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What is the central question of this study?

We assessed the utility of a novel metric for quantifying ventilatory acclimatization to high altitude, derived from differential ascent and descent steady-state cardiorespiratory variables (i.e., hysteresis). Furthermore, we aimed to investigate whether the magnitude of cardiorespiratory hysteresis was associated with the development of acute mountain sickness.

What is the main finding and its importance?

Hysteresis in steady-state cardiorespiratory variables quantifies ventilatory acclimatization to high altitude. The magnitude of cardiorespiratory hysteresis during ascent to and descent from high altitude was significantly related to the development of acute mountain sickness symptoms. Hysteresis in steady-state chemoreflex drive can provide a simple, non-invasive method of tracking ventilatory acclimatization to high altitude.

Abstract

Maintenance of arterial blood gases is achieved through sophisticated regulation of ventilation, mediated by central and peripheral chemoreflexes. Respiratory chemoreflexes are important during exposure to high altitude due to the competing influence of hypoxia and hypoxic hyperventilation-mediated hypocapnia on steady-state ventilatory drive. Inter-individual variability exists in ventilatory acclimatization to high altitude, potentially affecting the development of acute mountain sickness (AMS). We aimed to quantify ventilatory acclimatization to high altitude by comparing differential ascent and descent values (i.e. hysteresis) in steady-state cardiorespiratory variables. We hypothesized that (a) the hysteresis area formed by cardiorespiratory variables during ascent and descent would quantify the magnitude of ventilatory acclimatization, and (b) larger hysteresis areas would be associated with lower AMS symptom scores during ascent. In 25 healthy, Diamox-free trekkers ascending to and descending from 5160m, cardiorespiratory hysteresis was measured in the

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pressure of end-tidal (P_{ET})CO_2, peripheral oxygen saturation (SpO_2), minute ventilation ($\dot{V}_E$), chemoreceptor stimulus index (SI; $P_{ET}CO_2/SpO_2$) and the calculated steady-state chemoreflex drive (SS-CD; $\dot{V}_E$/SI) using portable devices (capnograph, peripheral pulse oximeter and respirometer, respectively). AMS symptoms were assessed daily using the Lake Louise Questionnaire. We found that (a) ascent-descent hysteresis was present in all cardiorespiratory variables, (b) SS-CD is a valid metric for tracking ventilatory acclimatization to high altitude and (c) highest AMS scores during ascent were significantly, moderately and inversely-correlated to SS-CD hysteresis magnitude (r_s=-0.408, P=0.043). We propose that ascent-descent hysteresis is a novel and feasible way to quantify ventilatory acclimatization in trekkers during high altitude exposure.

Keywords: High altitude, hypoxia, respiratory chemoreflexes, ventilatory acclimatization, acute mountain sickness

Introduction

Chemoreceptors and Ventilatory Acclimatization

Arterial blood gases (PaO_2 and $PaCO_2$) are maintained via two interacting respiratory chemoreflex loops, which communicate to regulate ventilation (Boulet et al., 2016; Bruce et al., 2018; Pfoh et al., 2016). Central chemoreceptors (CCRs), located across several regions of the brainstem, hypothalamus and midbrain (Duffin, 2010; Nattie & Li, 2012), regulate ventilation in response to changes in CO_2 and/or [H^+] within brain tissue (Duffin, 2010; Pfoh et al., 2016; Natte & Li, 2012; Guyenet et al., 2010; Skow et al., 2014). In contrast, peripheral chemoreceptors (PCRs), located within the aortic and carotid bodies, regulate ventilation in response to changes in PaO_2, $PaCO_2$ and pH (Pfoh et al., 2016; López-Barneo et al., 2016). Both CCRs and PCRs regulate ventilation via afferent signaling to the respiratory control centers in the brainstem, which modulate ventilation accordingly. Both central and peripheral chemoreflexes are important in the context of high altitude exposure to mediate ventilatory acclimatization to chronic exposure to hypobaric hypoxia.

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Ventilatory acclimatization to hypoxia (VAH) can be described as the time-dependent increase in ventilation following chronic (days to weeks) exposure to hypoxia (Powell et al., 2000a). Typically, the assessment of ventilatory acclimatization is performed by determination of the hypoxic ventilatory response (HVR) (Pfoh et al., 2016 and 2017; Bruce et al., 2018; Steinback and Poulin, 2007; Teppema and Dahan, 2010).

Assessment of Chemoreflexes

The mode of assessment of respiratory chemoreflexes is dependent on the experimental question, experimental design and to some extent, feasibility within the context. Lab-based assessments are performed through bespoke gas challenges specific to chemoreflex responses under investigation (Duffin, 2007). For example, assessment of the central chemoreflex can be performed by incremental or progressive increases in CO_2 (hypercapnia) against a background of hyperoxia (Boulet et al., 2016; Duffin, 2011; Skow et al., 2014). The ventilatory response to steady-state increases in CO_2 is a result of augmented CCR activity, and the hyperoxia serves to dampen or silence PCR activity. In contrast, the peripheral chemoreflex can be elicited either through transient or steady-state gas challenges used to elicit acute and/or prolonged fluctuations in either PaO_2 and/or $PaCO_2$ (Pfoh et al., 2016; Koehle et al., 2009; Girard et al., 1959; Chua & Coats, 1995). Both steady-state and transient assessments of the peripheral chemoreflex are accompanied with several confounders which warrant consideration. Steady-state techniques are contaminated by: (a) autonomic, cerebrovascular and cardiovascular responses to chronic or prolonged hypoxia which may affect ventilation (Hoiland et al., 2015, Steinback & Poulin, 2007 and 2008), (b) high inter-subject variability and repeatability, calling into question their validity (Pfoh et al., 2016) and (c) the possibility of CCR and PCR interaction (Wilson and Teppema, 2016). In contrast, transient tests of peripheral chemoreflexes, which most likely specifically target the PCRs, do not elicit ventilatory

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response magnitudes equivalent to steady-state assessments (Pfoh et al., 2016; Teppema & Dahan, 2010). In short, the method of chemoreflex assessment should be specific to the experimental question under investigation, but isolating central or peripheral chemoreflexes in humans is challenging and likely confounded, particularly in fieldwork contexts.

