CEREBRAL HEMODYNAMIC AND VENTILATORY RESPONSES TO HYPOXIA, HYPERCAPNIA AND HYPOCAPNIA DURING 5 DAYS AT 4,350 M

Altitude and cerebral hemodynamics

Thomas Rupp, PhD[1,2], François Esteve, MD PhD[3,4], Pierre Bouzat MD PhD[3,4], Carsten Lundby, PhD[5], Stéphane Perrey, PhD[6], Patrick Levy, MD PhD[1,2], Paul Robach, PhD[1,2,7], Samuel Verges, PhD[1,2]

[1] INSERM U1042, Grenoble, F-38000, France

[2] HP2 laboratory, Joseph Fourier University, Grenoble, F-38000, France

[3] U836/team 6, INSERM, F-38000, Grenoble, France

[4] Grenoble Institute of Neurosciences, Joseph Fourier University, F-38000, Grenoble, France

[5] Institute of Physiology, University of Zurich, CH-8006, Zurich, Switzerland

[6] Movement To Health (M2H), Montpellier-1 University, Euromov, F-34090, Montpellier, France

[7] Ecole Nationale de Ski et d'Alpinisme, F-74400, Chamonix, France

Corresponding author:

Dr Verges Samuel, Laboratoire HP2 (INSERM U1042), UF Recherche sur l'Exercice, Hôpital Sud, Avenue Kimberley, 38 434 Echirolles, France.

Tel: +33 4 76 76 68 60; Fax: +33 4 76 76 89 21; Email: sverges@chu-grenoble.fr

Disclosure/Conflict of Interest

The authors have no disclosure or conflict of interest to declare.

Acknowledgments

We thank all the subjects for participating to this study, the SMTEC company for providing the gas mixing device, Philips and Atys Medical for providing the TCD devices and the Rhône-Alpes Region for financial support.

ABSTRACT

This study investigated changes in cerebral near-infrared spectroscopy (NIRS) signals, cerebrovascular and ventilatory responses to hypoxia and CO_2 during altitude exposure. At sea level (SL), after 24 h and 5 days at 4,350 m, 11 healthy subjects were exposed to normoxia, isocapnic hypoxia, hypercapnia and hypocapnia. The following parameters were measured: pre-frontal oxygenation index (TOI), oxy- (HbO_2), deoxy- and total hemoglobin (HbTot) concentrations with NIRS, blood velocity in the middle cerebral artery (MCAv) with transcranial Doppler and ventilation. Smaller pre-frontal deoxygenation and larger ΔHbTot in response to hypoxia were observed at altitude compared to SL (day 5: ΔHbO_2 -0.6±1.1 vs. -1.8±1.3 µmol·cm·mmHg^{-1} and ΔHbTot 1.4±1.3 vs. 0.7±1.1 µmol·cm·mmHg^{-1}). The hypoxic MCAv and ventilatory responses were enhanced at altitude. Pre-frontal oxygenation increased less in response to hypercapnia at altitude compared to SL (day 5: ΔTOI 0.3±0.2 vs. 0.5±0.3 %·mmHg^{-1}). The hypercapnic MCAv and ventilatory responses were decreased and increased, respectively, at altitude. Hemodynamic responses to hypocapnia did not change at altitude. Short-term altitude exposure improves cerebral oxygenation in response to hypoxia but decreases it during hypercapnia. Although these changes may be relevant for conditions such as exercise or sleep at altitude, they were not associated with symptoms of acute mountain sickness.

Keywords: altitude illness, carbon dioxide, cerebral hemodynamic, near-infrared spectroscopy, oxygenation

INTRODUCTION

Sufficient oxygenation of brain tissue is critical to avoid severe symptoms and life-threatening consequences during hypoxic exposure[14]. Oxygen delivery to the brain is dependent on both arterial O_2 content and cerebral perfusion. During hypoxic exposure, the cerebral blood flow (CBF) greatly depends on the balance between the O_2 and the carbon dioxide (CO_2) cerebrovascular responses, since reduced arterial oxygenation is accompanied by hyperventilation-induced changes in arterial CO_2.

Global CBF at high altitude has been measured by using inert tracers such as the Kety-Schmidt nitrous oxide washout method[25,36] or the ^{133}xenon technique[18]. Cerebrovascular responses to O_2 and CO_2 have mostly been measured by using transcranial Doppler (TCD)[5]. Near-infrared spectroscopy (NIRS) has been proposed as an attractive and complementary methodology to TCD in order to measure cerebrovascular reactivity[37]. Compared to TCD, cerebral NIRS has the advantage to provide insights into local tissue hemodynamics compared to the assessment of perfusion in large vessels by TCD and to measure not only the amount of blood under the probe (total hemoglobin content, HbTot) but also the respective concentration changes of oxy- (HbO_2) and deoxy- (HHb) hemoglobin reflecting cerebral tissue oxygenation[29].

Cerebral oxygenation measured by NIRS is reduced when arterial oxygenation drops during acute hypoxic exposure (10 min - 10 h[2,20,28]). After 2-5 days of hypoxic exposure, the cerebrovascular response to hypoxia measured by TCD was shown to be enhanced, *i.e.* the middle cerebral artery blood flow velocity (MCAv) increased to a greater extent in response to hypoxia[19,31]. It remains to determine whether an enhanced hypoxic cerebrovascular response during prolonged hypoxic exposure (several days) may induce a better preservation

of cerebral oxygenation in response to hypoxia and consequently a better acclimatization to high altitude.

Controversial results are available regarding changes in cerebrovascular response to CO_2 following several days at high altitude, with unchanged[3,4,17], reduced[22] or increased[12,18] responses to hypercapnia, and reduced[3] or increased[8,17,22] responses to hypocapnia. Some studies showed that in response to hypercapnia at sea level, the enhanced MCAv is accompanied by parallel changes in NIRS signal, *i.e.* a large increase in HbO_2 and a small decrease in HHb concentrations[37,41]. Changes in cerebral tissue hemodynamics measured by NIRS in response to hyper- and hypocapnia during prolonged hypoxic exposure have never been investigated and may help to better understand cerebral adaptations and acclimatization to high altitude.

Cerebral oxygenation represents a vital parameter that can be challenged during prolonged hypoxic exposure such as at high altitude, under resting conditions but also during exercise and sleep that are able to induce profound changes in blood gases[16,33]. Whether cerebral oxygenation during changes in arterial CO_2 differs following prolonged hypoxic exposure is also important to determine since high altitude exposure is associated with hypocapnia (at rest and even more during physical exercise) but also with periods of relative hypercapnia (during sleep apnea as frequently observed at high altitude)[3]. In addition, changes in cerebral perfusion and oxygenation are connected with the ventilatory response which is an important factor for altitude acclimatization[1]. In a recent study[42], we showed by using arterial spin labeling magnetic resonance imaging at sea level that CBF and cerebrovascular response to hypercapnia were increased and reduced, respectively, immediately after 6 days at 4,350 m. By using NIRS during the stay at high altitude, the present study investigated in the same subjects after acute (24 h) and short-term (5 days) exposure to 4,350 m O_2- and CO_2-induced changes in cerebral perfusion and oxygenation. Based on previous reports regarding O_2- and

CO_2-induced changes in MCAv at altitude[3,19,22,42], we hypothesized that short-term exposure to high altitude would i) increase cerebral perfusion and as a consequence cerebral oxygenation in response to hypoxia and ii) reduce MCAv and therefore cerebral oxygenation in response to arterial CO_2 changes. To address the potential link between changes in cerebrovascular and ventilatory responses at altitude[3,22], we also tested the hypothesis that changes in cerebral perfusion and oxygenation would correlate with changes in ventilatory responses.

MATERIALS AND METHODS

Subjects. Eleven male subjects (mean ± SD age 28 ± 8 yrs, height 176 ± 7 cm, weight 71 ± 7 kg) participated in this study after giving written, informed consent. All participants underwent full medical screening before inclusion to rule out respiratory, cardiovascular and cerebrovascular diseases. Subjects were usual recreational climbers with no history of severe acute mountain sickness (AMS) during previous high-altitude ascents and were unacclimatized to high altitude (no sojourn above 1,500 m over the past 3 months). They received no treatment to prevent or treat AMS throughout the study. The study was approved by the local ethics committee (CPP Grenoble Sud Est V) and was performed according to the Declaration of Helsinki (Clinical trial registration: NCT01565603).

