the endothelium into the VSMC. Vasoactive compounds released from the endothelium include constrictors such as endothelin-1 as well as dilators including nitric oxide (NO) (Sandoo et al., 2015). NO is released in response to increased shear stress and is an important regulator of vascular tone in the delivery of blood flow to tissue (Sandoo et al., 2015). Shear stress applied to endothelial cells is translated through the cytoskeleton of endothelial cells (Chatzizisis et al., 2007) inducing calcium release

from the endothelial endoplasmic reticulum, as well as phosphorylating the enzyme endothelial nitric oxide synthase (eNOS), releasing NO from endothelial cells (Xiao, Zhang, Ranjan, & Diamond, 1997). As shear stress increases, endothelial cells increase eNOS activity, increasing nitric oxide synthesis and consequent vasodilation (Figure 1). Flow mediated dilation (FMD) is a measure of the ability of the endothelium lining the brachial artery to respond to a post-ischemic increase in shear stress (Green et al., 2014). Healthy endothelial cells will react and significantly dilate the brachial artery, whereas dysfunctional endothelial cells will respond with a poorer dilatory reaction. This reaction to flow changes is quantifiable via ultrasound imaging of the brachial artery, allowing flow and brachial artery diameter changes to be visualized and analyzed (Thijssen et al., 2019).

Endothelial Nitric Oxide Synthase

Nitric oxide (NO) is a thermodynamically unstable molecule that can rapidly diffuse along the vasculature (Kelm, 1999). It has significant vasodilatory effect on smooth muscle and plays an important role in the maintenance of basal vasodilatory tone. In the endothelium, eNOS synthesizes NO by oxidizing the amino acid L-Arginine into L-citrulline and NO. eNOS can be activated by an agonist such as acetylcholine, as well as by physical stimuli including pulsatile stretch, and shear stress (Fleming & Busse, 2003). Classified as a calcium-calmodulin dependent enzyme, agonist activation of eNOS requires an increase in intracellular calcium concentration, however shear stress activation does not rely on increased calcium and can promote eNOS activity at resting calcium levels (Dimmeler et al., 1999). Transduction of the shear stress force through the endothelial cytoskeleton activates phosphatidylinositol 3-kinase and subsequently protein kinase B. Serine phosphorylation of eNOS by protein kinase B activates the enzyme,

resulting in a high output of NO (Figure 1) (Fisslthaler, Dimmeler, Hermann, Busse, & Fleming, 2000).

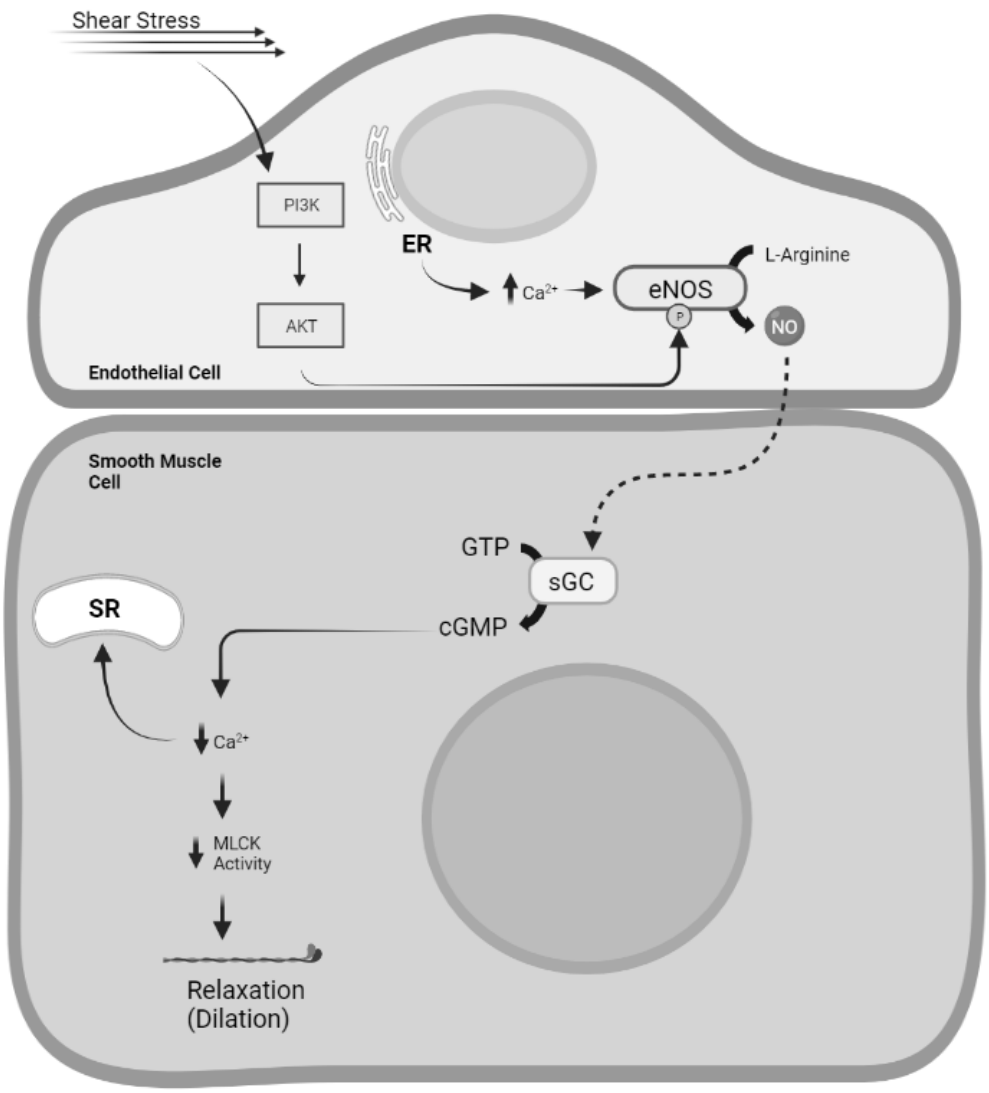

Figure 1: *Signalling pathway in endothelial cells and vascular smooth muscle cells for shear-stress induced vascular smooth muscle relaxation.*

PI3K = Phosphatidylinositol 3-kinase; AKT = Protein Kinase B; ER = Endoplasmic Reticulum; eNOS = Endothelial nitric oxide synthase; NO = Nitric Oxide; sGC = Soluble guanylyl cyclase; GTP = Guanosine triphosphate; cGMP = Cyclic guanosine monophosphate; SR = Sarcoplasmic Reticulum; MLCK = Myosin light chain kinase

Reactive Hyperemia

Shear stress within arterial walls activate endothelial-dependent vasodilator synthesis, causing vascular smooth muscle relaxation. Rapid increases in flow and shear stress can be caused by release from occlusion, such as a blood pressure cuff, exercise, or pharmaceutically induced. This reactive increase in blood flow is a phenomenon known as reactive hyperemia (Crecelius, Richards, Luckasen, Larson, & Dinenno, 2013). Reactive hyperemia acts as the stimulus for endothelial flow mediated dilation, and peak hyperemic velocity has been shown to have an independent association with cardiovascular events (Anderson et al., 2011). The most frequently encountered stimulus for increased shear stress and blood flow in humans is exercise (Tremblay & Pyke, 2018). Exercise-induced vasodilation begins at the activated muscle and can conduct into feed arteries and conducts proximally up the arterial tree. Activation of these feed arteries is integral to full expression of functional hyperemia (Segal, 2000). Exercise induced hyperemia therefore may provide a more functional stimulus, as it is a common physiological occurrence, and may provide functional relevance for exercise perfusion ability (Tremblay & Pyke, 2018). However, ischemic reactive hyperemia caused by occlusion-release and exercise induced hyperemia have been suggested to reflect distinct aspects of endothelial function, and future research including both is required to compare the two stimuli (Tremblay & Pyke, 2018).