Caveats of Chemoreflex Assessment during High Altitude Exposure

Although lab-based techniques are heavily utilized for chemoreflex assessment, several issues arise when implementing these techniques in field-based studies, such as high altitude expeditions. Primarily, lab-based assessments of chemoreflexes typically involve the use of sophisticated and expensive equipment such as end-tidal forcing systems, gas analyzers and calibration gas tanks (Koehle et al., 2009; Steinback and Poulin, 2007; Tymko et al., 2015). In certain circumstances, it may not always be feasible or wise for the transportation of such bespoke equipment during high altitude expeditions due to high transport costs as well as risk of damage. In addition, the process of eliciting a hypoxic stressor in an environment where participants are already hypoxic could prove both uncomfortable and potentially dangerous for participants. Typically, the process of measuring chemoreflexes is by identifying the peak ventilatory response to a given stimulus. Previous literature has examined the ventilatory response to both isocapnic and poikilocapnic hypoxia (Steinbeck & Poulin, 2007), the latter being representative of the environment at altitude. The results showed a blunted peak ventilatory response during poikilocapnic hypoxia compared with isocapnic hypoxia, due to the HVR-mediated hypocapnia and the known CO_2-O_2 stimulus interaction at the carotid body (Lahiri and DeLaney, 1975). Of note, after twenty minutes of exposure to poikilocapnic hypoxia, ventilation returns to near baseline (Steinback and Poulin, 2007). Exposure to high altitude incurs HVR-induced hypocapnia and resultant respiratory alkalosis. This respiratory alkalosis is offset by compensatory metabolic acidosis, through augmented bicarbonate

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excretion by the renal system (Dempsey et al., 2014; Goldfarb-Rumyantzev & Alper, 2014; Krapf et al., 1991). This reduction in buffering capacity could also increase CCR sensitivity to alterations in CO_2 (Bruce et al., 2018; Forster et al., 1971). The acid-base and CO_2 changes associated with exposure to altitude likely alter basal CCR and PCR chemoreceptor activity, thus making it difficult to compare chemoreflex response magnitude at altitude with unacclimated sea-level measures.

Proposal of a Novel Steady-State Chemoreflex Drive Metric

To combat the caveats of traditional lab-based assessments of chemoreflexes at altitude, our group proposes the utility of a novel metric termed steady-state chemoreflex drive (SS-CD). SS-CD can serve as an alternative to traditional lab-based assessments of PCR and CCR chemoreflexes by providing a feasible, portable and relatively inexpensive method of quantifying VAH by measuring specific steady-state cardiorespiratory parameters. In addition, unlike peak chemoreflex tests, the measurement of steady-state cardiorespiratory parameters is likely a better representation of the ventilatory acclimatization status of a participant for a given combination of prevailing O_2, CO_2 and pH stimuli whilst at altitude (Bruce et al., 2018). SS-CD can be quantified by the measurement of steady-state end-tidal CO_2 ($P_{ET}CO_2$), peripheral oxygen saturation (SpO_2) and minute ventilation ($\dot{V}_E$). Both $P_{ET}CO_2$ and SpO_2 are used to derive a stimulus index (SI: $P_{ET}CO_2$/ SpO_2; Bruce et al., 2018; Pfoh et al., 2017). SS-CD is then quantified by indexing $\dot{V}_E$ against SI ($\dot{V}_E$/SI; Bruce et al., 2018; Pfoh et al., 2017). Both $P_{ET}CO_2$ and SpO_2 are utilized to calculate SI as these metrics are positively and inversely linearly related to ventilation, respectively (Duffin, 2011; Rebuck & Campbell, 1974). In addition, SS-CD incorporates the cumulative effect of both hypocapnia (inhibitory) and hypoxia (excitatory) on

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steady-state ventilation, to provide a comprehensive observation of the effects of both CCR and PCR activity on ventilation in the resting steady-state at altitude.

Aims and Hypotheses

The aim of this study was to (a) assess the utility of SS-CD to characterize and quantify ventilatory acclimatization to hypoxia in the context of ascent to and descent from high altitude (i.e., hysteresis) and (b) assess whether SS-CD hysteresis magnitude during ascent was associated with the development of acute mountain sickness (AMS). We hypothesized that SS-CD ascent-descent hysteresis provides a feasible and portable metric for quantifying VAH at high altitude. Secondly, we reasoned that the magnitude of VAH at high altitude will be inversely related to the development of AMS, suggesting a role for ventilatory acclimatization in protecting against the development of AMS symptoms.

METHODS

Ethical Approval

This study abided by the Canadian Government Tri-Council policy on research ethics with human participants (TCPS2) and the Declaration of Helsinki, except for registration in a public database. Ethical approval was received in advance through Mount Royal University Human Research Ethics Board (Protocol 100012) and the Nepal Health Research Council (Protocol 109-2017). All participants were recruited via verbal communication and provided written and informed consent prior to voluntary participation in the study. This study took place in the context of a large research and field-course expedition to high altitude in the Nepal Himalaya. However, the specific study design, research question and data collection were planned *a priori*.

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Participant Recruitment and Inclusion Criteria

Twenty-nine healthy participants (14 females) were recruited as part of a research expedition to Everest base camp, Nepal Himalaya. The research expedition involved a slow, incremental ascent/descent profile to 5160m over a period of 18 days (see Figure 1). Participants were non-smokers and had no pre-existing cardiovascular, pulmonary, neurological or cerebrovascular medical conditions. Furthermore, in order to be included for analysis in this study, participants had to complete the entire research expedition without the aid of acetazolamide (Diamox). Acetazolamide is a pharmaceutical agent commonly prescribed for high altitude exposure, acting to increase ventilation through renal carbonic anhydrase blockade (Leaf & Goldfarb, 2007; Low et al., 2012; Luks et al., 2012). Data collection was completed at rest prior to breakfast each morning (06:00-09:00) on non-trekking days. Female ovarian status was not accounted for due to the nature of fieldwork expeditions. All participants lived below 1400m and were non-acclimatized prior to ascent, and each participant spent a total of three days at Kathmandu (1400m) before ascent began.

