Title: Measuring the human ventilatory and cerebral blood flow response to CO₂: a technical consideration for the end-tidal-to-arterial gas gradient

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Running Head: End-tidal-to-arterial gas gradients

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ABSTRACT

Our aim was to quantify the end-tidal-to-arterial gas gradients for O₂ (PET-PaO₂) and CO₂ (Pa-PETCO₂), during a CO₂ reactivity test in order to determine their influence on the cerebrovascular (CVR) and ventilatory (HCVR) response in subjects with (PFO+, n=8) and without (PFO-, n=7) a patent foramen ovale (PFO). We hypothesized that 1) the Pa-PETCO₂ would be greater in hypoxia compared to normoxia, 2) the Pa-PETCO₂ would be similar while the PET-PaO₂ gradient would be greater in those with a PFO, 3) the HCVR and CVR would be underestimated when plotted against PETCO₂ compared with PaCO₂, and 4) previously derived prediction algorithms will accurately target PaCO₂. PETCO₂ was controlled by dynamic end-tidal forcing in steady-state steps of -8, -4, 0, +4, and +8 mmHg from baseline in normoxia and hypoxia. Minute ventilation (\(\dot{V}_E\)), internal carotid artery blood flow (\(\dot{Q}_{ICA}\)), middle cerebral artery blood velocity (MCAv) and temperature corrected end-tidal and arterial blood gases were measured throughout experimentation. HCVR and CVR were calculated using linear regression analysis by indexing \(\dot{V}_E\) and relative changes in \(\dot{Q}_{ICA}\), and MCAv against PETCO₂, predicted PaCO₂, and measured PaCO₂. The Pa-PETCO₂ was similar between hypoxia and normoxia and PFO+ and PFO-. The PET-PaO₂ was greater in PFO+ by 2.1 mmHg during normoxia (\(P = 0.003\)). HCVR and CVR plotted against PETCO₂ underestimated HCVR and CVR indexed against PaCO₂ in normoxia and hypoxia. Our PaCO₂ prediction equation modestly improved estimates of HCVR and CVR. In summary, care must be taken when indexing reactivity measures to PETCO₂ compared to PaCO₂.

Key words: cerebrovascular reactivity, dynamic end-tidal forcing, end-tidal-to-arterial gas gradients, hypercapnic ventilatory response, patent foramen ovale
The partial pressure of end-tidal gases is the sum of gases contained within the alveoli and physiological deadspace. Physiological deadspace contains air that does not participate in gas exchange and consists of two components: (I) alveolar deadspace from non-perfused but ventilated alveoli, and (II) anatomical deadspace from airway structures that do not contribute to gas exchange (i.e. trachea and bronchi). During hypercapnia with dynamic end-tidal forcing (DEF), a high concentration of administered CO₂ occupies physiological deadspace during inspiration and mixes with alveolar gas during expiration, inflating PETCO₂ in comparison to PaCO₂ (15, 30, 37). At rest, the end-tidal-to-arterial PCO₂ gradient (Pa-PETCO₂; calculated as PaCO₂-PETCO₂) is usually non-existent or slightly positive (4, 30, 39). Conversely, it has also been reported as being negative at sea-level (24, 26, 37) and high-altitude (37) and this negative gradient is thought to be due in part to CO₂ trapped in external deadspace (7). Interestingly, the Pa-PETCO₂ gradient increased in magnitude by approximately 2-fold after one-week exposure to high-altitude (37). This was attributed to a potential combination of increased alveolar deadspace due to hypoxic pulmonary vasoconstriction (HPV), and increased CO₂ administration during DEF to compensate for the increased ventilatory sensitivity associated with high altitude acclimatization (37). Nevertheless, the effect of acute hypoxia on the Pa-PETCO₂ gradient during DEF has yet to be investigated.

The end-tidal-to-arterial gas gradient can be altered with changes in body position (3), aging (23), exercise (16), low breathing frequencies (15), and with CO₂ administration (i.e. hypercapnia) (1, 30, 37). But, it is currently unknown whether the presence of a patent foramen ovale (PFO) could also influence the Pa-PETCO₂ or end-tidal-to-arterial O₂ gradient (PET-PaO₂; calculated as PETO₂-PaO₂). A PFO is detected in as many as 35-38% of the general population (6) and potentially results in venous admixture, causing a reduction in pulmonary gas exchange efficiency. While it is unlikely that a small intracardiac shunt caused by a PFO would have an appreciable effect on the Pa-PETCO₂ gradient, the typically larger arterial-to-mixed-venous difference for oxygen suggests that a small PFO could affect the PET-PaO₂ gradient. Any potential departure of end-tidal gases from arterial blood gases is important in the context of DEF, as all DEF systems assume that end-tidal gases are a surrogate measure for arterial blood gases, which is not always accurate.

INTRODUCTION
Dynamic end-tidal forcing is commonly used to measure the hypercapnic ventilatory response (HCVR) and cerebrovascular reactivity (CVR) to CO$_2$. Cerebrovascular reactivity to CO$_2$ and HCVR are normally quantified using linear regression analysis by plotting measures of cerebral blood flow or minute ventilation ($\dot{V}_E$) against PaCO$_2$, the true driving stimulus for CVR and HCVR, or by plotting against $P_{ET}CO_2$, a commonly used surrogate for PaCO$_2$ (33, 38). However, quantifying CVR and HCVR using $P_{ET}CO_2$ during DEF may lead to data misinterpretation, as $P_{ET}CO_2$ does not uniformly reflect PaCO$_2$ (37). It is unclear to the extent that the CVR and HCVR reactivity profiles are affected by the hypercapnic $P_{ET}CO_2$ gradient during DEF in normoxia, and in the background of acute hypoxia, which results in an augmentation of chemosensitivity (i.e. increase in ventilation) and potentially alveolar deadspace. Some research groups have offered algorithms for predicting PaCO$_2$ during exercise (12, 16), severe hypoxia (12), and during CO$_2$ reactivity tests using different levels of administered CO$_2$ (26), but these do not seem to be transferrable to DEF (37). Due to this, we derived PaCO$_2$ correction algorithms by multivariate regression analysis for specific use with DEF (37).

**Non-invasive PaCO$_2$ correction algorithm (37):**

1) Pred-PaCO$_2$ = 0.363 + (0.958 • $P_{ET}CO_2$)

**Invasive PaCO$_2$ correction algorithm (37):**

2) *Pred-PaCO$_2$ = 0.964 + (0.960 • $P_{ET}CO_2$) + (0.331 • Baseline Pa-P$_{ET}CO_2$)

However, these equations have yet to be validated in an independent sample and it is unclear if they could be useful for HCVR and CVR measurements during normoxia and/or hypoxia. Given the recent advancements in DEF forcing systems (17, 25, 34), and continuing advancements in science and technology, portable DEF systems will be more accessible for human physiology researchers interested in conducting their work in unique locations (such as high-altitude or during magnetic resonance imaging). For this reason, the purpose of this study was to expand our understanding on the methodological constraints of DEF by investigating their utility between different populations of people (e.g. PFO vs non-PFO) and in different environments (e.g. acute hypoxia). We hypothesized that 1) the Pa-
$P_{ET}CO_2$ gradient would be greater in the background of hypoxia compared to normoxia due to augmentation in alveolar deadspace and increased CO$_2$ administration, similar to previous high altitude observations (37). II) the Pa-$P_{ET}CO_2$ gradient would be similar, while the $P_{ET}$-PaO$_2$ gradient would be larger in PFO+ compared to PFO- participants, III) the HCVR and hypercapnic CVR would be significantly underestimated when indexed against $P_{ET}CO_2$ compared with PaCO$_2$ in normoxia and hypoxia due to the presence of a negative Pa-$P_{ET}CO_2$ gradient, and IV) our previously derived correction algorithms would improve the prediction of PaCO$_2$ and abolish the difference between HCVR and CVR measured using predicted versus actual PaCO$_2$. 
MATERIALS AND METHODS

Ethical approval. All experimental procedures and protocols were reviewed and approved by the Clinical Research Ethics Board at the University of British Columbia and conformed to the Declaration of Helsinki. All participants provided written informed consent prior to participation in this study.

Participants. All experimental sessions were conducted in the Cardiopulmonary Laboratory for Experimental and Applied Physiology at the University of British Columbia’s Okanagan Campus. Recruited participants (n=18) were required to fill out a health history questionnaire to ensure normal pulmonary, cerebrovascular and cardiovascular health. Each participant was pre-screened (see below) on two separate days prior to the experimental protocol. Participants were between the ages of 18-45 years and were excluded from the study if they had abnormal lung function (defined as showing signs of large or small airway obstruction or a FEV1/FVC ratio <0.70). Participants were excluded if they were obese (body mass index >30 kg/m²), pregnant, or hypertensive (i.e. systolic >140 mmHg, diastolic >90 mmHg). Participants included in mean data analysis (n=15; 3 female) were non-smokers, had no previous history of cardiovascular, cerebrovascular or respiratory diseases, and were not taking any medications during testing (except oral contraceptives). Three of the 18 subjects screened were excluded from mean data analysis for the following reasons: I) arterial catheter complications (n=2), and II) gas analyzer failure (n=1).

Pre-screening

Prior to experimentation, each participant went through the following pre-screening protocols and procedures on two separate occasions (one on each day).

Patent foramen ovale screening. In order to determine the influence of an intracardiac shunt on the end-tidal-to-arterial gas gradients all subjects were screened for a PFO by agitated saline contrast echocardiography technique at rest and upon the release of a Valsalva maneuver while the patient lie in the supine left lateral decubitus position, as previously described (5, 11). A 20-gauge intravenous catheter was placed into the antecubital vein of each subject and flushed with 0.9% saline. A three-way stopcock and two 10 mL syringes
were connected. One syringe contained 0.5 mL of air and the other syringe contained 4 mL of saline. To create the contrast microbubbles, the saline solution was rapidly flushed back and forth for at least 10 seconds from one syringe to the other. Immediately following agitation, the saline microbubbles were rapidly injected while recording an apical four-chamber image of the heart by echocardiography using a commercially available ultrasound system (Vivid E9, GE, Fairfield, CT, USA) and the same sonographer (AMW). The ultrasound system uses a broadband M5S 5 MHz probe for 2D imaging. Images were captured and saved for offline analysis using commercially available software (EchoPAC v.13, GE, Fairfield, CT, USA). Immediately following injection, contrast microbubbles were observed in the right side of the heart. Contrast microbubbles appearing within the left atrium at rest or after a provocative maneuver (upon release from a Valsalva maneuver) within four cardiac cycles suggests the presence of a PFO. A 4 beat cut-off for resting saline contrast echocardiography has previously been determined to provide optimal diagnostic performance for a PFO with 71% sensitivity and 94% specificity, and sensitivity is improved to 89% by using a Valsalva maneuver (9).

Pulmonary function testing. In order to determine whether our participants had normal healthy pulmonary function, we conducted a forced vital capacity (FVC) test to measure lung function, a vital capacity (VC) and inspiratory capacity (IC) maneuver to measure lung volumes, and a single breath carbon monoxide test to quantify diffusion capacity (DLCO) on each individual. All testing procedures were conducted in agreement with the American Thoracic Society and European Respiratory Society’s joint guidelines (21, 22). For each of these tests, participants sat within a body plethysmography box (V6200, Vmax Sensormedics, Yorba Linda, CA, USA) with a rigid upright posture and their feet flat on the ground, while breathing through a spirometer and bacteriological filter with their nose clamped. All pulmonary function measurements were compared against population-based normative predictions.