Flow Mediated Dilation

Flow mediated dilation (FMD) is a non-invasive ultrasound technique used to quantify the ability of an individual's endothelial cells to change the diameter of the brachial artery in response to a blood flow stimulus (Ras, Streppel, Draijer, & Zock, 2013). While not a widely used test in clinical context, a 1% higher FMD is associated with a relative risk for developing

cardiovascular disease of 0.9 (Rouyanne, Martinette, Richard, & Peter, 2013). To perform an FMD test, ultrasound is used to visualize brachial artery diameter before, during, and following a 5-minute ischemic occlusion of the downstream forearm vasculature via a blood pressure cuff inflated below the elbow to a suprasystolic pressure. Following this occlusion, reperfusion of the downstream microvasculature will increase conduit artery flow velocity and subsequently shear stress (Soares, Somani, Proctor, & Murias, 2019). Dilatory responses to this increased shear stress is endothelial-dependent and is nitric-oxide mediated (Green et al., 2014). Thus, FMD provides a quantifiable measure of endothelial function in human vasculature. The quantification of the artery dilatory response can be achieved with computer-aided continuous diameter tracking for the duration of the protocol, either in real time or offline. Baseline pre-occlusion diameter measurements are compared to post-occlusion dilatory peak diameters to get a quantification of the dilatory response to shear stress reported as absolute dilatory change (in millimeters) as well as percent change (Thijssen et al., 2019). To correct for population variation of baseline diameter, FMD values can be normalized mathematically to the baseline diameter with allometric scaling (Atkinson, Batterham, Thijssen, & Green, 2013), as well as to the causal shear stress stimulus, using the shear stress area under curve (SS_{AUC}) to FMD ratio (Pyke & Tschakovsky, 2007). Representative sample data for 1 participant is available in Figure 2.

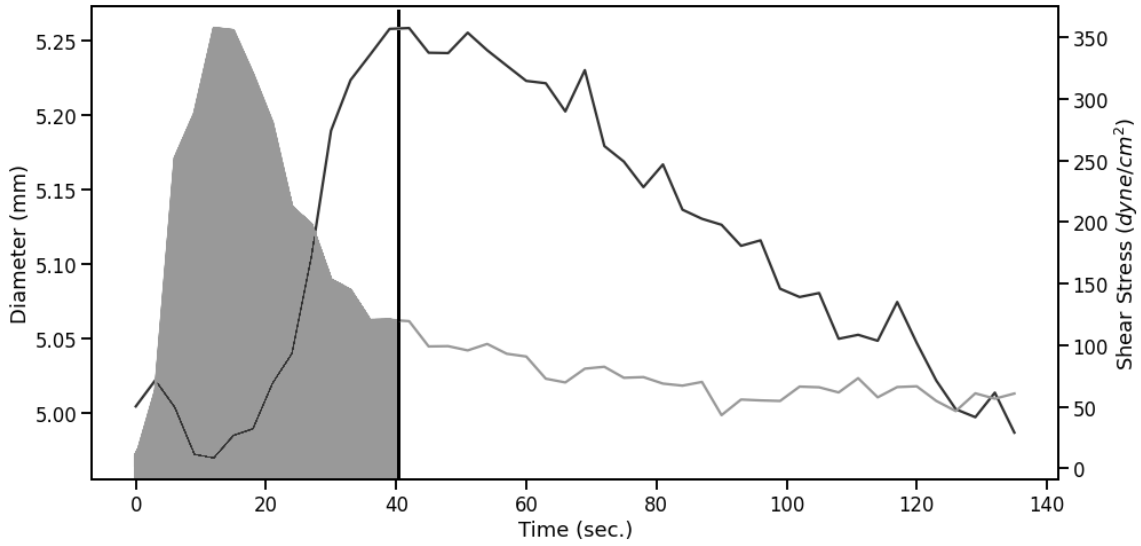

Figure 2: *Flow-mediated dilation and reactive hyperemia shear stress in one participant.* Increased shear stress following 5-min cuff occlusion of the forearm vasculature and changes in brachial artery diameter in one participant. Flow mediated dilation is quantified as the change in arterial diameter from baseline pre-occlusion to peak dilation post-release. Shaded area indicates shear stress area under curve (SS_{AUC}) used for quantification of the shear stress stimulus. Darker line represents diameter, lighter line represents shear stress. Shaded area represents shear stress area under curve, the quantified stimulus for FMD to the point of peak dilation, marked by the vertical black line.

Carbon Monoxide

Carbon monoxide (CO) is an odourless, colourless gas commonly known for its high toxicity in humans (Hess, 2017). Exposure to CO happens regularly for humans through many different avenues including air pollution (Poursafa et al., 2016), fuel combustion (Levels, 2010), and first and second-hand cigarette smoke (Weber, Al-Dissi, Marit, German, & Terletski, 2011). Exposure to gaseous pollutants is known to cause respiratory diseases and is associated with cardiovascular disease (Choi et al., 2018). CO binds to hemoglobin with an affinity 200-250 times stronger than that of oxygen, forming carboxyhemoglobin (COHb). Due to this high affinity, the binding of oxygen to hemoglobin is impaired, resulting in reduced oxygen diffusion and delivery (Stewart, 1975). Endogenous CO is also formed during normal metabolism, resulting in a resting baseline COHb percentage of 0.5-0.8%, however smoking can increase baseline levels to approximately 3-8% (Levels, 2010).

Endogenous Carbon Monoxide

Carbon Monoxide is produced endogenously in the vasculature by an enzyme called Heme-oxygenase 1 (HO-1). Heme molecules that are bound to HO-1 are subsequently bound to oxygen and NADPH, with resultant products of FeII, Biliverdin – IXα, and carbon monoxide (Ryter, Alam, & Choi, 2006). Endogenously generated CO has important intracellular effects as an anti-inflammatory, antiproliferative, and anti-apoptotic molecule through various mechanisms including activation sGC, and binding to intracellular heme proteins. Similar to NO, CO can bind to the heme group of sGC, elevating cyclic guanine monophosphate (cGMP) and subsequent vasodilation (Kim et al., 2011). CO has also been shown to have cGMP – independent

vasodilatory ability in rat tail arteries via calcium-activated K channels. This animal model is often used as a model of peripheral resistance vasculature (Zhao & Wang, 2001).

Exogenous Carbon Monoxide and the Endothelium

Smoke exposure has been shown to have a significant detrimental effect on endothelial function in humans (Poursafa et al., 2016; Weber et al., 2011). Previous flow mediated dilation studies suggested that decline in endothelial function associated with smoke exposure was correlated with CO levels in exposure chambers but not COHb, suggesting more research was required to determine the effect of CO specifically on endothelial function (Weber et al., 2011). Recently, Rezk-Hanna et al. have compared endothelial function in acute carbon monoxide exposure to hookah smoke in a smoking population, and found that 1000ppm carbon monoxide inhalation exposure acutely increased flow mediated dilation and masks detrimental effects of other aspects of hookah smoke (Rezk-Hanna et al., 2019). Microvascular dilation has also been observed following 250 ppm CO inhalation for 25 min (McRae et al., 2019), potentially impacting forearm vascular conductance and post-ischemic shear stress. These human studies combined with previous *in vitro* and animal studies suggest that CO can improve endothelial function and is not responsible for endothelial dysfunction associated with air pollution or tobacco smoke exposure (Rezk-Hanna et al., 2019; Zhao & Wang, 2001). Looking more closely at the mechanistic effect of CO on endothelial function, application of exogenous CO solution has been shown to increase release of intracellular heme bound NO in endothelial cells, and inhibition of eNOS completely abolished vasodilatory properties of exogenous CO application, suggesting CO-mediated vasodilation is at least in part endothelium-dependent (Hangai-Hoger, Tsai, Cabrales, Suematsu, & Intaglietta, 2007; Thorup et al., 1999). Binding of CO to sGC in smooth muscle (Kim et al., 2011), and release of heme-bound NO (Thorup et al., 1999) may

provide insight into potential pathways of increased endothelial-dependent vasodilation in acute carbon monoxide exposure.