Instrumentation and Data Collection

Participants were instrumented for resting, steady-state measurements in a lodge between 06:00-09:00 after at least one night at each altitude: 1400m (day 0), 3440m (ascent; day 3), 4240m (ascent; day 7), 5160m (ascent; day 10), 4240m (descent, day 12), 3440m (descent; day 15) and again at 1400m (day 18). Data was collected in the seated position, and participants were provided with a personal mouthpiece and nose clip for the duration of the expedition. Participants were provided with noise cancelling headphones and asked to close their eyes during data recording. Participants were instrumented with a portable capnograph (Masimo EMMA, Danderyd, Sweden) for measurement of the pressure of end-tidal (P_{ET})CO_2 (Torr; altitude adjusted), finger pulse oximeter

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(Masimo Pronto; Switzerland) on their left middle finger for measurement of SpO_2 (%) and a respirometer (nSpire, Haloscale respirometer, USA) for ventilation ($\dot{V}_E$; L/min). Once instrumentation was complete, the participants were instructed to close their eyes and after approximately three minutes, their values were recorded and archived. Recording of participants' self-reported AMS scores were incorporated in the daily measurements. AMS was quantified using the Lake Louise scoring system during ascent and descent (LLS; Roach et al., 1993). All participants followed the same trekking profile (Figure 1) and data collection protocol throughout the expedition.

Data and Statistical analysis

The daily ascent and descent cardiorespiratory variables (SpO_2, $P_{ET}CO_2$ and $\dot{V}_E$) were plotted using Graphpad prism 8 software. Statistical analysis was performed using SPSS v.26 statistical software. Stimulus index (SI) was derived through mathematical modelling of the recorded steady-state cardiorespiratory variables (SI: $P_{ET}CO_2/SpO_2$). Steady-state chemoreflex drive (SS-CD) was then determined by indexing steady-state ventilation ($\dot{V}_{E)}$ against SI (SS-CD = $\dot{V}_E$/SI). Hysteresis areas were calculated using SigmaPlot software (Systat, v14). The area between the curves was calculated using definite integrals, the upper and lower limits (x-axis) being the lowest and highest altitudes reached during the trek (1400m and 5160m). Hysteresis area for SS-CD was dichotomized using a median split, with values below the median representing low responders and values above the median representing high responders, with the median value discarded. AMS positive (+) and AMS negative (-) participants were dichotomized by separating into two groups, with AMS+ defined as LLS of 3 or higher and AMS- defined as LLS of 0-2.

Prior to statistical analysis, normal distribution was assessed using the Shapiro-Wilk test. In addition, normal distribution was further assessed by visual assessment of histogram and q-q plots.

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If data did not violate the assumption of normal distribution, a repeated measures ANOVA was used for between-location analysis of variables. If data violated the assumption of normal distribution, a Friedman ranks test was performed. Following the Friedman ranks test, a Wilcoxon-rank test was used for post-hoc analysis of between-location differences in variables. Manual Bonferroni adjustments were made for multiple comparisons during the Wilcoxon-rank test. The level of statistical significance was divided by the number of multiple comparisons made to mitigate the risk of a type 1 error. In this instance, 21 comparisons were made between locations; therefore, our adjusted p-value was 0.05/21 = 0.002. Under normally distributed data statistical significance was set at $p<0.05$. As per journal guidelines, non-parametric data was presented as median with interquartile range (25^{th} – 75^{th} percentile), whereas normally distributed data are presented as mean ± standard deviation. High vs. low responders, relative to SS-CD ascent-descent hysteresis area, were compared using an independent samples t-test (two-tailed). The SS-CD ascent-descent hysteresis area was compared between AMS (+) and AMS (-) participants using an independent samples t-test (two-tailed). The SS-CD hysteresis area was correlated with AMS scores (highest score in each participant) using a Spearman Rho correlation. Lastly, as data for AMS is presented on an ordinal scale, a non-parametric Mann Whitney-U test was used to test for differences between low and high responders.

RESULTS

Participant Demographics

Of the initial 29 participants recruited for this expedition, four were excluded from final analysis. Two participants took Diamox at 5160m (day 9) due to AMS symptoms (but completed the trek), one participant took Diamox and descended from 4910m (day 8) to a lower altitude due to

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severe AMS symptoms, and one participant was evacuated by helicopter during the descent due to an ankle injury. Twenty-five participants, all of whom completed an identical trekking ascent-descent profile and remained Diamox-free, were included in the final analysis (27.2±11.2 years; BMI 22.9±2.8 kg/m^2; 10 females).

Cardiorespiratory Parameters

End-tidal CO_2

Non-parametric Friedman ranks test was performed to assess for between-location differences in end-tidal CO_2. There was a statistically significant interaction between location and end-tidal CO_2, $\chi 2$ (6) = 116.34, $p<0.001$. A Wilcoxon signed-rank test was used for further post-hoc comparisons between locations (see Figure 2A). End-tidal CO_2 was significantly reduced from baseline (1400m) at 3440m (ascent; day 3), 4240m (ascent; day 7), 5160m (ascent; day 10), 4240m (descent, day 12) and 3440m (descent; day 15) (median (IQR range): 31 (29-33) vs. 24 (23-27), 23 (20-24), 21 (20-22) and 22 (20-24), respectively; Torr, $p<0.001$). There was no statistical difference between baseline (1400m) and 1400m (descent day 18) (median (IQR range) 31 (29-33) vs. 30 (28-31), Torr, $p=0.054$).

SpO_2

Non-parametric Friedman ranks test was performed to assess for between-location differences in SpO_2. There was a statistically significant interaction between location and SpO_2, $\chi 2(6)$ = 129.38, $p<0.001$. A Wilcoxon signed-rank test was used for further post-hoc comparisons between locations (see Figure 2C). SpO_2 was significantly reduced from baseline (1400m) at 3440m (ascent; day 3), 4240m (ascent; day 7), 5160m (ascent; day 10), 4240m (descent, day 12) and 3440m (descent; day 15) (median (IQR range): 97 (96-98) vs. 92 (90-94), 90 (87-92), 82 (76-85), 92 (88-94),

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94 (93-95), respectively; %, p<0.001). There was no statistical difference between baseline (1400m) and 1400m (descent day 18) (median (IQR range)), 97 (96-98) vs. 97 (96-98), %, p=0.674).