Participant instrumentation
Upon arrival on the experimental day, participants sat down in a semi-recumbent position for 10-minutes. An arterial catheter was inserted into the left radial artery by an anesthesiologist
under ultrasound guidance. The participant was then instructed to lay supine on a hospital bed where they were instrumented with a lead-II electrocardiogram to measure heart rate (HR), a nasopharyngeal temperature probe (RET-1, Physitemp Instruments, Clifton, NJ, USA), automated blood pressure cuff (HEM-775CAN, Omron Healthcare, Cannockburn, IL, USA), and a transcranial Doppler (TCD) ultrasound headpiece with the ultrasound probe insonating the right middle cerebral artery (MCA; PMD150B, Spencer Technologies, Redmond, WA, USA). The participant was then instructed to relax and breathe normally for approximately 30 minutes to ensure the body positional change in blood volume distribution was complete (13).

**Respiratory measurements.** All respiratory parameters were acquired at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO, USA) interfaced with a personal computer. Lab acquisition software was used to collect and analyze ventilatory and cardiovascular variables (LabChart V7.1, ADInstruments, Colorado Springs, CO, USA). Subjects breathed through a mouthpiece (with nose clamp), bacteriological filter, and a two-way non-rebreathing valve (2600 series, Hans Rudolph, Shawnee, KS, USA). Respired gas pressures were sampled near the mouth, dried with nafion tubing and desiccant, and analyzed for $P_{ETO2}$ and $P_{ETCO2}$ (ML206, ADinstruments, Colorado Springs, CO, USA). Inspiratory and expiratory tidal volume ($V_T$) were determined using an integral of the respiratory flow signal, breathing frequency ($F_B$) as the amount of breaths taken per minute, and minute ventilation ($V_E$) being the product of these two respiratory measurements. Gas analyzers were calibrated prior to experimentation with gases of known concentration using the same sample line used in the experiment. Gas analyzer calibration was confirmed immediately before and following each protocol to ensure accurate respiratory measurements. Measured $PO_2$ and $PCO_2$ were time-corrected for gas analyzer sample delay and the values corresponding to the end of expiration (i.e. when respiratory flow crossed zero in the positive to negative direction) were identified as the $P_{ETO2}$ and $P_{ETCO2}$. Respiratory flow was measured near the mouth using a pneumotachograph (HR 800L, HansRudolph, Shawnee, KS, USA) and a differential pressure transducer (ML141, ADInstruments, Colorado Springs, CO, USA), which was calibrated daily with a 3-liter syringe. End-tidal gases were adjusted for temperature corrected water vapour pressure. The
nasopharyngeal temperature probe was connected to a T-type pod (ML312, ADinstruments, Colorado Springs, CO, USA), calibrated daily, and inserted approximately 10 cm into the nasopharyngeal cavity to measure core body temperature continuously (20). At each stage of the CO2 test nasopharyngeal temperature was averaged over a 1-minute period.

**Dynamic end-tidal forcing.** \(P_{ET}O_2\) and \(P_{ET}CO_2\) were controlled by a portable DEF system as previously described (37). Briefly, this system uses independent gas solenoid valves for \(O_2\), \(CO_2\), and \(N_2\) and controls the volume of each gas being delivered to the inspiratory reservoir through both a mixing and a humidification chamber. \(P_{ET}O_2\), \(P_{ET}CO_2\), \(V_T\), \(F_B\), and \(V_E\) were determined for each breath online using custom software (Airforce V 4.8) programmed in Labview (Version 13.0, National Instruments, Austin, TX, USA). Using feedback information regarding \(P_{ET}O_2\), \(P_{ET}CO_2\), inspired \(V_T\), and expired \(V_T\) the DEF system adjusts the inspirate to bring end-tidal gas to the desired target values. Feed-forward control of the inspirate is based on estimates of baseline metabolic \(O_2\) consumption and \(CO_2\) production and employs the alveolar gas equation to determine the required partial pressure of inspired \(CO_2\) (\(P_{ICO2}\)) and \(O_2\) (\(P_{IO2}\)). Feedback control is accomplished using a proportional and integral error reduction control system. This system has been used previously to control end-tidal gases during physiological stressors (2, 27, 37). End-tidal steady state was achieved when end-tidal gases were within 1 mmHg of the desired target for at least 3 consecutive breaths and is normally achieved within 30s (37).

**Intracranial blood flow.** Unilateral TCD was employed to measure the intracranial cerebral blood flow velocity within the right MCA (MCAv) using previously described search techniques (40) (PMD150B, Spencer Technologies, Redmond, WA, USA). The same experienced sonographer insonated the right MCA for all subjects. Mean cerebral blood flow velocity was calculated from the envelope of the velocity tracing.

**Extracranial blood flow.** Simultaneous B-mode and Doppler ultrasonography were performed to assess continuous diameter and blood flow velocity recordings of the right internal carotid artery (ICA) using a 10 MHz linear array probe attached to a high-resolution ultrasound machine (Terason t3200, Teratech, Burlington, MA, USA). ICA blood flow
velocity and vessel diameter were measured at least 2 cm from the carotid bifurcation, while ensuring there was no evidence of turbulent or retrograde flow. In addition, insonation angle remained unchanged at 60 degrees throughout the entire protocol. The same experienced sonographer insonated the right ICA for all subjects. Ultrasound recordings were screened and saved for offline analysis. Blood flow analysis of the ICA was performed using edge-detection software, which allows for the integration of synchronous diameter and velocity measurements to determine the mean beat-to-beat flow at 30 Hz independent of investigator bias (42). Mean blood flow was determined as half of the time-averaged maximum velocity (8), multiplied by the cross-sectional luminal area for a minimum of 12 cardiac cycles.

**Arterial blood sampling and blood pressure.** After local anaesthesia (2% lidocaine), a 20-gauge catheter (radial artery catheter, Arrow International, Reading, PA, USA) was placed transcutaneously into the left radial artery using ultrasound guidance and a modified Seldinger technique (37). The catheter was connected to a commercially available arterial blood sampling kit (VAMP Adult, Edwards Lifescience, Irvine, CA, USA), allowing for repeated sampling and flushing with 0.9% saline and measurement of beat-by-beat arterial blood pressure. Before sampling, the deadspace volume was withdrawn and then an arterial sample (3 ml) was carefully collected into pre-heparinized syringes (safePICO syringes, Radiometer, Copenhagen, Denmark). Air bubbles were immediately evacuated from the syringe, the syringe was capped, and blood gas analysis was performed within 20 seconds of sampling with a gas analyzer (ABL90 FLEX, Radiometer, Copenhagen, Denmark). The blood gas analyzer was calibrated every 8 hours using manufacturer’s standard internal quality checks and external ampoule-based quality checks were routinely performed to confirm internal calibrations. Reported variables that were calibrated and analyzed included: PaO₂, PaCO₂, pH, and arterial oxyhaemoglobin saturation (SaO₂). Arterial blood was temperature corrected offline to nasopharyngeal temperature using previously derived constants and logarithmic equations (32).

**Experimental protocol**
This study was conducted in two parts: (I) normoxia CO$_2$ reactivity protocol (NX) and (II) hypoxia CO$_2$ reactivity protocols (HX), each separated by 20 minutes. The NX protocol was always conducted first to avoid any potential confounds involving carry-over effects of sympathetic nervous system activation associated with exposure to acute hypoxia (43). Prior to each experiment, all participants abstained from exercise and alcohol for 24h, and caffeine for 12h. Testing was conducted while participants lay in the supine position on a hospital bed.

Normoxia CO$_2$ reactivity protocol. This protocol was selected because it is often used to assess hypocapnic (HYPO) and hypercapnic (HYPER) CVR to CO$_2$ (37, 41). In addition, the hypercapnic portion of the protocol can be used to assess HCVR (NX-HCVR) (14). Subjects were instrumented and allowed to breathe quietly through a mouthpiece with their nose clamped. The protocol consisted of two baseline periods: baseline 1 (room air breathing; AB) and baseline 2 (resting end-tidal control by DEF; AB-DEF). Baseline 1 involved 10-minutes of eupnea through our breathing apparatus. Immediately following AB (time = 10 minutes), AB-DEF commenced when the DEF system was connected to the inspiratory port of the breathing apparatus, and P$_{ET}CO_2$ and P$_{ET}O_2$ were controlled at AB baseline values for five-minutes. Following AB-DEF, P$_{ET}O_2$ was maintained at AB values (isooxia) while P$_{ET}CO_2$ was controlled in a stepwise fashion at -8, -4, 0, +4, and +8 mmHg from individual baseline values. Targeted P$_{ET}CO_2$ in the hypocapnic range was achieved through active hyperventilation and controlled using the DEF system. Each step change in P$_{ET}CO_2$ lasted for approximately 4-minutes after steady-state was achieved and an arterial blood sample and Q$_{ICA}$ were collected during the final minute of each stage.

Hypoxia CO$_2$ reactivity protocol. Reasoning for this protocol design was to I) potentially increase alveolar deadspace (18), and II) increase CO$_2$ administration during hypercapnia compared to the NX protocol, due to an augmented ventilatory response (10) . In addition, the hypercapnic portion of the protocol can be used to assess the HCVR in the background of hypoxia (HX-HCVR). Subjects were instrumented and allowed to relax and breathe normally through a mouthpiece with their nose clamped. The protocol consisted of two baseline periods: baseline 1 (AB) and baseline 2 (HX-DEF). Baseline 1 involved 10-minutes
of eupneic breathing. Immediately following AB, HX-DEF commenced once our DEF system was connected to the inspiratory port of the breathing apparatus, and $P_{ETO_2}$ was lowered and controlled at 50 mmHg, while $P_{ETCO_2}$ was maintained isocapnic to AB values.

Baseline 2 (HX-DEF) in HX trials was longer (10-minutes vs 5-minutes) than baseline 2 (AB-DEF) in NX trials to minimize the influence of the peak hypoxic ventilatory response (29) and to allow the pulmonary vascular pressure response to stabilize (36). Following HX-DEF, $P_{ETO_2}$ was maintained at 50 mmHg while $P_{ETCO_2}$ was controlled in a stepwise fashion at -8, -4, 0, +4, and +8 mmHg from individual baseline values. Targeted $P_{ETCO_2}$ in the hypocapnic range was achieved through active hyperventilation and controlled by DEF. Each step change in $P_{ETCO_2}$ lasted for approximately 4-minutes after steady-state was achieved and an arterial blood sample and $Q_{ICA}$ were collected during the final minute of each stage.

Data analysis

Cerebrovascular reactivity. We quantified relative HYPO- and HYPER-CVR to CO$_2$ during both normoxia and hypoxia protocols by indexing MCAv and $Q_{ICA}$ against I) PaCO$_2$, II) $P_{ETCO_2}$, III) invasive Pred-PaCO$_2$, and IV) non-invasive Pred-PaCO$_2$ (see equation 1 and 2 in the introduction). Linear regression analysis was used to quantify the slope, an index of reactivity, for each participant.

Hypercapnic ventilatory responses. We quantified absolute NX-HCVR and HX-HCVR by indexing $V_E$ against I) PaCO$_2$, II) $P_{ETCO_2}$, III) invasive Pred-PaCO$_2$, and IV) non-invasive Pred-PaCO$_2$ and used linear regression analysis, an index of reactivity, separately for each participant.

