Title: End-tidal-to-arterial CO₂ and O₂ gas gradients at low- and high-altitude during dynamic end-tidal forcing.

Authors: Michael M. Tymko¹
Philip N. Ainslie¹
David B. MacLeod²
Chris K. Willie¹
Glen E. Foster¹

Affiliations: ¹Centre for Heart, Lung, and Vascular Health, School of Health and Exercise Science, University of British Columbia, Kelowna, Canada.
²Department of Anesthesiology, Duke University Medical Center, Durham, NC, USA.

Correspondence: Glen E. Foster, PhD.
School of Health and Exercise Science
Faculty of Health and Social Development
University of British Columbia.
3333 University Way,
Kelowna, BC, V1V 1V7
Telephone: 250-807-8224
Fax: 250-807-9665
Email: glen.foster@ubc.ca

Running head: End-tidal-to-arterial gas gradients during end-tidal forcing
ABSTRACT

We sought to characterize and quantify the performance of a portable dynamic end-tidal forcing (DEF) system in controlling the partial pressure of arterial CO₂ (P_{aCO₂}) and O₂ (P_{aO₂}) at low- (LA; 344m) and high-altitude (HA; 5050m) during an isooxic CO₂ test, and an isocapnic O₂ test, commonly used to measure ventilatory and vascular reactivity in humans (n=9). The isooxic CO₂ tests involved step changes in the partial pressure of end-tidal carbon dioxide (P_{ETCO₂}) of -10, -5, 0, +5 and +10 mmHg from baseline. The isocapnic O₂ test consisted of a 10-min hypoxic step (P_{ETO₂}=47mmHg) from baseline at LA, and a 5-min euoxic step (P_{ETO₂}=100mmHg) from baseline at HA. At both altitudes, P_{ETO₂} and P_{ETCO₂} were controlled within narrow limits (<1mmHg from target) during each protocol. During the isooxic CO₂ test at LA, P_{ETCO₂} consistently overestimated P_{aCO₂} (P<0.01) at both baseline (2.1±0.5mmHg) and hypercapnia (+5mmHg: 2.1±0.7mmHg; +10mmHg: 1.9±0.5mmHg). This P_{a}-P_{ETCO₂} gradient was approximately two-fold greater at HA (P<0.05). At baseline at both altitudes, P_{ETO₂} overestimated P_{aO₂} by a similar extent (LA: 6.9±2.1mmHg; HA: 4.5±0.9mmHg; both P<0.001). This overestimation persisted during isocapnic hypoxia at LA (6.9±0.6mmHg), and during isocapnic euoxia at HA (3.8±1.2mmHg). Step-wise multiple regression analysis, on the basis of the collected data, revealed that it may be possible to predict an individual’s arterial blood gases during DEF. Future research is needed to validate these prediction algorithms, and determining the implications of end-tidal-to-arterial gradients in the assessment of ventilatory and/or vascular reactivity.

Key words: End-tidal forcing, high-altitude, gas exchange
INTRODUCTION

The partial pressure of end-tidal gas, measured at the relatively flat portion of the expiratory phase, is often used as a surrogate for alveolar gas and considered a reasonable index for the partial pressure of arterial blood. Dynamic end-tidal forcing (DEF) provides a method of altering arterial blood gases by means of controlling end-tidal gases – an approach that has been used for investigating the control of breathing (10, 19) and cerebral (30, 47), pulmonary (46), and cardiac (5) vascular function. The DEF approach uses predictive feed-forward algorithms, feedback information, and error reduction algorithms to control the end-tidal partial pressure of carbon dioxide ($P_{ETCO_2}$) and the end-tidal partial pressure of oxygen ($P_{ETO_2}$) by adjusting the necessary fraction of inspired CO$_2$ ($F_{ICO_2}$) and O$_2$ ($F_{IO_2}$) on a breath-by-breath basis.

Several different DEF systems have been developed over the past few decades, each with their own strengths and weaknesses (21, 36, 39, 42). Most recently, a compact portable DEF system by Koehle et al. (21) has been developed. This system is portable and involves filling medical grade CO$_2$, N$_2$ and “air” into a reservoir bag by the time-dependent control of three gas solenoid valves (one for each mixing component). Similar to this DEF system (21), we recently developed a DEF system capable of controlling end-tidal gases while being portable. This system uses a mixture of three gases: O$_2$, CO$_2$, and N$_2$ to deliver the desired inspiratory gas volume into a 6L capacity reservoir bag. This DEF system has effectively controlled end-tidal gases independent of breathing frequency during isocapnic and poikilocapnic hypoxia (14, 32), and during a hyperthermic intervention to correct marked hypocapnia (1).

A common pitfall with DEF systems, first recognized by Swanson & Bellville (42), is the assumption that end-tidal gas partial pressures accurately represent arterial blood gas pressures in all conditions (arterial blood gases being the stimulus for changes in vascular tone and chemoreflex activity). However, even in otherwise healthy individuals, there is a gradient between end-tidal and arterial blood gases likely due to a combination of deadspace mixing (alveolar and anatomical), ventilation-perfusion ($V/Q$) mismatching, diffusion limitation, intrapulmonary and intracardiac shunting (11, 24, 41). The end-tidal-
to-arterial $P_{CO_2}$ gradient ($P_a-P_{ETCO_2}$) is usually positive, but can be altered with body position (4) and age (27). Moreover, in some cases this gradient can be negative (i.e. $P_{ETCO_2}>P_{aCO_2}$) with exercise (18), and low breathing frequencies (17). Caution has been advised by Robbins et al. (35) when interpreting $P_{ETCO_2}$ during hypercapnic conditions with DEF, as administered CO$_2$ occupies physiological deadspace during inspiration and mixes with alveolar gas during expiration, inflating $P_{ETCO_2}$ to a greater extent than $P_{aCO_2}$ (17, 35). The same effect can be observed with increases in apparatus deadspace (12). There is a vast amount of literature reporting CO$_2$ gradients clinically and during experimental chemosensitivity testing (6, 47, 50), and predictive correction equations for the end-tidal-to-arterial gradient have been previously derived in order to accurately predict $P_{aCO_2}$ during exercise and with fixed fraction inspirate but not DEF (18, 29). In contrast, there are few reports on the end-tidal-to-arterial $P_{O_2}$ gradient ($P_{ET-PaO_2}$; calculated as $P_{ETO_2}-P_{aO_2}$) in the context of chemoreflex or vascular (e.g., cardiac, pulmonary, cerebral, etc.) testing during DEF. It is well established that the alveolar-to-arterial difference is greater for O$_2$ compared to CO$_2$. This means that end-tidal gas may not reflect $P_{aO_2}$ accurately, in contrast to $P_{aCO_2}$ (40). Chemosensitivity and vascular function tests often manipulate O$_2$ to low levels (hypoxia), and could be potentially harmful in individuals with abnormally large $P_{ET-PaO_2}$ gradients.

Due to the compact nature of our DEF system, it was used on a high-altitude (HA) research expedition to 5050m (barometric pressure ~413 mmHg). However, it is unknown how well end-tidal gases reflect arterial blood gases at extreme HA during DEF, which is extremely important due to recent innovations in DEF systems making them more portable for HA physiology research. The strong humoral response of hypoxia elicits several physiological adaptations including heterogeneous hypoxic pulmonary vasoconstriction (HPV) (46), resulting in an increase in $\dot{V}/\dot{Q}$ ratio in some areas of the lung, which has been previously shown to increase physiological deadspace (22). In addition, because of HA-associated increases in chemosensitivity, a higher $F_{ICO_2}$ and lower $F_{IO_2}$ would be required to maintain $P_{ETCO_2}$ and $P_{ETO_2}$ during DEF (9, 37). The implications of a heightened
ventilatory sensitivity means that higher CO₂ and O₂ concentrations will occupy physiological deadspace at HA, potentially altering the end-tidal-to-arterial gas gradients.