Protocol. All subjects performed three experimental sessions. The first one (at sea level: SL) was performed at the Grenoble University Hospital (elevation: 212 m). Then, subjects underwent helicopter transport at midday (± 2 h) to be dropped 10 min later at 4,350 m (*Observatoire Vallot*, Mont Blanc, Chamonix, France). The second experimental session (D1) was performed the following day after one night spent at 4,350 m. The third session (D5) was performed after 5 days at 4,350 m. From D1 to D5, subjects stayed in the *Observatoire Vallot* without further ascent. During each experimental session, subjects were installed comfortably in a semi-recumbent position. They had to stay quiet and to avoid any movement for the entire test duration (about 90 min). Each subject performed the three experimental sessions at the same time of the day ± 2 h. Subjects had no heavy meal within 2 h before the tests and ingested no caffeine or alcohol at least 24h before the tests.

To assess ventilatory and cerebral hemodynamic responses, subjects inhaled gas mixtures with various inspiratory O_2 (FiO_2) and CO_2 ($FiCO_2$) fractions delivered by a

modified Altitrainer 200® (SMTEC, Nyon, Switzerland) via a face mask and were blinded for the gas mixture composition. FiO_2 and $FiCO_2$ were adjusted to reach the target values for end-tidal partial pressure of O_2 ($PetO_2$) and CO_2 ($PetCO_2$) according to the modified "Leiden proposal"[40]. The protocol (Fig. 1) consisted of eight consecutive 10-min phases. In phases 1, 3, 5 and 7 target $PetO_2$ was 100 mmHg and $FiCO_2$ was 0 (poikilocapnic normoxia). These phases allowed i) avoidance of prolonged sustained hypoxic or hypercapnic exposure that may induced some ventilatory or cerebrovascular adaptations (e.g. ventilatory decline[40]) and ii) repetitive measurement of baseline values to be compared with the subsequent phase (see below), therefore avoiding to compare measurements performed far from each other. In phases 2, 4 and 6, target $PetO_2$ was 55 mmHg (similar to values observed at ~4,300 m while breathing ambient air). Target $PetCO_2$ in phases 2, 4 and 6 was respectively 0, 5 and 12 mmHg above the value measured at the end of phase 1. These three phases thus represent isocapnic hypoxia, hypoxic hypercapnia +5 mmHg and hypoxic hypercapnia +12 mmHg conditions, respectively. In phase 8, to measure hypocapnic responses, subjects inhaled the same gas mixture as in phase 7 but were instructed and given verbal feedback to slightly hyperventilate to lower and then maintain $PetCO_2$ at 15 mmHg below the value measured at the end of phase 1.

Ventilation, $PetO_2$, $PetCO_2$, arterial oxygen saturation (SpO_2) by finger pulse oximetry and heart rate were continuously measured and recorded using an automated metabolic cart (Quark b2, Cosmed, Rome, Italy). Before each test ambient conditions were measured, then the flowmeter and gas sensors were calibrated by using a 2-L syringe and gases of known concentrations. All parameters were averaged over the last minute of each phase and used to calculate the hypoxic and hypercapnic ventilatory responses, *i.e.* the slope of the linear regression between changes in minute ventilation and the reduction in $PetO_2$ (in $l \cdot min^{-1} \cdot mmHg^{-1}$) or SpO_2 (in $l \cdot min^{-1} \cdot \%SpO_2^{-1}$) and the increase in $PetCO_2$ (in $\% \cdot mmHg^{-1}$),

respectively. TCD and cerebral NIRS signals were also continuously recorded during the experimental sessions. Room air temperature during the test sessions was 23 ± 2°C at sea level, 21 ± 2°C at D1 and 22 ± 1°C at D5 at altitude.

TCD. Two different Doppler devices were used to assess on one hand MCAv changes during periods of gas mixture inhalation and on the other hand absolute MCAv under ambient conditions at sea level and at altitude. MCAv changes in response to gas mixture inhalation were measured with a Doppler instrument operating at 2MHz (Waki[e], Atys Medical, Soucieu en Jarrest, France). With this device, the Doppler probe could be secured by a headband maintaining the same insonation position throughout the experimental session. In all subjects, the right MCA was insonated through the transtemporal window at a depth of 50 to 60 mm. Mean right MCAv (in $cm.s^{-1}$) was acquired over each heartbeat and averaged over the last minute of each phase of the protocol (see above). Measurements corresponding to isocapnic hypoxia, hypoxic hypercapnia + 5 and + 12 mmHg and hypocapnia were expressed as a percentage of the respective previous poikilocapnic normoxic periods and used to calculate the hypoxic, hypercapnic and hypocapnic cerebrovascular responses (TCD CVR), *i.e.* the slope of the linear regression between changes in MCAv and the reduction in $PetO_2$ (in $\%\cdot mmHg^{-1}$) or SpO_2 (in $\%\cdot\%SpO_2^{-1}$), the increase in $PetCO_2$ (in $\%\cdot mmHg^{-1}$) and the reduction in $PetCO_2$ (in $\%\cdot mmHg^{-1}$), respectively. Due to technical problem, TCD measurements at D1 were available in 5 subjects only.

Absolute MCAv while breathing ambient air was assessed using a 5 to 1 MHz two-dimensional Transducer CX-50 (Philips, Eindhoven, Netherlands). This device was used for absolute MCAv measurement under ambient air to have a better reliability for between-day comparisons of absolute TCD MCAv values at rest[23]. The clinoid process of the sphenoid bone and the brain stem were initially identified. Color-coded sonography allowed identifying

the circle of Willis. The M1 segment of the right MCA was identified and manual angle correction was applied to measure mean right MCAv (in $cm \cdot s^{-1}$) by the inbuilt software.

NIRS. Cerebral oxygenation was monitored with a four-wavelength (775, 810, 850, 905 nm) NIRS device (NIRO-300, Hamamatsu Photonics, Hamamatsu City, Japan). The optodes consisted of one emitter and one receiver (that includes three separate photo-detectors) housed in a black holder. The holder was stuck on the skin with double-sided adhesive tape to ensure no change in its relative position and to minimize the intrusion of extraneous light and the loss of transmitted NIR light from the field of interrogation. A differential optical pathlength factor (DPF) that takes into account light scattering in tissue is often inserted in the modified Lambert-Beer law used to describe optical attenuation. We decided not to use DPF values as they may vary from one wavelength to another, across subjects, and even over time for a given subject and tissue[11]. The optodes were positioned over the left prefrontal cortical area at the midpoint between Fp1-F3 landmarks of the international EEG 10-20 system electrode placement. The inter-optode distance was 4 cm and the optode holder was covered and maintained with a homemade black Velcro headband. Cerebral NIRS data were collected at time resolution of 0.5 s. Measurements corresponding to isocapnic hypoxia, hypoxic hypercapnia + 5 and + 12 mmHg and hypocapnia were expressed as relative concentration changes ($\Delta \mu mol \cdot cm$) from the respective previous poikilocapnic normoxic periods (see Fig. 2) and used to calculate the hypoxic, hypercapnic and hypocapnic NIRS responses, *i.e.* the slope of the linear regression between changes in NIRS signal and the reduction in $PetO_2$ (in $\mu mol \cdot cm \cdot mmHg^{-1}$) or SpO_2 (in $\mu mol \cdot cm \cdot \%SpO_2^{-1}$), the increase in $PetCO_2$ (in $\mu mol \cdot cm \cdot mmHg^{-1}$) and the reduction in $PetCO_2$ (in $\mu mol \cdot cm \cdot mmHg^{-1}$), respectively. The following NIRS parameters were measured: oxy-($\Delta[HbO_2]$), deoxy-($\Delta[HHb]$) and total hemoglobin ($\Delta[HbTot] = [HbO_2] + [HHb]$) changes. [HbTot] reflects changes in tissue blood volume within the illuminated area, [HHb] and $[HbO_2]$ are known to

be reliable estimator of changes in tissue oxygenation status[29]. In addition, the multidistance spatially resolved tissue oximeter we used (NIRO-300) could quantify tissue oxy-hemoglobin saturation directly as a tissue oxygenation index (TOI, expressed in %), which is a surrogate measure for cerebrovenous saturation when applied to the head.