Intracardiac shunt scoring. A scoring system has previously been established to estimate the volume of blood flow through a PFO based on the greatest density and spatial distribution of microbubbles in the left ventricle of a single cardiac cycle during the subsequent four cardiac cycles (9). The 0–5 scoring system assigns: ‘0’ for no microbubbles; ‘1’ for 1–3 microbubbles; ‘2’ for 4–12 microbubbles; ‘3’ for greater than 12 microbubbles bolus; ‘4’ for greater than 12 microbubbles heterogeneously distributed; and a ‘5’ for greater than 12
microbubbles homogeneously distributed (19). This scaling system is reproducible between independent blinded observers (19). All agitated saline contrast echocardiograms were reviewed and scored by two individuals.

Statistical analysis

All data were analyzed using SigmaStat V11.5 (Systat, Chicago, IL, USA). During both CO2 reactivity protocols (NX and HX) measurements were averaged over a 1-minute period at the end of each baseline in normoxia (i.e. AB and AB-DEF), hypoxia (AB and HX-DEF), and with each CO2 step (-8, -4, 0, +4, and +8 mmHg P_{ET}CO2), just prior to arterial blood sampling. All data is presented as means ± standard error of the mean (SEM).

Two-way analysis of variance was used to compare differences (P<0.05) in ventilatory, cardiovascular, cerebrovascular and arterial blood measurements for both protocols, including analysis between PFO+ and PFO- participants. To determine differences (P<0.05) between each reactivity measure when indexed against P_{ET}CO2, PaO2, invasive Pred-PaCO2, and non-invasive Pred-PaCO2 within NX and HX CO2 reactivity protocols, a one-way repeated measures analysis of variance was used. When significant F-ratios were detected, all post-hoc comparisons were made using Tukey’s HSD.

End-tidal-to-arterial gas gradients for both O2 and CO2 were determined by comparing end-tidal and arterial gas values using a paired t-test, significance was set at P<0.05. Bland-Altman relationships were used to assess the agreement between end-tidal and arterial blood gases. The 95% limits of agreement were calculated by using the mean ±1.96 x standard deviation (SD) of the bias (9).

Multiple forward stepwise regression analysis was used to determine which variables could accurately predict arterial blood gases based upon the current study data and to allow comparison of regression equations to previously published equations. The following independent variables were included in the regression analysis: \( \dot{V}_E \), \( V_T \), \( F_B \), \( P_{T}O_2 \), \( P_{T}CO_2 \), \( P_{ET}O_2 \), \( P_{ET}CO_2 \), the baseline \( P_{ET}-PaO2 \) gradient, and the baseline \( Pa-P_{ET}CO2 \) gradient. Inclusion of independent variables in the multiple forward stepwise regression model was determined with a P-value <0.05.
RESULTS

Participants. The participants (n=15) included in NX and HX protocol data analysis had a mean ± SEM age of 25.8 ± 1.5 years, height of 174.2 ± 2.1 cm, weight of 72.9 ± 3.7 kg, body mass index of 23.9 ± 0.9 kg/m², and the right MCA was insonated at a depth of 5.3 ± 1.1 cm. Of the participants included in the mean data analysis, eight tested positive for a PFO (two with a shunt score of ‘1’; six with a shunt score of ‘2’). Each PFO detected was only upon Valsalva release. Participants were normotensive (systolic blood pressure = 116 ± 2.2 mmHg, diastolic blood pressure = 68 ± 2.4 mmHg).

Recruited participants all had healthy lung function and demonstrated no signs of airway obstruction characterized by an irregular expiratory flow tracing during a FVC maneuver. Participants had an FVC of 5.3 ± 0.2 l (108.1 ± 3.3 % of predicted; % predicted values following each pulmonary measurement in parentheses), a FEV₁ of 4.2 ± 0.2 l (101.4 ± 3.8 %), and a FEV₁/FEV ratio of 79.2 ± 1.4%. They also had normal lung volumes with a functional residual volume of 3.0 ± 0.1 l (95.1 ± 3.8 %), alveolar volume of 6.2 ± 0.2 l (95.7 ± 2.3 %) and a total lung volume of 6.5 ± 0.3 l (101.3 ± 3.2 %). In addition, participants performed a single breath carbon monoxide transfer test and reported normal absolute values of 31.9 ± 1.6 ml/min/mmHg (93.5 ± 3.0 %), and after adjustment for alveolar volume 5.2 ± 0.2 ml/min/mmHg/l (98.5 ± 3.6 %).

Normoxia CO₂ protocol. Of the 15 participants included in this study, one participant was completely excluded from the NX protocol due to an arterial catheter failure in the middle of protocol (n=14). Table 1 displays ventilatory, and arterial blood gas data for both NX and HX CO₂ reactivity tests. The partial pressure of inspired CO₂ (PICO₂) was lower during AB and -8 mmHg PETO₂ step compared to AB-DEF, and was elevated during hypercapnia. Conversely, PICO₂ was higher during AB compared to AB-DEF, and was decreased during hypocapnia and hypercapnia. As expected, PETO₂ and PaCO₂ were both less during hypocapnia and elevated during hypercapnia. A positive Pa-PETO₂ gradient (i.e. PaCO₂ > PETO₂) was found during AB (non-controlled baseline), while a negative Pa-PETO₂ gradient (i.e. PETO₂ > PaCO₂) was observed during both hypercapnia stages (see Figure 1). Throughout all stages of the NX CO₂ reactivity protocol, PETO₂ was not different from AB-
DEF, however, PaO₂ was slightly elevated during +8 mmHg, compared to AB-DEF. The positive P<sub>ET</sub>-PaO₂ gradient was present at all protocol stages, and was reduced by 1.5 ± 0.9 mmHg at +8 mmHg P<sub>ET</sub>CO₂ step compared to AB-DEF baseline (see Table 1 and Figure 2).

Table 2 displays a comparison of ventilatory, cardiovascular, and cerebrovascular data between NX and HX CO₂ reactivity protocols. Minute ventilation and V<sub>T</sub> were both elevated during both hypercapnic steps, while participants F<sub>B</sub> was elevated only at the +8 mmHg P<sub>ET</sub>CO₂ step compared to AB-DEF. Heart rate was lower during the non-controlled AB baseline and was elevated during the -4 mmHg and +8 mmHg P<sub>ET</sub>CO₂ step, while MAP was only elevated during the +8 mmHg P<sub>ET</sub>CO₂ step compared to AB-DEF. Middle cerebral artery velocity and Q̇<sub>ICA</sub> were 61.1 ± 2.7 cm/s and 265.5 ± 15.8 ml/min during AB, respectively, and remained unchanged compared to the AB-DEF baseline. During hypocapnia, MCAv and Q̇<sub>ICA</sub> were 20.9 ± 1.1% and 27.0 ± 1.7% lower at -8 P<sub>ET</sub>CO₂ mmHg, but were not different from AB-DEF during the -4 mmHg P<sub>ET</sub>CO₂ step. During hypercapnia, absolute MCAv and Q̇<sub>ICA</sub> were 11.8 ± 1.7% and 20.8 ± 5.0% higher at +4 P<sub>ET</sub>CO₂ mmHg, and 30.8 ± 1.8% and 53.7 ± 6.0% higher at +8 P<sub>ET</sub>CO₂ mmHg from AB-DEF, respectively. No differences were found in nasopharyngeal temperature and SaO₂ throughout the CO₂ reactivity protocol.

**Hypoxia CO₂ protocol.** Of the 15 participants included in NX and HX protocol data analysis, one participant was completely excluded due to incomplete data in the HX protocol. Table 1 displays ventilatory and arterial blood gas data for both NX and HX isooxic CO₂ reactivity tests. The P<sub>CO₂</sub> was lower during AB and hypocapnia and elevated during hypercapnia compared to HX-DEF, while the P<sub>O₂</sub> was elevated during AB and reduced only during +8 mmHg P<sub>ET</sub>CO₂ step. As expected, the P<sub>ET</sub>CO₂ and PaCO₂ were both lower during hypocapnia and elevated during hypercapnia. Similar to the NX CO₂ reactivity protocol, a positive Pa-P<sub>ET</sub>CO₂ gradient was present during AB, whilst a negative Pa-P<sub>ET</sub>CO₂ gradient was present during both +4 and +8 mmHg P<sub>ET</sub>CO₂ steps (see Figure 1). Throughout all stages of the HX CO₂ reactivity protocol, P<sub>ET</sub>O₂ and PaOₐ were not different from HX-DEF with the exception of AB where the subject was breathing room air as opposed to hypoxic air. The P<sub>ET</sub>-PaO₂ gradient was present and consistent throughout all protocol stages (see Figure 2).
As seen in Table 2, \( \dot{V}_E \) and \( V_T \) were lower during AB compared to HX-DEF when hypoxia was administered and were elevated compared to HX-DEF in the hypercapnic range, while \( F_B \) was only elevated during the +8 mmHg \( P_{\text{ETCO}_2} \) step. Heart rate was lower during AB and hypocapnia, and HR and MAP were both elevated during the +8 mmHg \( P_{\text{ETCO}_2} \) step compared to HX-DEF. Middle cerebral artery velocity and \( \dot{Q}_{\text{ICA}} \) were 61.8 ± 3.0 cm/s and 278.1 ± 14.4 ml/min during AB and remained the same during HX-DEF. Absolute MCAv and \( \dot{Q}_{\text{ICA}} \) were reduced by 21.6 ± 1.3% and 26.0 ± 2.6% at -8 \( P_{\text{ETCO}_2} \) mmHg. During hypercapnia, absolute MCAv and \( \dot{Q}_{\text{ICA}} \) were increased by 20.8 ± 2.8% and 30.2 ± 3.8% at +4 \( P_{\text{ETCO}_2} \) mmHg, and 39.0 ± 3.7% and 64.5 ± 5.5% at +8 \( P_{\text{ETCO}_2} \) mmHg from HX-DEF, respectively. Nasopharyngeal temperature remained unchanged throughout the CO2 reactivity protocol when compared to HX-DEF baseline. Saturation level of oxygen of hemoglobin was reduced during HX-DEF from 97.9 ± 0.2% to 83.5 ± 0.6% compared to AB baseline. During the HX CO2 reactivity protocol the \( S_aO_2 \) was increased from HX-DEF during -8 mmHg \( P_{\text{ETCO}_2} \), and was reduced during the +8 mmHg \( P_{\text{ETCO}_2} \) step.

**Comparison of normoxia and hypoxia CO2 protocols.** We compared the results between NX and HX CO2 reactivity protocols (see Table 1 and Table 2). Between the two trials \( P_1 CO_2 \) was significantly lower overall during NX trial compared to HX, but only had an interaction effect at the DEF controlled baseline and +4 mmHg \( P_{\text{ETCO}_2} \). There was no interaction effect in \( P_1 CO_2 \) during the +8 mmHg \( P_{\text{ETCO}_2} \) step, which was unexpected. To no surprise, the \( P_1 O_2 \) was lower during the HX CO2 reactivity protocol when hypoxia was administered compared to the NX CO2 reactivity protocol. We also found that the \( P_{\text{ETCO}_2} \) was 0.8 mmHg greater during NX compared to HX CO2 reactivity protocols, while \( PaCO_2 \) and the Pa-P\( _{\text{ETCO}_2} \) gradient remained unchanged. A main effect was observed with variables \( P_{\text{ETO}_2} \) and \( PaO_2 \), as they were significantly higher during NX compared to HX CO2 reactivity protocol. Overall, the \( P_{\text{ET}}-PaO_2 \) gradient during the HX trial was 2.4 ± 0.4 mmHg larger compared to NX, however there were no interaction effects.