Minute Ventilation ($\dot{V}_E$)

Non-parametric Friedman ranks test was performed to assess for between-location differences in $\dot{V}_E$. There was a statistically significant interaction between location and $\dot{V}_E$, $\chi2(6) = 48.394$, p<0.001. A Wilcoxon signed-rank test was used for further post-hoc comparisons between locations (see Figure 2E). $\dot{V}_E$ was significantly increased from baseline (1400m) at 5160m (ascent; day 10), 4240m (descent, day 12) and 3440m (descent; day 15) only (median (IQR range): 9.2 (7.3-10.7) vs. 10.9 (9.4-13.2), 11.6 (9.8-13.1), 11.8 (9.8-13.5), respectively; L/min, p<0.001). There was no statistical difference between baseline (1400m) and 1400m (descent day 18) (median (IQR range), 9.2 (7.3-10.7) vs. 9.9 (7.5-11.4), L/min, p=0.242).

Stimulus index (SI)

Data was normally distributed and did not violate Mauchly's test of sphericity. A repeated measures ANOVA demonstrated a statistically significant interaction between location and stimulus index (SI) $F(_{6,144}) = 59.19$, p<0.001, $Np^2 = 0.71$. Post-hoc analysis was performed to test for further significant differences between locations (see Figure 3A). SI was significantly reduced from baseline (1400m) to 3440m (ascent; day 3), 4240m (ascent; day 7), 5160m (ascent; day 10), 4240m (descent, day 12) and 3440m (descent; day 15) (mean ± SD: 0.32 ± 0.03 vs. 0.26 ± 0.03, 0.25 ± 0.03, 0.26 ± 0.03, 0.22 ± 0.02, 0.23 ± 0.02 respectively, p<0.001). There was no statistical difference between baseline (1400m) and 1400m (descent day 18) (mean ± SD: 0.32 ± 0.03 vs. 0.31 ± 0.02, p=0.674).

Steady-state chemoreflex drive (SS-CD)

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Non-parametric Friedman ranks test was performed to assess for between-location differences in SS-CD. There was a statistically significant interaction between location and SS-CD, $\chi2(6) = 87.857$, $p<0.001$. A Wilcoxon signed-rank test was used for further post-hoc comparisons between locations (see Figure 3C). SS-CD was significantly increased from baseline (1400m) at 3440m (ascent; day 3), 4240m (ascent; day 7), 5160m (ascent; day 10), 4240m (descent, day 12) and 3440m (descent; day 15) (median (IQR range): 28.03 (21.5-33.7) vs. 36.4 (27.6-44.8), 39.9 (29.5-51.9), 45.4 (35.4-51.5), 50.3 (43.5-68.8), 49.3 (44.3-56.6) respectively; $p<0.001$). There was no statistical difference between baseline (1400m) and 1400m (descent day 18) (median (IQR range), 28.0 (21.5-33.7) vs. 34.3 (23.3-39.4), $p=0.093$).

SS-CD Ascent-Descent Hysteresis

A non-parametric Mann Whitney-U test was performed to compare the highest self-reported AMS score between high and low responders (see Figure 4D). A statistically significant difference was found for the highest self-reported AMS score and group ($U = 37.5$, $p = 0.04$). SS-CD ascent-descent hysteresis area was compared between high and low responders (see Figure 4C). An independent samples t-test was performed to compare SS-CD ascent-descent hysteresis in both groups. A statistically significant difference was found for SS-CD ascent-descent hysteresis area in high vs. low responders (mean ± SD: 48883 ± 13061 vs. 16060 ± 8128, a.u., $t(22) = 7.39$, $p<0.001$).

SS-CD and Acute Mountain Sickness (AMS)

SS-CD ascent-descent hysteresis area in AMS (+) vs. AMS (-)

An independent samples t-test was used to compare SS-CD ascent-descent hysteresis area in AMS (+) and AMS (-) participants. No statistical difference was found for SS-CD ascent-descent

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hysteresis area in AMS (+) vs. AMS (-) participants (mean ± SD; 23985 ± 16861 vs. 39652 ± 19921, a.u., *t*(22) = -2.057, p=0.052, see Figure 5A).

SS-CD ascent-descent hysteresis area and AMS correlation

A Spearman rho correlation found a moderate inverse relationship between SS-CD ascent-descent hysteresis area and self-reported AMS, which was statistically significant ($r_s(23) = -0.408$, p = 0.043, see Figure 5B).

DISCUSSION

Primary Outcomes and Key Findings

We aimed to assess the utility of a novel metric, ascent-descent hysteresis in SS-CD, to quantify ventilatory acclimatization in the context of incremental ascent to and descent from high altitude (5160m) in the Nepal Himalaya. The main findings of this study are (a) SS-CD hysteresis appears to be useful for tracking ventilatory acclimatization during high altitude ascent and descent and (b) SS-CD hysteresis magnitude is inversely related to the development of AMS symptoms. These findings suggest that ventilatory acclimatization can be quantified in the steady-state using simple and portable devices. Furthermore, these findings suggest a relationship between ventilatory acclimatization and the development of AMS during incremental ascent to high altitude.

Cardiorespiratory Changes at Altitude

We observed several hallmark changes in cardiorespiratory variables during exposure to high altitude. Our findings demonstrate significant reductions in both end-tidal CO_2 and SpO_2 in conjunction with a significant increase in $\dot{V}_E$ during ascent, which were reversed as expected with descent. The reductions observed in both end-tidal CO_2 and SpO_2 are similar to those observed previously with identical ascent profiles (e.g., Bruce et al., 2018; Leacy et al., 2018; Zouboules et al.,

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2018). The changes observed in the cardiorespiratory system during exposure to high altitude are a result of the HVR, in part. Initially, low PaO_2 levels are detected by the peripheral chemoreceptors (Teppema and Dahan. 2010) with resultant increases in afferent signaling along the carotid sinus nerve to the respiratory control centers in the brainstem, causing an increase in ventilation (Teppema and Dahan, 2010; Gonzalez et al., 1994). There is also evidence to support the existence of hypoxic sensitive central chemosensors, which contribute to the HVR (Angelova et al., 2015; Gourine and Funk, 2017; Powell et al., 2000b). Thus, it is likely that the increase in steady-state ventilatory drive observed during high altitude exposure is derived from both peripheral and central input with time-dependent changes in the key mechanisms involved (Pamenter & Powell, 2016).

