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Title: Time Course and Magnitude of Ventilatory and Renal Acid-Base Acclimatization Following Rapid Ascent to and Residence at 3,800 m over Nine Days

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Running head: Integrated Ventilatory and Renal Acid-Base Acclimatization

ABSTRACT

Rapid ascent to high altitude imposes an acute hypoxic and acid-base challenge, with ventilatory and renal acclimatization countering these perturbations. Specifically, ventilatory acclimatization improves oxygenation, but with concomitant hypocapnia and respiratory alkalosis. A compensatory, renally-mediated relative metabolic acidosis follows via bicarbonate elimination, normalizing arterial pH(a). The time-course and magnitude of these integrated acclimatization processes are highly variable between individuals. Using a previously-developed metric of renal reactivity (RR), indexing the change in arterial bicarbonate concentration ($\Delta[\text{HCO}_3^-]_a$; renal response) over the change in arterial pressure of CO_2 (ΔPaCO_2 ; renal stimulus), we aimed to characterize changes in RR magnitude following rapid ascent and residence at altitude. Resident lowlanders (n=16) were tested at 1,045 m (Day [D]0) prior to ascent, on D2 within 24-hours of arrival, and D9 during residence at 3,800 m. Radial artery blood draws were obtained to measure acid-base variables: PaCO_2 , $[\text{HCO}_3^-]_a$ and pH_a. Compared to D0, PaCO_2 and $[\text{HCO}_3^-]_a$ were lower on D2 ($P<0.01$) and D9 ($P<0.01$), whereas significant changes in pH_a ($P>0.058$) and RR ($P=0.056$) were not detected. As pH_a appeared fully compensated on D2 and RR did not increase significantly from D2 to D9, these data demonstrate renal acid-base compensation within 24-hours at moderate steady-state altitude. Moreover, RR was strongly and inversely correlated with ΔpH_a on D2 and D9 ($r\leq-0.95$; $P<0.0001$), suggesting that a high-gain renal response better protects pH_a. Our study highlights the differential time-course, magnitude, and variability of integrated ventilatory and renal acid-base acclimatization following rapid ascent and residence at high altitude.

Key Words: High Altitude, Hypoxia, Ventilatory acclimatization, Acid-base, Renal compensation

NEW & NOTEWORTHY

- We assessed the time-course, magnitude, and variability of integrated ventilatory and renal acid-base acclimatization with rapid ascent and residence at 3,800 m.
- Despite reductions in PaCO_2 upon ascent, pH_a was normalized within 24-hours of arrival at 3,800 m through renal compensation (i.e., bicarbonate elimination).
- Renal reactivity (RR) was unchanged between days 2 and 9, suggesting a lack of plasticity at moderate steady-state altitude.
- RR was strongly-correlated with ΔpH_a , suggesting that a high-gain renal response better protects pH_a .

INTRODUCTION

Sojourners to high altitude ($\geq 2,500$ m) are exposed to reductions in atmospheric pressure, leading to hypobaric hypoxia and resultant hypoxemia. Initial high altitude exposure reduces arterial partial pressure of oxygen (PaO_2), arterial blood oxygen saturation (SaO_2) and arterial oxygen content (CaO_2), triggering a cascade of integrated physiological responses. Acute responses include the hypoxic ventilatory response (HVR), whereby increases in alveolar ventilation increases the amount of oxygen carried in the blood (Goldfarb-Rumyantzev & Alper, 2013), but with concomitant respiratory alkalosis (Krapf et al., 1990; Zouboules et al., 2018). With chronic hypoxic exposure to high altitude, acclimatization processes are mounted in respiratory, hematological, and renal systems, improving oxygenation and countering hyperventilation-induced acid-base imbalances (Beidleman et al., 2017; Dempsey et al., 2014; Goldfarb-Rumyantzev & Alper, 2013; Powell, 2007).

Peripheral chemoreceptors (i.e., carotid bodies) initially detect hypoxemia and elicit the HVR (Dempsey & Forster, 1982; Teppema & Dahan, 2010). The HVR increases resting ventilation in attempt to partially restore PaO_2 while simultaneously decreasing the arterial partial pressure of carbon dioxide ($PaCO_2$) causing hypocapnia and respiratory alkalosis. The resultant hypocapnia dampens both central and peripheral respiratory chemoreceptor activation, blunting ventilatory drive (e.g., Daristotle et al., 1987; Lahiri & Delaney, 1975; Steinback et al., 2007). Thus, for the HVR to successfully increase oxygenation, the inhibitory effects of concomitant hypocapnia must be countered. This is accomplished by ventilatory acclimatization to chronic hypoxia (VAH; Ainslie et al., 2013; Dempsey et al., 2014), which increases sensitivity of the HVR through carotid body type I glomus cell hyperplasia within the first three days of

sustained hypoxia (Wang et al., 2008), and subsequent increases in carotid body sensitivity to hypoxia (Vizek et al., 1987). Further, plasticity is observed within the central nervous system, through integration of afferent signalling from the carotid bodies (Moya et al., 2020). The net result is an increase in ventilatory motor output for a given hypoxic stimulus (Powell, 2007; Dempsey et al., 2014; Leacy et al., 2020; Moya et al., 2020). VAH progressively increases ventilation and PaO_2 , which partially restores oxygenation, but concurrently amplifies the initial hypocapnia and respiratory alkalosis (Dempsey & Forster, 1982; Robbins, 2007).

Previously, we developed a metric of steady-state chemoreflex drive (SS-CD) in contexts of acute hypoxia in a laboratory setting (Pfoh et al., 2017) and during incremental ascent to high altitude (Bruce et al., 2018, Leacy et al., 2020). The SS-CD metric takes into account the integrated steady-state ventilatory response to prevailing respiratory chemostimuli at rest, and likely captures integrated ventilatory acclimatization through the contributions of both central and peripheral chemoreceptors (Bruce et al., 2018; Leacy et al., 2020). However, this SS-CD metric has yet to be characterized during rapid ascent to and residence at a single altitude over time.

With sustained exposure to hypoxia, the HVR-induced hypocapnia and respiratory alkalosis causes an acute acid-base dysregulation (e.g., Krapf et al., 1991). Two renal compensatory mechanisms arise to counteract this dysregulation: 1) minimizing renal acid ($[\text{H}^+]$) excretion (by reducing secretion) and 2) increasing bicarbonate (HCO_3^-) excretion into the urine (by reducing reabsorption; Ge et al., 2006; Gledhill et al., 1975; Hamm et al., 2015; Hohne et al., 2001; Forster et al., 1975; Krapf et al., 1991; Swenson, 2016). These two interrelated renal tubular cellular mechanisms return arterial blood pH (pH_a) to homeostatic values ($\text{pH}_a \sim 7.4$) through compensatory relative metabolic acidosis (Swenson, 2016; Zouboules et al., 2018).

However, this bicarbonate diuresis increases central chemoreceptor sensitivity to a given change in PaCO_2 (e.g., breath hold) by reducing buffering capacity within the respiratory control centers where the central chemoreceptors reside (Dempsey et al., 2014; Fan et al., 2010; Forster et al., 1975). In addition, the relative metabolic acidosis sensitizes central respiratory chemoreceptors and unbrakes peripheral chemoreceptor blunting resulting from hypocapnia and respiratory alkalosis, likely contributing to ventilatory acclimatization in addition to carotid body plasticity.

To describe the gain of the renal response, we previously introduced renal reactivity (RR), an index of the relative change in arterial bicarbonate concentration ($\Delta[\text{HCO}_3^-]_a$; i.e., renal response) over the relative change in arterial pressure of CO_2 (ΔPaCO_2 ; i.e., renal stimulus), during incremental ascent to high altitude (Zouboules et al., 2018). We showed that RR increased with incremental ascent from 3,440 m to 3,820 m from days 3 to 5 and plateaued with further ascent (4,240 m on day 7 and 5,160 m on day 10). We also showed that at all altitudes, RR was significantly and inversely correlated with relative changes in pH (ΔpH_a), with an increase in correlative magnitude (i.e., larger r value) with time spent at and further ascent to altitude, suggesting those with greater renal responses to sustained hypocapnia and acute respiratory alkalosis were better able to protect pH_a .