Minute ventilation and \( V_T \) were lower during NX compared to HX trial, while there was no difference in participants \( F_B \). There was a main effect for HR, MAP, MCAv, and \( \dot{Q}_{\text{ICA}} \), where they were significantly higher during HX compared to NX, but no interactions were observed for each of these variables. The \( S_aO_2 \) was higher during NX compared to the
HX protocol. Lastly, there was a main effect of nasopharyngeal temperature, as it was slightly lower during HX compared to NX CO₂ reactivity protocol.

**PFO+ vs PFO- participants.** Tables 3 and 4 display ventilatory and arterial blood data for PFO+ and PFO- participants during the NX and HX CO₂ reactivity protocols. Fifteen participants were recruited for this study, however, for each protocol one subject was excluded due to incomplete data. Since the excluded participants were not the same between NX and HX trials, there was a difference in the number of PFO+ participants between trials (7 PFO+ included in NX trial analysis; 8 PFO+ included in HX trial analysis). During NX and CO₂ reactivity protocol there were no differences found in V̇E, PETCO₂, PaCO₂, the Pa-PETCO₂ gradient, PETO₂, PaO₂ and nasopharyngeal temperature between PFO+ (n=7) and PFO- (n=7) participants. However, PFO+ participants presented with a greater PET-PaO₂ gradient compared to PFO- participants across all stages (5.0 ± 1.2 mmHg vs 2.9 ± 1.2 mmHg, P=0.003). During HX CO₂ reactivity protocols, no differences were found in V̇E, PETCO₂, PaCO₂, the Pa-PETCO₂ gradient, PETO₂, PaO₂, the PET-PaO₂ gradient, and nasopharyngeal temperature between PFO+ (n=8) and PFO- (n=6) participants.

In addition to these findings, for both NX and HX CO₂ reactivity protocols there were no differences in HCVR (P=0.437 and P=0.505, respectively), relative HYPO-Q̇ICA CVR (P=0.292 and P=0.096, respectively), relative HYPER-Q̇ICA CVR (P=0.839 and P=0.502, respectively), relative HYPO-MCAv CVR (P=0.250 and P=0.441, respectively), and relative HYPER-MCAv CVR (P=0.417 and P=0.197, respectively) between PFO+ and PFO- participants.

**Hypercapnic ventilatory reactivity.** Figure 3A illustrates the linear regression plotted between V̇E and PaCO₂ for one representative subject during normoxic and hypoxic CO₂ tests. For all subjects the Pearson correlation coefficient (r) were linear (i.e. r>0.7). The NX- and HX-HCVR plotted against ṖETCO₂, PaCO₂, *Pred-PaCO₂, and Pred-PaCO₂ (calculated using our previously derived correction algorithms (37) is displayed in Figure 4. During the NX reactivity protocol, ṖETCO₂ HCVR was lower than PaCO₂ HCVR (true HCVR) by 0.5 ± 0.1 l/min/mmHg, *Pred-PaCO₂ HCVR, and Pred-PaCO₂ HCVR (P<0.001, P=0.027, and P<0.001, respectively). The *Pred-PaCO₂ HCVR was greater compared to ṖETCO₂ HCVR...
by 0.3 ± 0.0 l/min/mmHg (P=0.003). During the HX reactivity protocol, PaCO₂ HCVR was
greater than P_{ET}CO₂ HCVR by 0.4 ± 0.2 l/min/mmHg, but not different from *Pred-PaCO₂
nor Pred-PaCO₂ HCVR reactivity (P=0.007, P=0.596 and P=0.143, respectively).

**Internal carotid artery reactivity.** Figure 3B illustrates the linear regression plotted between
\dot{Q}_{\text{ICA}} and PaCO₂ for one representative subject during normoxic and hypoxic CO₂ tests. For
all subjects the Pearson correlation coefficient (r) were linear (i.e. r>0.7). Figure 5 displays
relative HYPO-\dot{Q}_{\text{ICA}} and relative HYPER-\dot{Q}_{\text{ICA}} reactivity data during NX and HX CO₂
reactivity protocols. Of the 14 participants included in normoxia mean data, two were
excluded from the \dot{Q}_{\text{ICA}} analysis due to incomplete data (n=12). During the NX protocol
there was no difference between PaCO₂, P_{ET}CO₂, *Pred-PaCO₂, and Pred-PaCO₂ \dot{Q}_{\text{ICA}}
reactivity in the hypocapnic range (P=0.091). In the hypercapnic range, P_{ET}CO₂, *Pred-
PaCO₂, and Pred-PaCO₂ \dot{Q}_{\text{ICA}} reactivity were lower by 1.5 ± 0.3 %/mmHg (P<0.001), 0.8 ±
0.2 %/mmHg (P=0.007), and 1.2 ± 0.3 %/mmHg (P<0.001) compared to PaCO₂ \dot{Q}_{\text{ICA}}
reactivity, respectively. In addition, *Pred-PaCO₂ \dot{Q}_{\text{ICA}} reactivity was greater compared to
P_{ET}CO₂ \dot{Q}_{\text{ICA}} reactivity (P=0.016).

Due to incomplete data sets, four participants were removed from \dot{Q}_{\text{ICA}} analysis for
the HX CO₂ protocol due to inadequate images (n=10). During the HX protocol, there were
no differences between P_{ET}CO₂, PaCO₂, *Pred-PaCO₂, and Pred-PaCO₂ relative \dot{Q}_{\text{ICA}}
reactivity in the hypocapnic range (P=0.632). In the hypercapnic range, P_{ET}CO₂ \dot{Q}_{\text{ICA}}
reactivity was lower by 0.9 ± 0.3 %/mmHg (P<0.001) compared to PaCO₂ \dot{Q}_{\text{ICA}} reactivity. In
addition, the *Pred-PaCO₂ \dot{Q}_{\text{ICA}} reactivity was greater compared to P_{ET}CO₂ \dot{Q}_{\text{ICA}} reactivity
(P=0.025), and PaCO₂ \dot{Q}_{\text{ICA}} reactivity was greater compared to Pred-PaCO₂ \dot{Q}_{\text{ICA}} reactivity
(P=0.020).

**Middle cerebral artery reactivity.** Figure 3C illustrates the linear regression plotted between
\dot{Q}_{\text{ICA}} and PaCO₂ for one representative subject during normoxic and hypoxic CO₂ tests. For
all subjects the Pearson correlation coefficient (r) were linear (i.e. r>0.7). Figure 6 displays
relative HYPO-MCAv and relative HYPER-MCAv reactivity data during NX and HX CO₂
reactivity protocols. During the NX protocol there were no differences in PaCO₂, P_{ET}CO₂,
*Pred-PaCO₂, and Pred-PaCO₂ MCAv reactivity in the hypocapnic range (P=0.116). In the
hypercapnic range, $P_{ET}CO_2$, *Pred-PaCO$_2$, and Pred-PaCO$_2$ MCAv reactivity were lower compared to PaCO$_2$ MCAv reactivity by $0.7 \pm 0.1 \%/\text{mmHg}$ (P<0.001), $0.3 \pm 0.1 \%/\text{mmHg}$ (P=0.002), and $0.5 \pm 0.1 \%/\text{mmHg}$ (P<0.001), respectively. In addition, the *Pred-PaCO$_2$ MCAv reactivity was greater compared to $P_{ET}CO_2$ and Pred-PaCO$_2$ MCAv reactivity (P=0.031).

During the HX CO$_2$ reactivity protocol, $P_{ET}CO_2$ relative MCAv reactivity was lower by $-0.3 \pm 0.1 \%/\text{mmHg}$ compared to PaCO$_2$ relative MCAv reactivity in the hypocapnic range (P=0.011). In the hypercapnic range, $P_{ET}CO_2$ relative MCAv reactivity was lower compared to PaCO$_2$ and *Pred-PaCO$_2$ relative MCAv reactivity (P<0.001 and P=0.007, respectively).

**Multivariate Regression Analysis.** Equations 3 and 4 provide non-invasive prediction algorithms during normoxia, while equations 5 and 6 provide non-invasive prediction algorithms during hypoxia. Interestingly, in the current data set, the addition of tidal volume further improved the prediction of PaCO$_2$.

**Normoxic non-invasive PaCO$_2$ correction algorithms:**

3) $\text{PaCO}_2 = 2.189 + (0.941 \times P_{ET}CO_2); R^2 = 0.95; P<0.001$

4) $\text{PaCO}_2 = 3.196 + (0.948 \times P_{ET}CO_2) - (0.768 \times V_T); R^2 = 0.98; P<0.001$ 

**Hypoxic non-invasive PaCO$_2$ correction algorithms:**

5) $\text{PaCO}_2 = 2.531 + (0.931 \times P_{ET}CO_2); R^2 = 0.97; P<0.001$

6) $\text{PaCO}_2 = 2.551 + (0.955 \times P_{ET}CO_2) - (0.493 \times V_T); R^2 = 0.97; P<0.001$
DISCUSSION

The purpose of the current study was to I) measure any differences in the end-tidal-to-arterial gradient between NX and HX CO2 reactivity protocols, II) determine whether having a PFO had an effect on both CO2 and O2 end-tidal-to-arterial gradients, III) quantify differences (if any) between HCVR and CVR to CO2 when indexed against PaCO2 and PETCO2 in normoxia and hypoxia, and IV) determine whether our previously derived PaCO2 correction algorithms accurately predict measured PaCO2 during DEF. The findings from this study were I) there were no differences in the Pa-PETCO2 gradient between normoxia and hypoxia, however, the PET-PaO2 gradient was larger in hypoxia compared to normoxia during a CO2 reactivity test, II) the presence of a PFO increased the PET-PaO2 gradient during the normoxia CO2 reactivity protocols, but not during the hypoxia CO2 reactivity protocols, III) HCVR, relative MCAv, and relative QICA to CO2 profiles were significantly underestimated when plotted against PETCO2 compared to PaCO2 by 16.1 ± 2.6%, 15.8 ± 2.4%, and 19.5 ± 2.5% during the NX CO2 reactivity protocol, respectively, and by 8.4 ± 2.7%, 8.2 ± 2.9%, and 11.7 ± 3.3% during the HX CO2 reactivity protocol, respectively. (IV) Our previously derived correction algorithm showed modest improvement in the prediction of measured PaCO2.

The end-tidal-to-arterial CO2 gradient. Similar to previously reported data (4, 30, 39), our study found that there was positive Pa-PETCO2 gradient during eupneic air breathing, not controlled using DEF (Baseline 1, AB), which was in contrast to our previously published work (37). A novel finding was a significant decrease in the Pa-PETCO2 gradient between the unforced air-breathing baseline (baseline 1, AB), and the forced air-breathing baseline (baseline 2, AB/HX-DEF) in both NX and HX CO2 reactivity protocols, suggesting that DEF in itself has an effect on the Pa-PETCO2 gradient. This could be attributed to the increases in \( \dot{V}_E \) during DEF air breathing, which led to a greater \( P_{\text{ICO2}} \) that occupies physiological deadspace, possibly elevating end-tidal values upon expiration. Although participants are being controlled at the same \( P_{\text{ETCO2}} \) and \( P_{\text{ETO2}} \) during the DEF baseline, almost all participants increased their \( \dot{V}_E \) slightly due to pseudo-stimulation likely from the noise emitted by the DEF solenoid valves when triggered. By this logic, one would expect that the Pa-PETCO2 gradient be larger during hypoxic CO2 reactivity protocol during DEF isocapnic
hypoxia air breathing (HX-DEF) compared to DEF isocapnic normoxia air breathing (AB-DEF), however, this was not observed.