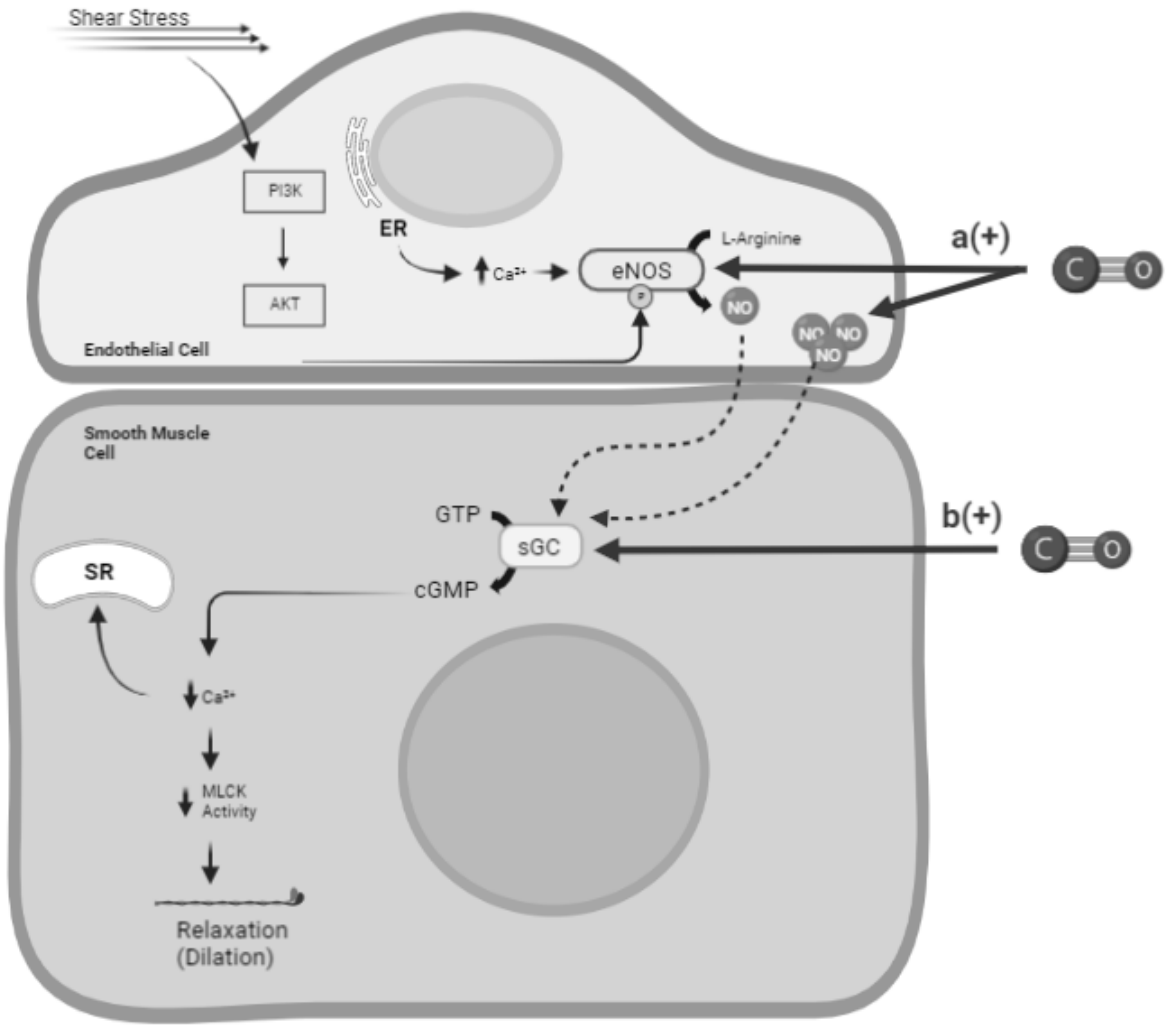

Figure 3: *Potential mechanisms of action of Carbon Monoxide in Endothelial and Vascular Smooth Muscle Cells*

Potential sites of interaction of endogenous and exogenous CO in endothelial and vascular smooth muscle cells. a(+) indicates potential endothelial-dependent vasodilatory interaction via eNOS upregulation, or via release of intracellular heme-bound NO (Hangai-Hoger et al., 2007; Thorup et al., 1999). b(+) indicates potential vascular smooth muscle interaction of CO via binding to sGC, increasing vasodilation.

Current literature poses carbon monoxide as a potent vasodilatory signalling molecule in endothelial and vascular smooth muscle cells. Inhibition of eNOS, a primary source of endothelial nitric oxide, has been shown to abolish vasodilatory effects of CO in animal models (Hangai-Hoger et al., 2007). FMD, which is endothelial-dependent and nitric-oxide mediated has also been shown to increase following exogenous inhaled carbon monoxide exposure in a smoking population (Rezk-Hanna et al., 2019). Studies showing increased microvascular dilation of forearm vasculature in humans following CO exposure (McRae et al., 2019) suggests a potential increase in the forearm vascular conductance, as well as post-ischemic shear stress. Further studies are required to understand the pathways and mechanisms of action of inhaled CO on endothelial function in healthy individuals.

Aims

This study aims to observe the effect of mild, acute CO exposure on resting conduit artery flow, conductance and flow mediated dilation. Observation of FMD will allow for further understanding of how CO affects endothelial-dependent vasodilation.

Hypothesis

In this study, I hypothesized FMD would increase following inhalation of CO, as it has been shown to increase endothelial-dependent vasodilation as well as interact with endothelial nitric oxide pathways in different populations, as well as in animal models (Rezk-Hanna et al., 2019; Wang et al., 1997).

Methods

Ethical Approval

This trial was under supervision of the University of Alberta Research Ethics Office (Pro00096251) and Health Canada (NOL 241154). This trial is in full adherence with the principles of the Declaration of Helsinki, including registration in a public database (NCT04928183). Each subject completed the informed consent form and health history questionnaire approved by the Human Research Ethics Board at the University of Alberta.

Study Design

A randomized, single-blinded crossover design was used in this trial, with participants tested on 2 consecutive trial days; one day with CO rebreathing procedure (Schmidt & Prommer, 2005), and one with the same procedure with a room air (sham) rebreathe. Exposure day was randomized using an online randomization service (Geoffrey C. Urbaniak, 2013). Testing time of day for participants was consistent on consecutive days to reduce diurnal variation. Participants were instructed to avoid strenuous exercise, alcohol, and caffeine 12 hrs prior to their visit, and to avoid a large meal within 2 hrs of their visit. All participants completed the full study protocol.

Participants

A total of 19 participants were recruited for this study (10 male, 9 female). A convenience sample of healthy volunteer students and alumni of the University of Alberta in an age range of 20-31 was used. Participants were excluded if they had any existing cardiovascular disease, respiratory disease, nervous system disorders, metabolic disease, were pregnant, post menopausal, obese, daily smokers, or taking any monoamine inhibitor or tricyclic antidepressant

medications. Female participants were not excluded due to menstrual cycle stage, or for active contraceptive use. Participants served as their own controls. Descriptive statistics for participant demographics are available in Table 1.

Table 1: *Participant Characteristics*

Variable	**Mean ± SD or n(%)**
Sex (n=19)	
Male	9 (47.4)
Female	10 (52.6)
Age	25 ± 3
Height (m)	1.73 ± 0.08
Weight (kg)	69.4 ± 11.8
BMI (kg/m^2)	23.1 ± 2.8

Participant Characteristics. Values are stated as means ± standard deviation or n(%)

Protocol

An intravenous catheter was placed in an antecubital vein to allow for blood sampling. Participants were instrumented with a non-invasive blood pressure monitor (NIBP, ADInstruments), and continuous 3-lead ECG (ADInstruments). A 10-minute baseline period was completed in a supine position. Heparinized blood samples were taken for blood viscosity (DVNext Rheometer, Brookfield Ametek) and baseline COHb (ABL80 FLEX, Radiometer) analyses.

For FMD measurements, a sphygmomanometric cuff was placed around the forearm and inflated to a supra-systolic pressure (200 mmHg) to occlude forearm blood flow for 5 min. After this time period, the cuff was rapidly deflated (~1 s) according to best recommendations (Thijssen et al., 2019) with continuous ultrasound recording (12MHz linear array probe, GE Vivid 7; DVI2USB, Epiphan Systems) at an insonation angle of 60° of the brachial artery. Recording consisted of a 3-minute baseline, 5-minute forearm occlusion, and 3-minutes of post-occlusion imaging. Baseline brachial intensity weighted mean velocity (qDAT, Penn State) and diameter (Brachial Analyzer, Medical Imaging Applications) were analyzed for 1 minute before cuff inflation, and for 3.5 min beginning 30 s prior to cuff release. Following the first FMD, participants completed the optimized CO-rebreathe protocol (Schmidt & Prommer, 2005). This procedure included a single bolus inhalation of CO or sham air at a dose of 1 mL/kg body weight mixed with oxygen in a rebreather apparatus containing a carbon-dioxide scrub. The first breath was held for 10 seconds, followed by a 1 min 50 second rebreathe period. Participants were then instructed to visit the washroom to avoid prolonged supine lying (Soto-Rodríguez, Cabañas, & Pérez-Mármol, 2022). After re-instrumentation and a 5 min rest period, a second blood sample

was collected for COHb measurements, and another FMD was performed. Mean time between FMD recordings was 82±17 minutes for CO day, and 73±12 minutes for the sham day (p=0.2)