Symptoms of acute mountain sickness and cardiorespiratory parameters under ambient air. Subjects were asked every morning at altitude to complete self-reported questionnaires for AMS evaluation according to the Lake Louise Score (LLS, 5 items)[32] and the cerebral subscore of the Environmental Symptom Questionnaire (ESQ-III AMS-C, 11 items)[34]. The presence of AMS was defined as LLS > 3 and AMS-C $\geq$ 0.7. Heart rate and non-invasive blood pressure (Dinamap, GE Medical Systems Inc., Milwaukee, WI) and SpO_2 using finger-pulse oximetry (Biox 3740 Pulse Oximeter, Ohmeda, Louisville, CO) were measured under resting conditions while breathing ambient air.

Statistical analysis. Analysis of the statistical significance of temporal changes during the study period (SL, D1, D5) was performed using one-way analysis of variance for repeated measurements (StatView SE program, SAS Institute, Cary, NC). When a significant main effect was found, post-hoc analysis was performed with Fisher's LSD tests. Correlations were performed using linear regression and Pearson's coefficient. For all statistical analyses, a two-tailed alpha level of 0.05 was used as the cut-off for significance. All descriptive statistics presented are mean values ± SD.

RESULTS

Hypoxic responses (Fig. 3 and Fig. 1 and 2 in supplementary online data). The hypoxic ventilatory response was significantly increased at D5 compared to SL and D1

(ANOVA main effect of time: F = 6.92, p = 0.005, when expressed per mmHg $PetO_2$ reduction; F = 8.14, p = 0.003, when expressed per % SpO_2 reduction). The hypoxic TCD CVR was significantly increased at D1 and D5 compared to SL (F = 4.17, p = 0.031, per mmHg $PetO_2$ reduction; F = 5.30, p = 0.014, per % SpO_2 reduction). The hypoxia-induced HbO_2 decrease per mmHg of $PetO_2$ reduction was significantly smaller at D5 compared to SL and D1 (F = 3.94, p = 0.036) and a similar tendency was observed for TOI (F = 2.98, p = 0.074). The increase in HbTot per % of SpO_2 reduction was significantly larger at D5 compared to SL and D1 (F = 5.90, p = 0.027). No other significant difference in NIRS parameters was observed for hypoxic responses.

At D1, the ventilatory response was significantly correlated with TOI (per % SpO_2 reduction; r^2 = -0.60, p = 0.005) and HbTot (per mmHg $PetO_2$ reduction; r^2 = -0.48, p = 0.004) responses, *i.e.* the greater the ventilatory response, the greater the TOI reduction and the smaller the HbTot increase. At D5, the TCD CVR was significantly correlated with HbO_2 (per mmHg $PetO_2$ reduction; r^2 = -0.58, p = 0.007) and HbTot (per mmHg $PetO_2$ reduction; r^2 = -0.39, p = 0.039) responses, *i.e.* the greater the TCD CVR, the larger the HbO_2 decrease and the smaller the HbTot increase.

Hypercapnic responses (Fig. 4 and Fig. 3 in supplementary online data). The hypercapnic ventilatory response was significantly increased at D5 compared to SL and D1 (F = 14.60, p = 0.001). The hypercapnic TCD CVR was significantly reduced at D1 (and tended to be reduced at D5) compared to SL (F = 5.90, p = 0.027). The HbO_2 increase per mmHg of $PetCO_2$ increase was significantly smaller at D1 compared to SL (F = 4.26, p = 0.029), the TOI increase per mmHg of $PetCO_2$ increase was significantly smaller at D1 and D5 compared to SL (F = 4.14, p = 0.031) and the HHb reduction per mmHg of $PetCO_2$ increase was

significantly smaller at D5 compared to SL ($F = 3.85$, $p = 0.041$). No other significant difference in NIRS parameters was observed for hypercapnic responses.

At D5, the TCD CVR was significantly correlated with the TOI response ($r^2 = 0.71$, $p = 0.002$), *i.e.* the smaller the TCD CVR, the smaller the TOI increase.

Hypocapnic responses (Fig. 5 and Fig. 4 in supplementary online data). The hypocapnic TCD CVR and NIRS responses did not change significantly throughout the protocol.

Symptoms, cardiorespiratory parameters, TOI and MCAv under ambient air (Table 1). Symptoms of AMS were significantly increased at D1 compared to SL. At altitude while breathing ambient air, $PetO_2$, $PetCO_2$, SpO_2 and TOI were significantly reduced while TCD MCAv values at rest, heart rate and mean arterial pressure were significantly increased. No significant correlation was observed between symptoms of AMS or cardiorespiratory parameters under ambient air conditions and ventilatory, TCD and NIRS responses to hypoxia, hypercapnia or hypocapnia.

DISCUSSION

This study reports changes in cerebral NIRS signals together with ventilatory and TCD responses during hypoxic, hyper- and hypocapnic gas inhalation at high altitude. Compared to sea level, high-altitude exposure induced i) smaller prefrontal deoxygenation in response to isocapnic hypoxia, and lower prefrontal oxygenation increase in response to hypercapnia, ii) larger hypoxic and smaller hypercapnic TCD CVR, and iii) greater hypoxic and hypercapnic ventilatory responses. In contrast, no change in NIRS and TCD hypocapnic responses was

observed at high altitude. These results suggest that after several days at altitude brain oxygenation is better preserved when arterial oxygenation drops while it is reduced during hypercapnia. Although these changes may be relevant for conditions such as exercise or sleep at altitude, their relationships with symptoms of AMS remain to be clarified.

Hypoxic responses

The isocapnic hypoxic ventilatory and TCD responses show robust increases after 5 days at high altitude. Similar results were observed after several days in hypoxia (*e.g.*[19,31,35]). This increase was observed both as a function of $PetO_2$ (per mmHg, Fig 1) and SpO_2 (per %, Fig. 1 in supplementary online data). Since O_2 arterial saturation is not the stimulus to the oxygen sensors at the carotid bodies or cerebrovascular levels, it appears more appropriate to express these responses as a function of mmHg $PetO_2$[40]. The present study indicates that prefrontal oxygenation (HbO_2) is less impaired during an isocapnic hypoxic challenge after 5 days (but not after 24 h) at 4,350 m. Only one study[21] measured cerebral deoxygenation during hypoxic breathing by NIRS before and after five nocturnal hypoxic exposures (8h/day, simulated altitude 4,300 m) and reported no significant change in prefrontal deoxygenation (assessed from an index of regional oxygen saturation, SrO_2). This suggests that the amount of time spent in hypoxia may have been insufficient to induce significant improvement in cerebral oxygenation and/or that indexes of cerebral oxygenation (TOI in the present study and SrO_2 in Kolb et al. study) are less sensitive to altitude-induced changes in cerebral oxygenation than HbO_2 concentration.