The time-course and magnitude of the integrated respiratory and renal acclimatization phenotypes are variable between individuals, particularly following rapid ascent. We aimed to assess the integration between respiratory and renal acclimatization during rapid ascent and residence at high altitude (3,800 m) over 9 days. We hypothesized that (a) steady-state chemoreflex drive would increase following ascent with ventilatory acclimatization, (b) with subsequent renal compensation, RR magnitude would increase with duration at altitude, and (c)

RR would be inversely-correlated with relative changes in pH ($\Delta p\text{H}_a$), improving with time spent at a single altitude.

MATERIAL AND METHODS

Ethics and Participant recruitment

This study abided by the Canadian Government Tri-Council policy on research ethics with human participants (TCPS2) and it adhered to the standards set by the latest revision of the *Declaration of Helsinki*, except for registration in a database. Ethical approval was received in advance through University of Calgary Conjoint Human Research Ethics Board (Protocol REB18-0374) and MRU Human Research Ethics Board (Protocol 101879), with subsequent harmonization as required with each investigator's institutional Research Ethics Board.

Healthy participants were recruited on a voluntary basis to undergo cardiorespiratory and arterial blood gas measurements during a research expedition to the Barcroft Research Station (3,800 m) in the Sierra Nevada mountains in California, USA in August 2019. Participants were recruited via verbal communication and provided voluntary, informed, verbal and written consent prior to participation in the study.

All members of the expedition consulted with their family physicians for a medical check prior to participating in this expedition. We pre-screened and recruited 16 healthy participants for inclusion in the present study with no self-reported medical history of cardiopulmonary, neurological, or metabolic disease. Participants were excluded if they had a body mass index $>35 \text{ kg/m}^2$, had a history of smoking, or were taking prescription medication other than hormonal birth control. Inclusion criteria included adult participants between the ages of 18-60 years who planned to stay at the research station for the entire 10-day period. Each participant completed a pre-screening questionnaire and consent form for documentation.

The participant recruitment, specific study design, research questions and data collection were planned *a priori*. All participants were native lowlanders who had not been exposed to high altitude (>3,000 m) for at least one year prior to the study. No participants included in the study took carbonic anhydrase inhibitors (e.g., acetazolamide) or any corticosteroids for AMS prevention or treatment at any point during this study.

Study protocol and ascent profile

Baseline measurements were conducted at 1,045 m (Calgary, Canada; Day 0; D0) over a week. Participants were then flown to, and spent one night (less than 12 hours) at 610 m (Las Vegas, Nevada, USA) before driving up to 3800 m (Barcroft Research Station, California, USA) the following day over 5-6 hours for a study on acute ascent and sustained altitude exposure. Participants resided at 3,800 m for 10 days and nights to study acclimatization to altitude. Early exposure and late exposure arterial blood gas measurements were obtained on days 2 (D2; within 24 hours of arrival), and day 9 (D9), respectively.

Measurements

Daily Ancillary Cardiorespiratory Measures

Every morning at high altitude and once at low altitude between 06:00-09:00 local time, fasted cardiorespiratory measures were taken to characterize steady-state physiological responses. Participants self-reported their acute mountain sickness (AMS) scores using the standard Lake Louise Questionnaire (Roach et al., 2018). Hemoglobin concentration [Hb] was obtained via finger capillary blood sample using sterile lancets (AccuChek, Softclix) with standard practice and universal precautions, measured via hemoglobinometer (Hemocue Hemoglobin System, Hb201+ with microcuvettes; Ängelholm, Sweden). All cardiorespiratory measures were obtained at rest in the supine position with eyes closed and white noise played through

headphones to limit external distraction. Three brachial blood pressure measurements were obtained via an automated blood pressure monitor (model BP786n; Omron, San Ramon, CA, USA) then averaged. A peripheral pulse oximeter (Masimo SET[®] Rad-5, Danderyd, Sweden) was placed on the participant's left middle finger for measurement of peripheral oxygen saturation (SpO₂; %) and heart rate (HR; bpm). Respiratory measures were obtained using a personal mouthpiece, bacteriological filter, and nose clip. We instrumented participants with a portable capnograph (EMMA, Masimo, Danderyd, Sweden) for measurement of end-tidal PCO₂ (P_{ET}CO₂; mmHg) and breathing frequency (f_B; breathes/min), and a respirometer (nSpire Haoscale, Colorado, USA) for ventilation ($\dot{V}_E$; L/min). Once instrumentation was complete, the participants laid down with their eyes closed and after approximately three minutes, we recorded and archived their values. Steady-state chemoreflex drive (SS-CD) was calculated as previously described (Bruce et al., 2018; Leacy et al., 2020; Pfoh et al., 2017). First, we calculated a steady-state stimulus index (SI; P_{ET}CO₂/SpO₂; mmHg/%), which represents the prevailing central and peripheral chemoreceptor stimulus. We then divided $\dot{V}_E$ by the SI to calculate SS-CD each day to characterize ventilatory acclimatization over time at 3,800 m.

Arterial blood gas and electrolytes

Arterial blood samples were obtained in Calgary (1,045 m) and on days 2 and 9 of the expedition at 3,800 m during the day (08:00 - 15:00). Arterial blood samples were acquired percutaneously from the radial artery using a pre-heparinized, self-filling arterial blood syringe (PICO50, Radiometer) while the participants were resting in the supine position. Standard procedures involved topical sterilization and local anesthesia (i.e., EMLA topical cream; 2.5% lidocaine /2.5% prilocaine cream) with universal precautions, including a modified Allen's test to ensure redundant circulation of the hand. Samples were then immediately analyzed using a

cartridge-based system for measurement/calculation of PaO_2 (mmHg), SaO_2 (%), PaCO_2 (mmHg), $[\text{HCO}_3^-]_a$ (mM), pH_a , hematocrit (%), hemoglobin concentration (g/L), and [creatinine] (μM), using a portable analyzer and cartridges (Abbott iSTAT, CG4+ and CHEM8+ cartridges; Abbott, Mississauga, Ontario, Canada). All samples were subject to thermal correction and atmospheric pressure calibration.

Data Analysis

Atmospheric pressure for each altitude was calculated from https://baillielab.net/critical_care/air_pressure/ and partial pressure of inspired oxygen (P_{IO_2}) was calculated as:

Equation (1):

$$P_{\text{IO}_2} = (P_{\text{ATM}} - 47 \text{ mmHg}) \times 0.21\#$$

where 47 mmHg is water vapor pressure at normal body temperature (37°C) and 0.21 is the fraction of inspired oxygen.

Strong ion difference (SID; meq/L) was calculated according to the traditional Stewart approach (Stewart, 1983).

Equation (2):

$$\text{SID} = ([\text{Na}^+] + [\text{K}^+] + 2[\text{Ca}^{2+}]) - ([\text{Cl}^-] + [\text{Lactate}])$$

Serum osmolality (mOsmol kg^{-1}) was calculated from the recommended formula in Martin-Calderon *et al.* (2015).

Equation (3):

$$\text{Osmolality} = 1.86([\text{Na}^+] + [\text{K}^+]) + 1.15 \left(\frac{[\text{Glucose}]}{18} \right) + \left(\frac{[\text{Urea}]}{6} \right) + 14$$

Arterial oxygen content (CaO_2) was calculated as:

Equation (4):

$$CaO_2 = \left(1.36 \times [Hb] \times \frac{SaO_2}{100} \right) + (0.003 \times PaO_2) \#$$

270 where [Hb] = hemoglobin (g/dL); SaO₂ = arterial oxygen saturation (%; or SpO₂); and PaO₂ =
 271 partial pressure of arterial oxygen (mmHg). 1.36 is the binding capacity of oxygen to
 272 hemoglobin, and 0.003 is the fraction of free oxygen dissolved in blood (at 37°C). In cases where
 273 we did not have PaO₂ (Table 1), CaO₂ was estimated without it (using only [Hb] and SpO₂),
 274 given the negligible contribution of PaO₂ when [Hb] is normal or high.