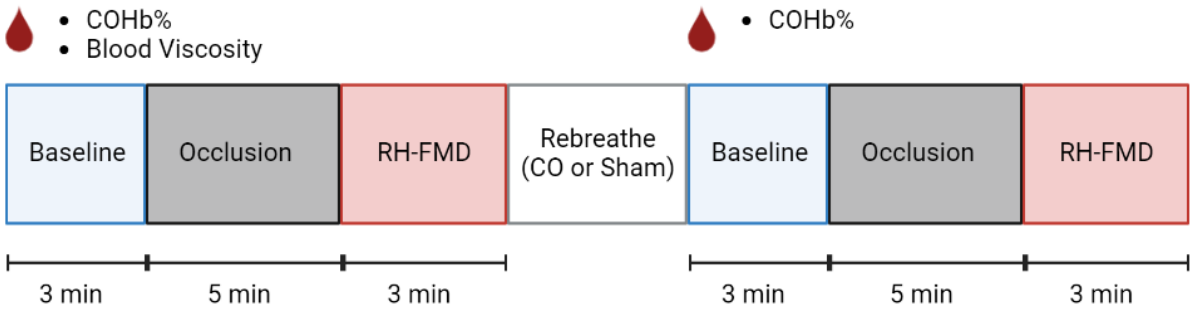

Figure 4: *Protocol Diagram*

Experiment timeline for 1 day of testing. Blood samples were collected prior to the beginning of the standardized FMD protocol. Ultrasound recordings spanned the entirety of the FMD, including baseline, occlusion, and during the RH-FMD. RH-FMD represents the post-occlusion phase of reactive hyperemia where peak brachial artery diameter measurements were recorded. The rebreathe procedure was completed with either sham or room air, and a second FMD was performed

Figure 5: *Sample FMD Set-Up*

Sample FMD setup of participant arm. All measurements were completed on the right arm with a 12mHz linear array probe. Distal cuff was inflated to 200mmHg for all participants for 5 minutes for the occlusion period.

Analysis

Blood Viscosity

Blood viscosity measurements were completed in a 5-step protocol, with viscosity measured at shear rates of 150, 187.5, 225, 262.5, and 300 (s^{-1}) at 36°C for 1 minute each using a cone-plate rheometer (DVNext Rheometer, Brookfield Ametek). Raw values at 225 s^{-1} are reported in centipont (cP) were used for the determination of shear stress (Tremblay et al., 2019). Hematocrit values were also collected using the ABL80 Blood Gas analyzer (ABL80 FLEX, Radiometer).

Vascular Flow and Conductance

Baseline and peak arterial flow (Q_A) were calculated as the product of blood flow velocity (V_A) and arterial cross-sectional area. Forearm vascular conductance (FVC) was calculated for as Q/Mean arterial Pressure (MAP) and was used as a measure of changes to downstream microvasculature.

Brachial Diameter and Flow Mediated Dilation

Ultrasound recordings were recorded in video format for later offline diameter analysis (OBS Studio, Open Broadcaster Software). All brachial artery diameter (D_A) analysis was measured at a resolution of 0.01mm by an offline, blinded observer at 30 Hz. Baseline arterial diameter was calculated as the mean arterial diameter for 1 minute of pre-occlusion. Peak arterial diameter was calculated as peak 3-second mean arterial diameter post-occlusion.

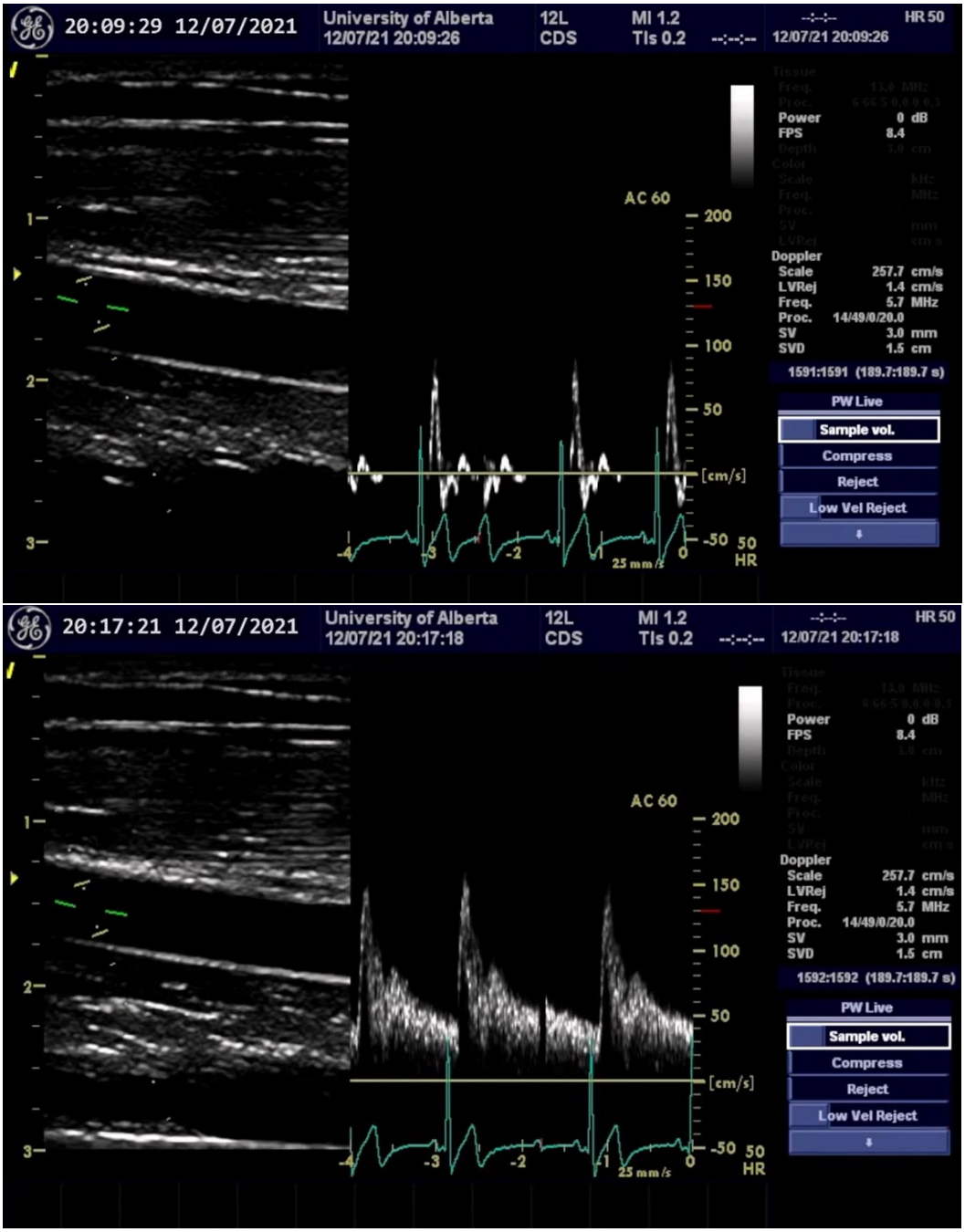

Figure 6: *Sample ultrasound images*

Sample ultrasound images from baseline phase (top) and following occlusion release (bottom).

Images were recorded at 30hz for offline analysis of diameter.

Flow mediated dilation was calculated as the change in arterial diameter from baseline to peak, as well as FMD% ($PeakD_A/BaselineD_A$ *100). Normalization of FMD to shear stress stimulus to peak dilation (SS_{AUC}) was calculated using the FMD:SS_{AUC} ratio, as well as to FMD% (FMD%:SS_{AUC}).

Vascular Shear Rate and Stress

Vascular shear rate was calculated as $Shear\ Rate = 8Q_A/D_A$ (Thijssen et al., 2019). Vascular shear stress was calculated as the product of shear rate, and blood viscosity at $225s^{-1}$ (Tremblay et al., 2019). Shear quantification of area under curve to peak diameter was used to normalize the FMD response to the causal shear stress stimulus (Pyke & Tschakovsky, 2007).

Statistics

Statistical tests were performed using SigmaPlot 14.0 (Systat Software). All participants acted as their own controls, and any differences in values pre/post rebreathe were identified using a two-way repeated measures ANOVA, with interaction of timepoint (pre vs. post rebreathe) and treatment (CO vs sham exposure) significance used to identify CO-rebreathe dependent changes. Post-hoc Pearson correlation analyses were performed to identify any relationships between COHb% and FMD. Significant differences were further assessed using Tukey's post hoc tests. Sex differences were identified using a one-way ANCOVA with sex as the covariate. Statistical significance was defined as p-value <0.05 for all statistical tests.

Results:

Baseline Hemodynamics

Baseline hemodynamic values for the sample can be found in Table 2. Timepoint p-values correspond to pre- vs post- measurements, Treatment p-values correspond to the CO vs sham air day exposure.