Changes in NIRS hemoglobin concentrations expressed as a function of $PetO_2$ can be influenced by changes in the oxygen dissociation curve (ODC) as observed at high altitude. These changes typically consist in a left shift due to hypocapnia and alkalosis associated with hyperventilation during acute exposure followed by a progressive return to sea level values or

even a right shift with, among other mechanisms, an increase in 2,3-DPG during full acclimatization[44]. Since the ODC could not be measured within the present study, one can only speculate about a left shift of the ODC at D1 while at D5 it was probably more similar to sea level. HbO_2 (and TOI) reduction was significantly smaller at altitude when expressed as a function of $PetO_2$ (Fig. 1) but this effect did not reach significance when expressed as a function of SpO_2 (Fig. 1 in supplementary online data). This suggests that part of the improved NIRS cerebral oxygenation in response to hypoxia at D5 was due to the smaller % SpO_2 decrease during the hypoxic challenge (-9 ± 2% at SL, -7 ± 3% at D1 and -5 ± 1% at D5, $p < 0.001$) when $PetO_2$ was reduced from 100 to 55 mmHg. Regarding HbTot however, a larger increase in hypoxia when expressed as a function of % SpO_2 reduction (and a similar trend when expressed per mmHg $PetO_2$, $p = 0.14$) suggests an increased local perfusion in response to hypoxia after 5 days of high-altitude exposure. Overall, similar tendencies were observed regarding changes in hypoxic NIRS responses when expressed as a function of $PetO_2$ or SpO_2 (Fig. 3 and Fig. 1 in supplementary online data), suggesting that other factors than modifications of the oxygenation dissociation curve probably underlie the improved prefrontal oxygenation and perfusion in response to hypoxia at altitude. Systemic cardiovascular adaptations (as shown by the increased heart rate and mean arterial pressure, Table 1), neuronal pathways, circulating and endothelium-derived vasoactive stimuli are other potential mechanisms able to explain changes in cerebrovascular reactivity at high altitude[5,9].

The HbO_2 and HbTot isocapnic hypoxic responses at D5 correlated with the TCD CVR but these correlations were somehow unexpected and it is difficult to explain why subjects with small NIRS responses have high TCD CVR. TCD evaluates changes in flow in a large artery (MCA) supplying several regions of the brain in addition to the frontal lobe. In contrast, NIRS HbTot is a volumetric index that is not directly dependent on blood flow but is rather influenced by local capillary vasodilation and venous compartment compliance.

Peltonen et al.[28] observed no correlation between MCAv and HbTot responses to acute hypoxia. The absence of correlation between TCD CVR and NIRS signals at SL and D1 shows that both responses are overall poorly related.

The correlations between the hypoxic ventilatory response and the NIRS signals at D1 indicate that a large cerebral deoxygenation is associated with brisk hypoxic ventilatory response. Although the ventilatory response to hypoxia is mostly initiated by the carotid bodies, central oxygen sensors may also be involved[40]. The latters exposed to more severe hypoxia as shown in the prefrontal cortex may stimulate ventilation to a greater extent. This is however hypothetical and it remains unclear why such a relationship was obtained at D1 only. Peltonen et al.[28] reported at sea level no correlation between hypoxic ventilatory, MCAv and NIRS responses. The present results suggest that this is also essentially true at high altitude and that each of these responses relies on distinct mechanisms and regional adaptations (*e.g.* peripheral *versus* central chemoreceptors, large cerebral artery *versus* regional cortical oxygenation). Interestingly, the change in hypoxic TCD CVR occurred as soon as D1 (TCD CVR at D1 was available in 5 subjects only who had all larger values at D1 compared to SL) while significant changes in ventilatory and NIRS responses were observed at D5 only. Early cerebral adaptations may mostly depend on changes in blood gases, cerebrospinal fluid pH and autonomic nervous system, while later adaptations may also include hematological modifications, angiogenesis and alterations in cerebral metabolic rate[9]. Smith et al.[39] recently reported an increase in O_2 cerebral metabolic rate ($CMRO_2$) assessed by MRI methods during sustained hypoxia at high altitude (2 days at 3,800 m) that might be driven by the reduced arterial CO_2 content associated with the hypoxic ventilatory response. In the present study, since $PetCO_2$ was reduced at altitude, the cerebral responses were possibly measured from a larger initial $CMRO_2$ at altitude compared to sea level. Because NIRS signal is affected by cerebral perfusion but also by O_2 extraction and metabolic rate, altitude-induced changes in

$CMRO_2$ might have influenced changes in NIRS signal at altitude. However, altered $CMRO_2$ at high altitude is not a universal finding[25,36] and since we measured changes in NIRS signals relative to normoxic measurements made at the same altitude (i.e. at sea level or at 4,350 m), the effect of changes in $CMRO_2$ on the present results remains hypothetical. The present study does not provide direct evidence in support of any particular mechanisms regulating ventilatory and cerebral hypoxic responses at high altitude.

The improved tissue oxygenation in response to transient isocapnic hypoxic challenge did not translate into better prefrontal oxygenation under ambient air from D1 to D5 (based on TOI values, Table 1). This may be due to the insufficient sensitivity of TOI to changes in cerebral oxygenation (compared to HbO_2 for instance, as indicated above) or to the concomitant influence of hypoxia and hypocapnia on cerebral oxygenation under ambient air at altitude. The increase in hypoxic TCD CVR may be one of the mechanisms leading to increased MCAv under ambient air at altitude (Table 1). Although both hypoxic TCD CVR and ambient air MCAv were significantly increased as soon as D1, no correlation was observed between both parameters. In a study parallel to the present one, we have observed that absolute CBF (measured in the same subjects by arterial spin labeling magnetic resonance imaging) is still enhanced when measured at sea level within a few hours (6 ± 2 h) after descent from several days at high altitude[42], suggesting that hypoxia *per se* is not the only factor influencing CBF changes at altitude.

CO_2 responses

The hypercapnic ventilatory response was significantly increased after 5 days at 4,350 m in accordance with previous observations in chronic hypoxia[4,12,22]. The literature provides contrasted results regarding the effect of prolonged hypoxic exposure on the TCD CVR to CO_2 (see Introduction). This may be due at least in part to differences in methodological

approaches, with for instance inverse changes observed within the same altitude stay when measured with a rebreathing[12] or a steady-state[22] protocol. In the present study we assessed steady-state hypercapnic responses under hypoxia in order to provide data relevant to conditions at altitude (*e.g.* sleep, exercise) while several other studies have measured the hypercapnic TCD CVR in hyperoxia (*e.g.*[19,22,31]).

An increase in CO_2 ventilatory and cerebrovascular responses after several days at high altitude has been suggested to occur due to the decline in cerebrospinal fluid HCO_3^-[10,13] which would result in larger pH reduction per mmHg of PCO_2 increase[12,19]. Despite this potential effect on the cerebrovascular tone, Lucas et al.[22] recently reported a reduced hypercapnic TCD CVR (measured in hyperoxia with a steady-state protocol) upon initial arrival and after 2 weeks at 5,050 m. One potential mechanism for reduced vasodilatory response to CO_2 as in the present study and in Lucas et al.[22] may be the increased sympathetic nervous activity known to occur at altitude[7]. The sympathetic control of cerebral perfusion remains however debated[6] and further studies are needed to elucidate the mechanisms responsible for changes in hypercapnic CVR at altitude.

The present study described for the first time alterations in NIRS signal in response to changes in arterial CO_2 at altitude. Smielewski et al.[37] demonstrated that NIRS parameters react to alterations in arterial CO_2, changes in HbO_2 and HHb being significantly correlated to the change in TCD flow velocity measured nearby at the temporal window. The same authors showed the clinical value of NIRS responses to hypercapnia that were related to the severity of stenosis in patients with carotid artery diseases[38]. In the present study, the TOI and HbO_2 increase and the HHb reduction in response to hypercapnia were blunted at altitude. The correlation between TCD CVR and TOI responses at D5 suggest that reduced prefrontal oxygenation during CO_2 inhalation may be due to a smaller increase in CBF. Hence, the NIRS data further suggest that the hypercapnic cerebral responses are altered after several days at

high altitude as previously suggested[22,42]. We recently reported a reduced normoxic hypercapnic CVR measured by arterial spin labeling magnetic resonance imaging at sea level within a few hours after descent from several days at high altitude[42], showing that altitude-induced reduction in hypercapnic CVR observed in the present study does not depend on the hypoxic background during the measurement.