275 Relative delta values were calculated with respect to baseline (1045 m) using:

276 Equation (5):

$$277 \Delta x = x_{altitude} - x_{baseline} \#$$

278 where x represents any variable.

279 RR was calculated for both nights at altitude (D2 and D9) following the formula reported
 280 in Zouboules *et al.* (2018):

281 Equation (6):

$$RR = \frac{\Delta[HCO_3^-]_a}{\Delta PaCO_2} \#$$

282 where [HCO₃⁻]_a is arterial bicarbonate (mM; i.e., renal response) and PaCO₂ (mmHg; i.e., renal
 283 stimulus) is partial pressure of arterial carbon dioxide.

284 **Statistical Analysis**

285 All continuous data is represented as mean ± SD in table format unless otherwise stated.
 286 Data were assessed for normality and variance using the Shapiro-Wilk and the Brown-Forsythe
 287 test. One-factor repeated measures ANOVAs were used to assess changes in daily ancillary
 288 cardiorespiratory and hematological variables, and arterial blood oxygenation, acid-base and
 289 electrolyte metrics. Where significant F-ratios were detected, Student-Newman-Keuls *post hoc*

tests were used for multiple pair-wise comparisons between testing days. Student's two-tailed t-tests were used to evaluate the difference between delta changes from baseline on days 2 and 9 for RR and delta pHa. A Pearson product moment correlation was used to assess RR against the change in pHa from baseline. A Cohen's d statistic was used to calculate effect size for the t-test comparing RR between D2 and D9. In all cases, statistical significance was assumed at $P < 0.05$ (SigmaPlot v10, Systat Software, Inc., San Jose, CA, USA).

RESULTS

In the final analysis, we included 16 participants where we had repeated measures on all metrics (5F, 11M, BMI = 26.0 ± 3.8 kg/m²; Age = 31.3 ± 11.7 yrs).

Daily cardiorespiratory measures and AMS scores from D0 to D9 are presented in Table 1. In addition, comprehensive arterial blood gas and electrolyte values from D0, D2 and D9 are presented in Table 2.

Figure 1 illustrates metrics of oxygenation with rapid ascent (D2) and residence (D9) at altitude. PaO₂ and SaO₂ were lower on D2 and D9 compared to D0 ($P < 0.01$; Figure 1A & 1B) but were higher on D9 than D2 ($P < 0.01$; Figure 1B). [Hb] was greater on D9 compared to D0 and D2 ($P < 0.01$; Figure 1C). CaO₂ decreased from D0 to D2 ($P < 0.01$) but then was partially corrected from D2 to D9 ($P < 0.01$) such that D0 and D9 were not different (Figure 1D).

Figure 2 illustrates metrics of acid-base homeostasis with rapid ascent (D2) and residence (D9) at altitude. PaCO₂ and [HCO₃⁻]_a were lower on D2 compare to D0 ($P < 0.01$), and lower still on D9 ($P < 0.01$; Figure 2A & 2B). pHa was not different on D2 or D9 from D0 ($P > 0.058$; Figure 2C).

Figure 3 illustrates renal reactivity (RR) and the change (Δ) in pHa on D2 and D9. RR was not significantly increased from D2 to D9 ($P = 0.056$; Effect size 0.61; Figure 3A). Δ pHa

was also unchanged between D2 and D9 ($P = 0.4$; Figure 3B). The magnitude of RR was negatively correlated with ΔpHa on D2 ($r = -0.95$, $P < 0.0001$; Figure 3C) and D9 ($r = -0.98$, $P < 0.0001$; Figure 3D).

Figure 4 illustrates Davenport diagrams on D0, D2 and D9 (Figure 4A, B and C). Although these plots are qualitative, they illustrate the integrated changes in PaCO_2 (right hand Y axis), pHa (X axis) and $[\text{HCO}_3^-]_a$ (left hand Y axis; same data as Figure 2). Qualitatively, it is evident that as PaCO_2 and $[\text{HCO}_3^-]_a$ values are incrementally lower with time at altitude (D2 and D9) compared to D0, and that pHa was relatively well-maintained throughout, despite what appears to be an initial alkalinisation within 24 hours of arrival on D2. Mean data in Figure 4D illustrate the integrated disturbances and compensation over time.

DISCUSSION

We aimed to characterize the time course and magnitude of integrated ventilatory and renal acclimatization following rapid ascent to and residence at 3,800 m for nine days. The principal findings were (a) compared to low altitude (D0), PaCO_2 and $[\text{HCO}_3^-]_a$ were lower on D2 and D9, (b) pHa was unchanged at D2 or D9 due to renal compensation, (c) RR did not increase significantly from D2 to D9 ($P = 0.056$), suggesting that renal acclimatization was in full effect within 24 hours of rapid ascent and (d) there was a strong, negative correlation between RR and ΔpHa from baseline on both D2 and D9. Our findings highlight the differential time-course, magnitude, and variability of integrated ventilatory and renal acclimatization following rapid ascent to and residence at high altitude.

Ventilatory Acclimatization

Previous studies have assessed ventilatory acclimatization using transient peak HVR tests before and after ascent (e.g., Powell et al., 2000; Teppema & Dahan, 2010). However, we argue

these methods are confounded due in part to the lack of equivalency between the HVR response at sea level with the HVR at altitude, given (a) PaCO_2 is lower at high altitude, resulting from the hypoxic ventilatory response in the steady-state, (b) PaCO_2 continues to be reduced over time resulting from ventilatory acclimatization and (c) renally-mediated bicarbonate elimination and relative metabolic acidosis sensitizes both central chemoreceptors and ‘unbrakes’ the hypocapnia-mediated blunting of peripheral chemoreceptors, given the stimulus interaction between O_2 and $\text{CO}_2/[\text{H}^+]$ at the carotid body (Lahiri & Delaney, 1975; Fitzgerald & Delghani, 1982). In addition, a transient peak response test may not capture the steady-state ventilatory strategy given the prevailing chemostimuli acting on both chemoreceptor compartments. Thus, we recently developed an index of steady-state chemoreflex drive (Pfoh et al., 2017), which we suggest captures ventilatory acclimatization in the steady-state during ascent (Bruce et al., 2018; Leacy et al., 2020). Specifically, we showed that SS-CD increased with incremental ascent to high altitude (Bruce et al., 2018; Leacy et al., 2020), and thus captured ventilatory acclimatization during rest with ascent to (Bruce et al., 2018; Leacy et al., 2020) and descent from 5,160 m (Leacy et al., 2020). In the present study, we assessed SS-CD during rapid ascent to and residence at 3,800 m for 9 days. We showed that both $\dot{V}_E$ and SS-CD increased upon immediate ascent to 3,800 m, and both further increased by 9 days of residence at this altitude. These data suggest that an immediate HVR was elicited with rapid ascent and that ventilatory acclimatization was responsible for further hypocapnia over time at 3,800 m (Table 1 and 2, Figure 2). These data further validate the utility of the SS-CD metric in high altitude fieldwork contexts in assessing an integrated central and peripheral chemoreflex for resting steady-state while residing at altitude. In addition, they illustrate respiratory-induced alkalosis with hypoxic exposure, which is a stimulus for renal compensation.