The purpose of the present study was to quantify the end-tidal-to-arterial gas gradient for both CO₂ and O₂ at LA and HA using a portable DEF system during (1) an isooxic CO₂ protocol, and (2) an isocapnic O₂ protocol. To our knowledge, end-tidal gas control systems have not been used above 4300m, and end-tidal-to-arterial gas gradients have not been directly measured during DEF at or above 5050m (28). We hypothesized that PETCO₂ would overestimate PaCO₂ to a greater extent during a CO₂ protocol at HA, while PETO₂ would overestimate PaO₂ at a lesser extent at HA due to a reduction in the arterial-venous gas gradient.
MATERIALS AND METHODS

*Ethical approval.* All experimental procedures and protocols were approved by the clinical research ethics board at the University of British Columbia and the Nepal Health Medical Research Council, and conformed to the Declaration of Helsinki. All participants provided written informed consent prior to participation in this study. This study was part of a larger research expedition conducted in April-June in 2012 (14, 23). As such, participants took part in a number of studies conducted in Kelowna, BC and during three weeks at the Ev-K2 CNR Pyramid Laboratory located near Mt. Everest basecamp at 5050m.

*Participants.* To assess pulmonary and cardiovascular health, altitude naïve participants (n=12) were initially screened at LA (elevation = 344m, Kelowna, BC) for normal pulmonary function (FEV/FVC >0.75), normal resting blood pressure (systolic blood pressure <140 and diastolic blood pressure <90 mmHg), and were required to fill out a medical history questionnaire. Three of these subjects were unable to participate in the HA protocols due to time constraints and one case of appendicitis; these subjects were excluded from our mean data analysis. Participants (n=9; all male) included in mean data analysis were non-smokers, had no previous history of cardiovascular, cerebrovascular or respiratory diseases, were not taking any medications during testing, and had an age (mean ± SEM) of 32.0 ± 1.9 years, height of 180.0 ± 1.1 cm, weight of 81.9 ± 3.7 kg, and body mass index of 25.3 ± 1.6 kg/m². All participants took acetazolamide (125 mg, 3xday) during the trek to HA to minimize the risk of acute mountain sickness but medication was discontinued 24-hours before ascending from Pheriche (4300m) to the Pyramid lab, which was at least 5 days prior to experimentation. The 8-10 day ascent profile to 5050m has been described in detail elsewhere (14, 23, 48).

*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. Commercially available software was used to analyze ventilatory and cardiovascular variables (LabChart V7.1, ADInstruments,
Colorado Springs, CO, USA). Throughout all procedures, subjects breathed through a
mouthpiece (with nose clip), a bacteriological filter and a two-way non-rebreathing valve
(7900 series, Hans Rudolph, Shawnee, KS, USA). The resistance to airflow for this
breathing apparatus is 0.80 and 0.73 cmH2O/l/s at flow rates of 1.5 and 3.0 l/s, respectively.
Respired gas pressures were sampled at the mouth, dried with nafton tubing, and analyzed
for $P_{ETO_2}$ and $P_{ETCO_2}$ (ML206; ADinstruments, Colorado Springs, CO, USA). Gas
analyzers were calibrated before each experiment with gases of known concentration using
the same sample line used in the experiment. Measured $P_{O_2}$ and $P_{CO_2}$ 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 $P_{ETO_2}$ and $P_{ETCO_2}$. $F_{ICO_2}$ and $F_{IO_2}$ delivered to the subject for each breath was determined
by our DEF system (see below) and recorded in a text file for subsequent analysis.
Respiratory flow was measured near the mouth using a pneumotachograph (HR 800L,
HansRudolph, Shawnee, KS, USA) and a differential pressure amplifier (ML141,
ADinstruments, Colorado Springs, CO, USA), which was calibrated with a 3 l syringe at a
variety of expected flow rates. Total apparatus deadspace was measured at 250 ml.

**End-Tidal Forcing.** The $P_{ETO_2}$ and $P_{ETCO_2}$ were controlled by a portable DEF system. 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 a mixing and
humidification chamber. $P_{ETO_2}$, $P_{ETCO_2}$, tidal volume ($V_T$), breathing frequency ($F_B$), and
minute ventilation ($\dot{V}_E$) was determined for each breath online using specifically designed
software (Labview 13.0, National Instruments, Austin, TX, USA). Using feedback
information regarding $P_{ETO_2}$, $P_{ETCO_2}$, 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 $F_{ICO_2}$ and $F_{IO_2}$. While
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 (1, 14, 32). End-tidal steady-state was determined once values were within one mmHg of the desired target point for at least three consecutive breaths.

**Arterial Blood Sampling.** After local anaesthesia (2% lidocaine), a 20-gauge catheter (Radial artery catheter, Arrow International, Reading, PA, USA) was placed transcutaneously into the radial artery using ultrasound guidance and a modified Seldinger technique. 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. Before sampling, the deadspace volume was withdrawn and an arterial sample (3mL) was collected in pre-heparanized syringes (safePICO syringes, Radiometer, Copenhagen, Denmark). Air bubbles were immediately evacuated from the syringe and blood gas analysis was performed within 30-seconds of sampling with a gas analyser (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: $P_aO_2$, $P_aCO_2$, and arterial oxyhaemoglobin ($S_aO_2$). Following cannulation, all subjects rested quietly in a supine position breathing room air for at least 30-minutes to ensure normal baseline measurements prior to beginning the experimental protocol.

**Experimental Design**

This study was conducted in two parts: LA and HA investigations. Prior to each experiment, all participants abstained from exercise and alcohol for 24 hrs, and caffeine for 12 hrs. All testing was conducted while participants lay in supine position. Baseline measurements are made during the last two minutes of a five-minute period of eupnea breathing room-air.

**Low-Altitude Protocols (344m).** LA studies involved both CO$_2$-LA and O$_2$-LA protocols. A minimum of 15-minutes separated each protocol, and protocols were not randomized.
(CO₂ protocol completed first) to avoid any confounds involving carry-over effects of sympathetic nervous activity associated with exposure to acute hypoxia (49).

Protocol CO₂-LA. This protocol was selected because it can be used to assess the ventilatory and cerebral vascular response to CO₂ (47). PETO₂ was controlled immediately after baseline at 100 mmHg for the duration of the protocol. Following 5-minutes of baseline, an arterial blood gas sample was collected and then PETCO₂ was altered and controlled in a stepwise fashion at -10, -5, 0, +5, +10, and +15 mmHg from individual baseline values. Targeted PETCO₂ in the hypocapnic range was achieved through active hyperventilation and controlled by the DEF system. Each step change in PETCO₂ lasted for approximately 3-minutes after steady-state was reached and an arterial blood sample was carefully collected during the final 30-seconds of each stage.

Protocol O₂-LA. This protocol was selected because it is often used to determine both the ventilatory and vascular responses to isocapnic hypoxia (46, 47). In this protocol, PETCO₂ was controlled at +1 mmHg above baseline values for the entire duration of the protocol except for baseline. Following 5-minutes of baseline, an arterial blood sample was collected and then PETO₂ was decreased to 47 mmHg. This value (PETO₂ = 47 mmHg) was selected based on previous research at the same location showing that subjects on average have a baseline PETO₂ of 47 mmHg at 5050m and this facilitated a comparison to the baseline state in the HA condition (13). After PETO₂ reached its target value (time = 0-minutes), arterial blood samples were collected at 7- and 10-minutes.

High-Altitude Protocols. HA studies involved two DEF protocols to CO₂ and to relief of hypoxia (protocols CO₂-HA and O₂-HA respectively). A minimum of 15-minutes separated each protocol with the CO₂ protocol conducted first.