Heart Rate and Blood Pressure

No significant change in heart rate was observed (timepoint p=0.791, treatment p=0.761, interaction p=0.467). Resting mean arterial pressure (timepoint p=0.186, treatment p=0.187, interaction p=0.099) and diastolic blood pressure (timepoint p=0.905, treatment p=0.729, interaction p=0.270) also did not change between conditions. Resting systolic blood pressure was significantly lower pre sham rebreathe (timepoint p=0.011, treatment p=0.10, interaction p=0.046) than in the other conditions.

Baseline Arterial Diameter, Flow and Conductance

Baseline brachial artery diameter (timepoint p=0.567, treatment p=0.344, interaction p=0.483) and flow (timepoint p=0.084, treatment p=0.932, interaction p=0.089) were not significantly different between conditions. Baseline forearm vascular conductance was also not significantly different between conditions (timepoint p=0.094, treatment p=0.865, interaction p=0.078)

Carboxyhemoglobin

Carboxyhemoglobin was significantly higher in the post-CO rebreathe condition (timepoint $p<0.001$, treatment $p<0.001$, interaction $p<0.001$) compared to baseline and post sham rebreathe.

Blood Viscosity

Blood viscosity was not found to be significantly different between testing days (treatment $p=0.773$) and hematocrit did not change between conditions (timepoint $p=0.243$, treatment $p=0.296$, interaction $p=0.213$).

Table 2: *Baseline Hemodynamics*

	Sham		CO		p-value		
Variable	*Pre*	*Post*	*Pre*	*Post*	*Timepoint*	*Treatment*	*Interaction*
Heart Rate	57.20 ± 8.42	56.62 ± 9.31	56.55 ± 9.52	56.79 ± 9.99	0.791	0.761	0.467
Mean Arterial Pressure (mmHg)	80.41 ± 7.40	82.43 ± 7.30	82.92 ± 8.43	82.46 ± 8.66	0.186	0.187	0.099
Systolic Blood Pressure (mmHg)	108.43 ± 9.08	112.23 ± 9.88	113.18 ± 10.39	113.85 ± 10.63	**0.011**	**0.010**	**0.046**
Diastoilc Blood Pressure (mmHg)	66.40 ± 7.23	67.54 ± 7.50	67.80 ± 8.62	66.76 ± 8.94	0.905	0.729	0.270
Baseline Flow (ml/min)	39.20 ± 25.16	34.48 ± 36.90	48.85 ± 37.46	31.73 ± 19.29	0.084	0.932	0.089
Baseline Diameter (mm)	3.84 ± 0.75	3.79 ± 0.76	3.77 ± 0.73	3.77 ± 0.81	0.567	0.344	0.483
Baseline FVC	0.50 ± 0.32	0.47 ± 0.52	0.63 ± 0.52	0.38 ± 0.24	0.094	0.865	0.078
COHb(%)	1.50 ± 0.19	1.46 ± 0.28	1.43 ± 0.23	5.64 ± 1.24	**<0.001**	**<0.001**	**<0.001**
Hematocrit	41.54 ± 3.67	41.54 ± 3.67	41.27 ± 4.01	40.82 ± 3.85	0.243	0.296	0.213
Viscosity (cP)	3.79 ± 0.36	-	3.81 ± 0.40	-	-	0.773	-

Baseline hemodynamic results stated as means ± standard deviation. Baseline values were collected before cuff occlusion phase of the FMD protocol. Timepoint refers to the p-value of Pre vs Post rebreathe protocol. Treatment refers to the exposure of CO vs sham air.

Flow Mediated Dilation

Summary FMD Data is available in Table 3. Absolute FMD was not significantly different between treatment, time-point or interaction (timepoint p=0.191, treatment p=0.827, interaction p=0.690). FMD% was also not significantly different between conditions (timepoint p=0.319, treatment p=0.754, interaction p=0.840). Individual participant FMD% values for each condition can be viewed in Figure 7. FMD:ssAUC (timepoint p=0.345, treatment p=0.577, interaction p=0.200) and FMD%:ssAUC (timepoint p=0.382, treatment p=0.464, interaction p=0.227) were also not significantly different conditions (Figure 8). Regression analysis of COHb% and FMD% indicated no significant relationship between these variables (p=0.493) (Figure 9).

Additional Brachial Artery Measurements

Peak brachial artery diameter was not significantly different between conditions (timepoint p=0.064, treatment p=0.158, interaction p=0.415). Peak shear stress (timepoint p=0.398, treatment p=0.116, interaction p=0.372), shear stress area under curve (timepoint p=0.058, treatment p=0.957, interaction p=0.878), and change in forearm vascular conductance (timepoint p=0.374, treatment p=0.166, interaction p=0.287) were not significantly different between conditions.

Table 3: *Reactive Hyperemia Results*

	Sham		CO		p-value		
Variable	*Pre*	*Post*	*Pre*	*Post*	*Timepoint*	*Treatment*	*Interaction*
FMD (mm)	0.31 ± 0.24	0.23 ± 0.20	0.28 ± 17	0.236 ± 0.22	0.191	0.827	0.690
FMD%	8.12 ± 5.36	6.69 ± 6.36	7.47 ± 4.34	6.46 ± 5.891	0.319	0.754	0.840
FMD:ssAUC	3.53e-5 ± 3.87e-5	2.61e-5 ± 1.94e-5	2.70e-5 ± 2.14e-5	2.82e-5 ± 3.55e-5	0.345	0.577	0.200
FMD%:ssAUC	8.90e-4 ± 8.51e-4	7.50e-4 ± 6.40e-4	7.06e-4 ± 5.50e-4	7.36e-4 ± 9.03e-4	0.382	0.464	0.227
Peak Diameter (mm)	4.15 ± 0.84	4.03 ± 0.72	4.05 ± 0.79	4.006 ± 0.849	0.064	0.158	0.415
Peak Shear Stress ($dyne/cm^2$)	340.54 ± 165.63	342.39 ± 86.24	435.18 ± 205.14	340.54 ± 80.22	0.398	0.116	0.372
ssAUC	12082.79 ± 4271.53	9366.23 ± 3283.95	11694.16 ± 5245.46	9381.81 ± 2976.68	0.957	0.058	0.878
Δ FVC	3.64 ± 1.89	3.12 ± 1.41	4.05 ± 1.80	3.813 ± 1.87	0.374	0.166	0.287

Reactive hyperemia results stated as means ± standard deviation. Values were collected following 5- minute cuff occlusion phase of the FMD protocol. Timepoint refers to the p-value of Pre vs Post rebreathe protocol. Treatment refers to the exposure of CO vs sham air

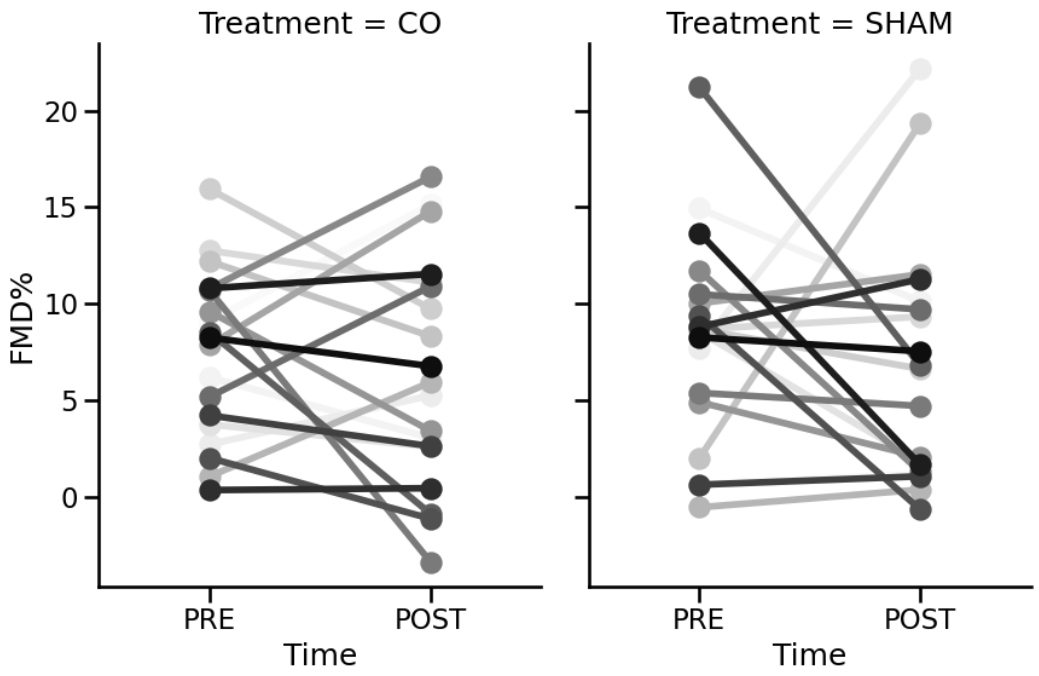

Figure 7: *Individual FMD% values across conditions.*

Flow-mediated dilation values for individual participants in each condition. Treatment indicates the exposure received during the rebreathe protocol (CO or sham) and Time indicates if the measurement was recorded before or after the rebreathe protocol. Timepoint p=0.319, treatment p=0.754, interaction p=0.840