Renal Acclimatization and Acid-Base Regulation

Previous studies that assessed longitudinal acid-base regulation at altitude investigated PaCO_2 , $[\text{HCO}_3^-]_a$ and pH_a following rapid ascent to and residence at $\sim 4,300$ m over 7 days (Steele et al., 2020) and 10/11 days (Forster et al., 1975). These investigators found that pH_a compensation was incomplete, due in part to disproportionate reductions in both PaCO_2 and $[\text{HCO}_3^-]_a$. Participants were still alkalotic at day 7 ($\text{pH} = 7.45$; Steele et al., 2020) and days 10/11 ($\text{pH} = 7.449$; Forster et al., 1975). Although Forster et al. (1975) tested a limited sample size ($n = 5$), the consistency of the reported acid-base variables for these two studies suggests that pH_a remained alkalotic following 10/11 days of high altitude exposure. In contrast, Zouboules et al. (2018) found during incremental ascent to high altitude that although there was alkalosis at 3,440 m following 3 days of ascent, pH_a was normalized to baseline values at 3,820 m on day 5 and 4,240 m on day 7. However, with further ascent, a larger prevailing hypocapnic stimulus relative to bicarbonate compensation was observed, as participants were alkalotic again at 5,160 m on day 10. Similar relative alkalosis when ascending from 3,647 m to 4,554 m has been demonstrated in a small cohort ($n = 5$; Limmer et al., 2020). Conversely, pH_a was normalized within 24 hours upon arrival at 3,800 m in the present study, suggesting that pH maintenance is largely driven by the capacity of the renal system to detect and respond to dynamic changes in PaCO_2 during incremental and/or sustained residence-style ascent profiles.

Studies of chronic hypercapnia suggest that the severity of the hypercapnic stimulus determines whether the kidneys are able to facilitate a full compensatory metabolic alkalosis. Under conditions of mild chronic hypercapnia (1.5% inspired), renal compensatory mechanisms fully corrected pH_a (Schaefer et al., 1963), while more severe chronic hypercapnia (3% and 6% inspired) elicited only a partial renal compensation (Schaefer, 1949; Crosby et al., 2003; Burgraff

et al., 2018). In fact, in a severe hypercapnic environment (6% CO₂ inspired), pH_a was not fully compensated, even after 30 days of exposure (Burgraff et al., 2018). Further, when inspiring 3% CO₂, urine bicarbonate excretion was nonexistent until 6/7 days after returning to breathing room air, suggesting maximal bicarbonate retention was employed to combat the respiratory acidosis (Schaefer, 1949). When viewed together, these data suggest that there is a threshold for the efficacy of renal responses to mitigate PaCO₂ challenges. Similarly, in the context of high altitude hypoxia and hypocapnia, there may be a threshold of hypocapnic stimulus below which full renal compensation cannot be achieved, likely at an altitude between 3,800 m and 4,300 m.

Time-Course, Magnitude and Variability in Renal Responses

Our data suggest that there was an immediate HVR with ascent, which reduced PaCO₂, as expected (Tables 1 and 2 and Figure 3A). However, we did not expect to find that pH_a was fully compensated for within ~24 hours upon arrival (Day 2 of exposure; see Figure 3C), due to bicarbonate excretion (Figure 3B). This highlights a remarkably rapid renal compensation within our sample population. This may not be surprising however, as rapid compensatory metabolic alkalosis (Crosby et al., 2003) and acidosis (Ge et al., 2006) have been previously-shown to begin to occur within 24 hours of exposure to respiratory perturbations. In any case, as evidenced by our scatter plots on Day 2 (Figure 3), there appears to be distinct ventilatory and renal phenotypes along a continuum within our group of participants, based on the differential and integrated ventilatory and renal responses within the first 24 hours of exposure to high altitude. In one group, there was minimal HVR, and thus no renal hypocapnic stimulus, and pH remained unchanged. In yet another group, there was a robust HVR and concomitant hypocapnia, with no early renal compensation. Lastly, in another group, there was a robust HVR and concomitant hypocapnia, with early renal compensation. On balance, early HRV, hypocapnia and subsequent

early renal compensation dominated the statistical analysis in our study of 16 participants, bringing mean group pHa statistically back to baseline values within 24 hours of arrival to high altitude. Unfortunately, with only three time points (i.e., low altitude, day 2 and day 9 at 3,800 m) and 16 participants, our resolution is low to confirm the existence of three distinct phenotypes that participants move through with variable time courses and magnitudes with time spent at altitude. Future studies should aim to explore these potentially distinct 24-hour post-ascent phenotypes more systematically to determine if they play a role in both short and longer term acclimatization. In addition, the fact that (a) we were unable to maintain strict water and electrolyte ingestion in this field study (e.g., Biollaz et al., 2021; Gougoux et al., 1975) and (b) there was heterogeneity in BMI, age and ovarian hormone status (e.g., Dick and Prince, 2001; Gohar et al., 2017) in our participant pool, these factors that may introduce additional variability, and should be better-controlled for in future studies to address phenotypical variability in renal acid-base regulation at altitude.

The Davenport diagrams (Figure 4) represent a graphical demonstration of the Henderson–Hasselbalch relationships. They depict primary acid-base disturbances and their corresponding secondary compensations, including PaCO_2 isopleths (stimuli), pHa (as the controlled variable), $[\text{HCO}_3^-]_a$ (response) and the non- $[\text{HCO}_3^-]_a$ buffer slope (Davenport, 1974). Here, we plotted Davenport diagrams on D0 (1,045 m), D2 within 24 hours of arrival at 3,800 m, and D9 at 3,800 m. As expected, we observed that on D2, both PaCO_2 and $[\text{HCO}_3^-]_a$ are reduced, with pHa slightly, but not statistically elevated. On D9, both PaCO_2 and $[\text{HCO}_3^-]_a$ are reduced further and pHa appears to be fully corrected to baseline values. The superimposed mean data in Figure 4D shows the trajectory of disturbance-compensation relationship from D0, to D2, to D9. To our knowledge, this is the first time Davenport diagrams have been used to demonstrate acid-

base variables with rapid ascent and residence to high altitude. This further expands our understanding of acid-base disturbances and their respective renal compensations during ascent, particularly the extent of variability between a large group of participants.

Considerations and Reconciliation with Previous Reports

In a previous study from our group, Zouboules et al. (2018) developed and characterized a novel index of renal reactivity (RR) during incremental ascent to high altitude (5,160 m) whereby the change in [bicarbonate] (i.e., renal response) was indexed against the change in PaCO_2 (i.e., renal stimulus). We demonstrated plasticity in renal responses with incremental ascent, where RR increased with increasing altitude from 3,440 m (day 3) to 3,820 m (day 5) but plateaued thereafter. This plasticity may have been a consequence of incremental increases in altitude, which increased the HVR-mediated hypocapnic stimuli to the renal tubules and stimulated larger renal responses over time, until the full response was realized. In the current study, we employed a rapid ascent and steady-state model, where participants ascended from 610 m to 3,800 m over 5-6 hours and resided at this altitude for 10 days before descending. Thus, the present study differed from Zouboules et al. (2018) in two important ways. First, we did not increase the magnitude of the hypoxic stimulus over the approximately same time course. In fact, as evidenced by the increase in PaO_2 , SpO_2 , [Hb] and CaO_2 between days 2 and 9 in our study, our ascent and steady-state residence model eliminated the confounder of progressive, incremental hypoxic stimulation with further ascent that occurred in Zouboules et al. (2018). Second, given the VAH observed between days 2 and 9, as evidenced by the increased SS-CD and continued decreases in P_{ETCO_2} and PaCO_2 , the hypocapnic stimulus progressively increased over the duration of our study (from ~ 33 to 30 mmHg). However, RR did not increase significantly from Day 2 (0.46 ± 0.28) to Day 9 (0.59 ± 0.14 ; $P = 0.056$) suggesting that there

were concomitant, proportionate decreases in both PaCO_2 and $[\text{HCO}_3^-]$, without affecting RR. In comparison, Forster et al. (1975) had participants reside at 4,300 m for 10 days. A calculation from their mean data suggests similar RR values, from ~ 0.45 on day 2 to ~ 0.6 on day 10, similar to the present study. This is further corroborated by Limmer et al. (2020) during incremental ascent where a calculated mean RR of ~ 0.64 on day 3 (3,425 m) increased to ~ 0.71 on day 7 (4,554 m). These values from Limmer et al. appear to be slightly higher than other studies highlighted for two reasons. First, RR was calculated on day 3 of ascent, allowing time for more relative bicarbonate excretion to occur. Second, baseline measurements were obtained at 100 m, thereby giving comparatively larger baseline bicarbonate values (~ 25.4 mM) and thus a potentially larger bicarbonate excretion capacity. These results contrast with the findings of Steele et al. (2020) where an increase in RR from day 1 (0.098) to day 7 (0.54) was demonstrated. These considerations illustrate the biphasic nature of RR as ventilatory and renal compensations differ temporally. The low RR on day 1 for Steele et al. can be attributed to a predominance of Phenotype B (HVR-mediated hypocapnia, but with no appreciable renal response) when the measures were taken only 12 hours after ascent. As the acute HVR precedes the renal acclimatization process, there will be a point in time (e.g., <12 hours post-ascent) where there will be relatively minor changes in bicarbonate but concurrently large changes in PaCO_2 elicited by the HVR. Under these conditions, the numerator remains small (i.e., no change in bicarbonate), while the denominator will increase dramatically (i.e., large change in PaCO_2), resulting in a very low RR. Thus, RR should be interpreted taking into context the temporal differences between both HVR-mediated changes in PaCO_2 and the subsequent renal compensation. By contrast, in Limmer et al. (2020), a larger RR was observed by day 3 as the

full renal response is realized, and participants moved toward Phenotype C, where a HVR-mediated hypocapnia had elicited a renal compensation.