Protocol CO₂-HA. Similar to protocol CO₂-LA, CO₂-HA was used to investigate cerebral vascular and chemoreflex sensitivity to step changes in CO₂. PETO₂ was controlled at
baseline values for the entire duration of the protocol (46.8 ± 1.0 mmHg). Following a 5-minute baseline, an arterial blood sample was collected and then $P_{\text{ETCO}_2}$ was controlled in a stepwise fashion at -10, -5, 0, +5, and +10 mmHg from baseline values. Targeted $P_{\text{ETCO}_2}$ in the hypocapnic range was achieved through active hyperventilation and controlled by the DEF system. Due to heightened chemosensitivity at HA, a step change of +15 mmHg $P_{\text{ETCO}_2}$ was too uncomfortable for most participants to endure, and was therefore excluded from this protocol. Each step change in $P_{\text{ETCO}_2}$ lasted for 3-minutes after steady-state was reached and an arterial blood sample was carefully collected during the final 30-seconds of each stage.

**Protocol O2-HA.** O2-HA was used to address the changes in end-tidal to arterial gradient with the relief of environmental hypoxia. In this protocol, $P_{\text{ETCO}_2}$ was controlled at +1 mmHg above baseline values for the entire duration of the protocol. In addition, to prevent the rise in $P_{\text{ETCO}_2}$ during hypoxia relief, subjects were asked to maintain slightly elevated and driven ventilation. Following a 3-minute baseline, an arterial blood sample was collected, and then $P_{\text{ETO}_2}$ was controlled at 100 mmHg (relative hyperoxia). This value was selected to restore $P_{\text{ETO}_2}$ to LA values. After end-tidal gases were stable (time = 0-minutes), arterial blood samples were collected at 4- and 5-minutes. The O2 protocol was shorter at HA compared to LA in order to conserve limited gas supply at the field-based laboratory.

**Statistical Analysis**
To ensure an adequate power (>0.80) to detect a change in $P_a-P_{\text{ETCO}_2}$ gradient from rest to hypercapnia a sample size calculation was conducted based on the $P_{\text{ETCO}_2}$ and $P_{\text{aCO}_2}$ values reported by Peebles et al. (2007). To increase our power of detection for the comparison of LA and HA parameters and to account for the possibility of subject illness and dropout at HA the sample size was increased to n=12. All data were analysed using SigmaStat V11.5 (Systat, Chicago, IL, USA). For each protocol baseline measurements were averaged over
two minutes immediately prior to the start of the test.

During the CO₂ protocols (CO₂-LA and CO₂-HA), all measurements were averaged over a one minute period at the end of each CO₂ stage, just prior to arterial blood sampling. During the O₂ protocols (O₂-LA and O₂-HA), all measurements were averaged one minute prior to minute seven and ten at LA, and at minute four and five at HA, in association with when the arterial blood samples were collected. These two measurements, taken near the end of the O₂ protocol for both LA and HA, were subsequently averaged together.

Two-way (Altitude: LA vs HA X PĒTCO₂ step: -10, -5, 0, +5, +10) repeated measures analysis of variance (RM ANOVA) was used to compare differences (P<0.05) in respiratory, cardiovascular and arterial blood measurements between LA and HA for CO₂ tests. One-way (PĒTCO₂ step: -10, -5, 0, +5, +10, +15) RM ANOVA was used to compare differences (P<0.05) in respiratory, cardiovascular and arterial blood measurements within LA data during the CO₂ test, as the additional +15 mmHg PĒTCO₂ step at LA hindered comparisons to HA. When significant F-ratios were detected, post-hoc comparisons within LA and HA were made using Tukey’s HSD. Paired T-tests were used to determine 1) differences between LA and HA for all outcome measures during the CO₂ tests, 2) differences between end-tidal gases and arterial blood gases for both CO₂ and O₂ tests within LA and HA, and 3) differences between baseline and O₂ condition within LA (isocapnic hypoxia) and HA (isocapnic euoxia). Bland-Altman plots were used to assess the agreement between end-tidal and arterial blood gases. The 95% limits of agreement were calculated by using ±1.96 x S.D. of the bias (8). Multiple forward stepwise regression analysis was used to determine which variables could accurately predict arterial blood gases. For each condition, regression equations were determined for both PₐCO₂ and PₐO₂ as the dependent variable. The following independent variables were included in the regression analysis: V̇E, Vₜ, Fₜ, F₁O₂, F₁CO₂, PₑEO₂, PₑCO₂ and the baseline Pₑ-PₐO₂ and Pₐ-PₑCO₂ gradient. P<0.05 was used to determine the tolerance for including independent variables in the regression model. All data are expressed as mean values ± SEM.
RESULTS

CO₂-LA Test. Table 1 displays ventilatory data for the isooxic CO₂ test at both LA and HA. As expected, \( \dot{V}_E \), \( V_T \), and \( F_B \) were elevated compared to baseline throughout hypocapnia (-10 and -5 mmHg \( P_{ETCO₂} \) step) and hypercapnia (+5, +10, and +15 mmHg \( P_{ETCO₂} \) step) ranges. \( F_{ICO₂} \) was elevated, while \( F_{IO₂} \) was decreased from baseline in both hypo- and hyper-capnic ranges (P<0.05). The average difference between measured \( P_{ETCO₂} \) and target \( P_{ETCO₂} \) (target \( P_{ETCO₂} = -10, -5, 0, +5, +10, \) and +15 mmHg from baseline) was 0.6 ± 0.3 mmHg, and the difference between measured \( P_{ETO₂} \) and target \( P_{ETO₂} \) (target \( P_{ETO₂} = 100 \) mmHg) was -0.2 ± 0.1 mmHg throughout the protocol. Table 2 displays end-tidal and arterial blood gas values at baseline and during the isooxic CO₂ test at both LA and HA. At LA, significant (P<0.01) \( P_a-P_{ETCO₂} \) gradients were present at baseline, +5 mmHg and +10 mmHg \( P_{ETCO₂} \) steps. Multiple stepwise regression analysis determined that \( P_{ETCO₂} \) (P<0.001), and the \( P_a-P_{ETCO₂} \) gradient at baseline (P=0.08) were the best predictors of \( P_{aCO₂} \) during DEF at LA. Linear regression analysis identified the following equations for accurately predicting \( P_{aCO₂} \):

\[
(1) \quad P_{aCO₂} = 0.363 + (0.958 \times P_{ETCO₂})
\]
\[R^2 = 0.95; \ p<0.001\]

\[
(2) \quad P_{aCO₂} = 0.964 + (0.960 \times P_{ETCO₂}) + (0.331 \times \text{Baseline } P_a-P_{ETCO₂})
\]
\[R^2 = 0.95; \ p<0.001\]

We found that there was a \( P_{ET}-P_{aO₂} \) gradient at LA baseline (P<0.001), and +15 mmHg (P=0.02) \( P_{ETCO₂} \) step (see Table 2). The \( P_{ET}-P_{aO₂} \) gradient was reduced during DEF (i.e. during the \( P_{ETCO₂} \) steps) compared to the uncontrolled baseline (Table 2; P<0.001). Based upon these findings it was deemed unnecessary to correct for the \( P_{ET}-P_{aO₂} \) gradient during the CO₂ test at LA.
**CO₂-HA Test.** As illustrated in table 1, $V̇_E$ and $F_B$ were significantly elevated compared to baseline throughout both hypocapnic (-10 and -5 mmHg $P_{ETCO₂}$ steps) and hypercapnic (+5 and +10 mmHg $P_{ETCO₂}$ steps) ranges. $V_T$ remained unchanged during the hypocapnic range but was significantly increased during hypercapnia. $FICO₂$ was elevated from baseline at each $P_{ETCO₂}$ step with the exception of -10 mmHg, while $FIO₂$ was decreased from baseline in both hypo- and hyper-capnic ranges (P<0.05). The average difference between measured $P_{ETCO₂}$ and target $P_{ETCO₂}$ ($P_{ETCO₂} = -10, -5, 0, +5, and +10$ mmHg from baseline) was $0.4±0.2$ mmHg, and the difference between measured $P_{ETO₂}$ and target $P_{ETO₂}$ (target $P_{ETO₂} = 46.8$ mmHg) was $0.1±0.3$ mmHg throughout the protocol. Displayed in table 2, we found that there was a $Pₐ-P_{ETCO₂}$ gradient (P<0.01) not only at baseline, but also during each stage of hypocapnia and hypercapnia. In addition, throughout the CO₂ protocol the $Pₐ-P_{ETCO₂}$ gradient was significantly greater (i.e. more negative) during the hypercapnic steps compared to the hypocapnic steps (P<0.05). Multiple stepwise regression analysis determined that $P_{ETCO₂}$ (P<0.001), and the baseline $Pₐ-P_{ETCO₂}$ gradient (P<0.001) were the best predictors of $PₐCO₂$ when using DEF at HA. Using linear regression analysis including these variables the following equations were derived which accurately predict $PₐCO₂$:

\[
\begin{align*}
(3) \quad PₐCO₂ &= 0.143 + (0.861 \times P_{ETCO₂}) \\
R^2 &= 0.92; \ p<0.001 \\
(4) \quad PₐCO₂ &= 2.899 + (0.861 \times P_{ETCO₂}) + (0.675 \times \text{Baseline } Pₐ-P_{ETCO₂}) \\
R^2 &= 0.97; \ p<0.001
\end{align*}
\]

We also found that there was a $P_{ET-PₐO₂}$ gradient present during baseline (P<0.001) and all stages of hypocapnia (P<0.01) and hypercapnia (P<0.001), as seen in table 2. This gradient remained unchanged from baseline throughout the CO₂ protocol meaning that the $P_{ET-PₐO₂}$
Gradient can be corrected for during the CO₂ protocol using the individual’s baseline P_{ET-PaO₂} gradient (P<0.001).

**Comparison of CO₂-LA and CO₂-HA Tests.** Differences between HA and LA for respiratory and arterial blood gases are reported in tables 1 and 2. Breathing frequency was significantly elevated at HA compared to LA during baseline, hypocapnia, and hypercapnia. \( V_E \) and \( V_T \) were unchanged at baseline and during active hyperventilation between LA and HA, but were higher at HA during hypercapnia. The \( FICO₂ \) was significantly greater at HA compared to LA at -10 mmHg, +5 mmHg, and +10 mmHg (P<0.05). The \( FIO₂ \) was unchanged between LA and HA in the hypocapnic range, but was lower during HA in the hypercapnic range (P<0.05). The \( P_a-PETCO₂ \) gradient (P<0.05) was significantly greater (i.e. more negative) at HA compared to LA at baseline, +5, and +10 PETCO₂ steps (see table 2, and figures 1 and 2). As mentioned, there was no \( P_a-PETCO₂ \) gradient found during hypocapnia at LA, nor did we achieve a +15 mmHg PETCO₂ step at HA; thus, these comparisons were not made.

The \( PET-PaO₂ \) gradient (P<0.05) was significantly greater (i.e. more positive) at HA compared to LA during DEF at -10, -5, +5, and +10 mmHg PETCO₂ steps (see table 2).

**O₂-LA Test.** Table 3 displays ventilatory and arterial blood data during the isocapnic O₂ test at both LA and HA. \( \dot{V}_E \), \( V_T \), and \( F_B \) significantly increased from baseline during isocapnic hypoxia. We found that there was a PET-PaO₂ gradient at baseline (6.9±2.1 mmHg, P<0.001) and during isocapnic hypoxia (6.9±0.6 mmHg, P<0.001). The average difference between measured PETO₂ and target PETO₂ (target PETO₂ = 47 mmHg) was 0.1±0.2 mmHg, and the difference between measured PETCO₂ and target PETCO₂ (target PETCO₂ = +1 mmHg from baseline) was -0.4±0.3 mmHg throughout the protocol. The FIO₂ was lower during isocapnic hypoxia compared to baseline, while the FICO₂ was higher during isocapnic hypoxia compared to baseline (P<0.05). There was no difference of the PET-PaO₂
gradient between baseline and isocapnic hypoxia (see table 3). Multiple stepwise regression analysis identified $P_{ETO2}$ ($P<0.001$), and the baseline $P_{ETO2}-P_{aO2}$ gradient ($P<0.001$) as suitable predictors of $P_{aO2}$ during DEF at LA. Linear regression analysis identified the following equations for accurately predicting $P_{aO2}$:

$$P_{aO2} = -6.024 + (0.986 \times P_{ETO2})$$

$R^2 = 0.94; \ p < 0.001$

$$P_{aO2} = -2.520 + (0.995 \times P_{ETO2}) - (0.592 \times \text{Baseline } P_{ET-P_{aO2}})$$

$R^2 = 0.98; \ p < 0.001$

Whilst there was a $P_{a-PETCO2}$ gradient during the $O_2$ protocol at baseline (-2.0 ± 0.4 mmHg, $P<0.001$), this gradient was abolished during isocapnic hypoxia (table 3).

**O2-HA Test.** In table 3, $V_E$ and $V_T$ did not change from baseline after administration of isocapnic euoxia, but $F_B$ was elevated. The difference between measured $P_{ETO2}$ and target $P_{ETO2}$ (target $P_{ETO2} = 100$ mmHg) was -0.1 ± 0.2 mmHg, and the difference between measured $P_{ETCO2}$ and target $P_{ETCO2}$ (target $P_{ETCO2} = 100$ mmHg) was -0.5 ± 0.3 mmHg from our target ($P_{ETCO2} = +1$ mmHg from baseline) throughout the protocol. Also shown in table 3, we found that there was a $P_{ET-P_{aO2}}$ gradient at baseline, and during isocapnic euoxia. The $F_{IO2}$ and $F_{ICO2}$ were higher during isocapnic euoxia compared to baseline ($P<0.05$). There was no difference in the $P_{ET-P_{aO2}}$ gradient between these conditions. Multiple stepwise regression analysis identified $P_{ETO2}$ ($P<0.001$), and the baseline $P_{ET-P_{aO2}}$ gradient ($P<0.001$) as the best predictors of $P_{aO2}$ when using DEF at HA. Linear regression analysis derived the following equations which could accurately predict $P_{aO2}$:

$$P_{aO2} = -4.826 + (1.010 \times P_{ETO2})$$
R^2 = 0.99; p < 0.001

\( P_{aO_2} = -1.880 + (1.012 \times P_{ETO_2}) - (0.695 \times \text{Baseline } P_{ET-PaO_2}) \)

\( R^2 = 1.0; p < 0.001 \)

In addition, we found that there was a \( P_{a-PETCO_2} \) gradient at baseline and isocapnic euoxia, displayed in table 3. This gradient remained unchanged from baseline throughout the O_2 protocol meaning that \( P_{aCO_2} \) can be accounted for based on the individual’s \( P_{ET-PaCO_2} \) gradient during the O_2-HA protocol (P<0.001).

**Comparison of O_2-LA and O_2-HA Tests.** There were no significant differences when comparing the \( P_{ET-PaO_2} \) gradient between baseline at LA to isocapnic euoxia at HA (P=0.15), and between isocapnic hypoxia at LA to baseline at HA (P=0.08), where \( P_{ETO_2} \) values were similar to one another. It was deemed unreasonable for the O_2 tests conducted at LA and HA to be directly compared to one another outside of baseline since the two tests were fundamentally different from one another and as a result we did not conduct any other statistical analysis between LA and HA.
DISCUSSION

The primary purpose of this study was to quantify the end-tidal-to-arterial gradients for both $O_2$ and $CO_2$ at LA and, for the first time, HA during an isooxic $CO_2$ and an isocapnic $O_2$ protocol. The main findings from this study are that (1) The $P_a$-$P_{ETCO_2}$ gradient is greater at HA (i.e. more negative) compared to LA, especially during hypercapnia; (2) The $P_{ET}$-$P_{aO_2}$ gradient remained the same between LA and HA at baseline, and was unchanged during isocapnic hypoxia (at LA) and relative hyperoxia (at HA); and (3) our recently developed computer-controlled portable DEF system effectively targets $P_{ETO_2}$ and $P_{ETCO_2}$ at both LA and HA. Based upon the results from this study, correction equations were derived using multiple stepwise regression analysis. This approach was utilized in order to improve predictions of arterial blood gases at SL and HA during DEF.