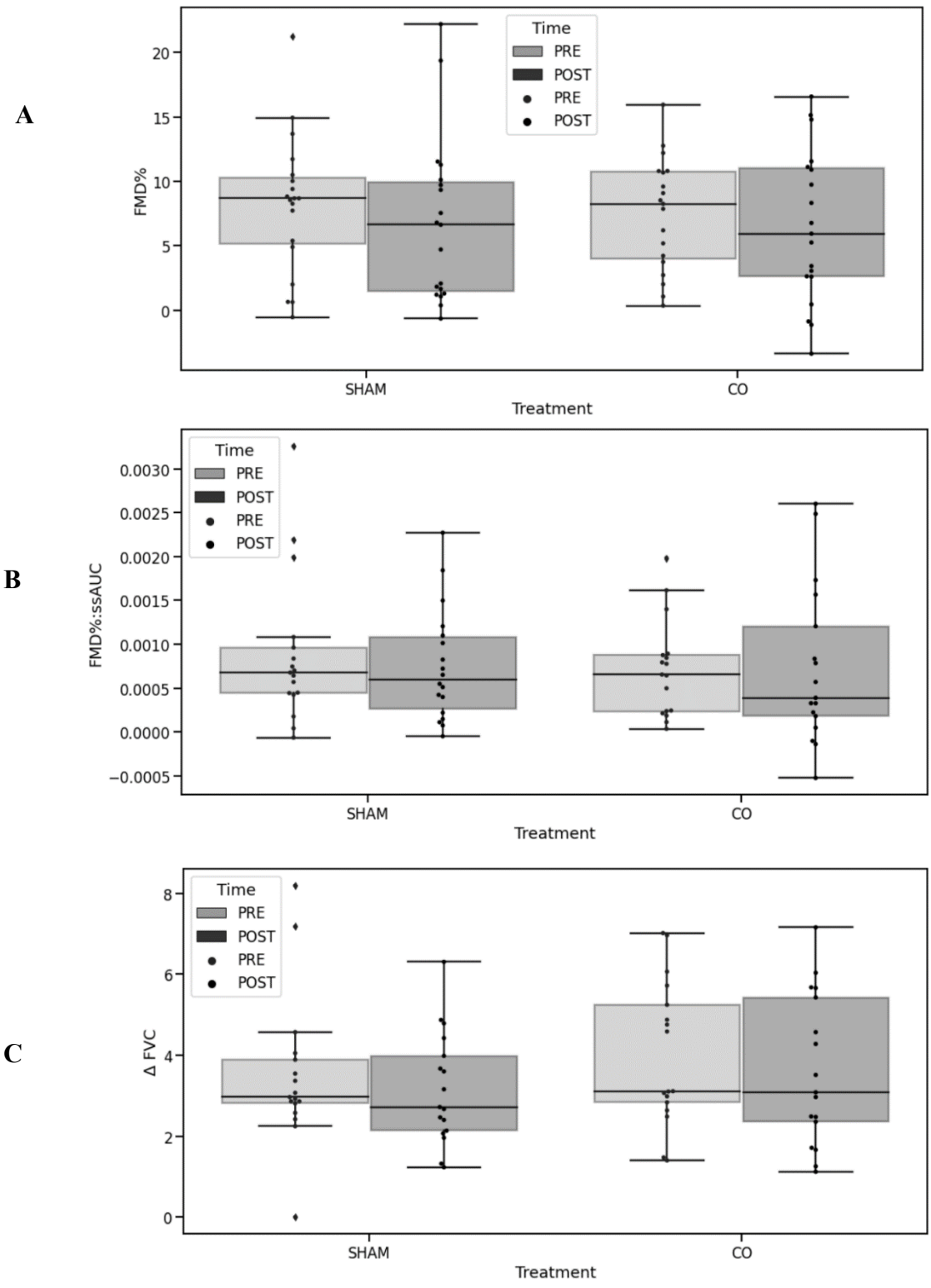

Figure 8: *Boxplots of reactive hyperemia responses across conditions*

Boxplot distribution of A) flow-mediated dilation (%), B) shear area-under-curve corrected FMD%, and C) change in forearm vascular function across rebreathe conditions. Treatment

indicates the exposure received during the rebreathe protocol (CO or sham) and Time indicates if the measurement was recorded before or after the rebreathe protocol. No significance was identified in timepoint, treatment, or interaction for these variables.

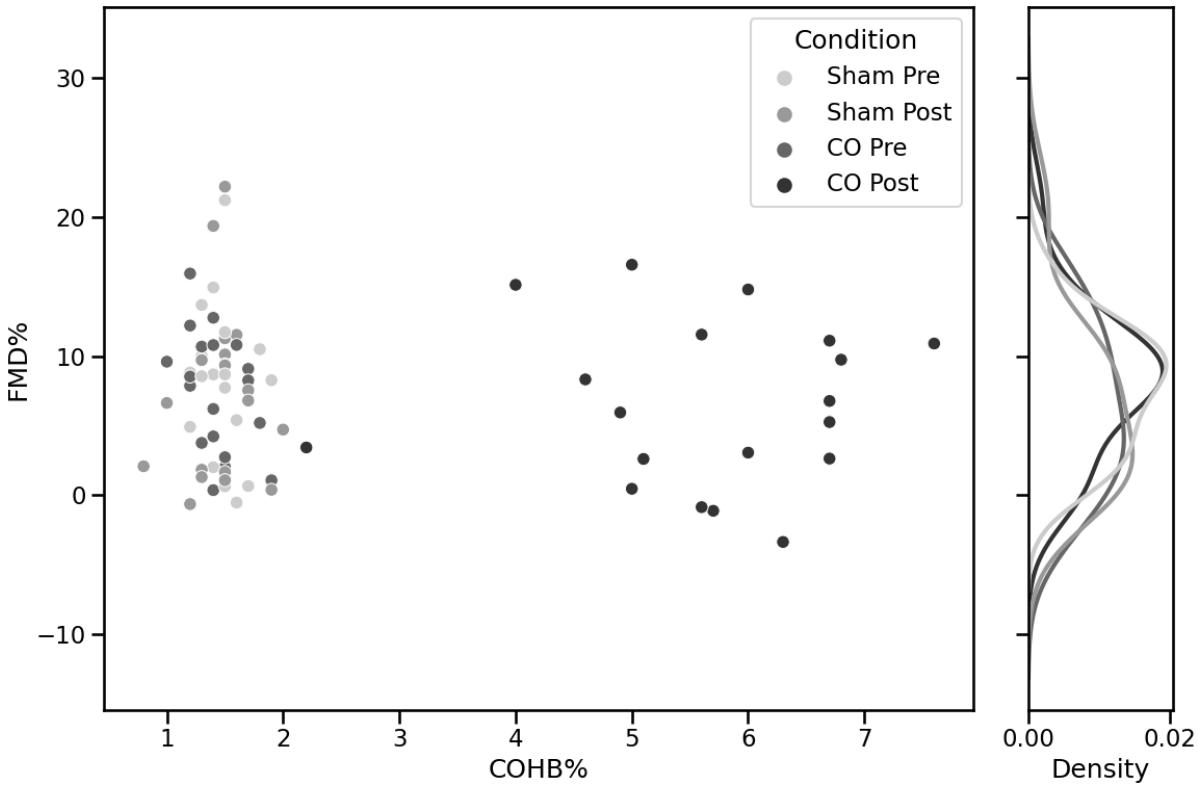

Figure 9: *Flow-mediated dilation and carboxyhemoglobin*

Left: Scatterplot of flow-mediated dilation (%) and carboxyhemoglobin (%) for all participants. Condition represents the different time points (pre or post rebreathe protocol) and exposure (CO or sham air). Right: Density is representative of kernel density estimate distribution of FMD% values for each condition. No relationship between FMD% and COHb% was observed (p=0.493)

Discussion

This trial examined the effect of a single bolus inhalation and 2-minute rebreathe of carbon monoxide on brachial artery endothelial function. Despite an acute increase in COHb%, no change was observed in baseline hemodynamics, vascular flow, or reactive hyperemic responses.