An alternative explanation for the plasticity noted in Zouboules et al. (2018) may have been due to progressive exposure to superimposed hypoxia and hypocapnia. Interestingly, in the present study, the increase in hypocapnic stimulus was larger (Figure 2) and oxygenation was improved (Figure 1) compared with the period RR plasticity was observed in Zouboules et al. (2018). There, PaCO_2 was not decreased from Day 3 (30.7 mmHg) to Day 5 (30.3 mmHg) and participants were hypoxic which suggests that a dose-dependent interaction may exist between sustained hypoxia and hypocapnia in driving renal plasticity. There is some evidence to support the hypothesis that sustained hypoxia interacts with hypocapnia to enhance renal compensation. In Gledhill et al. (1975), participants voluntarily hyperventilated for 26 hours in either room air or in a hypobaric hypoxia chamber simulating 3,100 m with roughly equivalent PaCO_2 between the two trials over time. The hypoxic-hypocapnic trial ($n = 8$) appeared to show more complete pH_a compensation over time compared to the normoxic-hypocapnic trial, although the authors report that these observed differences in pH_a were not significant. However, a calculation from their mean data between groups at the 9-hour time point suggests that RR was higher by ~45% in the hypoxic-hypocapnic trial compared to the hypocapnic trial alone (0.29 vs. 0.20, respectively). This potential difference between renal responses to sustained hypocapnia between hypoxia and normoxia in the same individuals hints that the comparable, iso-hypoxic stimulus between Day 2 and 9 in the present study may have attenuated the magnitude of RR compared to the incremental ascent model (Zouboules et al., 2018). There, PaO_2 and SaO_2 were maintained at approximately 50 mmHg and 85%, respectively between days 3 and 5, and RR appeared to show plasticity. Whereas in the present study, the continual rise in PaO_2 , SaO_2 , $[\text{Hb}]$ and CaO_2 (Tables 1 and 2;

Figure 1) over 7-days may have attenuated plasticity in RR, despite sustained hypocapnic stimulus due to VAH (Figure 3).

With respect to the effects of systemic hypoxia on the renal system, a previous report suggests that the HVR magnitude was related to diuresis and natriuresis in response to sustained (6 hours) of steady-state hypoxia (Swenson et al., 1995). Indeed, anatomical substrates are already known to exist whereby both extrinsic and intrinsic systems may account for the effects of hypoxia on the renal tubules. First, renal sympathetic nerves innervate not only the renal vasculature but also the renal tubular network (Johns et al., 2011), and hypoxia enhances sympathetic outflow (Keir et al., 2019; Prasad et al., 2020; Steinback et al., 2009), likely through carotid body activation (Dempsey & Morgan, 2015). Second, there is a known peritubular capillary system involved in oxygen sensing and hematological acclimatization through local HIF-1 α expression and subsequent erythropoietin release (e.g., Haase et al., 2010; Rosenburger et al., 2002). Thus, the intriguing possibility exists that hypoxia interacts with hypocapnia to augment renal acid-base acclimatization through bicarbonate elimination and acid retention, potentially through upregulation of membrane transporters in intercalated cells (Al-Awqati, 2003; Hamm et al., 2015; Roy et al., 2015; Schwartz et al., 2002; Welsh-Bacic et al., 2011). This hypothesis remains to be fully elucidated and requires further experimental attention in both animal and human models.

CONCLUSION

We characterized the time course, magnitude, and variability of integrated ventilatory and renal acclimatization following rapid ascent to and residence at 3,800 m for nine days. We found that compared to D0 (i.e., baseline), PaCO₂ and [HCO₃⁻]_a were lower on D2 and D9, and that pHa was unchanged at D2 or D9 due to renal compensation, suggesting a rapid time course of

renal compensation (within 24 hours). We also found that RR did not increase from D2 to D9. This indicates a short time course of renal acclimatization followed by maintenance of pH_a with progressive hypocapnia following rapid ascent and a subsequent 9-day residence at steady-state altitude (3,800 m). Similar to a previous study during incremental ascent, there was a strong negative correlation between RR and Δ pH_a from baseline on both D2 and D9 which suggests that carbon dioxide and bicarbonate are the dominant buffer system for acid-base homeostasis at high altitude, and those with larger renal compensation better protect pH_a in the context of sustained progressive hypocapnia. Our findings highlight the differential time course, magnitude, and variability of ventilatory and renal acclimatization following rapid ascent to and residence at high altitude, and suggest future work investigating the capacity for renal acclimatization and the role of renal-specific hypoxia on acid-base homeostasis.

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- 717

TABLE LEGENDS

Table 1. Daily ancillary measures of cardiorespiratory and hematological variables before and during rapid ascent to and residence: days (D)0 (1,045 m) and D1-D9 at 3,800 m). P_{ATM} , atmospheric pressure; P_{IO_2} , partial pressure of inspired oxygen; HR, heart rate; MAP, mean arterial pressure; $\dot{V}_E$, expiratory ventilation; P_{ETCO_2} , partial pressure of end-tidal carbon dioxide; SpO_2 , peripheral oxygen saturation; SS-CD, steady-state chemoreflex drive; [Hb], concentration of hemoglobin; CaO_2 , arterial oxygen content (calculated using SpO_2 and without PaO_2); AMS, acute mountain sickness scores. * denotes significant difference from baseline (1,045 m), $P < 0.05$. ‡ denotes significant difference from previous day, $P < 0.05$. Unless otherwise stated, $n = 15$. ^a $n = 14$. ^b $n = 13$.

Table 2. Arterial blood gas and electrolyte data during rapid ascent to and residence at 3,800 m on days (D)0, (D)2 and (D)9. PaO_2 , partial pressure of arterial oxygen; SaO_2 , arterial oxygen saturation; [Hb], concentration of hemoglobin; Hct, hematocrit; CaO_2 , arterial oxygen content; $PaCO_2$, partial pressure of arterial carbon dioxide; $[HCO_3^-]_a$, concentration of arterial bicarbonate; pH_a , arterial pH; RR, renal reactivity; SID, strong ion difference. * denotes significant difference from baseline (1,045 m), $P < 0.05$. ‡ denotes significant difference from previous day, $P < 0.05$. Unless otherwise stated, $n = 16$. ^a $n = 15$.