In addition to changes in peripheral chemosensitivity that are crucial for the development of ventilatory acclimatization as shown by changes in hypoxic ventilatory responses, changes in CO_2 sensitivity is also relevant and can be potentially influenced by alteration in CVR. Hence, a reduction in CO_2 CVR at altitude may enhance the CO_2 ventilatory response by reducing cerebrospinal H^+ washout while arterial CO_2 increases[22]. Changes in CO_2 cerebral and ventilatory responses were however not correlated in the present study. A reduction in CO_2 CVR may also promote breathing instability and the development of periodic breathing as frequently observed in newcomers to high altitude[3,22]. The reduction in cerebral oxygenation observed in the hypercapnic range at altitude might also suggest that the brain could be at risk of neurological injuries during sleep apnea for instance.

As opposed to hypercapnic responses, we found no change in hypocapnic responses at high altitude. Cerebrovascular responses to CO_2 are known to differ and possibly to involve distinct mechanisms in the hypercapnic and hypocapnic ranges[15]. Previous studies have reported changes in TCD CVR to hypocapnia at high altitude[3,22]. These studies measured the hypocapnic TCD CVR while breathing ambient air at sea level and at high altitude. It has been recently suggested[26] that the comparison of hypocapnic reactivity may be influenced by the PaO_2 increase associated with hyperventilation that could affect CBF differently when starting from normoxia (at sea level) or pre-existing hypoxia (at altitude). To eliminate this effect, we measured hypocapnic NIRS and TCD responses both at sea level and at altitude while breathing a normoxic gas (leading to $PetO_2$ = 100 mmHg for 10 min before starting

hyperventilation). The absence of change in NIRS and TCD responses to hypocapnia after several days at high altitude suggests that altitude-induced changes in CO_2 CVR differ between the hypocapnic and hypercapnic range, the later only showing significant alterations. Finally, no correlation was observed between changes in hypoxic or CO_2 cerebral responses and AMS severity. The present study was not designed to assess potential relationships between changes in cerebral perfusion and oxygenation and AMS severity since only subjects with no AMS history (and therefore considered as AMS resistant) were included and because the demonstration of such a relationship would require a larger sample size.

Methodological considerations

The hypercapnic responses were assessed under hypoxic conditions in order to mimic altitude conditions, *i.e.* to evaluate cerebral responses to CO_2 as sojourners at high altitude would face while breathing ambient air. Because of the interaction between hypoxic and hypercapnic stimuli[1], the ventilatory and cerebrovascular hypercapnic responses measured in the present study may differ from measurements under normoxic or hyperoxic conditions.

Several limitations inherent to NIRS measurement should be noted. First, we measured cerebral oxygenation over the prefrontal cortex. CBF may be heterogeneously distributed under hypoxia[27] and regional differences in cerebral oxygenation could not be detected within the present study. Second, although we sought to minimize the effects of near-surface blood flow by controlling room air temperature, by using a 4-cm inter-optode distance and by measuring the TOI provided by the NIRO-300 using NIR spatially resolved spectroscopy, we cannot rule out the possibility that superficial layers blood flow affected cerebral oxygenation measured by NIRS. Whether changes in blood gases affect skin blood flow similarly at sea level and after several days at high altitude is unknown. Third, slight variations in probe placement could affect cerebral oxygenation measurements. To counteract

this possibility, we carefully placed the probe holder at the same position on each experimental day.

The validity of TCD to measure variation in CBF depends critically on the assumption of a constant diameter of the investigated artery. Previous reports suggest that under the conditions of the present study (<5,000 m), MCA diameter remains constant[21,30,43], while at higher altitude this may not be the case[43]. Further studies with sensitive measurements of MCA diameter are however needed to confirm that absolute values and acute changes in MCAv as measured in the present study can be considered as a good estimate of absolute CBF and CBF responses at altitude. Because of the poor between-day reliability of standard TCD absolute MCAv assessment[24], we measured absolute MCAv under ambient air at SL and at altitude by using 2D transcranial color-coded sonography that enables precise identification of the M1 segment of the right MCA and insonation angle correction when determining blood flow velocities[23].

Conclusion

The present study shows that changes in hypoxic and hypercapnic ventilatory and cerebrovascular responses at high altitude are associated with significant alterations in prefrontal NIRS responses, cerebral oxygenation being less impaired in response to isocapnic hypoxia while reactivity of NIRS oxygenation variables to hypercapnia is smaller. The absence of change in hypocapnic responses suggests that the effect of altitude on cerebral responses to CO_2 may differ between the hypercapnic and hypocapnic ranges. Based on correlations and kinetics of changes over the 5 days at altitude, ventilatory, TCD and NIRS responses appear to rely, at least in part, on distinct mechanisms. Further studies are needed to clarify their respective roles regarding altitude acclimatization.

Supplementary information is available at the Journal of Cerebral Blood Flow & Metabolism website – www.nature.com/jcbfm

Disclosure/Conflict of Interest

The authors have no disclosure or conflict of interest to declare.

REFERENCES

1. Ainslie PN, Poulin MJ. Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. *J Appl Physiol* 2004; **97**: 149-59.

2. Ainslie PN, Barach A, Murrell C, Hamlin M, Hellemans J, Ogoh S. Alterations in cerebral autoregulation and cerebral blood flow velocity during acute hypoxia: rest and exercise. *Am J Physiol Heart Circ Physiol* 2007; **292**: H976-83.

3. Ainslie PN, Burgess K, Subedi P, Burgess KR. Alterations in cerebral dynamics at high altitude following partial acclimatization in humans: wakefulness and sleep. *J Appl Physiol* 2007; **102**: 658-64.

4. Ainslie PN, Burgess KR. Cardiorespiratory and cerebrovascular responses to hyperoxic and hypoxic rebreathing: effects of acclimatization to high altitude. *Respir Physiol Neurobiol* 2008; **161**: 201-9.

5. Ainslie PN, Ogoh S. Regulation of cerebral blood flow in mammals during chronic hypoxia: a matter of balance. *Exp Physiol* 2009; **95**: 251-62.

6. Ainslie PN, Tzeng YC. On the regulation of the blood supply to the brain: old age concepts and new age ideas. *J Appl Physiol* 2010; **108**: 1447-9.

7. Ainslie PN, Lucas SJ, Fan JL, Thomas KN, Cotter JD, Tzeng YC, Burgess KR. Influence of sympathoexcitation at high altitude on cerebrovascular function and ventilatory control in humans. *J Appl Physiol* 2012; **113**: 1058-67.

8. Blaber AP, Hartley T, Pretorius PJ. Effect of acute exposure to 3660 m altitude on orthostatic responses and tolerance. *J Appl Physiol* 2003; **95**: 591-601.

9. Brugniaux JV, Hodges AN, Hanly PJ, Poulin MJ. Cerebrovascular responses to altitude. *Respir Physiol Neurobiol* 2007; **158**: 212-23.

10. Dempsey JA, Forster HV, Gledhill N, doPico GA. Effects of moderate hypoxemia and hypocapnia on CSF [H+] and ventilation in man. *J Appl Physiol* 1975; **38**: 665-74.

11. Duncan A, Meek JH, Clemence M, Elwell CE, Fallon P, Tyszczuk L, Cope M, Delpy DT. Measurement of cranial optical path length as a function of age using phase resolved near infrared spectroscopy. *Pediatr Res* 1996; **39**: 889-94.

12. Fan JL, Burgess KR, Basnyat R, Thomas KN, Peebles KC, Lucas SJ, Lucas RA, Donnelly J, Cotter JD, Ainslie PN. Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO2. *J Physiol* 2010; **588**: 539-49.

13. Forster HV, Dempsey JA, Chosy LW. Incomplete compensation of CSF [H+] in man during acclimatization to high altitude (48300 M). *J Appl Physiol* 1975; **38**: 1067-72.

14. Hornbein TF. The high-altitude brain. *J Exp Biol* 2001; **204**: 3129-32.

15. Ide K, Eliasziw M, Poulin MJ. Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *J Appl Physiol* 2003; **95**: 129-37.

16. Imray CH, Myers SD, Pattinson KT, Bradwell AR, Chan CW, Harris S, Collins P, Wright AD. Effect of exercise on cerebral perfusion in humans at high altitude. *J Appl Physiol* 2005; **99**: 699-706.