Baseline hemodynamics and flow

Baseline hemodynamic values including resting heart rate, mean arterial pressure, diastolic blood pressures, and resting brachial artery diameter were not significant following CO or sham rebreathe. However, systolic blood pressure was increased following both sham and CO rebreathes, as well as being higher on CO days. Interaction of timepoint and condition were also found to be statistically significant for systolic blood pressure. Although systolic blood pressure was increased, mean arterial pressure and diastolic blood pressure were unchanged. Mean arterial pressure was used for the calculation of vascular conductance, so differences in systolic pressure had only minor cardiovascular influence. Changes in resting heart rate and blood pressure might be expected if changes in systemic resistance is experienced, as resistance will directly impact blood pressure, which can modulate heart rate via baroreceptor reflex (Laude et al., 2004). Systemic resistance in turn would be expected to decrease if the application of exogenous CO resulted in VSMC relaxation or microvascular dilation. Previous studies have shown decreases in diastolic blood pressure following low-dose, 45 minute exposure to CO (Lee et al., 2017). CO has the potential to interact with the cGMP pathway, the target for NO released from endothelial cells. Endogenous carbon monoxide remains an important vasodilatory molecule within a functional VSMC cGMP cascade (Wang et al., 1997), and has been shown in animal models to act as a potent vasodilator in VSMC independent of endothelial cells (Zhao & Wang, 2001).

Baseline brachial artery flow and vascular conductance also showed no change following either the CO or sham rebreathe. Changes to baseline arterial flow and vascular conductance would be expected if changes to downstream microvascular dilation were experienced following CO rebreathe. These results further indicate the applied rebreathe method did not interact with the cGMP cascade within VSMCs in both peripheral conduit arteries and downstream microvasculature. The ability for CO to act within the VSMC cGMP pathway (Wang et al., 1997) would result in vasodilation of downstream microvasculature, and subsequently increase brachial arterial flow and vascular conductance at rest. In previous studies, low dose CO inhalation over 25 minutes has been shown to increase microvascular vasorelaxation (McRae et al., 2019) in non-smoking, non-pregnant women. Since COHb% was elevated in all participants, circulating carboxyhemoglobin does not appear to impact the cGMP pathway in peripheral conduit arteries or downstream microvasculature at rest.

Reactive Hyperemia and Endothelial Function

Brachial artery endothelial function measured using FMD was not different following the CO rebreathe protocol. Vasodilatory pathways of CO have been shown to be endothelial-dependent and nitric-oxide mediated, and improvements in FMD following CO exposure have been observed before in young adult hookah smokers (Rezk-Hanna et al., 2019), suggesting interaction of exogenous CO inhalation and endothelial function. Inhibition of eNOS in endothelial cells has been shown to abolish vasodilatory effects of exogenous CO, suggesting eNOS activity is vital to the vasodilatory effect of CO (Hangai-Hoger et al., 2007). Application of low dose CO to isolated renal resistance arteries induced release of NO from intracellular storage, which would in turn increase bioavailable NO, an important vasodilatory molecule released from endothelial cells responsible for FMD responses (Green et al., 2014). Increased

NO release from endothelial cells *in vivo* would result in increased NO bioavailability in VSMCs, increasing vasodilation via the cGMP pathway. Improvements in endothelial function would be suggestive of increased NO release from endothelial cells, specifically via eNOS activation as a result of shear stress stimulus following cuff occlusion, resulting in increased endothelial-dependent vasodilation, which would be indicated by increased FMD values. FMD, FMD% or shear-corrected FMD values values were not significantly different following CO rebreathe, indicating there was no impact of this CO exposure on endothelial-dependent vasodilatory eNOS or intracellular heme-bound NO pathways. This could be a result of the delivery method and duration of CO exposure in this trial.

Endogenous vs. Exogenous Carbon Monoxide and Carbon Monoxide diffusion

Endogenous CO produced within VSMC is a potent vasodilatory molecule and is important in a properly functioning vasculature, and excess endogenous CO is removed via hemoglobin binding due to the high binding affinity of CO and hemoglobin (Mao et al., 2021). This high binding affinity is also suggested to be protective in the presence of exogenous CO, as the binding affinity for CO and hemoglobin is higher than that of CO and intracellular storage components, shuttling excess CO away from tissue and to the lungs where it can be expelled (Coburn, 1970; Mao et al., 2021). This CO shuttling is protective of tissue, as increased COHb itself is not toxic (Goldbaum, Orellano, & Dergal, 1976). As the rebreathe protocol utilized in the study was very short (2 min), high binding affinity of COHb could prevent tissue CO levels to increase to a clinically significant level. Observed increases in COHb with no change to endothelial function or reactive hyperemia indicates carboxyhemoglobin concentration may not directly influence reactive hyperemia shear stress, or endothelial-mediated vasodilation.

Considerations

Strengths of this study include a single-blinded crossover design, allowing participants to serve as their own controls, as well as a sex-balanced sample. Inclusion of blood viscosity measurement allows for the shear stimulus to be more accurately calculated and is more representative of shear stimulus experienced on a participant-to-participant basis. Some limitations to the present study have also been identified. Endothelial-independent vasodilatory capacity via sodium nitroprusside administration or oral GTN tablets was not collected due to supply shortages during the COVID-19 pandemic. This would have allowed to differentially observe endothelial-dependent and endothelial-independent vasodilation following CO exposure, which would have given more information on the interaction of vasodilatory pathways (endothelium-dependent vs. independent) and CO. Meals for participants were not controlled, and a fasting period of 2 hours is shorter than the recommended 6 hours (Thijssen et al., 2019), and meal composition was not controlled, potentially impacting FMD results (Fewkes, Kellow, Cowan, Williamson, & Dordevic, 2022).

Implications

This study explored the effect of mild, acute CO exposure on endothelium-dependent vasodilation. The exposure utilised in the protocol is a common technique in research for the measurement of blood volume (Schmidt & Prommer, 2005). This data suggests this method of blood volume measurement does not impact resting hemodynamics, vasodilation, or endothelial function in a non-smoking population. Other sources of CO (pollution, smoke, second hand cigarette smoke, etc.) will likely have a similar lack of impact on vascular function as long as exposure concentrations and timing is not different. Healthy, non-smoking populations are seemingly well protected against mild, acute CO exposure by the high binding affinity of COHb.

With CO emerging as a potential anti-inflammatory therapeutic target, delivery of CO to target tissue remains an important and complicated discussion (Hess, 2017).

Future Considerations

Future research considerations should include a more direct physiological measure of CO in endothelial and VSMC tissue. Although difficult and invasive for human studies, application of either gas chromatography or the emerging hemoCD-1 techniques (Mao et al., 2021) to identify tissue levels of CO would provide valuable insight to the physiological exposure to CO in the target tissue, as well as the relationship between CO exposure timing and tissue CO levels. With CO emerging as a potential anti-inflammatory therapeutic target (Rochette, Cottin, Zeller, & Vergely, 2013), it is important to understand the delivery mechanics of CO in inhalation to control dosages to targeted tissue.

Increasing exposure time and CO concentrations would result in higher COHb and allow for a longer diffusion period for CO into tissue at a higher CO tension. This in turn would allow for increased effect of CO on intracellular function, potentially increasing the effect of CO on endothelial-dependent vasodilatory pathways.

Carboxyhemoglobin remains an important measurement in the quantification of environmental CO exposure but does not indicate physiological levels of CO within tissue and does not impact brachial artery endothelial function. Further studies exploring the delivery of carbon monoxide to vascular endothelial cells would be valuable in the exploration of the impact of CO on endothelial function.

Summary

No significant changes to baseline hemodynamic, arterial flow, or reactive hyperemia responses following either CO or sham rebreathe were observed. These results suggest carboxyhemoglobin does not have a direct impact on vascular function at rest or in response to a shear stress stimulus. While previous studies have shown significant changes in vascular function in response to CO exposure (Lee et al., 2017; McRae et al., 2019; Rezk-Hanna et al., 2019) the present study shows COHb% may not be a direct indication of the physiological effect of CO. CO toxicity has also been shown to be independent of COHb% (Goldbaum et al., 1976), and may not be appropriate for quantifying the physiological effect of CO inhalation (Mao et al., 2021). Observing tissue CO concentration would provide a more complete picture of CO exposure within the target tissue, and a more appropriate quantification for observing the effect of CO on physiological function. The delivery method used in this trial is a standardized technique developed by Schmidt & Prommer for the assessment of blood volume (Schmidt & Prommer, 2005). This method effectively delivers carbon monoxide quickly into the blood, indicated by the increased COHb% following CO rebreathe. However the ability of exogenous CO to penetrate and stay within tissue following a single bolus inhalation may be compromised due to minimal CO leakage into the extravascular space (Schmidt & Prommer, 2005). Additionally, maintaining any CO in tissue may be prevented due to the scavenging ability of hemoglobin (Mao et al., 2021). The rebreathe protocol used for this trial may not have effectively distributed CO to the target tissue. Exposure time of CO in the rebreathe method used was lower compared to previous human trials observing FMD (Rezk-Hanna et al., 2019), and inhalation parameters may affect carbon monoxide uptake in tissue (Mao et al., 2021).