Steady-State Chemoreflex Drive

Our results offer strong support for the utility of SS-CD in quantifying VAH during high altitude exposure. As observed in Figure 3C, SS-CD increases incrementally during immediate ascent to altitude, returning to baseline levels following descent to 1400m. Of interest, we observed continued elevations in SS-CD at 3440m and 4240m during descent, compared with respective ascent values. This observation could be due in part to carotid body and central plasticity following acclimatization to chronic hypoxia (Dempsey et al., 2014). Numerous studies have demonstrated a heightened O_2 sensitivity at the carotid body following chronic exposure to hypoxia (Barnard et al., 1987; Nielsen et al., 1988; Vizek et al., 1987). This plasticity is not limited to the carotid body. Further evidence suggests central plasticity following chronic exposure to hypoxia (Dwinell and Powell, 1999). Plasticity, or increased gain, within the central nervous system (CNS) is due to changes in integration of afferent signaling with resultant increases in ventilatory motor output (Powell, 2007; Dempsey et al., 2014). Thus, increased carotid body and central sensitization to hypoxia likely

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contributes to the sustained elevation in SS-CD and ventilation observed at 3440m and 4240m during descent, compared with ascent values, following exposure to higher altitudes (5160m).

AMS and Respiratory Chemoreflexes

Several studies and reviews have attempted to determine the role of chemoreflexes and ventilatory acclimatization in the development of AMS through the simulation of high altitude environments, as well as comparing chemoreflex assessments at both sea-level and high altitude (e.g., Burtscher et al., 2008; Milledge et al., 1988; Smith et al., 2017; Grant et al., 2002). In general, the findings from these studies relating respiratory chemoreflexes and the development of AMS are inconsistent. Two conclusions can be drawn from these observations: (a) either there is no relationship between the development of AMS and chemoreflex acclimatization to altitude, or (b) the hitherto method of quantifying ventilatory acclimatization and chemoreflexes at altitude is inadequate. Our study suggests the latter. Due to the aforementioned concerns of utilizing lab-based assessments of chemoreflex at altitude (e.g., Bruce et al., 2018; Pfoh et al., 2016, 2017) we suggest our metric of SS-CD provides a more comprehensive analysis of the ventilatory strategy of individuals for a given O_2, CO_2 and acid-base status. Our results indicate a significant and inverse relationship in SS-CD hysteresis magnitude and the development of AMS symptoms, suggesting that greater ventilatory acclimatization to sustained hypoxia is associated with a moderately reduced susceptibility to AMS. From a physiological standpoint, larger SS-CD hysteresis magnitude could be a product of greater individual ventilatory sensitivity to hypoxia, resulting in more profound peripheral and central plasticity during chronic hypoxic exposure. The inverse relationship between SS-CD hysteresis magnitude and self-reported AMS could highlight the importance of VAH in preserving oxygenation and health during high altitude exposure. VAH could prove crucial for mitigating the risks of further deoxygenation during progressive ascent to altitude.

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Methodological Considerations

There are several limitations, which must be considered when interpreting these findings. Firstly, the requirement for both ascent as well as descent cardiorespiratory values in order to calculate SS-CD hysteresis calls into question the utility of this metric as a predictor variable for AMS in advance of an expedition. Ideally, simple physiological assessments and metrics, which can be performed either in a lab-setting at sea-level or progressively during ascent would have greater utility as a predictor variable for AMS. Unfortunately, such a metric or assessment has yet to be determined or developed. However, our findings suggest the importance of ventilatory acclimatization in preserving oxygenation and health during high altitude ascent, and our study advances a simple and portable technique for assessing ventilatory acclimatization in large groups of trekkers. In addition, our study provides normative values for SS-CD hysteresis for comparison for future trekkers and climbers as they ascend to and descend from similar altitudes.

The acid-base status of our participants was not recorded during ascent-descent. However, in a previous study by our group incorporating an identical ascent profile we showed that arterial pH is remarkably stable during incremental ascent up to 5160m (Leacy et al., 2018; Zouboules et al., 2018), suggesting adequate renal compensation during an 11-day incremental ascent model to 5160m. Unlike traditional lab-based peak chemoreflex assessments, SS-CD does not provide independent assessment of peripheral and/or central chemoreflex gain. Instead, it provides an assessment of overall steady-state ventilatory drive considering a conjugate of both central and peripheral chemoreflexes and prevailing chemostimuli. We are not suggesting that SS-CD should replace traditional lab-based techniques used in peripheral or central chemoreflex assessments, where these techniques are warranted for particular mechanistic studies.

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Lastly, the time-course of ascent and descent profiles was not uniform. Ascent from 1400m to 5160m was achieved over 11 days. In contrast, descent from 5160m to 1400m was achieved over 9 days. How this might have affected cardiorespiratory hysteresis remains unclear. One could speculate that a longer descent profile would afford more time for both peripheral and central plasticity to chronic hypoxia. This could potentially increase the hysteresis area formed by cardiorespiratory variables. In any case, unlike ascent to high altitude, there are no guidelines regarding the descent from altitude, and a more rapid descent profile may have made our assessment impossible, given that we made measurements at identical altitudes during descent as during ascent (i.e., 3440m, 4240m).

Conclusions

In conclusion, our findings demonstrate a novel metric for the assessment of chemoreflex drive during incremental ascent to and descent from high altitude, namely calculation of ascent-descent hysteresis magnitude of cardiorespiratory variables (SS-CD). The simplicity and portability of the SS-CD measurement illustrates the feasibility of its use in fieldwork contexts, thus providing utility to large research and/or commercial expeditions to high altitude. These findings provide valuable insight into the importance of ventilatory acclimatization in the protection against AMS symptoms. Future studies should examine the utility of this metric across various ascent profiles as well as prolonged exposure to altitude, where individuals spend extended periods at high altitudes before descending, where we anticipate that SS-CD hysteresis will be larger than the present study. Additionally, given the utility of carbonic anhydrase inhibitors during high altitude expeditions, and their effects on ventilation, it would be of interest to measure SS-CD hysteresis in participants receiving Diamox prophylaxis and/or treatment. Presumably, given that Diamox acts as a respiratory stimulant, it may serve to increase SS-CD magnitude in the context of high altitude ascent.