17. Jansen GF, Krins A, Basnyat B. Cerebral vasomotor reactivity at high altitude in humans. *J Appl Physiol* 1999; **86**: 681-6.

18. Jensen JB, Wright AD, Lassen NA, Harvey TC, Winterborn MH, Raichle ME, Bradwell AR. Cerebral blood flow in acute mountain sickness. *J Appl Physiol* 1990; **69**: 430-3.

19. Jensen JB, Sperling B, Severinghaus JW, Lassen NA. Augmented hypoxic cerebral vasodilation in men during 5 days at 3,810 m altitude. *J Appl Physiol* 1996; **80**: 1214-8.

20. Kolb JC, Ainslie PN, Ide K, Poulin MJ. Protocol to measure acute cerebrovascular and ventilatory responses to isocapnic hypoxia in humans. *Respir Physiol Neurobiol* 2004; **141**: 191-9.

21. Kolb JC, Ainslie PN, Ide K, Poulin MJ. Effects of five consecutive nocturnal hypoxic exposures on the cerebrovascular responses to acute hypoxia and hypercapnia in humans. *J Appl Physiol* 2004; **96**: 1745-54.

22. Lucas SJ, Burgess KR, Thomas KN, Donnelly J, Peebles KC, Lucas RA, Fan JL, Cotter JD, Basnyat R, Ainslie PN. Alterations in cerebral blood flow and cerebrovascular reactivity during 14 days at 5050 m. *J Physiol* 2011; **589**: 741-53.

23. Martin PJ, Evans DH, Naylor AR. Measurement of blood flow velocity in the basal cerebral circulation: advantages of transcranial color-coded sonography over conventional transcranial Doppler. *J Clin Ultrasound* 1995; **23**: 21-6.

24. McMahon CJ, McDermott P, Horsfall D, Selvarajah JR, King AT, Vail A. The reproducibility of transcranial Doppler middle cerebral artery velocity measurements: implications for clinical practice. *British journal of neurosurgery* 2007; **21**: 21-7.

25. Moller K, Paulson OB, Hornbein TF, Colier WN, Paulson AS, Roach RC, Holm S, Knudsen GM. Unchanged cerebral blood flow and oxidative metabolism after acclimatization to high altitude. *J Cereb Blood Flow Metab* 2002; **22**: 118-26.

26. Moller K. Every breath you take: acclimatisation at altitude. *J Physiol* 2010; **588**: 1811-2.

27. Pagani M, Salmaso D, Sidiras G, Jonsson C, Jacobsson H, Larsson SA, Lind F. Impact of acute hypobaric hypoxia on blood flow distribution in brain. *Acta Physiol* 2011; **In press**.

28. Peltonen JE, Kowalchuk JM, Paterson DH, DeLorey DS, duManoir GR, Petrella RJ, Shoemaker JK. Cerebral and muscle tissue oxygenation in acute hypoxic ventilatory response test. *Respir Physiol Neurobiol* 2007; **155**: 71-81.

29. Perrey S. Non-invasive NIR spectroscopy of human brain function during exercise. *Methods* 2008; **45**: 289-99.

30. Poulin MJ, Robbins PA. Indexes of flow and cross-sectional area of the middle cerebral artery using doppler ultrasound during hypoxia and hypercapnia in humans. *Stroke* 1996; **27**: 2244-50.

31. Poulin MJ, Fatemian M, Tansley JG, O'Connor DF, Robbins PA. Changes in cerebral blood flow during and after 48 h of both isocapnic and poikilocapnic hypoxia in humans. *Exp Physiol* 2002; **87**: 633-42.

32. Roach RC, Bartsch P, Hackett PH, Oelz O. The lake louise acute mountain sickness scoring system. In: *Hypoxia and Molecular Medicine* 1993; (Sutton JR, Coates J, S. HC, eds): Queen City Printers, 272-4.

33. Salvaggio A, Insalaco G, Marrone O, Romano S, Braghiroli A, Lanfranchi P, Patruno V, Donner CF, Bonsignore G. Effects of high-altitude periodic breathing on sleep and arterial oxyhaemoglobin saturation. *Eur Respir J* 1998; **12**: 408-13.

34. Sampson JB, Cymerman A, Burse RL, Maher JT, Rock PB. Procedures for the measurement of acute mountain sickness. *Aviat Space Environ Med* 1983; **54**: 1063-73.

35. Sato M, Severinghaus JW, Powell FL, Xu FD, Spellman MJ, Jr. Augmented hypoxic ventilatory response in men at altitude. *J Appl Physiol* 1992; **73**: 101-7.

36. Severinghaus JW, Chiodi H, Eger EI, 2nd, Brandstater B, Hornbein TF. Cerebral blood flow in man at high altitude. Role of cerebrospinal fluid pH in normalization of flow in chronic hypocapnia. *Circ Res* 1966; **19**: 274-82.

37. Smielewski P, Kirkpatrick P, Minhas P, Pickard JD, Czosnyka M. Can cerebrovascular reactivity be measured with near-infrared spectroscopy? *Stroke* 1995; **26**: 2285-92.

38. Smielewski P, Czosnyka M, Pickard JD, Kirkpatrick P. Clinical evaluation of near-infrared spectroscopy for testing cerebrovascular reactivity in patients with carotid artery disease. *Stroke* 1997; **28**: 331-8.

39. Smith ZM, Krizay E, Guo J, Shin DD, Scadeng M, Dubowitz DJ. Sustained high-altitude hypoxia increases cerebral oxygen metabolism. *J Appl Physiol* 2013; **114**: 11-8.

40. Teppema LJ, Dahan A. The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis. *Physiol Rev* 2010; **90**: 675-754.

41. Terborg C, Gora F, Weiller C, Rother J. Reduced vasomotor reactivity in cerebral microangiopathy : a study with near-infrared spectroscopy and transcranial Doppler sonography. *Stroke* 2000; **31**: 924-9.

42. Villien M, Bouzat P, Rupp T, Robach P, Lamalle L, Tropres I, Esteve F, Krainik A, Levy P, Warnking JM, Verges S. Changes in cerebral blood flow and vasoreactivity to CO(2) measured by Arterial Spin Labeling after 6 days at 4,350 m. *Neuroimage* 2013; **72**: 272-9.

43. Wilson MH, Edsell ME, Davagnanam I, Hirani SP, Martin DS, Levett DZ, Thornton JS, Golay X, Strycharczuk L, Newman SP, Montgomery HE, Grocott MP, Imray CH. Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia-an ultrasound and MRI study. *J Cereb Blood Flow Metab* 2011; **31**: 2019-29.

44. Winslow RM. The role of hemoglobin oxygen affinity in oxygen transport at high altitude. *Respir Physiol Neurobiol* 2007; **158**: 121-7.

LEGENDS TO FIGURES

Figure 1. Description of the main phases of the protocol to measure ventilatory and cerebral hemodynamic responses with simultaneous changes in end-tidal O_2 ($PetO_2$) and CO_2 ($PetCO_2$) partial pressures at sea level and at high altitude. $PetCO_2$ values illustrate typical sea level measurements. During poikilocapnic conditions (phases 1, 3, 5, 7) $PetCO_2$ was uncontrolled, during the isocapnic condition (phase 2) $PetCO_2$ was maintained at the same level as the end of phase 1, while during hypercapnic conditions (phases 4 and 6) $PetCO_2$ was increased by +5 and +12 mmHg above the value at the end of phase 1. See Materials and Methods for more details.

Figure 2. Typical changes in prefrontal oxy-(HbO_2), deoxy-(HHb) and total haemoglobin (HbTot) concentrations in response to hypoxic isocapnia (A), hypoxic hypercapnia +5 (B) and +12 mmHg (C), and normoxic hypocapnia (D), from a baseline in poikilocapnic normoxia. HbO_2 is black, HHb is dark grey and HbTot is light grey. Raw data are presented for a representative subject at sea level.