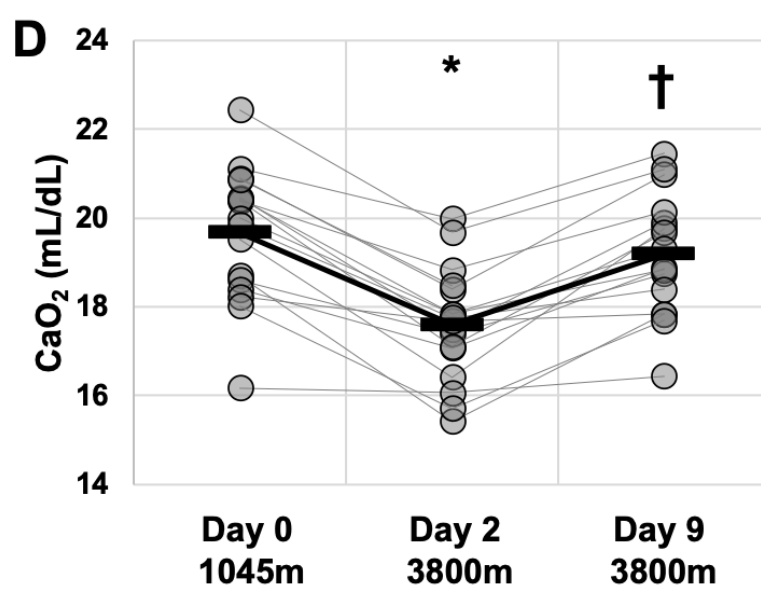
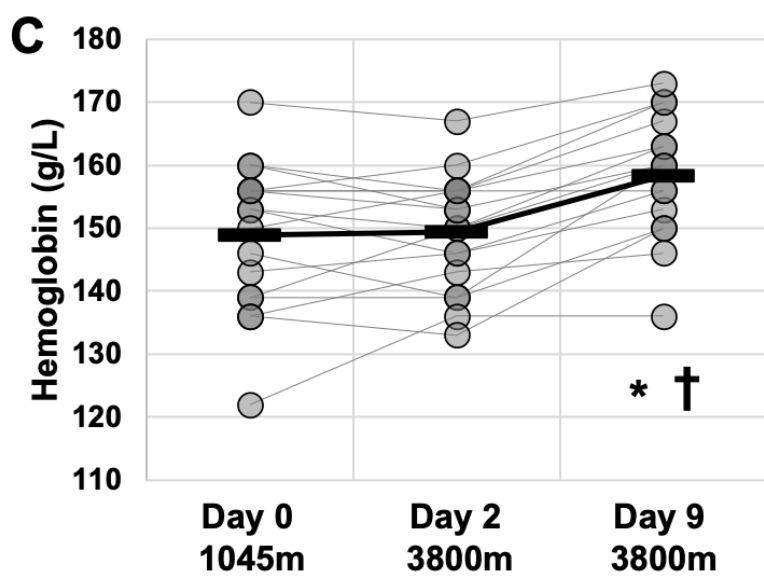
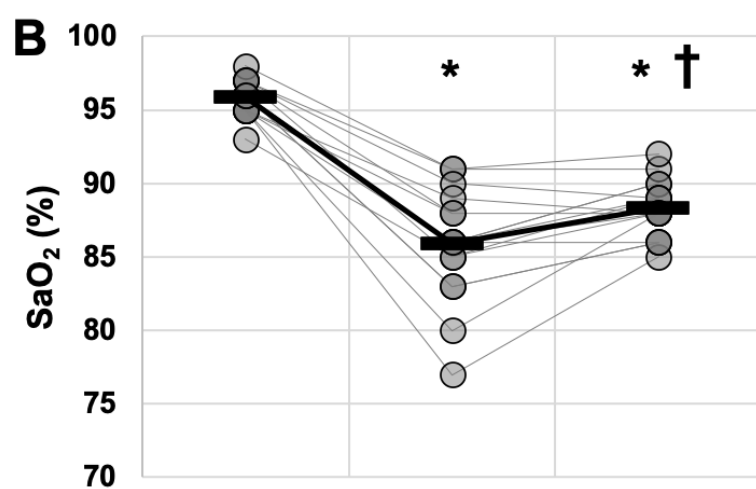
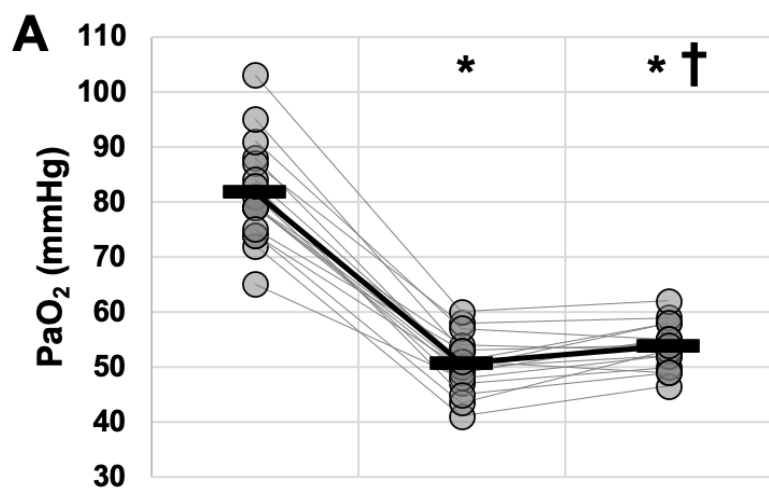
FIGURE LEGENDS

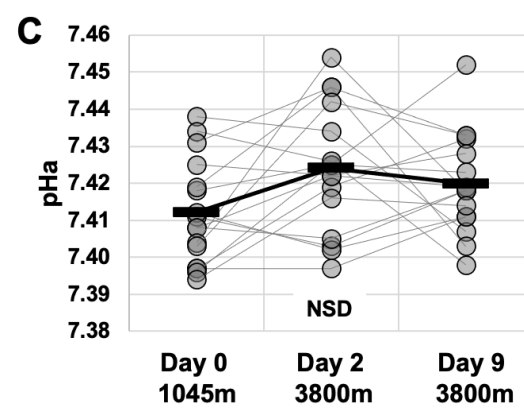
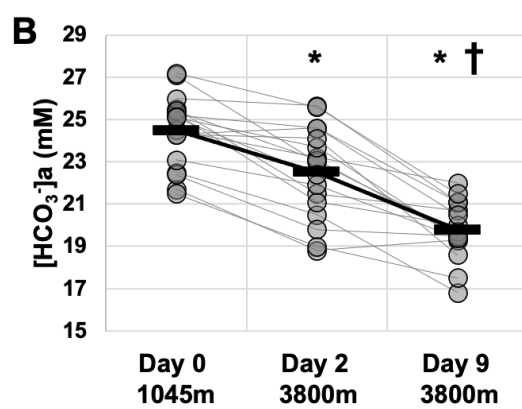
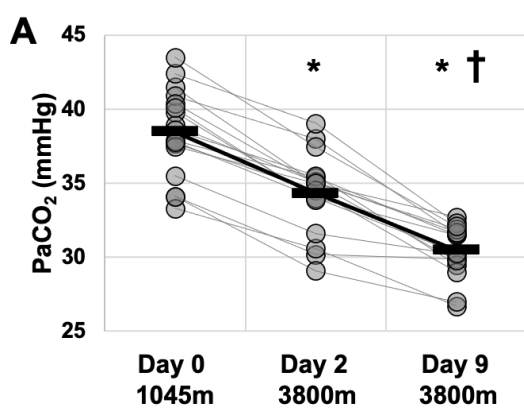
Figure 1. Oxygenation during rapid ascent and residence at 3800m. Data were obtained via arterial blood gas/electrolyte measures at 1,045 m (day 0), prior to ascent, and at 3,800 m on days 2 (within 24 hours of arrival by rapid ascent) and 9. A. PaO_2 , pressure of arterial oxygen. B. SaO_2 , arterial oxygen saturation. C. $[\text{Hb}]$, hemoglobin concentration and D. CaO_2 , arterial oxygen content. * denotes different than day 0. † denotes different than previous day. Presented as mean $\pm$ SD. $n = 16$.