Consistent with previous findings (37), no Pa-P_{ET}CO_2 gradient was detected during normoxia CO_2 reactivity protocols in the hypocapnic range, and this also held true during the hypoxia CO_2 reactivity protocols. During the hypercapnic steps (+4 and +8 mmHg P_{ET}CO_2), a negative Pa-P_{ET}CO_2 gradient (P_{ET}CO_2 overestimating PaCO_2) was present in both normoxia and hypoxia, and in opposition to one of our hypotheses, there was no difference in the Pa-P_{ET}CO_2 gradients between NX and HX CO_2 reactivity protocols.

Previous work has shown a widening (i.e. more negative) Pa-P_{ET}CO_2 gradient present at high altitude compared to sea level (37). Initially, it was thought that the effect was simply due to the heightened ventilatory response associated with hypobaric hypoxia (atm ~413 mmHg), which would require increased administered CO_2 in order to maintain a given P_{ET}CO_2 during DEF. This effect paired with the potential increase in alveolar deadspace at high altitude due to HPV could potentially be responsible for the difference in the Pa-P_{ET}CO_2 gradient between sea level and high altitude (18, 37). In the current study, an acute isocapnic bout of hypoxia (10-minutes) was administered prior to conducting the CO_2 reactivity test in order to elicit a HPV response to potentially increase alveolar deadspace. However, even though V̇_E mildly increased during hypoxic hypercapnia compared to normoxic hypercapnia, there was no difference in the Pa-P_{ET}CO_2 gradient between the two protocols. We speculate that the reasoning behind the negative finding was that we failed to deliver a powerful enough respiratory stimulus in order to achieve a difference in the Pa-P_{ET}CO_2 gradient between normoxia and hypoxia CO_2 protocols. Although V̇_E increased during the hypoxia trials compared to normoxia, P_{i}CO_2 remained similar which was not expected. Considering our previous report (37), there was a difference in the Pa-P_{ET}CO_2 gradient between sea level and high altitude during the +10 mmHg hypercapnia condition where the V̇_E response was nearly 2-fold greater at high altitude. This V̇_E response at high altitude necessitated a fraction of inspired CO_2 (F_{i}CO_2) delivery of 9.1 ± 0.1% compared to 6.7 ± 0.1%. In order to see a difference in the Pa-P_{ET}CO_2 gradient between normoxia and hypoxia trial, we likely would have needed to deliver a more severe hypoxic stimulus to further increase V̇_E.

However, the knowledge that the Pa-P_{ET}CO_2 gradient remains relatively unchanged within a
~15-20 l/min difference in $V_E$ between the normoxia and hypoxia CO$_2$ reactivity protocols is extremely valuable for future application of our DEF system.

**The end-tidal-to-arterial O$_2$ gradient.** Surprisingly, there is little literature available with respect to the $P_{ET}$-PaO$_2$ gradient during changes in P$_{ET}$CO$_2$ conducted by DEF. Although experiments investigating the alveolar-to-arterial oxygen difference (AaDO$_2$) exist, it is difficult to assess the AaDO$_2$ during DEF due to breath-by-breath fluctuations in inspired fractions of O$_2$ and CO$_2$, necessitating the study of the $P_{ET}$-PaO$_2$ gradient in its place. However, literature regarding the AaDO$_2$ are inconsistent suggesting that the AaDO$_2$ can widen or narrow following hypoxia, and is heavily dependent on the length and severity of exposure (18, 28, 35). Throughout the normoxic CO$_2$ reactivity protocol, the $P_{ET}$-PaO$_2$ gradient remained significantly unchanged from the DEF baseline but was trending in the negative direction, and is consistent with previous findings (37). This novel observation was not expected and reasoning for this may be due to a combination of I) increased $V/Q$ matching from the hypercapnia stimulus, and II) decreased P$_{IO2}$ due to the increased $V_E$ during hypercapnia. The latter theory likely being the most probable, as during hyperventilation less P$_{IO2}$ is required to maintain a given P$_{ETO2}$ level, minimizing the difference between P$_{ETO2}$ and PaO$_2$. In contrast, the effect of the $P_{ET}$-PaO$_2$ gradient decreasing during hypercapnia was not observed during our HX CO$_2$ reactivity protocol, perhaps providing evidence that alveolar deadspace is increasing with acute hypoxia, countering the effects seen during the NX protocol and maintaining the $P_{ET}$-PaO$_2$ gradient. An alternative explanation may lie within the much greater reduction in P$_{IO2}$ delivery between stage 0 mmHg and +8 mmHg P$_{ET}$CO$_2$ during the NX trial compared to the HX trial. In addition, the $P_{ET}$-PaO$_2$ gradient was larger during hypoxia compared to the normoxia CO$_2$ reactivity protocols. This supports the notion that our acute isocapnic hypoxia stimulus increased alveolar deadspace within the lung, and provides further evidence that the $P_{ET}$-PaO$_2$ gradient has a higher potential of being affected compared to the Pa-P$_{ET}$CO$_2$ gradient.

**Comparison of PFO+ and PFO- participants.** Although possible, it is unlikely that a small to moderately sized PFO (PFO score of 1-3) would have a significant effect on the Pa-P$_{ET}$CO$_2$ gradient, but this has yet to be established. In the current study, participants that
presented with a PFO were only detected upon release from a Valsalva maneuver and were small in magnitude (i.e. shunt scores of “1” and “2”). In both NX and HX CO₂ reactivity protocols, the \( \Delta \text{Pa-PECO₂} \) gradient was the same between PFO+ and PFO- participants. Due to the large resting gradient of \( \text{O₂} \) between atmospheric air and venous blood returning to the lung, it was more likely that the presence of a PFO may alter the \( \Delta \text{PET-\text{PaO₂}} \) gradient. We found that the \( \Delta \text{PET-\text{PaO₂}} \) gradient was significantly larger in PFO+ participants compared to PFO- participants during the normoxia CO₂ reactivity protocols. This was somewhat unexpected due to the small magnitude of the PFO’s that were screened, but it is possible that the magnitude of the PFO’s became even larger with the increased intra-thoracic pressure swings associated with increased \( \dot{V}_\text{E} \) during active hyperventilation (used to achieve hypocapnia), and during increased chemosensitivity with hypercapnia. However, the greatest difference in the \( \Delta \text{PET-\text{PaO₂}} \) gradient between our PFO+ and PFO- participants was during baseline, when the magnitude of the PFO was expected to be small. Based on this, the difference we found on the \( \Delta \text{PET-\text{PaO₂}} \) between PFO+ and PFO- participants should be cautiously interpreted, as the effect could be explained by our small sample size. In contrast, the effect was not observed during the HX protocol as the \( \Delta \text{PET-\text{PaO₂}} \) gradient was the same between PFO+ and PFO- participants. This was likely due to the narrowing of the gradient between the amount of \( \text{O₂} \) administered to maintain a \( \text{PETO₂} \) of 50 mmHg, and the venous blood returning to the lung (5, 37).

**Hypercapnic ventilatory reactivity.** The NX-HCVR was indexed against \( \text{PaCO₂}, \text{PETCO₂}, \) \*\( \text{Pred-PaCO₂} \), and \( \text{Pred-PaCO₂} \). As expected, HCVR was underestimated when plotted against \( \text{PETCO₂} \) compared to \( \text{PaCO₂} \) (actual stimulus) for both NX and HX CO₂ reactivity protocols. These results have already been observed when using a Douglas bag to administer CO₂, however, these results had yet to be validated using DEF, and during hypoxia (26). Similar results were found between NX and HX CO₂ reactivity protocols, in which HX-HCVR was greater when plotted against \( \text{PaCO₂} \) compared to \( \text{PETCO₂} \). While our correction algorithms were inconsistent at predicting \( \text{PaCO₂} \), both equations were still better predictors of \( \text{PaCO₂} \) compared to \( \text{PETCO₂} \).
Internal carotid artery blood flow reactivity. To our knowledge this is the first study comparing relative $Q_{ICA}$ responses when indexing against measured PaCO$_2$ and $P_{ETCO_2}$, and our corrected PaCO$_2$ values with our previously derived correction algorithms. During the NX and HX CO$_2$ reactivity protocol, there was no difference between HYPO-$Q_{ICA}$ $P_{ETCO_2}$ CVR and PaCO$_2$ CVR to CO$_2$. This was expected, as there were no Pa-P$E_{TCO_2}$ gradients present in the hypocapnic range.

HYPER-$Q_{ICA}$ $P_{ETCO_2}$ CVR was lower compared to PaCO$_2$ CVR; this was consistent and expected throughout both NX and HX protocols as a significant Pa-P$E_{TCO_2}$ gradient was present. HYPER-$Q_{ICA}$ Pred-PaCO$_2$ CVR was greater than $P_{ETCO_2}$ CVR in normoxia and hypoxia trials. Much like our HCVR data, the correction algorithms were inconsistent at predicting PaCO$_2$, however, both equations were an intermediate predictor of PaCO$_2$ as they performed better than $P_{ETCO_2}$ $Q_{ICA}$ CVR.

Middle cerebral artery velocity reactivity. Middle cerebral artery blood velocity was indexed against PaCO$_2$, $P_{ETCO_2}$, *Pred-PaCO$_2$, and Pred-PaCO$_2$. During the NX and HX CO$_2$ reactivity protocols no differences were found within HYPO-MCAv. Similar to Peebles et al. (2007) and what we observed with our $Q_{ICA}$ reactivity results, $P_{ETCO_2}$ CVR was lower compared to PaCO$_2$ CVR during the normoxia CO$_2$ reactivity protocol. Consistent with our HCVR and $Q_{ICA}$ CVR to CO$_2$ findings, both of our prediction algorithms were better at predicting PaCO$_2$ compared to $P_{ETCO_2}$ rendering them moderately effective.

Efficacy of our PaCO$_2$ correction algorithms. One of the purposes of this study was to validate our previously derived correction algorithm that involved non-invasive measurements (i.e. respiratory gases, see equation 1) and invasive measurements (i.e. arterial blood sampling, see equation 2). We quantified the performance of our algorithms by comparing the predicted PaCO$_2$ value and the actual PaCO$_2$. Non-invasive correction algorithms have been proposed for predicting PaCO$_2$ by Tymko et al. 2015a, during a similar CO$_2$ reactivity test using the same DEF system, and Peebles et al. (2007) during hypocapnia (through active hyperventilation) and hypercapnia (increased fixed F$J_{CO_2}$ inspirate). As highlighted in Figure 7, it was found that the correction algorithms proposed by Peebles et al. (2007) could not accurately predict PaCO$_2$ during the DEF protocols in normoxia and
hypoxia, and this was likely due to the different experimental conditions. However, the non-invasive equation proposed by Tymko et al. (2015a) significantly improved both slope (b[1]) and y-intercept (b[0]) during both the normoxia and hypoxia CO2 reactivity protocols when plotting $P_{ETCO2}$ vs $PaCO2$, suggesting that this algorithm is additionally useful when predicting $PaCO2$ based on $P_{ETCO2}$ during a CO2 reactivity test during DEF, and specifically advantageous as it does not require invasive measures.