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COMPETING INTERESTS

No competing interests to declare.

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AUTHOR CONTRIBUTIONS

All measurements were taken during a research expedition to Everest base camp, Nepal Himalaya. Initial data analysis and interpretation was performed in Mount Royal University, Calgary, Canada. Further analysis and interpretation as well as manuscript formation was completed at University College Cork, Cork, Ireland. Acquisition of data was completed by ZR, BH, LM, JS, ST, JB, and AK. Data analysis was performed by SZ, ZR and JL. Further data analysis, interpretation as well as drafting the manuscript and critically revising it was completed by JL, AL, KOH and TD. Design of work/study was completed by TD and TB. Nepal ethical approval was supported by MS. All authors have approved the final version of this manuscript and agree to be accountable for all aspects of the manuscript formation and data analysis relating to accuracy and integrity. Furthermore, all persons designated as authors on this manuscript qualify to do so.

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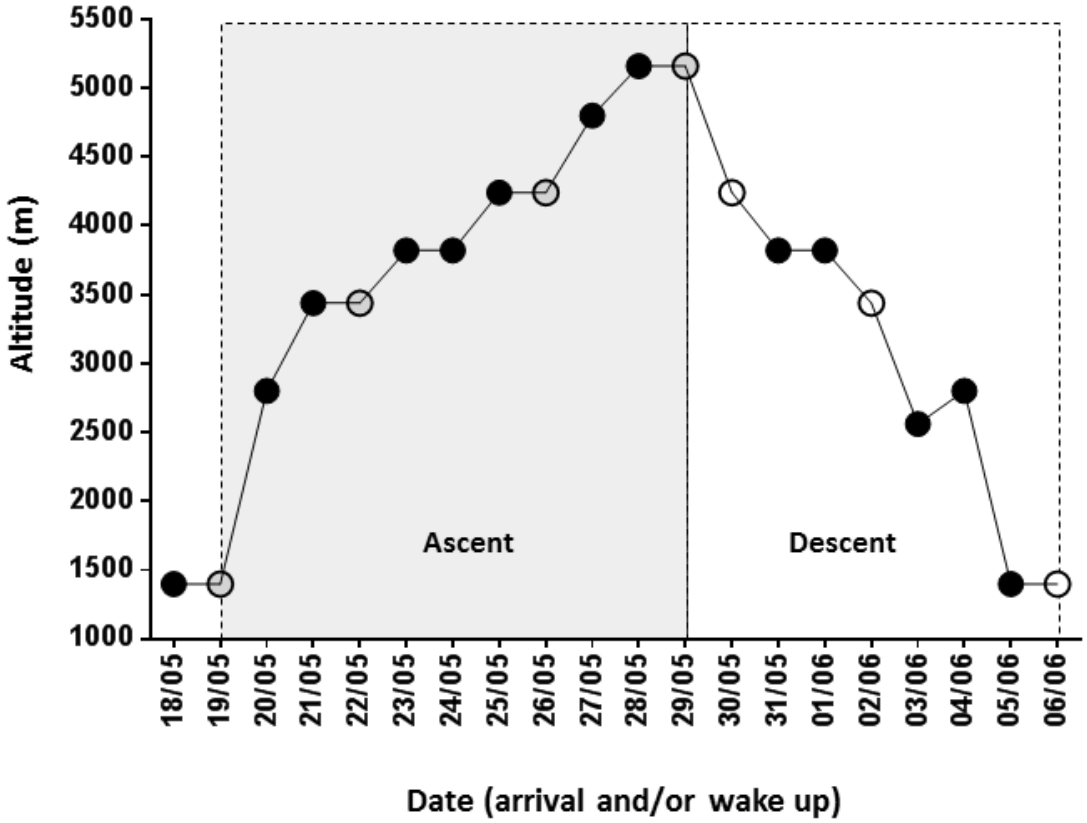


Figure 1. Ascent-descent altitude trekking profile (date and altitude) of the expedition group. Measurements were made on participants every morning (~06:00-09:00) on indicated rest days during ascent and descent, including 1400m (day 0), 3440m (ascent, day 3), 4240m (ascent, day 7), 5160m (ascent, day 10), 4240m (descent, day 12), 3440m (day 15), 1400m (day 18). Grey shaded circles indicate measurement days during ascent and white/clear circles indicate measurement days during descent.