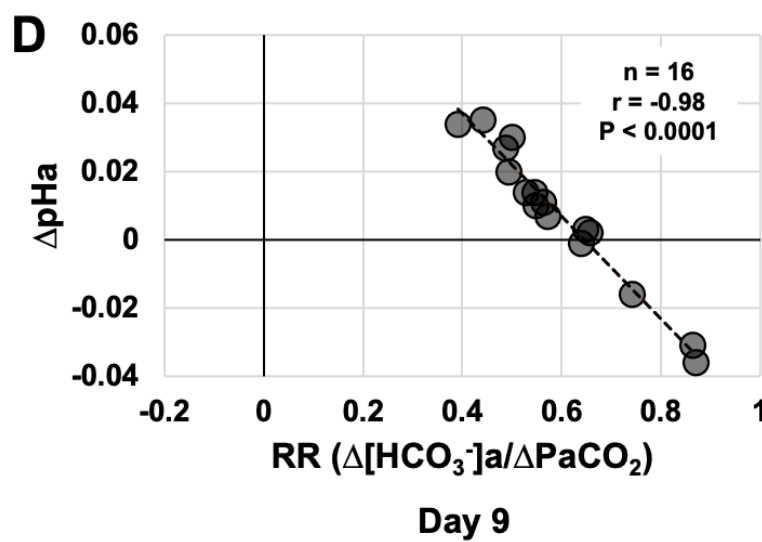
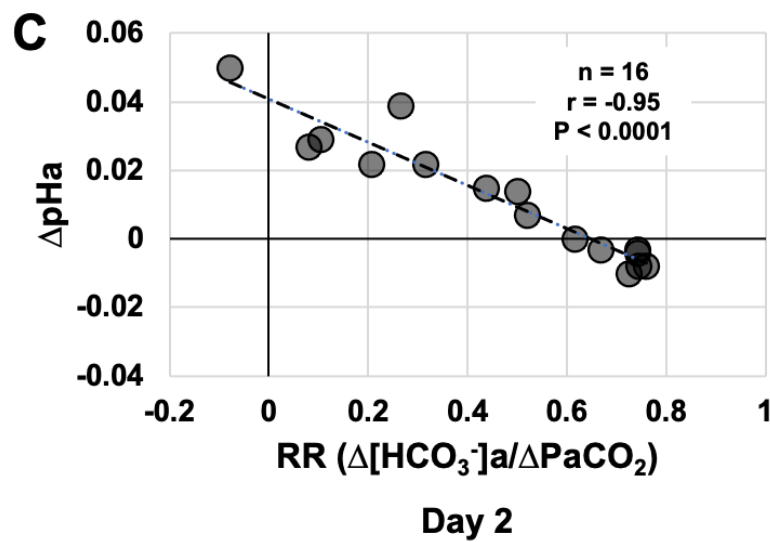
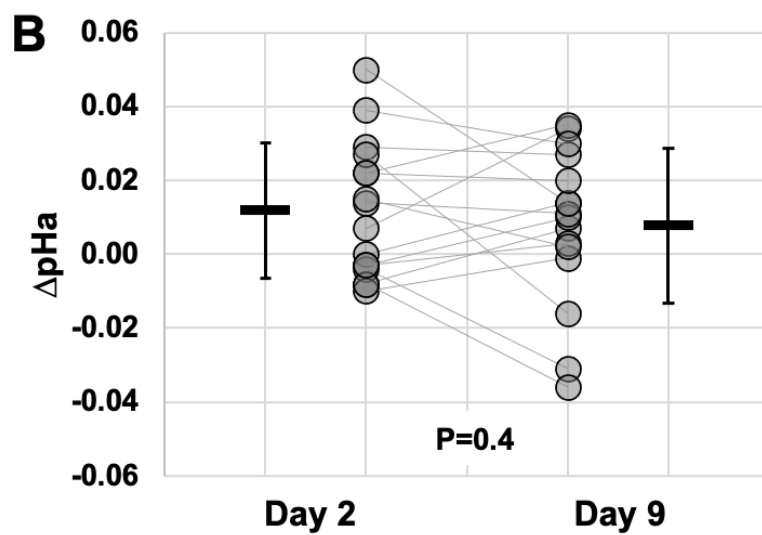
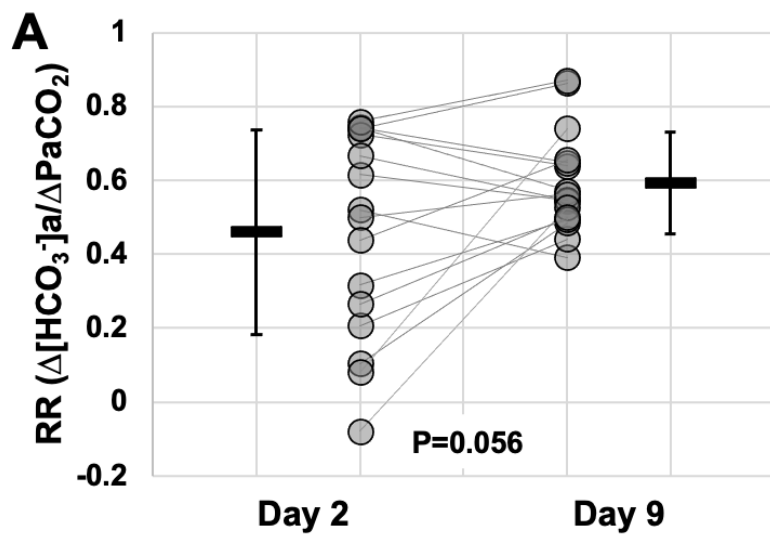
Figure 2. Carbon dioxide and acid-base variables during rapid ascent to and residence at 3800m. Data were obtained via arterial blood gas/electrolyte measures at 1,045 m (day 0), prior to ascent, and at 3,800 m on days 2 (within 24 hours of arrival by rapid ascent) and 9. A. PaCO_2 , partial pressure of arterial carbon dioxide. B. pH_a , arterial pH. C. $[\text{HCO}_3^-]_a$, arterial bicarbonate concentration. * denotes different than day 0. † denotes different than previous day. Presented as mean $\pm$ SD. $n = 16$.

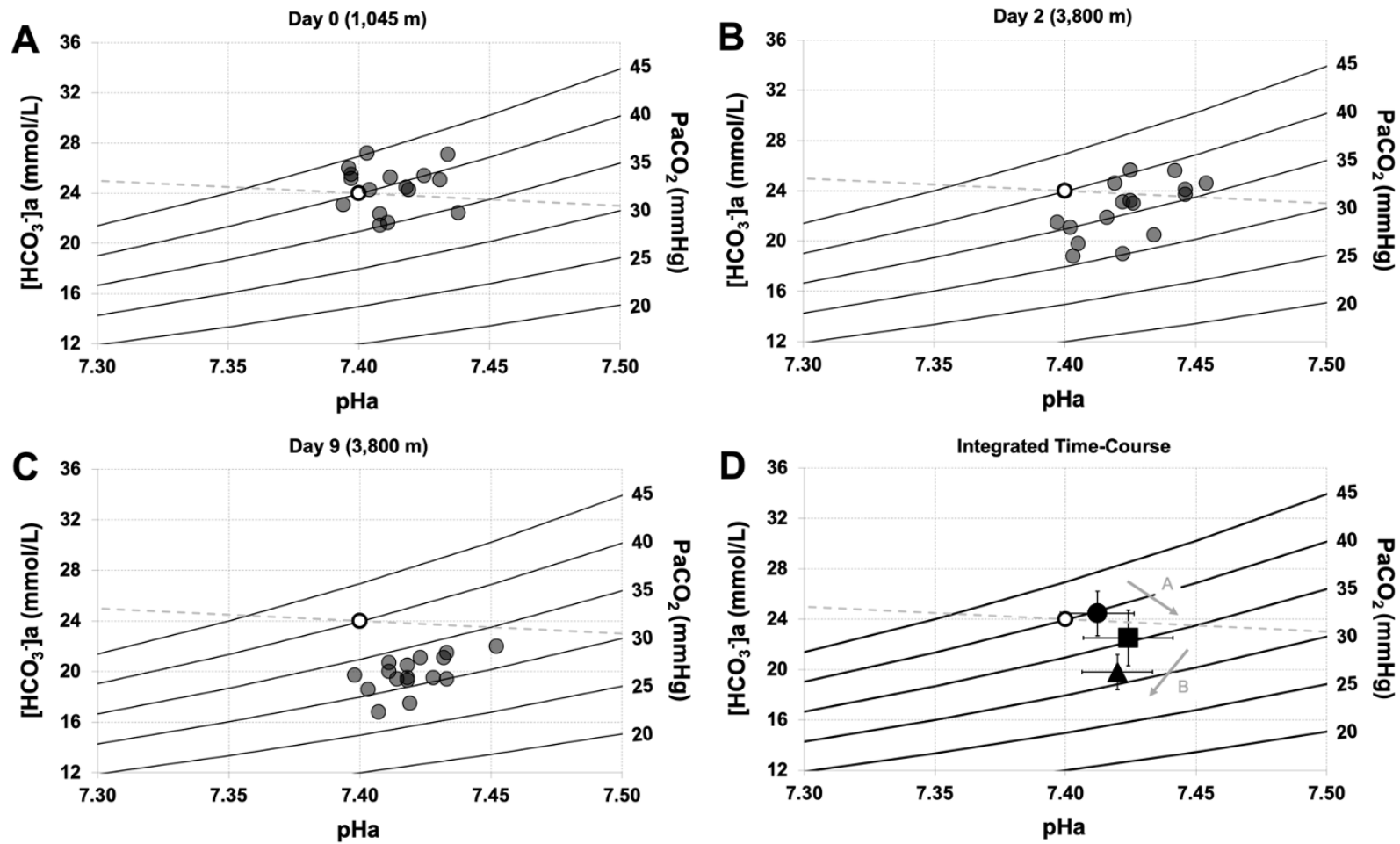
Figure 3. Renal reactivity and changes in pH_a during rapid ascent and residence at 3,800 m. Data were calculated from PaCO_2 , $[\text{HCO}_3^-]_a$ and pH_a variables reported in Figure 2. A. Renal reactivity (RR) calculated on days 2 (within 24 hours of arrival by rapid ascent) and 9 at 3,800 m, compared to D0 (1,045 m; $P = 0.056$). B. Delta pH_a calculated on days 2 and 9 at 3,800 m, compared to D0 (1,045 m; $P = 0.4$). C. Correlation between RR vs. pH_a Day 2 at 3,800 m. D. Correlation between RR vs. pH_a Day 2 at 3,800 m. For correlations (C and D), r , P and n values are presented on each graph. $n = 16$.