In addition to quantifying the performance of our previously derived $PaCO2$ prediction algorithms, we ran multiple stepwise regression analysis in the current data set to see how the ‘ideal’ prediction equations between studies compared (see equations 3-6). The $PaCO2$ prediction algorithms were similar to each other between normoxia and hypoxia trials, which were not surprising since there was no statistical difference in the $Pa-P_{ETCO2}$ gradient between the two reactivity protocols. The non-invasive $PaCO2$ prediction equation derived from our previous study (equation 1) and the new non-invasive $PaCO2$ prediction equations (equations 3 and 5) were similar in performance ($R^2 = 0.95$ versus 0.95). However, in the current data set, $VT$ also contributed significantly to the prediction of $PaCO2$ (equations 4 and 6), which is similar to previous work during exercise (16). Reasoning for not previously finding $VT$ as a significant variable for non-invasively predicting $PaCO2$ could have been due to our smaller sample size in our previous study.

**Methodological considerations.** One of the prediction algorithms we used (see equation 2) showed promise in predicting $PaCO2$ based on $P_{ETCO2}$ and the individual’s baseline $Pa-P_{ETCO2}$ gradient. Although this equation proved to be valuable by considering the $Pa-P_{ETCO2}$ gradient, it may not be as impactful since it involves the collection of a reliable baseline arterial blood measurement (i.e. arterial puncture) while simultaneously collecting respiratory measurements to calculate the $Pa-P_{ETCO2}$ gradient. The latter being improbable to achieve due to the likelihood of participant hyperventilation from discomfort. In the event where arterial blood measurements are not possible, our non-invasive $PaCO2$ correction algorithm also showed utility in the current study (see equation 1).

A second consideration was our method of measuring body temperature. Traditionally, core body temperature is usually measured using esophageal or rectal body temperature. Given the nature of the experimental protocols where the CO2 stages were only
four minutes in length, it is likely that any changes in core body temperature would not be reflected in rectal temperature due to time delay. Initially, our methodology involved measuring esophageal temperature, however, due to the length of our experimental protocol (~6-hours) we found that nasopharyngeal temperature was a more appropriate measure to minimize participant discomfort. Nasopharyngeal temperature has been considered a reliable measure of core body temperature (31), and likely a good measure of brain temperature as the ICA is located nearby (20).

Perhaps the most concerning criticism of our methodology was the reliability of the arterial blood gas measurements. The root mean squared coefficient of variation was 1.45% and 1.66% between two consecutive arterial blood gas measurements (n=28) taken on the exact same sample for PaCO2 and PaO2, respectively. The approximate variation between the two measurements with our blood analyzer was ±2 mmHg and ±4 mmHg for PaCO2 and PaO2, respectively. The magnitude of the mean Pa-PETCO2 gradient is well within the variability of the blood gas machine, but the mean bias of the Pa-PETCO2 gradient was significantly different from zero, meaning that these end-tidal-to-arterial gradients do exist and are not a product of instrument error.

**Conclusion.** The correction algorithms derived are specific to the CO2 and O2 protocols employed, and possibly only our DEF system. However, these previously derived algorithms have now been validated in the current study in an independent sample. Although the Pa-PETCO2 gradient was not different between normoxia and acute normobaric hypoxia, our results demonstrate that data interpretation of both HCVR and cerebrovascular reactivity to CO2 is misleading when indexed against PETCO2 compared to PaCO2 (true stimulus). In light of these findings, the end-tidal-to-arterial gas gradients must be considered when performing common ventilatory and vascular reactivity protocols while using DEF. In addition to this, we confirmed that a small PFO has little effect on the end-tidal-to-arterial gradients. It is prudent to have a complete understanding of the methodological limitations of DEF, a device commonly used in human physiology research, it is now known that the ability to control end-tidal gases using DEF is not hindered by the presence of a small PFO.
**Author Contributions:** Conception and design of experiments: MMT and GEF. Data Collection: MMT, RLH, TK, LMB, JCT, AMW, and GEF. Data analysis and interpretation: MMT, LMB, GEF, and BKP. Manuscript first draft: MMT and GEF. Critical revisions of manuscript for important intellectual content: MMT, RLH, TK, LMB, JCT, BKP, AMW and GEF. Approval of final draft: MMT, RLH, TK, LMB, JCT, BKP, AMW and GEF.

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**Disclosures:** The authors have no conflict of interest
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10.111/sms.12545


Table 1: Comparison of ventilatory and arterial blood gas data between NX and HX CO2 reactivity tests.

<table>
<thead>
<tr>
<th>Trial</th>
<th>AB</th>
<th>AB/HX-DEF</th>
<th>-8 mmHg</th>
<th>-4 mmHg</th>
<th>0 mmHg</th>
<th>+4 mmHg</th>
<th>+8 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NX vs HX: P&lt;0.001; Stage: P&lt;0.001; Interaction: P&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>PICO2 (mmHg)</td>
<td>NX</td>
<td>3.8 ± 0.7*</td>
<td>19.1 ± 2.1</td>
<td>12.3 ± 1.7*</td>
<td>19.1 ± 1.3</td>
<td>22.1 ± 1.2</td>
<td>33.1 ± 1.1*</td>
</tr>
<tr>
<td></td>
<td>HX</td>
<td>4.2 ± 0.6*</td>
<td>27.9 ± 0.7‡</td>
<td>15.1 ± 1.4*</td>
<td>20.7 ± 1.2*</td>
<td>28.8 ± 0.7</td>
<td>36.5 ± 0.5‡*</td>
</tr>
<tr>
<td>PETCO2 (mmHg)</td>
<td>NX</td>
<td>40.2 ± 0.7</td>
<td>40.7 ± 0.7</td>
<td>33.3 ± 0.9*</td>
<td>36.9 ± 0.9*</td>
<td>41.4 ± 0.7</td>
<td>44.4 ± 0.7*</td>
</tr>
<tr>
<td></td>
<td>HX</td>
<td>40.0 ± 0.6</td>
<td>40.0 ± 0.7</td>
<td>32.3 ± 0.7*</td>
<td>36.4 ± 0.7*</td>
<td>40.1 ± 0.6</td>
<td>43.5 ± 0.7*</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>NX</td>
<td>139.0 ± 0.9*</td>
<td>125.4 ± 2.5‡</td>
<td>119.5 ± 1.6‡</td>
<td>121.1 ± 1.5‡</td>
<td>122.8 ± 1.3</td>
<td>116.4 ± 1.6‡*</td>
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<tr>
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<td>HX</td>
<td>138.2 ± 0.6*</td>
<td>68.9 ± 1.0</td>
<td>73.7 ± 1.5</td>
<td>73.7 ± 1.5</td>
<td>68.6 ± 0.9</td>
<td>63.8 ± 0.5</td>
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<td>Pa-PETCO2 (mmHg)</td>
<td>NX</td>
<td>95.6 ± 1.1</td>
<td>94.3 ± 1.3‡</td>
<td>94.2 ± 1.1‡</td>
<td>93.5 ± 1.2‡</td>
<td>93.5 ± 1.1</td>
<td>94.4 ± 1.0‡</td>
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<td>HX</td>
<td>94.4 ± 0.6*</td>
<td>50.8 ± 0.1</td>
<td>50.6 ± 0.3</td>
<td>50.8 ± 0.1</td>
<td>50.8 ± 0.1</td>
<td>50.9 ± 0.1</td>
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<tr>
<td>PETO2 (mmHg)</td>
<td>NX</td>
<td>88.5 ± 1.4</td>
<td>90.8 ± 1.4‡</td>
<td>90.1 ± 1.4‡</td>
<td>89.8 ± 1.4‡</td>
<td>88.8 ± 1.8</td>
<td>91.5 ± 1.1‡</td>
</tr>
<tr>
<td></td>
<td>HX</td>
<td>87.4 ± 1.1*</td>
<td>44.1 ± 0.5</td>
<td>45.0 ± 0.6</td>
<td>44.4 ± 0.3</td>
<td>44.1 ± 0.7</td>
<td>45.0 ± 0.4</td>
</tr>
<tr>
<td>PET-PaO2 (mmHg)</td>
<td>NX</td>
<td>7.0 ± 0.8†</td>
<td>3.5 ± 0.9†</td>
<td>4.1 ± 0.9†</td>
<td>3.5 ± 0.8†</td>
<td>4.7 ± 0.9</td>
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<tr>
<td></td>
<td>HX</td>
<td>6.9 ± 1.0†</td>
<td>6.7 ± 0.5†</td>
<td>5.6 ± 0.5†</td>
<td>6.4 ± 0.3†</td>
<td>6.7 ± 0.7</td>
<td>5.9 ± 0.4†</td>
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</tbody>
</table>

Definition of Abbreviations: PICO2, partial pressure of inspired CO2; PETCO2, end-tidal partial pressure of CO2; PaCO2, arterial partial pressure of CO2; Pa-PETCO2, end-tidal-to-arterial CO2 difference; PTO2, partial pressure of inspired O2; PETO2, end-tidal partial pressure of O2; PaO2, arterial partial pressure of O2; PET-PaO2, end-tidal-to-arterial O2 difference. *P<0.05, compared to AB/HX-DEF. ‡P<0.05, interaction effect between NX and HX. Bolded HX or NX, main effect between NX and HX, bolded value is significantly larger. †P<0.05, presence of an end-tidal-to-arterial gradient. Values are mean ± SEM.
Table 2: Comparison of ventilatory cardiovascular, and cerebrovascular data between NX1 and HX1 CO2 reactivity tests.