A DEF system for controlling end-tidal gases at LA and HA.

Our DEF system has been previously used to control end-tidal gases in other recent studies (1, 14, 32), but its performance has not yet been formally quantified. This DEF system is inexpensive and portable making it possible to perform studies in remote HA environments as well as at LA. To our knowledge, this is the first study to use a computer controlled DEF system above 5000m (28). On average this DEF system was able to control end-tidal gases within 1-mmHg from our target end-tidal values. In addition, there were no differences in the ability of our DEF system to target $P_{ETO_2}$ and $P_{ETCO_2}$ between LA and HA environments (see Figures 1 and 3).

Relationship between $P_{ETCO_2}$ and $P_{aCO_2}$ at LA and HA.

There are a number of reported conditions where $P_{ETCO_2}$ does not accurately reflect $P_{aCO_2}$, such as: exercise, aging, body position, and in patients with abnormal lung conditions such as pulmonary oedema and pneumonia (4, 18, 25, 27, 31). In contrast to some previously reported data (7, 35), our study found that there was a negative $P_a$-$P_{ETCO_2}$ gradient at baseline similar to that reported by Peebles et al. (29). Apparatus deadspace could
potentially explain our negative $P_a-P_{ETCO_2}$ gradient at baseline, but perhaps a better explanation is that we found large negative $P_a-P_{ETCO_2}$ gradients in three of our subjects at LA shifting our mean bias enough to become statistically significant. In addition, we found a negative $P_a-P_{ETCO_2}$ during hypercapnia (+5, and +10 mmHg) at LA. No gradient presented itself in the +15 mmHg $P_{ETCO_2}$ step at LA, however the $P_a-P_{ETCO_2}$ gradient was close to significance ($P=0.071$). In contrast, at HA there was a $P_a-P_{ETCO_2}$ gradient at baseline and during both hypocapnia and hypercapnia, and these gradients were of greater magnitude (i.e. more negative) when compared to the gradients found at LA. The widening of the $P_a-P_{ETCO_2}$ gradient between LA and HA could potentially be the result of a diffusion limitation from subclinical mild HA pulmonary oedema (44). However, the participants in our study had no signs or symptoms of acute mountain sickness at the time of experimentation (experimentation took place between days 5-10 at HA). In addition, there was no change in the $P_{ET}-P_{aO_2}$ gradient between LA and HA, which further supports the belief that diffusion limitation remains unchanged. The most likely explanation for the differences in the $P_a-P_{ETCO_2}$ gradient between LA and HA is increased alveolar deadspace (26, 44). The $P_a-P_{ETCO_2}$ gradient was not only greater at HA compared to LA, but it also widened progressively (i.e. became more negative) during hypercapnia at HA, this trend can be observed in figure 2. One explanation for this phenomenon is the heightened ventilatory response at HA necessitates an increased $F_{ICO_2}$ to maintain a given $P_{ETCO_2}$ during DEF, increasing the fraction of CO$_2$ trapped in physiological and apparatus deadspace and thereby inflating the measured $P_{ETCO_2}$. In addition to chronic hypoxia leading to an increase in alveolar deadspace, there is also evidence demonstrating that administered $P_{CO_2}$ can cause small airway dilation and pulmonary vasoconstriction augmenting the HPV response, potentially further increasing physiological deadspace during hypercapnia which could explain the $P_a-P_{ETCO_2}$ gradient differences between hypocapnia and hypercapnia (2, 3, 20, 38). A controversial mechanism suggested by Gurtner & Traystman (16) for negative gas gradients observed during steady-state hypercapnia is referred to as the ‘charged membrane hypothesis’. Here, the suggested mechanism is that the generation of an
electrical negative field near the capillary endothelial surface within the lung results in
dissociation of weak acids and elevates local hydrogen ion concentration. The increase in
local hydrogen ion concentration shifts the equilibrium of the bicarbonate buffering
relationship toward an increased P\textsubscript{CO\textsubscript{2}} (16). However, this hypothesis is based on 1)
anesthetized dogs during an extreme level of hypercapnia that is not tolerated by healthy
humans, and 2) measured alveolar-to-arterial gradients, in contrast to end-tidal-to-arterial
gradients. Therefore, we speculate that our observations are more likely due to an effect of
physiological deadspace on end-tidal gas sampling.

During our isocapnic O\textsubscript{2} test, participants endured a hypoxic step change at LA and
a euoxic step change at HA, while P\textsubscript{ETCO\textsubscript{2}} was controlled at +1 mmHg above baseline
values. We discovered that there was a P\textsubscript{a}-P\textsubscript{ETCO\textsubscript{2}} gradient at LA during our uncontrolled
baseline period, but this gradient was abolished during isocapnic hypoxia. In contrast, at
HA, there was a P\textsubscript{a}-P\textsubscript{ETCO\textsubscript{2}} gradient of the same magnitude at baseline and during isocapnic
euoxia. At baseline, P\textsubscript{ETCO\textsubscript{2}} overestimated P\textsubscript{aCO\textsubscript{2}} at HA to a greater degree compared to LA.
Our data indicate that during an acute isocapnic hypoxia test at LA the P\textsubscript{a}-P\textsubscript{ETCO\textsubscript{2}} gradient is
negligible, but is maintained during hypoxia (eg. high-altitude, normobaric hypoxia).

**Differences in P\textsubscript{ETO\textsubscript{2}} and P\textsubscript{aO\textsubscript{2}} at LA and HA.**

Surprisingly, there is little literature available with respect to the P\textsubscript{ET}-P\textsubscript{aO\textsubscript{2}} gradient during
DEF at LA or HA. Although experiments investigating the alveolar-to-arterial oxygen
difference (AaDO\textsubscript{2}) between LA and HA exist, it is difficult to assess the AaDO\textsubscript{2} during
DEF necessitating the study of the P\textsubscript{ET}-P\textsubscript{aO\textsubscript{2}} gradient in its place. However, literature
regarding the AaDO\textsubscript{2} is inconsistent suggesting that the AaDO\textsubscript{2} can widen or narrow
following ascent to HA at baseline (22, 33, 45). During DEF, inspired gas concentrations
are constantly changing making it difficult to assess alveolar P\textsubscript{O\textsubscript{2}}. The P\textsubscript{ET}-P\textsubscript{aO\textsubscript{2}} gradient
differs from the AaDO\textsubscript{2} because it includes some mixing of alveolar gas with physiological
deadspace gas.
During the isocapnic hypoxia test at LA, both baseline measures and measurements taken near the end of the protocol showed that $P_{ETO2}$ consistently overestimated $P_{aO2}$, and these gradients measured at LA were unchanged from each other. Intuitively, the $P_{ET-PaO2}$ gradient would increase with isocapnic hypoxia due to increased alveolar deadspace from hypoxic pulmonary vasoconstriction, however, the hypoxic stimulus we administered (10-minutes) would have resulted in a modest change in pulmonary artery pressure compared to that at high altitude (15).