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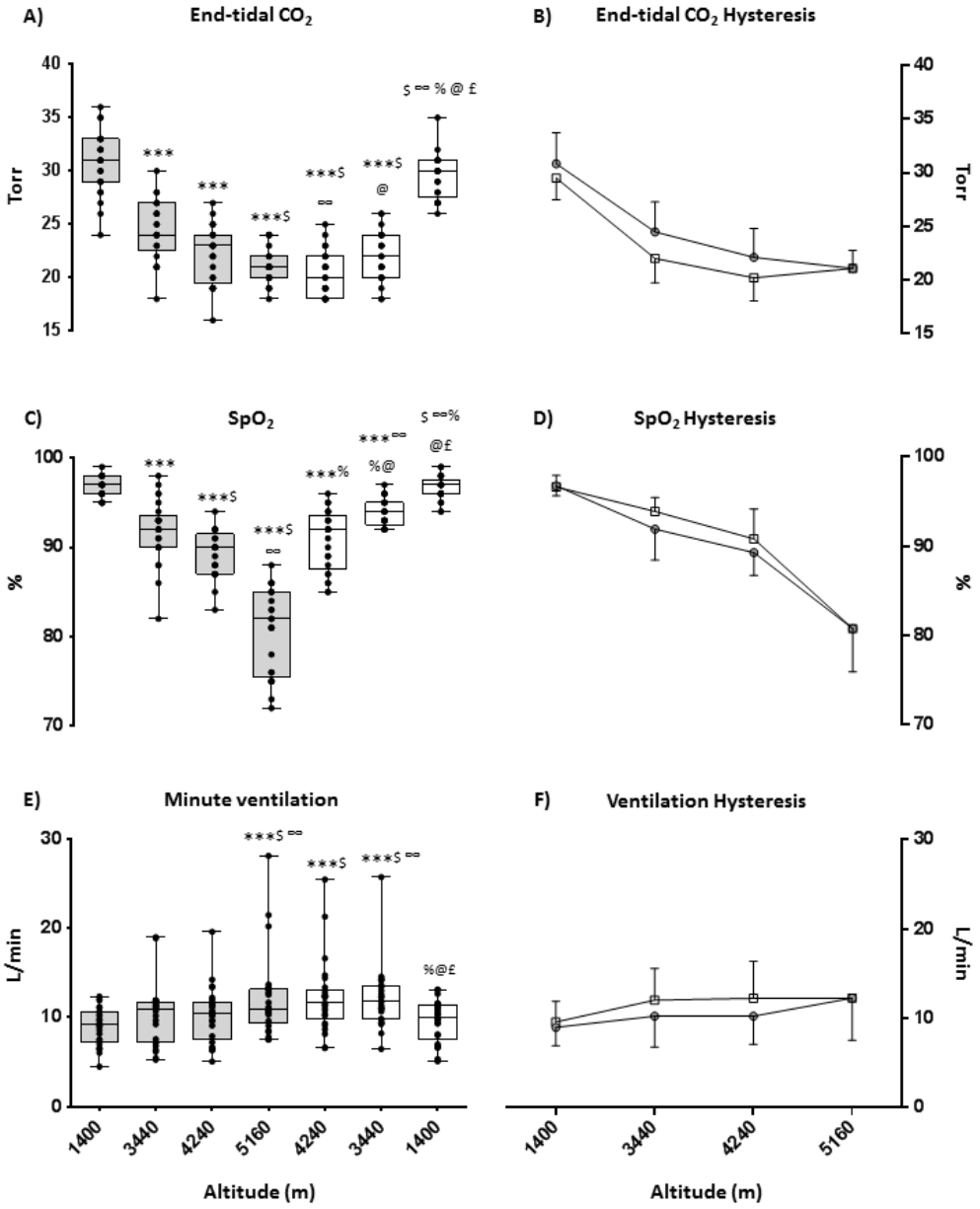


Figure 2. Cardiorespiratory measures during ascent and descent, illustrating hysteresis. Grey box and whiskers and circles (hysteresis) denote ascent. White/clear box and whisker and squares (hysteresis) denote descent. (A) Pressure of end-tidal (P_{ET})CO_2 (Torr) during ascent and descent. (B) $P_{ET}CO_2$ hysteresis during ascent and descent. (C) Peripheral oxygen saturation (SpO_2; %) during ascent and descent. (D) SpO_2 hysteresis during ascent and descent. (E) Minute Ventilation ($\dot{V}_E$; L/min) during ascent and descent. (F) $\dot{V}_E$ hysteresis during ascent and descent. All values are presented in box and whisker format. *** = P<0.001 from 1400m. $ = P<0.05 from 3440m. ∞ = P<0.05 from 4240m. % = P<0.05 from 5160m. @ = P<0.05 from 4240m (descent). £ = P<0.05 from 3440m (descent).

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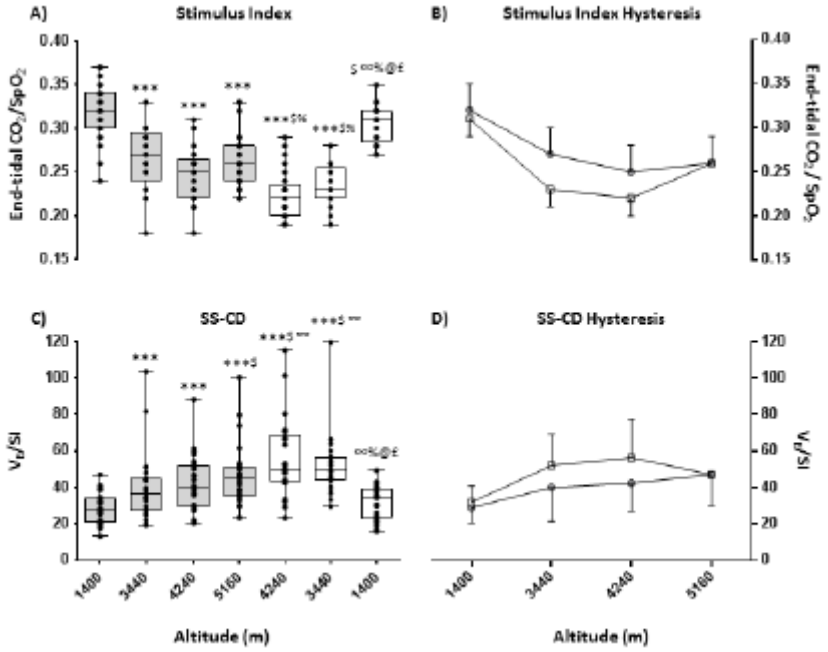


Figure 3. Stimulus Index and steady state chemoreflex drive during ascent and descent, illustrating hysteresis. Grey box and whiskers and circles (hysteresis) denote ascent. White/clear box and whisker and squares (hysteresis) denote descent. (A) Stimulus index ($P_{ET}CO_2/SpO_2$) during ascent and descent. (B) SI hysteresis during ascent and descent. (C) Steady-state chemoreflex drive (SS-CD; $\dot{V}_E$/SI) during ascent and descent. (D) SS-CD hysteresis during ascent and descent. All values are presented in box and whisker. *** = P<0.001 from 1400m. $ = P<0.05 from 3440m. ∞ = P<0.05 from 4240m. % = P<0.05 from 5160m. @ = P<0.05 from 4240m (descent). £ = P<0.05 from 3440m (descent).