<table>
<thead>
<tr>
<th>Trial</th>
<th>AB</th>
<th>AB/HX-DEF</th>
<th>-8 mmHg</th>
<th>-4 mmHg</th>
<th>0 mmHg</th>
<th>+4 mmHg</th>
<th>+8 mmHg</th>
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<tr>
<td>V̇E</td>
<td>NX1</td>
<td>12.4 ± 0.5</td>
<td>16.9 ± 1.0</td>
<td>22.2 ± 1.3</td>
<td>20.6 ± 1.1</td>
<td>17.7 ± 0.8</td>
<td>26.5 ± 2.2*</td>
</tr>
<tr>
<td>(l/min)</td>
<td>HX1</td>
<td>12.8 ± 0.5</td>
<td>27.4 ± 1.5*</td>
<td>22.2 ± 1.2</td>
<td>22.2 ± 1.3</td>
<td>26.7 ± 1.3</td>
<td>40.5 ± 2.6‡*</td>
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<tr>
<td>VT</td>
<td>NX1</td>
<td>0.9 ± 0.0*</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.1*</td>
</tr>
<tr>
<td>(l)</td>
<td>HX1</td>
<td>0.9 ± 0.0*</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>2.3 ± 0.1*</td>
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<tr>
<td>FB</td>
<td>NX1</td>
<td>14.3 ± 1.0</td>
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<td>15.8 ± 2.1</td>
<td>14.8 ± 1.1</td>
<td>17.3 ± 2.0</td>
</tr>
<tr>
<td>(/min)</td>
<td>HX1</td>
<td>15.1 ± 0.9</td>
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<td>16.0 ± 1.9</td>
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<tr>
<td>HR</td>
<td>NX1</td>
<td>61.3 ± 2.5*</td>
<td>63.7 ± 2.5</td>
<td>66.8 ± 2.2</td>
<td>65.1 ± 1.9*</td>
<td>64.1 ± 2.5</td>
<td>69.6 ± 2.9</td>
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<tr>
<td>(/min)</td>
<td>HX1</td>
<td>61.5 ± 2.4*</td>
<td>76.0 ± 2.7</td>
<td>73.2 ± 2.6</td>
<td>70.5 ± 2.9*</td>
<td>72.8 ± 2.6</td>
<td>77.1 ± 2.8</td>
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<tr>
<td>MAP</td>
<td>NX1</td>
<td>83.9 ± 2.1</td>
<td>85.2 ± 1.8</td>
<td>85.2 ± 1.7</td>
<td>86.1 ± 1.7</td>
<td>87.4 ± 1.6</td>
<td>91.8 ± 2.2</td>
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<tr>
<td>(mmHg)</td>
<td>HX1</td>
<td>86.5 ± 1.7</td>
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<td>87.8 ± 2.1</td>
<td>90.5 ± 2.0</td>
<td>93.0 ± 2.0</td>
<td>95.9 ± 2.0</td>
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<tr>
<td>MCAv</td>
<td>NX1</td>
<td>61.1 ± 2.7</td>
<td>64.5 ± 3.1</td>
<td>50.8 ± 2.1*</td>
<td>57.1 ± 2.5</td>
<td>65.6 ± 3.1</td>
<td>72.3 ± 3.9*</td>
</tr>
<tr>
<td>(cm/s)</td>
<td>HX1</td>
<td>61.8 ± 3.0</td>
<td>68.1 ± 2.8</td>
<td>53.3 ± 2.0*</td>
<td>61.1 ± 2.4</td>
<td>69.6 ± 2.8</td>
<td>81.9 ± 3.3*</td>
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<tr>
<td>Q̇ICA</td>
<td>NX1</td>
<td>265.5 ± 15.8</td>
<td>277.7 ± 11.9</td>
<td>203.5 ± 12.1*</td>
<td>234.3 ± 13.8</td>
<td>289.3 ± 14.7</td>
<td>334.9 ± 19.7*</td>
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<tr>
<td>(ml/min)</td>
<td>HX1</td>
<td>278.1 ± 14.4</td>
<td>312.1 ± 15.6</td>
<td>229.4 ± 11.0*</td>
<td>268.2 ± 11.6</td>
<td>326.5 ± 18.0</td>
<td>407.0 ± 26.4*</td>
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<tr>
<td>Nasal Temp</td>
<td>NX1</td>
<td>36.5 ± 0.1</td>
<td>36.5 ± 0.1</td>
<td>36.5 ± 0.1</td>
<td>36.5 ± 0.1</td>
<td>36.5 ± 0.1</td>
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<tr>
<td>(°C)</td>
<td>HX1</td>
<td>36.5 ± 0.1</td>
<td>36.3 ± 0.1</td>
<td>36.5 ± 0.1</td>
<td>36.4 ± 0.1</td>
<td>36.3 ± 0.1</td>
<td>36.2 ± 0.1</td>
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<tr>
<td>SaO2</td>
<td>NX1</td>
<td>97.7 ± 0.2</td>
<td>97.7 ± 0.3‡</td>
<td>98.2 ± 0.2‡</td>
<td>97.9 ± 0.3‡</td>
<td>97.6 ± 0.3</td>
<td>97.6 ± 0.2‡</td>
</tr>
<tr>
<td>(%)</td>
<td>HX1</td>
<td>97.9 ± 0.2*</td>
<td>83.5 ± 0.6</td>
<td>87.1 ± 0.4*</td>
<td>84.7 ± 0.5</td>
<td>83.0 ± 0.5</td>
<td>82.1 ± 0.6</td>
</tr>
</tbody>
</table>

Definition of Abbreviations: V̇E, minute ventilation; V̇T, tidal volume; FB, breathing frequency; HR, heart rate; MAP, mean arterial pressure; MCAv, middle cerebral artery blood flow velocity; Q̇ICA, internal carotid artery blood flow; SaO2, oxygen saturation of hemoglobin. *P<0.05, compared to AB/HX-DEF. ‡P<0.05, interaction effect between NX and HX. Bolded HX or NX, main effect between NX and HX, bolded value is significantly larger. Values are mean ± SEM.
Table 3: Ventilatory and arterial blood gas data between PFO+ and PFO- participants during NX CO2 reactivity test.

<table>
<thead>
<tr>
<th></th>
<th>NX</th>
<th>AB</th>
<th>AB-DEF</th>
<th>-8 mmHg</th>
<th>-4 mmHg</th>
<th>0 mmHg</th>
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<th>+8 mmHg</th>
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<td><strong>V̇ E (l/min)</strong></td>
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<tr>
<td>PFO+</td>
<td>12.5 ± 0.8</td>
<td>16.2 ± 1.4</td>
<td>21.6 ± 2.0</td>
<td>20.3 ± 1.6</td>
<td>16.8 ± 0.7</td>
<td>25.1 ± 2.1*</td>
<td>34.5 ± 3.0*</td>
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</tr>
<tr>
<td>PFO-</td>
<td>12.3 ± 0.7</td>
<td>17.7 ± 1.4</td>
<td>22.8 ± 1.7</td>
<td>20.9 ± 1.5</td>
<td>18.5 ± 1.3</td>
<td>27.8 ± 3.9*</td>
<td>43.3 ± 5.3*</td>
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<tr>
<td><strong>PETCO2 (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>39.3 ± 0.8*</td>
<td>40.7 ± 0.8</td>
<td>32.4 ± 1.0*</td>
<td>36.8 ± 0.8*</td>
<td>41.0 ± 0.8</td>
<td>44.8 ± 0.8*</td>
<td>48.8 ± 0.8*</td>
<td></td>
</tr>
<tr>
<td>PFO-</td>
<td>39.7 ± 1.2*</td>
<td>41.3 ± 1.0</td>
<td>33.2 ± 1.0*</td>
<td>37.3 ± 1.1*</td>
<td>41.3 ± 1.1</td>
<td>45.3 ± 1.0*</td>
<td>49.3 ± 1.1*</td>
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<td><strong>PaCO2 (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>40.2 ± 0.9</td>
<td>40.6 ± 0.8</td>
<td>32.6 ± 1.0*</td>
<td>36.6 ± 1.1*</td>
<td>41.5 ± 0.8</td>
<td>44.1 ± 0.9*</td>
<td>47.8 ± 0.8*</td>
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</tr>
<tr>
<td>PFO-</td>
<td>40.2 ± 1.2</td>
<td>40.7 ± 1.2</td>
<td>34.0 ± 1.3*</td>
<td>37.1 ± 1.4*</td>
<td>41.3 ± 1.1</td>
<td>44.6 ± 1.1*</td>
<td>48.1 ± 1.3*</td>
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<tr>
<td><strong>Pa-PETCO2 (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>0.8 ± 0.5†*</td>
<td>0.0 ± 0.2</td>
<td>0.2 ± 0.3</td>
<td>-0.2 ± 0.4</td>
<td>0.6 ± 0.4</td>
<td>-0.7 ± 0.2†</td>
<td>-1.0 ± 0.2†</td>
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<tr>
<td>PFO-</td>
<td>0.6 ± 0.4†*</td>
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<td>-0.2 ± 0.4</td>
<td>0.0 ± 0.2</td>
<td>-0.7 ± 0.3†</td>
<td>-1.3 ± 0.3†</td>
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<td><strong>PETO2 (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>95.1 ± 1.2</td>
<td>93.3 ± 1.2</td>
<td>93.8 ± 1.1</td>
<td>92.4 ± 0.9</td>
<td>92.7 ± 0.8</td>
<td>93.7 ± 0.7</td>
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<tr>
<td>PFO-</td>
<td>96.0 ± 1.7</td>
<td>95.4 ± 2.2</td>
<td>94.5 ± 1.9</td>
<td>94.2 ± 2.2</td>
<td>94.3 ± 1.9</td>
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<td>95.1 ± 1.8</td>
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<td><strong>PaO2 (mmHg)</strong></td>
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<td>PFO+</td>
<td>86.9 ± 1.7</td>
<td>88.0 ± 1.3</td>
<td>88.4 ± 1.5</td>
<td>88.0 ± 1.4</td>
<td>86.5 ± 1.6</td>
<td>90.6 ± 0.9</td>
<td>91.0 ± 0.9</td>
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<tr>
<td>PFO-</td>
<td>90.2 ± 2.1</td>
<td>93.6 ± 2.0</td>
<td>91.8 ± 2.0</td>
<td>91.7 ± 2.3</td>
<td>91.1 ± 2.9</td>
<td>92.4 ± 2.0</td>
<td>93.5 ± 2.0</td>
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<tr>
<td><strong>PET-PaO2 (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>8.2 ± 1.1†*</td>
<td>5.3 ± 1.0†</td>
<td>5.4 ± 1.2†</td>
<td>4.4 ± 1.0†</td>
<td>6.2 ± 1.0</td>
<td>3.1 ± 0.4†</td>
<td>2.5 ± 0.7†</td>
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</tr>
<tr>
<td>PFO-</td>
<td>5.8 ± 0.9†*</td>
<td>1.8 ± 1.0†</td>
<td>2.7 ± 1.2†</td>
<td>2.5 ± 1.1†</td>
<td>3.2 ± 1.3</td>
<td>2.7 ± 1.1†</td>
<td>1.6 ± 0.5†</td>
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</tr>
</tbody>
</table>

Definition of Abbreviations: V̇ E, minute ventilation; PETCO2, end-tidal partial pressure of CO2; PaCO2, arterial partial pressure of CO2; Pa-PETCO2, end-tidal-to-arterial CO2 difference; PETO2, end-tidal partial pressure of O2; PaO2, arterial partial pressure of O2; PET-PaO2, end-tidal-to-arterial O2 difference. *P<0.05, main effect of CO2 stage vs AB-DEF. Bolded PFO+, main effect between PFO+ and PFO-, bolded value is significantly larger. †P<0.05, presence of an end-tidal-to-arterial gradient. Values are mean ± SEM.
Table 4: Ventilatory and arterial blood gas data between PFO+ and PFO- participants during HX CO₂ reactivity test.