The isocapnic O$_2$ protocol used at HA was fundamentally different than the one used at LA. The HA isocapnic O$_2$ test involved administering higher amounts of O$_2$ from baseline to values that were similar to those seen during baseline at LA (euoxia). For this reason we did not compare the $P_{ET-PaO2}$ gradient between the LA and HA outside of our baseline measures, as we technically delivered different stimuli. The unchanged $P_{ET-PaO2}$ gradient seen at baseline between LA and HA in healthy subjects was somewhat unexpected as there are likely HA-related changes in alveolar deadspace volume. However, this is likely explained by the reduction in atmospheric O$_2$ at HA, making the inspiratory O$_2$ to $P_{aO2}$ difference lower, which will minimize the difference between end-tidal and arterial O$_2$. The concomitant changes of alveolar deadspace and the reduction of atmospheric O$_2$ associated with HA may oppose each other in such a manner that the net result is an unchanged $P_{ET-PaO2}$ gradient observed during baseline between LA and HA. Changes in alveolar deadspace not only occur during hypoxia (acute and chronic), but it may also occur during hyperoxia (i.e. increased FiO$_2$) (50). During hyperoxia, there is a redistribution of pulmonary blood from poorly perfused alveoli to highly perfused alveoli due to pulmonary vasodilation potentially further increasing physiological deadspace during hypercapnia (50). The end result is an increase in alveolar deadspace volume which has the potential of widening the $P_{ET-PaO2}$ gradient. However, we did not observe this effect at HA, as the $P_{ET-PaO2}$ gradient was unchanged between baseline and isocapnic euoxia ($P_{ETO2} = 100$ mmHg). It is possible that our euoxia stimulus was not strong enough or applied for too short a time period to elicit a significant response.
Can $P_{aCO_2}$ be predicted from $P_{ETCO_2}$ during end-tidal forcing?

Previous equations have been proposed for predicting $P_{aCO_2}$ by Jones et al. (18) during exercise, and Peebles et al. (29) during hypocapnia (through active hyperventilation) and hypercapnia (increased fixed $F_{ICO_2}$ inspirate). However, as highlighted in figure 5, we found that the correction algorithms proposed by Jones et al. (18) and Peebles et al. (29) could not accurately predict $P_{aCO_2}$ during our DEF protocols (likely due to the different experimental conditions); therefore, multiple stepwise regression analysis was used to determine which variables might predict $P_{aCO_2}$ at LA and HA. Based on these variables we conducted linear regression analysis with $P_{aCO_2}$ as our dependent variable to better predict $P_{aCO_2}$ at LA and HA. Our results suggest that a correction algorithm that includes $P_{ETCO_2}$ as a variable will more accurately predict $P_{aCO_2}$.

Methodological Considerations.

Acetazolamide (a carbonic anhydrase inhibitor), decreases the risk of high-altitude sickness by increasing central chemoreflex drive and improving arterial oxygenation (43). Acetazolamide administration has been proven useful for safe acclimatization to extreme altitude, and effectively reduces the risk of pulmonary edema (thus diffusion limitation), and alveolar deadspace due to improved $V/Q$ matching (44, 46). Therefore, if acetazolamide had long-lasting effects (i.e. 5-days beyond its discontinuation), it could be argued that our results have underestimated the true difference in end-tidal-to-arterial gradient between LA and HA during DEF. This possibility would seem unlikely, however, given the rapid clearance of acetazolamide (<36 hrs) (34).

Despite the advantages of our DEF system being portable, cost effective (due to low gas requirements), and capable of controlling end-tidal gases within narrow limits, it, similar to other systems is affected by methodological constraints. First, similar to all DEF systems, hypocapnic steps can only be achieved by asking participants to actively hyperventilate, driving their $P_{ETCO_2}$ below desired targets where the DEF is then capable of
controlling and fine tuning $P_{ETCO_2}$ by adding CO$_2$ into the breathing circuit. Thus, with hypoxia, end-tidal gas control is dependent upon the ability of the participant to maintain a constant rate of ventilation. Second, similar to the DEF system developed by Koehle et al. (21), our DEF system functions by mixing inspirate into a reservoir bag on a breath-by-breath basis. In an ideal world the reservoir bag would be emptied with each breath so that only the inspirate for the next breath is available to the subject at any given time. However, in order to deal with variation in $V_T$ with each breath a small amount of extra air remains in the reservoir bag to prevent its complete emptying. This safety volume is normally 100 ml more than the current $V_T$ but can be adjusted by the DEF operator to maintain the reservoir volume as small as possible. In addition, the algorithm for the system estimates the volume of each gas remaining in the reservoir and takes these volumes into account before injecting the next volume of gases to better target the correct fractions of inspired O$_2$ and CO$_2$. Nonetheless, inaccuracies in this prediction or inflation in the estimated reservoir volume can lead to a sluggish response or a delay in the systems response to a perturbation. Third, our DEF system uses 100% CO$_2$, O$_2$, and N$_2$ compressed gas to fill our inspirate reservoir. Although avoiding pre-mixed gases is usually less expensive, it does present some potential safety issues as it is possible for the participant to be administered 100% N$_2$ during a severe hypoxic step. Therefore it is crucial to employ a well-trained DEF device operator to recognize these potential issues. If a problem arises in which the participant’s end-tidal values are not being controlled properly, 100% O$_2$ can be delivered to the participant for safety reasons. Finally, similar to all DEF system, our DEF system assumes the end-tidal gases are a surrogate for arterial blood gases.

**Perspectives and Significance.**

We hypothesized that $P_{ETCO_2}$ would overestimate $P_{aCO_2}$ to a greater extent during a CO$_2$ protocol at HA, while $P_{ETO_2}$ would overestimate $P_{aO_2}$ to a lesser extent at HA due to a reduction in the arterial-venous gas gradient. In agreement with our hypothesis, we demonstrated that during DEF $P_{aCO_2}$ is systematically overestimated by $P_{ETCO_2}$ at baseline, hypoxia and hypercapnia at HA, but only at baseline and hypercapnia at LA. This
overestimation of $P_{aCO2}$ is exacerbated at HA compared to LA. In contrast to our hypothesis, we found that $P_{ETO2}$ overestimates $P_{aO2}$ by the same magnitude at LA and HA, and these gradients remained unchanged throughout the O2 protocols. The correction algorithms derived are specific to our experimental conditions including our study sample, altitude, the CO2 and O2 protocols employed, and possibly only our DEF system. However, these algorithms need to be validated in an independent sample during similar protocols. We recommend that the end-tidal-to-arterial gas gradients be considered when performing common ventilatory and vascular reactivity protocols to CO2 and O2 as they could potentially effect the measured response. With future validation, it may be possible to use the regression equations reported here to estimate arterial blood gases in the event that invasive measurements are not feasible.
Acknowledgements: This study was carried out within the framework of the Ev-K2-CNR Project in collaboration with Nepal Academy of Science and Technology as foreseen in the Memorandum of Understanding between Nepal and Italy, and thanks to a contribution from the Italian National Research Council. The authors are grateful to the members of the UBC International Research expedition to the Pyramid Laboratory for invaluable help with organization and implementation of this research study.

Grants: This study was supported by the Natural Sciences and Engineering Research Council of Canada, and the Canadian Foundation for Innovation. C.K.W was supported by a Vanier Canada Graduate Scholarship.