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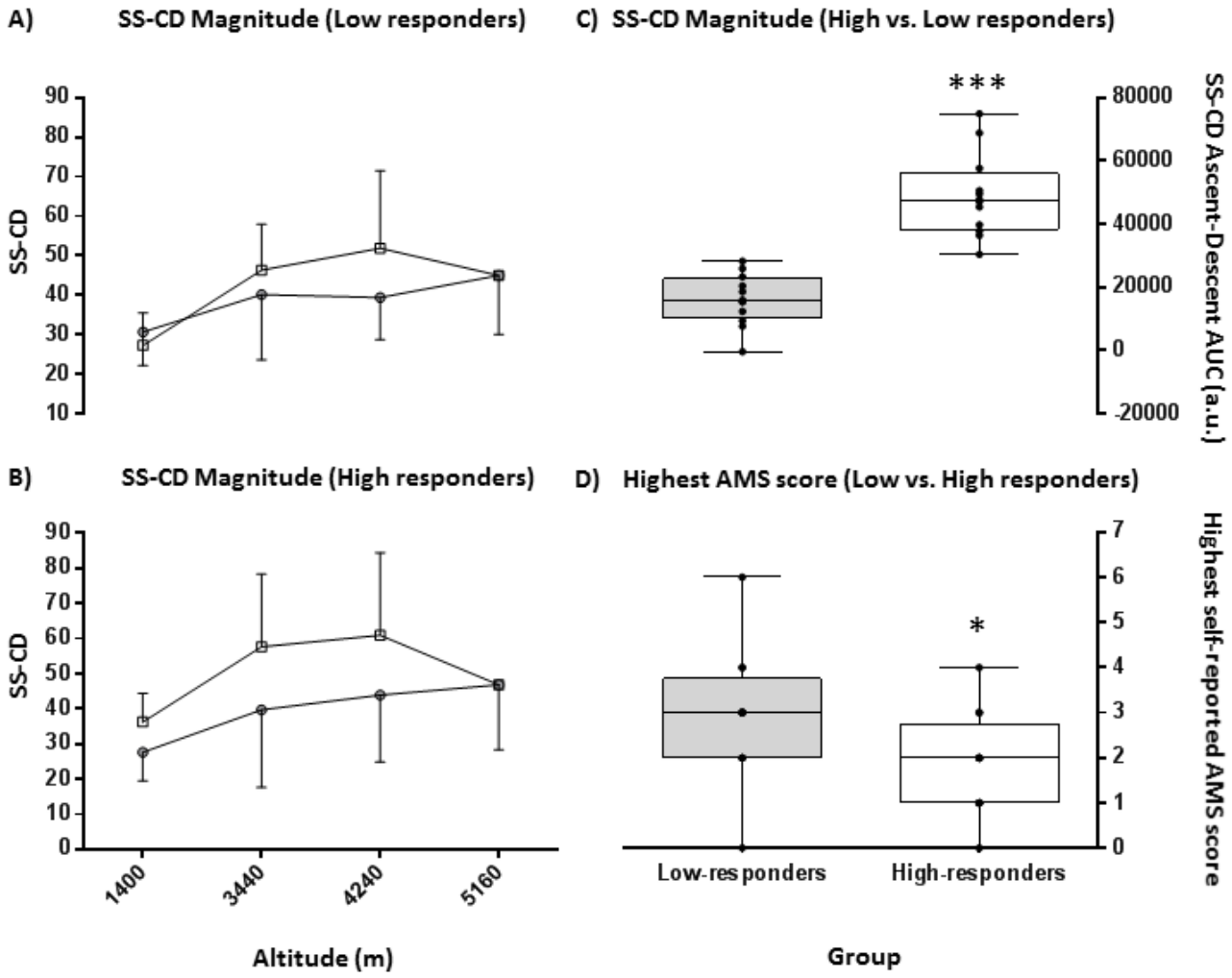


Figure 4. Dichotomization between high and low ventilatory acclimatization responders. (A) Steady-state chemoreflex drive (SS-CD) hysteresis during ascent and descent of low responders. (B) SS-CD hysteresis during ascent and descent of high responders. (C) Comparison of SS-CD hysteresis area for low and high responders. (D) Comparison of the highest self-reported AMS score in low and high responders. In A and B, grey circles denote ascent and white/clear squares denote descent. In C, SS-CD ascent-descent is presented in box and whisker format and compared between low (grey) and high (white/clear) responders. In D, the highest self-reported AMS score is compared between low (grey) and high (white/clear) responders, presented in box and whisker format. *** = P<0.001 from low responders, * = P<0.05 from low responders.

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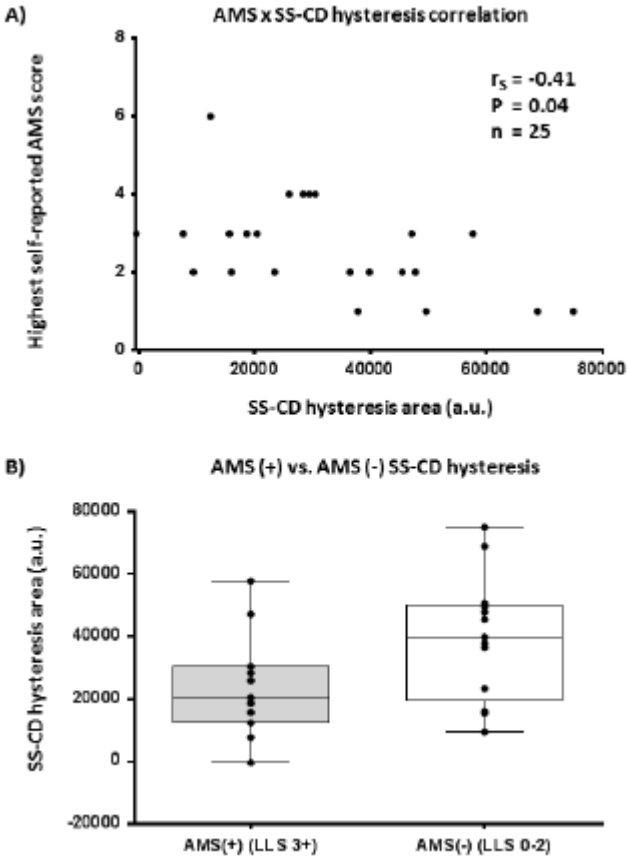


Figure 5. Relationship between steady-state chemoreflex drive (SS-CD) hysteresis area and AMS symptom severity. (A) Correlation between SS-CD AUC and highest AMS score during ascent. (B) Comparison of SS-CD AUC between AMS positive (highest score 3+; n=11) and AMS negative (highest score 0-2; n=13) participants (p = 0.052).

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