Figure 3. Hypoxic responses: Ventilatory (V_E, panel A), TCD (TCD CVR, panel B) and NIRS (oxyhemoglobin HbO_2, panel C; tissue oxygenation index TOI, panel D; deoxyhemoglobin HHb, panel E; total hemoglobin HbTot, panel F) responses to isocapnic hypoxia at sea level (SL), at day 1 (D1) and 5 (D5) at altitude. Changes in V_E, TCD CVR and NIRS variables are expressed per mmHg of change in end-tidal O_2 partial pressure. * significantly different from SL; $^+$ significantly different from D1.

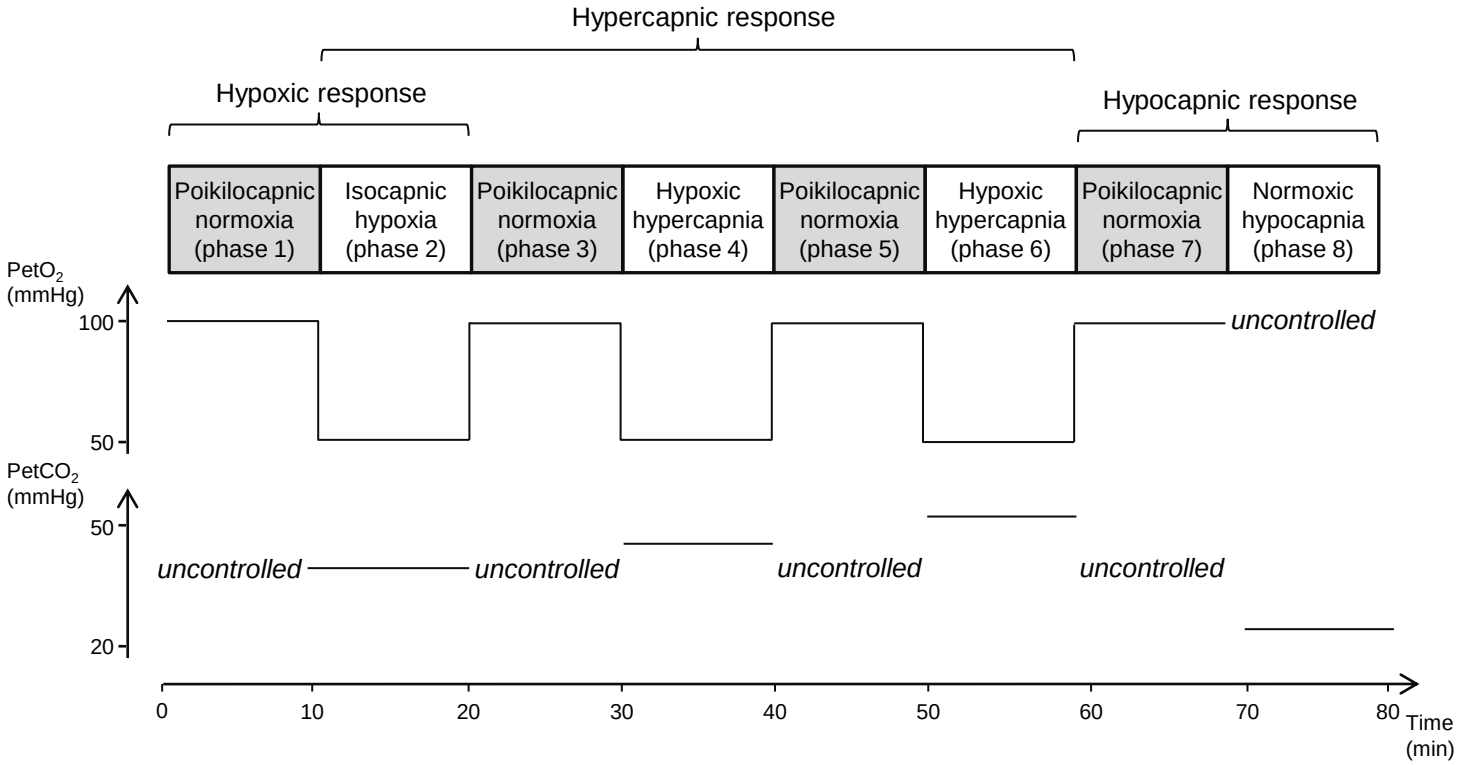
Figure 4. Hypercapnic responses: Ventilatory (V_E, panel A), TCD (TCD CVR, panel B) and NIRS (oxyhemoglobin HbO_2, panel C; tissue oxygenation index TOI, panel D; deoxyhemoglobin HHb, panel E; total hemoglobin HbTot, panel F) responses to hypercapnia (expressed per mmHg of change in end-tidal CO_2 partial pressure) at sea level (SL), at day 1 (D1) and 5 (D5) at altitude. * significantly different from SL; $^+$ significantly different from D1.

Figure 5. Hypocapnic responses: TCD (TCD CVR, panel A) and NIRS (oxyhemoglobin HbO_2, panel B; tissue oxygenation index TOI, panel C; deoxyhemoglobin HHb, panel D; total hemoglobin HbTot, panel E) responses to hypocapnia (expressed in mmHg of change in end-tidal CO_2 partial pressure) at sea level (SL), at day 1 (D1) and 5 (D5) at altitude.

Table 1. Symptoms, cardiorespiratory and cerebrovascular parameters while breathing ambient air at sea level and at altitude.

	SL	D1	D5
LLS	1 ± 1	5 ± 3*	1 ± 1^{+}
ESQ-c	0.01 ± 0.12	0.80 ± 0.85*	0.07 ± 0.10^{+}
$PetO_2$ (mmHg)	102 ± 4	47 ± 2*	50 ± 6*,$^{+}$
$PetCO_2$ (mmHg)	41 ± 5	34 ± 2*	31 ± 3*,$^{+}$
SpO_2 (%)	97 ± 1	87 ± 2*	88 ± 1*
TOI (%)	76 ± 4	65 ± 3*	66 ± 4*
TCD MCAv ($cm \cdot s^{-1}$)	55 ± 8	64 ± 8*	66 ± 9*
HR (bpm)	61 ± 8	73 ± 12*	78 ± 16*
MAP (mmHg)	104 ± 6	107 ± 7	116 ± 7*,$^{+}$

Values are mean ± SD; SL, sea level, D1, first day at 4,350 m; D5, fifth day at 4,350 m; LLS, Lake Louise score; ESQ-c, cerebral subscore of the environmental symptom questionnaire; $PetO_2$, end-tidal oxygen partial pressure; $PetO_2$, end-tidal carbon dioxide partial pressure; SpO_2, arterial oxygen saturation; TOI, cerebral tissue oxygenation index; TCD MCAv, middle cerebral artery blood flow velocity; HR, heart rate; MAP, mean arterial pressure. *, significantly different from SL, $^{+}$, significantly different from D1 ($p < 0.05$).


Hypercapnic response
Hypoxic response
Hypocapnic response
Poikilocapnic normoxia (phase 1)
Isocapnic hypoxia (phase 2)
Poikilocapnic normoxia (phase 3)
Hypoxic hypercapnia (phase 4)
Poikilocapnic normoxia (phase 5)
Hypoxic hypercapnia (phase 6)
Poikilocapnic normoxia (phase 7)
Normoxic hypocapnia (phase 8)
PetO2 (mmHg)
100
50
uncontrolled
PetCO2 (mmHg)
50
20
uncontrolled
uncontrolled
uncontrolled
uncontrolled
0
10
20
30
40
50
60
70
80
Time (min)