Disclosures: The authors have no conflict of interest.
References


Table 1. Ventilatory data during an isooxic CO2 test at LA and HA.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>-10 mmHg</th>
<th>-5 mmHg</th>
<th>0 mmHg</th>
<th>+5 mmHg</th>
<th>+10 mmHg</th>
<th>+15 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_E$ (l min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>14.3 ± 0.7</td>
<td>39.0 ± 5.0*</td>
<td>34.2 ± 4.8*</td>
<td>19.7 ± 2.2</td>
<td>21.5 ± 1.8*</td>
<td>44.5 ± 5.4*</td>
<td>57.6 ± 5.7*</td>
</tr>
<tr>
<td>HA</td>
<td>19.9 ± 1.0†</td>
<td>46.8 ± 3.5*</td>
<td>40.5 ± 4.4*</td>
<td>28.6 ± 2.6†</td>
<td>53.3 ± 7.2*†</td>
<td>84.2 ± 5.9*†</td>
<td></td>
</tr>
<tr>
<td>$P_{ETCO_2}$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Altitude$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Interaction$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_T$ (l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>1.0 ± 0.1</td>
<td>1.7 ± 0.3*</td>
<td>1.5 ± 0.3*</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.1*</td>
<td>2.2 ± 0.1*</td>
<td>2.5 ± 0.2*</td>
</tr>
<tr>
<td>HA</td>
<td>1.2 ± 0.1†</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>2.1 ± 0.1*†</td>
<td>2.6 ± 0.1*†</td>
<td></td>
</tr>
<tr>
<td>$P_{ETCO_2}$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Altitude$</td>
<td>0.210</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Interaction$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_B$ (breaths min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>15.1 ± 0.8</td>
<td>24.5 ± 2.3*</td>
<td>24.2 ± 3.0*</td>
<td>19.7 ± 2.7</td>
<td>15.2 ± 1.1</td>
<td>20.3 ± 2.7</td>
<td>24.1 ± 3.1*</td>
</tr>
<tr>
<td>HA</td>
<td>17.2 ± 1.0</td>
<td>31.5 ± 2.4*†</td>
<td>30.1 ± 2.8*</td>
<td>24.6 ± 2.4*</td>
<td>24.9 ± 2.5*†</td>
<td>32.5 ± 2.7*†</td>
<td></td>
</tr>
<tr>
<td>$P_{ETCO_2}$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Altitude$</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Interaction$</td>
<td>0.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$FICO_2$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>0.2 ± 0.0</td>
<td>1.9 ± 0.3*</td>
<td>3.4 ± 0.2*</td>
<td>2.9 ± 0.4*</td>
<td>5.0 ± 0.3*</td>
<td>6.7 ± 0.1*</td>
<td>7.4 ± 0.1*</td>
</tr>
<tr>
<td>HA</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.3†</td>
<td>3.2 ± 0.4*</td>
<td>4.0 ± 0.5*</td>
<td>7.3 ± 0.3*†</td>
<td>9.1 ± 0.1*†</td>
<td></td>
</tr>
<tr>
<td>$P_{ETCO_2}$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Altitude$</td>
<td>0.028</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Interaction$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$FIO_2$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>20.8 ± 0.1</td>
<td>17.1 ± 0.2*</td>
<td>17.4 ± 0.2*</td>
<td>19.7 ± 0.6</td>
<td>18.2 ± 0.3*</td>
<td>16.6 ± 0.1*</td>
<td>16.3 ± 0.1*</td>
</tr>
<tr>
<td>HA</td>
<td>21.0 ± 0.2</td>
<td>16.1 ± 0.2*†</td>
<td>16.6 ± 0.4*</td>
<td>18.4 ± 0.7*</td>
<td>15.8 ± 0.5*†</td>
<td>14.7 ± 0.2*†</td>
<td></td>
</tr>
<tr>
<td>$P_{ETCO_2}$</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Altitude$</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Interaction$</td>
<td>0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$V_E$, minute ventilation; $V_T$, tidal volume; $F_B$, breathing frequency; $FICO_2$, fraction of inspired carbon dioxide; $FIO_2$, fraction of inspired oxygen. Reported p-values represent differences between LA and HA. *P<0.05, vs baseline. †P<0.05 low-altitude vs. high-altitude. Values are mean ± SEM. The P-values for the main effects of $P_{ETCO_2}$ step and altitude, and the P-value of the interaction effect are displayed underneath each measured variable.
Table 2. End-tidal and arterial blood gases during an isoxic CO₂ test at LA and HA.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>-10 mmHg</th>
<th>-5 mmHg</th>
<th>0 mmHg</th>
<th>+5 mmHg</th>
<th>+10 mmHg</th>
<th>+15 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PₑTₑCO₂ (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>41.5 ± 0.4</td>
<td>31.9 ± 0.4*</td>
<td>37.0 ± 0.4*</td>
<td>42.4 ± 0.4</td>
<td>46.9 ± 0.6*</td>
<td>52.3 ± 0.4*</td>
<td>57.2 ± 0.4*</td>
</tr>
<tr>
<td>HA</td>
<td>28.7 ± 0.5†</td>
<td>19.0 ± 0.5*†</td>
<td>24.1 ± 0.5*†</td>
<td>29.1 ± 0.5†</td>
<td>34.2 ± 0.5*†</td>
<td>39.3 ± 0.5*†</td>
<td></td>
</tr>
<tr>
<td><strong>PₐCO₂ (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>39.4 ± 0.5</td>
<td>30.9 ± 0.6*</td>
<td>36.2 ± 0.5*</td>
<td>40.5 ± 0.8</td>
<td>44.9 ± 0.5*</td>
<td>50.4 ± 0.5*</td>
<td>55.9 ± 1.0*</td>
</tr>
<tr>
<td>HA</td>
<td>24.6 ± 0.9†</td>
<td>16.2 ± 0.7*†</td>
<td>21.2 ± 0.9*†</td>
<td>25.5 ± 0.7†</td>
<td>29.1 ± 0.7*†</td>
<td>34.1 ± 0.8*†</td>
<td></td>
</tr>
<tr>
<td><strong>Pa-PₑTₑCO₂ (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>-2.1 ± 0.5‡</td>
<td>-1.1 ± 0.6</td>
<td>-0.7 ± 0.4</td>
<td>-1.8 ± 0.7‡</td>
<td>-2.1 ± 0.7‡</td>
<td>-1.9 ± 0.5‡</td>
<td>-1.3 ± 0.9</td>
</tr>
<tr>
<td>HA</td>
<td>-4.1 ± 0.7‡†</td>
<td>-2.7 ± 0.4‡</td>
<td>-2.9 ± 0.7‡</td>
<td>-3.6 ± 0.7‡†</td>
<td>-5.1 ± 0.7‡†⊥</td>
<td>-5.2 ± 0.7‡⊥⊥</td>
<td></td>
</tr>
<tr>
<td><strong>PₑTO₂ (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>93.2 ± 1.1</td>
<td>100.0 ± 0.2*</td>
<td>98.7 ± 0.9*</td>
<td>99.9 ± 1.0*</td>
<td>100.0 ± 0.2*</td>
<td>100.0 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>46.8 ± 1.0†</td>
<td>46.6 ± 0.5†</td>
<td>46.7 ± 0.5†</td>
<td>46.6 ± 0.4†</td>
<td>46.5 ± 0.5†</td>
<td>46.8 ± 0.5†</td>
<td></td>
</tr>
<tr>
<td><strong>Pₑ-PₑO₂ (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>5.1 ± 1.4‡</td>
<td>0.2 ± 1.5</td>
<td>0.0 ± 0.9</td>
<td>3.5 ± 1.5‡</td>
<td>1.0 ± 0.8</td>
<td>-1.9 ± 0.6</td>
<td>-2.7 ± 0.9‡</td>
</tr>
<tr>
<td>HA</td>
<td>5.1 ± 1.0‡</td>
<td>3.8 ± 1.0‡</td>
<td>5.1 ± 1.0‡</td>
<td>5.4 ± 1.0‡</td>
<td>5.2 ± 0.8‡</td>
<td>5.0 ± 0.9‡</td>
<td></td>
</tr>
</tbody>
</table>

PₑTₑCO₂, end-tidal partial pressure of carbon dioxide; PₐCO₂, arterial partial pressure of carbon dioxide; Pa-PₑTₑCO₂, end-tidal-to-arterial gas gradient of carbon dioxide; PₑTO₂, end-tidal partial pressure of oxygen; PₑO₂, arterial partial pressure oxygen; Pₑ-PₑO₂, end-tidal-to-arterial gas gradient of oxygen. Reported p-values represent differences between LA and HA (statistical significance set at P<0.05). *P<0.05, vs baseline. †P<0.05 low-altitude vs high-altitude. ‡P<0.05, end-tidal vs arterial gases (i.e. significant gradient). ⊥P<0.05, Pa-PₑTₑCO₂ hypercapnia vs hypocapnia (-10 and -5 mmHg) at HA. Values are mean ± SEM. The P-values for the main effects of PₑTₑCO₂ step and altitude, and the P-value of the interaction effect are displayed in the last three columns.
Table 3. Ventilatory and arterial blood gas data during an isocapnic O₂ test at LA and HA.