764 **Figure 4. Davenport diagrams during rapid ascent to and residence at 3800m.** Individual
765 data demonstrating relationships between PaCO_2 , $[\text{HCO}_3^-]_a$ and pH_a at A. 1,045 m on Day 0 B.
766 3,800 m on Day 2 (within 24 hours of arrival by rapid ascent) and C. 3,800 m on Day 9. D. Mean
767 data demonstrating dynamic relationship of stimulus-response relationships between acid-base
768 variables and time at 3800m. Open circle represents reference value. For A, B and C, filled
769 circles represent individual data. For D, filled circle represents 1,045 m (Day 0), filled square
770 represents 3,800 m (Day 2), filled triangle represents 3,800 m (Day 9), and arrows denote the
771 group directional changes over time. Error bars represent standard deviation. $n = 16$.









Variable	Calgary (D0)	Barcroft Lab							
		(D2)	(D3)	(D4)	(D5)	(D6)	(D7)	(D8)	(D9)
Altitude (m)	1,045	3,800							
P _{ATM} (mmHg)	665	487							
P _I O ₂ (mmHg)	130	92							
Cardiorespiratory Variables									
HR (min ⁻¹)	65.5 ± 12.4	75.5 ± 9.5*	69.1 ± 11.0	72.3 ± 12.0	72.3 ± 12.9	69.5 ± 11.1	70.3 ± 9.0	69.1 ± 11.7	70.9 ± 9.2
MAP (mmHg)	90.3 ± 11.9	89.7 ± 12.8	87.5 ± 11.5	84.2 ± 11.5*	86.3 ± 11.2	86.3 ± 9.8	84.4 ± 8.9*	84.5 ± 9.4*	87.0 ± 11.0
Ṁ _E (L/min)	8.6 ± 2.8	11.0 ± 3.8	10.4 ± 1.9	10.0 ± 2.4	9.5 ± 1.4	9.6 ± 1.9	10.0 ± 1.6	9.9 ± 2.8	10.1 ± 1.9
P _{ET} CO ₂ (mmHg)	36.9 ± 3.1	30.8 ± 4.1*	30.3 ± 2.9*	29.3 ± 3.6*	28.9 ± 1.6*	28.3 ± 2.7*	28.8 ± 2.7*	26.3 ± 3.0*‡	28.2 ± 2.5*‡
SpO ₂ (%)	97.5 ± 1.6	87.5 ± 4.2*	88.9 ± 3.0*	88.3 ± 2.2*	87.9 ± 2.9*	89.6 ± 2.3*	89.2 ± 2.7*	89.4 ± 2.2*	90.6 ± 2.9*
SS-CD (a.u.)	22.8 ± 7.2	31.9 ± 11.9*	30.5 ± 5.7*	30.4 ± 8.2*	28.8 ± 4.2*	30.8 ± 8.6*	31.3 ± 5.3*	34.3 ± 11.9*	32.8 ± 7.7*
[Hb] (g/L)	149.9 ± 12.2	153.0 ± 11.7	151.2 ± 10.5 ^a	154.1 ± 15.4	162.2 ± 12.6*	168.5 ± 15.8*	166.5 ± 13.7*	166.4 ± 10.3*	162.7 ± 14.8*
CaO ₂ (mg/dL)	19.9 ± 1.5	18.2 ± 1.4*	18.3 ± 1.4* ^a	18.5 ± 2.0*	19.4 ± 1.7‡	20.5 ± 1.9	20.2 ± 1.7	20.2 ± 1.2	20.0 ± 1.6
AMS (Median, [Range])	0 [0,0]	1 [0,8]*	1 [0,4] ^a	0 [0,2]‡ ^a	0 [0,1]	0 [0,2]	0 [0,2]	0 [0,2]	0 [0,2] ^b

Variable (mean ± SD)	Calgary (D0)	Barcroft Lab (D2)	Barcroft Lab (D9)
Altitude (m)	1,045	3,800	
P _{ATM} (mmHg)	665	487	
P _I O ₂ (mmHg)	130	92	
Oxygenation and Acid-Base/Electrolyte Variables			
PaO ₂ (mmHg)	80.8 ± 8.8	50.5 ± 5.2*	53.8 ± 4.1*‡
SaO ₂ (%)	95.8 ± 1.2	85.8 ± 3.8*	88.3 ± 1.9*‡
[Hb] (g/L)	148.4 ± 12.0	149.1 ± 9.4	158.3 ± 9.8*‡
Hct (%)	43.7 ± 3.5	43.9 ± 2.8	46.6 ± 2.9*‡
CaO ₂ (mL/dL)	19.6 ± 1.5	17.5 ± 1.3*	19.2 ± 1.4‡
PaCO ₂ (mmHg)	38.4 ± 3.0	34.3 ± 2.8*	30.5 ± 1.8*‡
[HCO ₃ ⁻]a (mM)	24.4 ± 1.8	22.5 ± 2.2*	19.8 ± 1.4*‡
pHa	7.412 ± 0.014	7.424 ± 0.017	7.420 ± 0.013
RR (Δ[HCO ₃ ⁻]a/ΔPaCO ₂)		0.46 ± 0.28	0.59 ± 0.14
SID (meq/L)	41.0 ± 2.2 ^a	39.4 ± 1.7*	37.5 ± 1.5*‡
Base Excess (mM)	-0.13 ± 1.8	-1.8 ± 2.5*	-4.7 ± 1.5*‡
Anion Gap (mM)	15.8 ± 1.8	16.3 ± 1.3	16.5 ± 1.1
Osmolality (mOsmol/kg)	283.2 ± 3.2	283.8 ± 1.8	282.3 ± 2.5
Lactate (mM)	0.97 ± 0.5 ^a	0.98 ± 0.5	0.74 ± 0.3
Creatinine (μM)	73.3 ± 12.4	77.3 ± 12.4	81.6 ± 14.3*