<table>
<thead>
<tr>
<th></th>
<th>HX</th>
<th>AB</th>
<th>HX-DEF</th>
<th>-8 mmHg</th>
<th>-4 mmHg</th>
<th>0 mmHg</th>
<th>+4 mmHg</th>
<th>+8 mmHg</th>
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</thead>
<tbody>
<tr>
<td><strong>VE (l/min)</strong></td>
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<tr>
<td>PFO+</td>
<td>12.7±0.6*</td>
<td>27.8±2.5</td>
<td>21.4±1.6</td>
<td>22.6±1.6</td>
<td>28.5±1.7</td>
<td>41.7±3.7*</td>
<td>55.0±5.0*</td>
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<tr>
<td>PFO-</td>
<td>13.0±0.8*</td>
<td>26.9±1.4</td>
<td>23.1±1.8</td>
<td>21.7±2.2</td>
<td>24.4±1.4</td>
<td>39.1±3.4*</td>
<td>58.3±7.5*</td>
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<tr>
<td><strong>PETCO₂ (mmHg)</strong></td>
<td></td>
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<tr>
<td>PFO+</td>
<td>38.6±0.6*</td>
<td>39.9±0.7</td>
<td>31.4±0.7*</td>
<td>35.8±0.7*</td>
<td>39.8±0.7</td>
<td>43.8±0.7*</td>
<td>47.8±0.7*</td>
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<tr>
<td>PFO-</td>
<td>39.9±1.0*</td>
<td>40.7±0.9</td>
<td>32.5±1.0*</td>
<td>36.8±1.0*</td>
<td>40.9±1.0</td>
<td>44.7±0.9*</td>
<td>48.9±1.0*</td>
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<tr>
<td><strong>PaCO₂ (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>39.9±0.7</td>
<td>39.6±0.8</td>
<td>31.8±1.0*</td>
<td>35.8±0.8*</td>
<td>39.7±0.7</td>
<td>43.2±0.9*</td>
<td>47.1±0.9*</td>
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<tr>
<td>PFO-</td>
<td>40.2±1.2</td>
<td>40.6±1.1</td>
<td>33.0±1.0*</td>
<td>37.2±1.1*</td>
<td>40.6±1.0</td>
<td>43.8±1.0*</td>
<td>47.7±0.9*</td>
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<tr>
<td><strong>Pa-PETCO₂ (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>1.3±0.4†</td>
<td>-0.3±0.3</td>
<td>0.3±0.4</td>
<td>0.0±0.2</td>
<td>-0.1±0.3</td>
<td>-0.6±0.4†</td>
<td>-0.7±0.5†</td>
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<tr>
<td>PFO-</td>
<td>0.4±0.4‡</td>
<td>-0.1±0.4</td>
<td>0.5±0.5</td>
<td>0.4±0.4</td>
<td>-0.3±0.2</td>
<td>-0.9±0.4‡</td>
<td>-1.2±0.4‡</td>
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<tr>
<td><strong>PETO₂ (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>94.2±0.6*</td>
<td>50.9±0.1</td>
<td>50.5±0.5</td>
<td>51.0±0.2</td>
<td>50.9±0.1</td>
<td>50.8±0.1</td>
<td>50.9±0.1</td>
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<tr>
<td>PFO-</td>
<td>94.7±1.2*</td>
<td>50.7±0.2</td>
<td>50.7±0.3</td>
<td>50.6±0.4</td>
<td>50.7±0.2</td>
<td>51.0±0.1</td>
<td>51.0±0.2</td>
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<tr>
<td><strong>PaO₂ (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>86.7±0.7*</td>
<td>43.7±0.7</td>
<td>45.2±0.8</td>
<td>44.8±0.2</td>
<td>43.6±0.7</td>
<td>44.5±0.5</td>
<td>44.3±0.7</td>
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<tr>
<td>PFO-</td>
<td>88.5±2.4*</td>
<td>44.6±0.6</td>
<td>44.7±1.1</td>
<td>43.8±0.4</td>
<td>44.7±1.1</td>
<td>45.7±0.6</td>
<td>45.7±0.6</td>
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<tr>
<td><strong>PET-PaO₂ (mmHg)</strong></td>
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<tr>
<td>PFO+</td>
<td>7.5±0.7†</td>
<td>7.1±0.7†</td>
<td>5.4±0.4†</td>
<td>6.2±0.3†</td>
<td>7.3±0.7</td>
<td>6.3±0.5†</td>
<td>6.6±0.8†</td>
<td></td>
</tr>
<tr>
<td>PFO-</td>
<td>6.2±2.0†</td>
<td>6.1±0.6†</td>
<td>6.0±1.0†</td>
<td>6.8±0.6†</td>
<td>6.0±1.3</td>
<td>5.3±0.5†</td>
<td>5.3±0.8†</td>
<td></td>
</tr>
</tbody>
</table>

Definition of Abbreviations: VE, minute ventilation; PETCO₂, end-tidal partial pressure of CO₂; PaCO₂, arterial partial pressure of CO₂; Pa-PETCO₂, end-tidal-to-arterial CO₂ difference; PETO₂, end-tidal partial pressure of O₂; PaO₂, arterial partial pressure of O₂; PET-PaO₂, end-tidal-to-arterial O₂ difference. *P<0.05, main effect of CO₂ stage vs AB-DEF. †P<0.05, presence of an end-tidal-to-arterial gradient. Values are mean ± SEM.
Figure 1: Bland and Altman plot for agreement between PaCO2 and PETCO2 during NX (Panel A) and HX (Panel B) CO2 reactivity protocols. (o), -8 mmHg; (▼), -4 mmHg; (∆), 0 mmHg; (■), +4 mmHg; (□), +8 mmHg. Dotted lines represent the 95% confidence intervals, and the continuous lines represent the mean bias.

Figure 2: Bland and Altman plot for agreement between PaO2 and PETO2 during NX (Panel A) and HX (Panel B) CO2 reactivity protocols. (o), -8 mmHg; (▼), -4 mmHg; (∆), 0 mmHg; (■), +4 mmHg; (□), +8 mmHg. Dotted lines represent the 95% confidence intervals, and the continuous lines represent the mean bias.

Figure 3: Analysis of HCVR, relative Q̇ ICA and relative MCAv CVR to CO2 for one subject during NX and HX CO2 reactivity protocols. Dashed lines represent linear regression analysis in the hypocapnic range, solid lines represent linear regression analysis in the hypercapnic range.

Figure 4: Hypercapnic ventilatory reactivity data during NX (Panel A) and HX (Panel B) protocols. (black bar), HCVR indexed against PETCO2; (white bar), HCVR indexed against PaCO2; (grey bar), HCVR indexed against *Pred-PaCO2; (dark grey bar), HCVR indexed against Pred-PaCO2. Mean R² values for NX-HCVR was 0.96 ± 0.02 for PETCO2 HCVR, 0.94 ± 0.00 for PaCO2 HCVR, 0.95 ± 0.02 for *Pred-PaCO2 HCVR, and 0.96 ± 0.02 for Pred-PaCO2 HCVR. For the HX protocol the mean R² values for HCVR was 0.99 ± 0.01 for PETCO2 HCVR, 0.98 ± 0.01 for PaCO2 HCVR, 0.97 ± 0.01 for *Pred-PaCO2 HCVR, and 0.99 ± 0.01 for Pred-PaCO2 HCVR. *P<0.05, PaCO2 HCVR vs. PETCO2, *Pred-PaCO2, and Pred-PaCO2 HCVR. †P<0.05, vs. PETCO2 HCVR.

Figure 5: Q̇ ICA hypocapnia and hypercapnia reactivity data during NX (Panel A) and HX (Panel B) protocols. (black bar), ICA reactivity indexed against PETCO2; (white bar), ICA reactivity indexed against PaCO2; (grey bar), ICA reactivity indexed against *Pred-PaCO2; (dark grey bar), ICA reactivity indexed against Pred-PaCO2. For the NX protocol mean R² values for HYPO-ICA CVR was 0.93 ± 0.03 for PETCO2 CVR, 0.93 ± 0.03 for PaCO2 CVR, and 0.93 ± 0.03 for Pred-PaCO2 CVR. The mean R² values for NX HYPER-ICA CVR was 0.89 ± 0.02 for PETCO2 CVR, 0.90 ± 0.02 for PaCO2 CVR, and 0.89 ± 0.02 for Pred-PaCO2 CVR. For the HX protocol the mean R² values for HYPO-ICA CVR was 0.93 ± 0.02 for PETCO2 CVR, 0.92 ± 0.02 for PaCO2 CVR, and 0.93 ± 0.02 for Pred-PaCO2 CVR. The mean R² values for HX HYPER-ICA CVR was 0.96 ± 0.02 for PETCO2 CVR, 0.97 ± 0.02 for PaCO2 CVR, and 0.98 ± 0.03 for Pred-PaCO2 CVR. *P<0.05, PaCO2 HYPER-Q̇ ICA vs PETCO2, *Pred-PaCO2, and Pred-PaCO2 HYPER- Q̇ ICA reactivity. †P<0.05, vs. PETCO2 HYPER- Q̇ ICA reactivity. ‡P<0.05, vs Pred-PaCO2 HYPER- Q̇ ICA reactivity.

Figure 6: MCAv hypocapnia and hypercapnia reactivity data during NX (Panel A) and HX (Panel B) protocols. (black bar), MCAv reactivity indexed against PETCO2; (white bar), MCAv reactivity indexed against PaCO2; (grey bar), MCAv reactivity indexed against *Pred-PaCO2; (dark grey bar), MCAv reactivity indexed against Pred-PaCO2. For the NX trail the mean R² values for HYPO-MCAv CVR was 0.98 ± 0.0 for PETCO2 CVR, 0.98 ± 0.0 for PaCO2 CVR, and
0.99 ± 0.0 for Pred-PaCO2 CVR. The mean $R^2$ values for NX HYPER-MCAv CVR was 0.96 ±
0.0 for PETCO2 CVR, 0.95 ± 0.0 for PaCO2 CVR, and 0.96 ± 0.0 for Pred-PaCO2 CVR. For the
HX trial the mean $R^2$ values for HYPO-MCAv CVR was 0.98 ± 0.01 for PETCO2 CVR, 0.98 ±
0.02 for PaCO2 CVR, and 0.98 ± 0.02 for Pred-PaCO2 CVR. The mean $R^2$ values for HX
HYPER-MCAv CVR was 0.98 ± 0.02 for PETCO2 CVR, 0.97 ± 0.02 for PaCO2 CVR, and 0.98 ±
0.02 for Pred-PaCO2 CVR.*$P<0.05$, PaCO2 HYPER-MCAv reactivity vs. PETCO2, *Pred-PaCO2,
and Pred-PaCO2 HYPER-MCAv reactivity. †$P<0.05$, vs PETCO2 HYPER-MCAv reactivity.
**$P<0.05$, PETCO2 HYPO-MCAv reactivity vs PaCO2 HYPO-MCAv reactivity.

Figure 7: Assessment of raw and non-invasively corrected PaCO2 and PETCO2 relationship
during NX and HX CO2 reactivity protocols. Data points were obtained during the last
minute of each step change in PETCO2. Panel A, represents pooled linear regression for PaCO2
and PETCO2. Panel B, represents pooled linear regression for PaCO2 and PETCO2 from the
current study data that has been adjusted using an algorithm proposed by Tymko et al. in 2015
($e_{\text{Tymko}}$PaCO2 = 0.363 + (0.958 • PETCO2)). Panel C, represents pooled linear regression for
PaCO2 and PETCO2 from the current study data that has been adjusted with an algorithm
proposed by Peebles et al. in 2007 ($e_{\text{Peebles}}$PaCO2 = 2.367 + 0.884 • PETCO2). b[1], slope; b[0], y-
intercept. Dashed line represents the linear regression. Solid line indicates a line of identity (i.e.
x = y).
Figure 1.
Figure 2.
Figure 3.

A. $R^2 = 0.99$
   $b[1] = -5.92$

B. $R^2 = 0.99$
   $b[1] = -3.99$

C. $R^2 = 0.99$
   $b[1] = 4.70$

NX

Minute ventilation (l/min) vs. $P_{aCO_2}$

Relative QCA (%) vs. $P_{aCO_2}$

Relative MCA (%) vs. $P_{aCO_2}$

HX

Minute ventilation (l/min) vs. $P_{aCO_2}$

Relative QCA (%) vs. $P_{aCO_2}$

Relative MCA (%) vs. $P_{aCO_2}$
Figure 4.
Figure 5.
Figure 6.
Figure 7.

A. $R^2 = 0.97$
   $b[1] = 1.04$
   $b[0] = -1.2$

B. $R^2 = 0.97$
   $b[1] = 0.99$
   $b[0] = -0.8$

C. $R^2 = 0.97$
   $b[1] = 0.91$
   $b[0] = 1.3$

NX

$PETCO_2$ (mmHg) vs $PaCO_2$ (mmHg)

HX

$PETCO_2$ (mmHg) vs $PaCO_2$ (mmHg)