<table>
<thead>
<tr>
<th></th>
<th>Altitude</th>
<th>Baseline</th>
<th>Isocapnic Hypoxia</th>
<th>Altitude</th>
<th>Baseline</th>
<th>Isocapnic Euoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_{\text{ETCO}_2} ) mmHg</td>
<td>LA</td>
<td>40.6 ± 0.5</td>
<td>41.2 ± 0.4</td>
<td>HA</td>
<td>28.6 ± 0.6</td>
<td>29.1 ± 0.6</td>
</tr>
<tr>
<td>( P_{\text{a}CO_2} ) mmHg</td>
<td>LA</td>
<td>38.6 ± 0.7</td>
<td>41.4 ± 0.7*</td>
<td>HA</td>
<td>24.8 ± 0.8</td>
<td>26.0 ± 0.9</td>
</tr>
<tr>
<td>( P_{a-P_{\text{ETCO}_2}} ) mmHg</td>
<td>LA</td>
<td>-2.0 ± 0.4‡</td>
<td>0.1 ± 0.5</td>
<td>HA</td>
<td>-3.7 ± 0.7‡</td>
<td>-3.1 ± 0.6‡</td>
</tr>
<tr>
<td>( P_{\text{ETO}_2} ) mmHg</td>
<td>LA</td>
<td>82.9 ± 1.8</td>
<td>46.9 ± 0.2*</td>
<td>HA</td>
<td>45.7 ± 0.7</td>
<td>100.1 ± 0.4*</td>
</tr>
<tr>
<td>( P_{\text{a}O_2} ) mmHg</td>
<td>LA</td>
<td>76.0 ± 2.4</td>
<td>40.0 ± 0.5*</td>
<td>HA</td>
<td>41.2 ± 0.5</td>
<td>96.3 ± 1.3*</td>
</tr>
<tr>
<td>( P_{ET-P_{\text{a}O_2}} ) mmHg</td>
<td>LA</td>
<td>6.9 ± 2.1‡</td>
<td>6.9 ± 0.6‡</td>
<td>HA</td>
<td>4.5 ± 0.9‡</td>
<td>3.8 ± 1.2‡</td>
</tr>
<tr>
<td>( V_E ) l min⁻¹</td>
<td>LA</td>
<td>12.6 ± 0.7</td>
<td>37.3 ± 4.4*</td>
<td>HA</td>
<td>19.6 ± 1.5</td>
<td>25.3 ± 2.9*</td>
</tr>
<tr>
<td>( V_T ) l</td>
<td>LA</td>
<td>0.9 ± 0.0</td>
<td>1.8 ± 0.1*</td>
<td>HA</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>( F_B ) breaths min⁻¹</td>
<td>LA</td>
<td>14.9 ± 0.8</td>
<td>20.3 ± 2.2*</td>
<td>HA</td>
<td>16.3 ± 1.5</td>
<td>22.4 ± 2.7*</td>
</tr>
<tr>
<td>( F_{IO_2} ) %</td>
<td>LA</td>
<td>20.7 ± 0.1</td>
<td>9.2 ± 0.2*</td>
<td>HA</td>
<td>20.9 ± 0.2</td>
<td>34.2 ± 0.7*</td>
</tr>
<tr>
<td>( F_{ICO_2} ) %</td>
<td>LA</td>
<td>0.3 ± 0.0</td>
<td>4.5 ± 0.2*</td>
<td>HA</td>
<td>0.2 ± 0.1</td>
<td>2.4 ± 0.6*</td>
</tr>
</tbody>
</table>

\( P_{\text{ETCO}_2} \), end-tidal partial pressure of carbon dioxide; \( P_{\text{a}CO_2} \), arterial partial pressure of carbon dioxide; \( P_{a-P_{\text{ETCO}_2}} \), end-tidal-to-arterial gas gradient of carbon dioxide; \( P_{\text{ETO}_2} \), end-tidal partial pressure of oxygen; \( P_{\text{a}O_2} \), arterial partial pressure oxygen; \( P_{ET-P_{\text{a}O_2}} \), end-tidal-to-arterial gas gradient of oxygen; \( V_E \), minute ventilation; \( V_T \), tidal volume; \( F_B \), breathing frequency; \( F_{IO_2} \), fraction of inspired oxygen; \( F_{ICO_2} \), fraction of inspired carbon dioxide. *P<0.05, vs baseline. ‡P<0.05, end-tidal vs arterial gases (i.e., significant gradient). Values are mean ± SEM.
Figure Legends.

Figure 1. Representative tracing of isooxic CO₂ test at LA and HA. Open circles (○) represent P_{ETO₂}, end-tidal partial pressure of oxygen; closed circles (●) represent P_{ETCO₂}, end-tidal partial pressure of carbon dioxide. Top panels A (LA) and B (HA), represents mean data (15-second time bins) ± SEM from the isooxic CO₂ test in 9 participants. The numeric values below the P_{ETCO₂} data represents the mean end-tidal-to-arterial gas gradient for carbon dioxide. Bottom panels C (LA) and D (HA), represents raw breath-by-breath data from one participant from the isooxic CO₂ test.

Figure 2. Bland and Altman plot of differences between P_{aCO₂} and P_{ETCO₂} during an isooxic CO₂ test at LA and HA. (●), -10 mmHg; (○), -5 mmHg; (▼), 0 mmHg; (Δ), +5 mmHg; (■), +10 mmHg; (□), +15 mmHg. Dotted lines represent the 95% confidence intervals and the continuous lines represents the mean bias. Panel A., representing the end-tidal-to-arterial difference at LA, and Panel B, represents the end-tidal-to-arterial difference at HA during the CO₂ test.

Figure 3. Representative tracing of the isocapnic O₂ test at LA and HA. Open circles (○) represent P_{ETO₂}, end-tidal partial pressure of oxygen; closed circles (●) represent P_{ETCO₂}, end-tidal partial pressure of carbon dioxide. Top panels A (LA) and B (HA), represents mean data (15-second time bins) ± SEM from the isocapnic O₂ test in 9 participants. The numeric values above P_{ETO₂} data represents the end-tidal-to-arterial gas gradient for oxygen. Bottom panels C (LA) and D (HA), represents raw breath-by-breath data from one participant during the isocapnic O₂ test.

Figure 4. Bland and Altman plot of differences between P_{aO₂} and P_{ETO₂} partial pressures of O₂ during an isocapnic O₂ test at LA and HA. Dotted lines represent the 95% confidence intervals and the continuous lines represent the mean bias. Panel A., representing the end-tidal-to-arterial difference at LA, and Panel B., represents the end-tidal-to-arterial difference at HA.
Figure 5. Assessment of PaCO₂ and PETCO₂ relationship of CO₂ during an isooxic CO₂ test at LA and HA. Data points were obtained during the last minute of each step change in PETCO₂. Dotted lines represent 95% confidence intervals. 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 Jones et al. (1979) (ePaCO₂ = 5.5 + 0.9* PETCO₂ – VT). 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 (ePaCO₂ = 2.367 + 0.884* PETCO₂). b[1], slope; b[0], y-intercept.
Figure 1.
Figure 2.
Figure 3.
Figure 4.

A. B.
Figure 5.

A. SL

\[ R^2 = 0.92 \\ b[1] = 0.97 \\ b[0] = 3.09 \]

B. Peeples PaCO2

\[ R^2 = 0.87 \\ b[1] = 0.80 \\ b[0] = 7.96 \]

C. Jones PaCO2

\[ R^2 = 0.92 \\ b[1] = 0.86 \\ b[0] = 5.10 \]

A. HA

\[ R^2 = 0.92 \\ b[1] = 1.07 \\ b[0] = 5.10 \]

B. Peeples PaCO2

\[ R^2 = 0.91 \\ b[1] = 0.88 \\ b[0] = 5.89 \]

C. Jones PaCO2

\[ R^2 = 0.92 \\ b[1] = 0.95 \\ b[0] = 4.26 \]