Conclusion

This study aimed to observe the effects of mild, acute carbon monoxide exposure on endothelial-dependent vasodilation in 19 young, healthy participants. Baseline hemodynamic and vascular parameters were also recorded before and after the rebreathe protocol. No changes were observed in baseline hemodynamics, vascular parameters, or endothelial-dependent vasodilation. The Schmidt & Prommer rebreathe method for the measurement of blood volume effectively increased COHb%, however the ability for CO to diffuse and remain within tissue may be compromised due to the high affinity of CO and hemoglobin. Future studies are required to better understand the release of hemoglobin-bound carbon monoxide to tissue and the effect of exposure length on tissue CO levels. This work suggests carboxyhemoglobin does not impact baseline hemodynamics, resting vascular parameters, or endothelial-dependent vasodilation.

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APPENDIX

Approval Form

Date:	February 05, 2021
Principal Investigator:	Craig Steinback
Study ID:	Pro00096251
Study Title:	The Impact of Carbon Monoxide and Altitude on Vascular Function
Protocol Number:	N/A
Approval Expiry Date:	February 4, 2022

Date of Informed Consent:	Approval Date	Approved Document
	2/5/2021	Consent Form (lowlander version)
	2/5/2021	Consent Form (highlander version)
	2/5/2021	Optional Biobanking Consent Form
Funding/Sponsor:	NSERC - Natural Sciences And Engineering Research Council	

Thank you for submitting the above study to the Health Research Ethics Board - Biomedical Panel, which was reviewed by special sub-committee in March 2020. All issues arising from the reviews have been addressed. The study is now approved. The following documentation forms part of this approval:

- Protocol Version 4 (07 Jan 2021)
- Product Monograph for Sodium Nitroprusside (25 Jan 2011)
- Phenylephrine Label (Revised 11/2019)
- Consent Form_lowlander Version 4 (dated 04 Feb 2020, revisions uploaded 04 Feb 2021)
- Optional Biobanking Consent Form Version 3 (-2 Jul 2019)
- Consent Form_highlander Version 4 (dated 04 Feb 2020, revisions uploaded 04 Feb 2021)
- Epworth_Sleepiness_Scale
- Medical screening form
- lake louise and ESQ

We acknowledge receipt of the Health Canada No Objection Letter re: Protocol # PRO00096251 Revision 3 (25 Aug 2020)

Any proposed changes to the study must be submitted to the REB for approval prior to implementation. A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date (February 4, 2022), you will have to re-submit an ethics application.

The membership of the Health Research Ethics Board - Biomedical Panel complies with the membership requirements for research ethics boards as defined in Division 5 of the Food and Drug Regulations and the Tri Council Policy Statement. The HREB - Biomedical Panel carries out its functions in a manner consistent with Good Clinical Practices.

Inquiries regarding administrative approval, and operational approval for areas impacted by the research should be directed to the Alberta Health Services Research Administration office (Edmonton Zone) at nactrc.contracts@albertahealthservices.ca or Covenant Health Research Administration (research@covenanthealth.ca) as applicable.

Approval by the Research Ethics Board does not encompass authorization to recruit and/or interact with human participants at this time. Researchers still require operational approval as applicable (eg AHS, Covenant Health,

Therapeutic Products Directorate
5th Floor, Holland Cross, Tower B
Address Locator # 3105A
OTTAWA, Ontario
K1A 0K9

25 August 2020

Dr. Craig Steinback
Associate Professor
The Governors of the University of Alberta
1-059A Li Ka Shing Centre for Research
11203-87 Ave NW
EDMONTON, Alberta
T6G 2H5
(780) 492-5553

Your file Votre référence
HC6-24-c241154
Our file Notre référence

No Objection Letter RE: Protocol # PRO00096251 (Revision 3)

Dear Dr. Craig Steinback:

I am pleased to inform you that the information and material to support your Clinical Trial Application for **PHENYLEPHRINE HYDROCHLORIDE / SODIUM NITROPRUSSIDE / CARBON MONOXIDE**, control number **241154**, received on July 20, 2020, have been reviewed and we have no objection to your proposed study. I would remind you of the necessity of complying with the *Food and Drug Regulations,* Division 5, in the sale of this product for clinical testing. In addition, the regulations impose record keeping responsibilities on those conducting clinical trials. You are also reminded that all clinical trials should be conducted in compliance with the Therapeutic Products Directorate's *Guideline for Good Clinical Practice*.

Please note that Health Canada has implemented electronic reporting of adverse drug reactions and is currently in pilots with some sponsors. Those sponsors who have an established electronic connection with Canada Vigilance Production stream should submit their reports using the distribution rules provided to them by Health Canada, and reporting to multiple directorates is no longer required. For the sponsors who have not yet established this connection, they should continue submitting their reports to the applicable directorate by fax or by courier. The following website provides further clarification on Health Canada's adverse drug reactions reporting requirements for clinical trials: https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/health-canada-clinical-trials-database.html

Consistent with Health Canada's Notice - *Registration and Disclosure of Clinical Trial Information* of November 30, 2007, sponsors are encouraged to register their clinical trials within 21 days of the trial's onset, using a publicly available registry that conforms with international standards for registries such as: Clinicaltrials.gov (www.clinicaltrials.gov); Current Controlled Trials (www.controlled-trials.com).

Should you have any questions concerning this letter, please contact the Office of Clinical Trials (613) 941-2132.

Yours sincerely,
This document has been signed electronically using the Health Canada docuBridge system.

Larissa Lefebvre
Acting Manager, Submission Management Division
Office of Clinical Trials

LL/mw

Faculty of Kinesiology, Sport, and Recreation

1-052 Li Ka Shing Center

Edmonton, AB, Canada

Tel: 780.493.5553

PARTICIPANT CONSENT FORM

Title of Research Study: **The impact of carbon monoxide and altitude on vascular function**

Principal Investigator: **Dr. Craig Steinback, PhD** **craig.steinback@ualberta.ca**

Research Coordinators: **Nicholas Cheung, BSc** **nkcheung@ualberta.ca**
Scott Thrall, BSc **sthrall@ualberta.ca**

This letter provides the information you need to make an informed decision as to whether you wish to take part in our study. Before you make a decision, one of the researchers will go over this form with you. Please ask questions if you feel anything needs to be made clearer. You will be given a copy of this form for your records.

Why am I being asked to take part in this research study?

You are being asked to participate in this research study because you are healthy and of lowlander descent. The aim of our study is to assess how carbon monoxide and high altitude alter blood vessel health. In addition, we are interested in comparing these responses between lowlanders and Sherpa highlanders, who have adapted to high altitude for several thousand years.

What is the reason for doing the study?

At high altitude you are exposed to reduced oxygen. Low oxygen reduces the health of your blood vessels. During travel to high altitude, we may also be exposed to carbon monoxide. We may be exposed to carbon monoxide through vehicle fumes, incense, and second-hand smoke exposure. Carbon monoxide is viewed as a life-threatening toxic gas, and may also reduce the health of your blood vessels. Study of low oxygen and carbon monoxide together may help us understand how carbon monoxide affects our cardiovascular system at altitude and as we travel.

For the experimental protocol we will measure how your blood vessels respond to several tests. A doctor will place an intravenous (IV) catheter into your arm where we will give you very small doses of safe drugs that are commonly used in hospitals. For the first test we will use a blood pressure cuff to restrict blood flow for 5 minutes, then measure your blood vessels after it is released. For the second test we will measure your blood vessels while you squeeze a handgrip device and with a very small dose of safe drugs. The way that your blood vessels respond to these tests will give us information on the health of your blood vessels. Then we will have you take one breath of a safe amount of carbon monoxide, similar to amounts experienced through smoking, and repeat the tests. Throughout the protocol we will be measuring blood flow through your arm using ultrasound. The protocol will be completed at sea level (Edmonton, AB; 645m), and at high altitude for those joining our high-altitude expedition (Khumbu valley, Nepal, 3800m).

Am I eligible to take part in this study?

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RESULTS

1 Set of **24** Numbers
Range: From **1** to **2**

Set #1

2, 1, 1, 2, 2, 1, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 2, 1, 2, 2, 1, 2, 2, 1