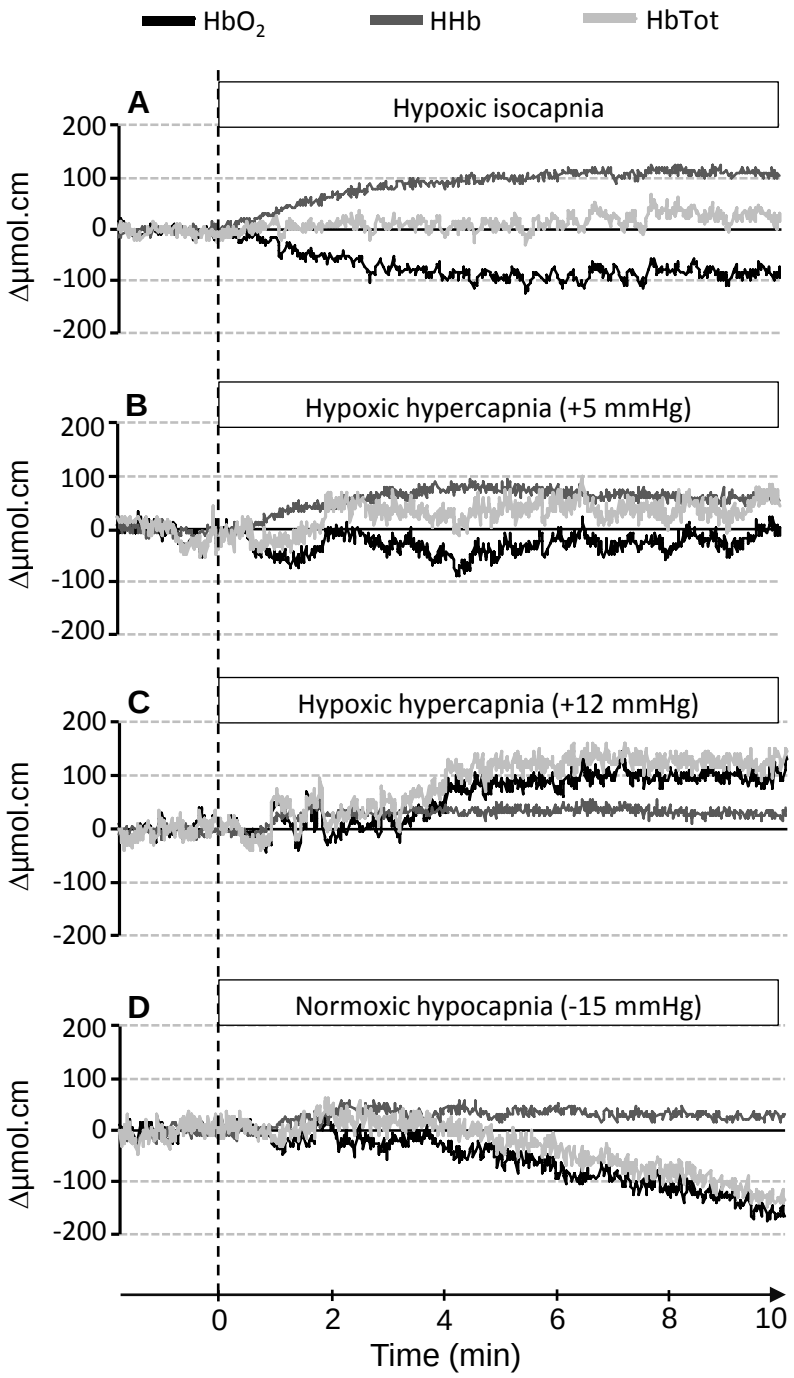

HbO2
HHb
HbTot
A
Hypoxic isocapnia
B
Hypoxic hypercapnia (+5 mmHg)
C
Hypoxic hypercapnia (+12 mmHg)
D
Normoxic hypocapnia (-15 mmHg)
Δμmol.cm
200
100
0
-100
-200
0
2
4
6
8
10
Time (min)

Fig. 3

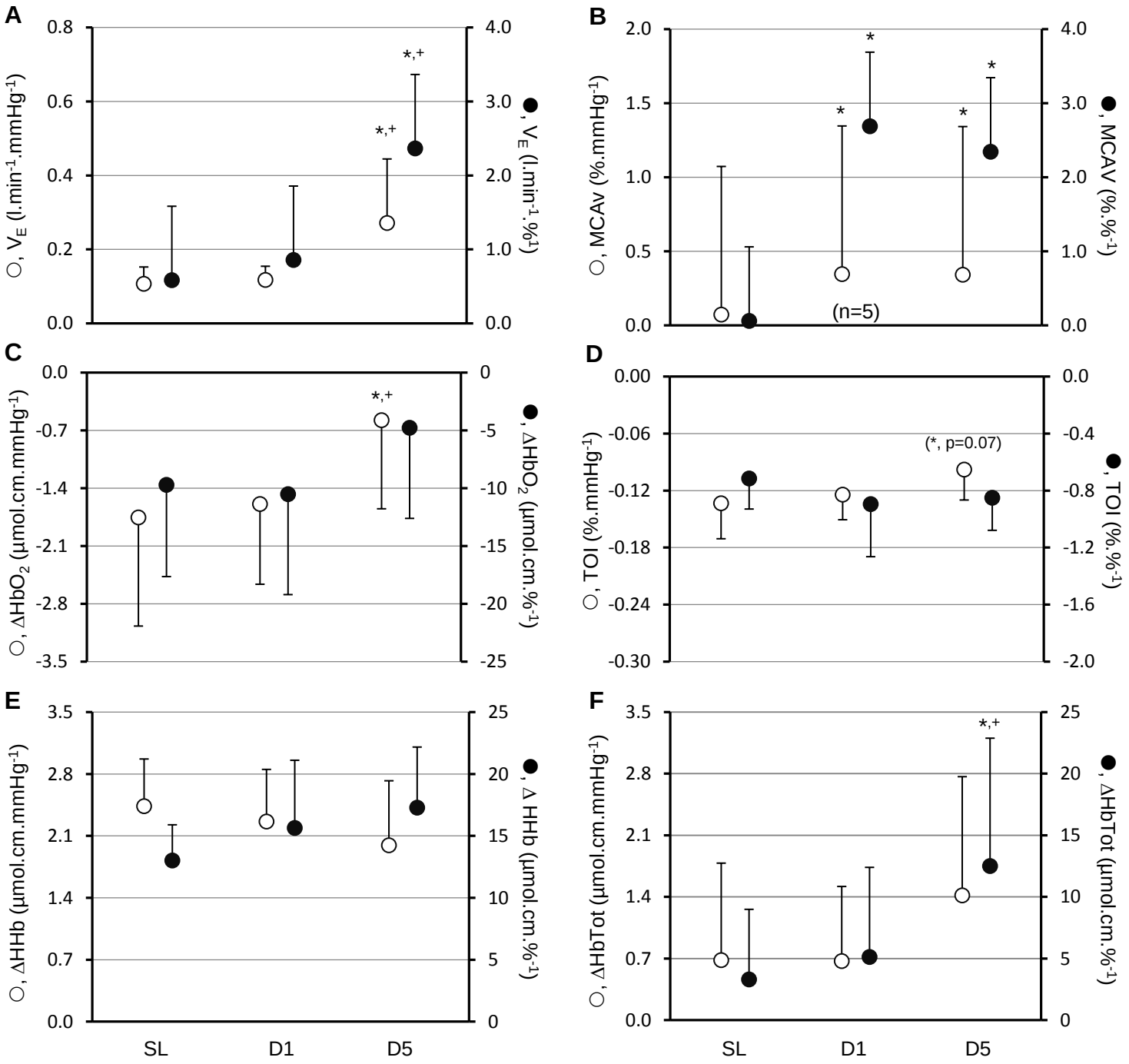

A
○, V_E (l.min^{-1}.mmHg^{-1})
●, V_E (l.min^{-1}.%$^{-1}$)
*,+
B
○, MCAv (%.mmHg^{-1})
●, MCAV (%.%$^{-1}$)
*
(n=5)
C
○, ΔHbO$_2$ (µmol.cm.mmHg^{-1})
●, ΔHbO$_2$ (µmol.cm.%$^{-1}$)
*,+
D
○, TOI (%.mmHg^{-1})
●, TOI (%.%$^{-1}$)
(*, p=0.07)
E
○, ΔHHb (µmol.cm.mmHg^{-1})
●, Δ HHb (µmol.cm.%$^{-1}$)
F
○, ΔHbTot (µmol.cm.mmHg^{-1})
●, ΔHbTot (µmol.cm.%$^{-1}$)
*,+
SL
D1
D5

Fig. 4

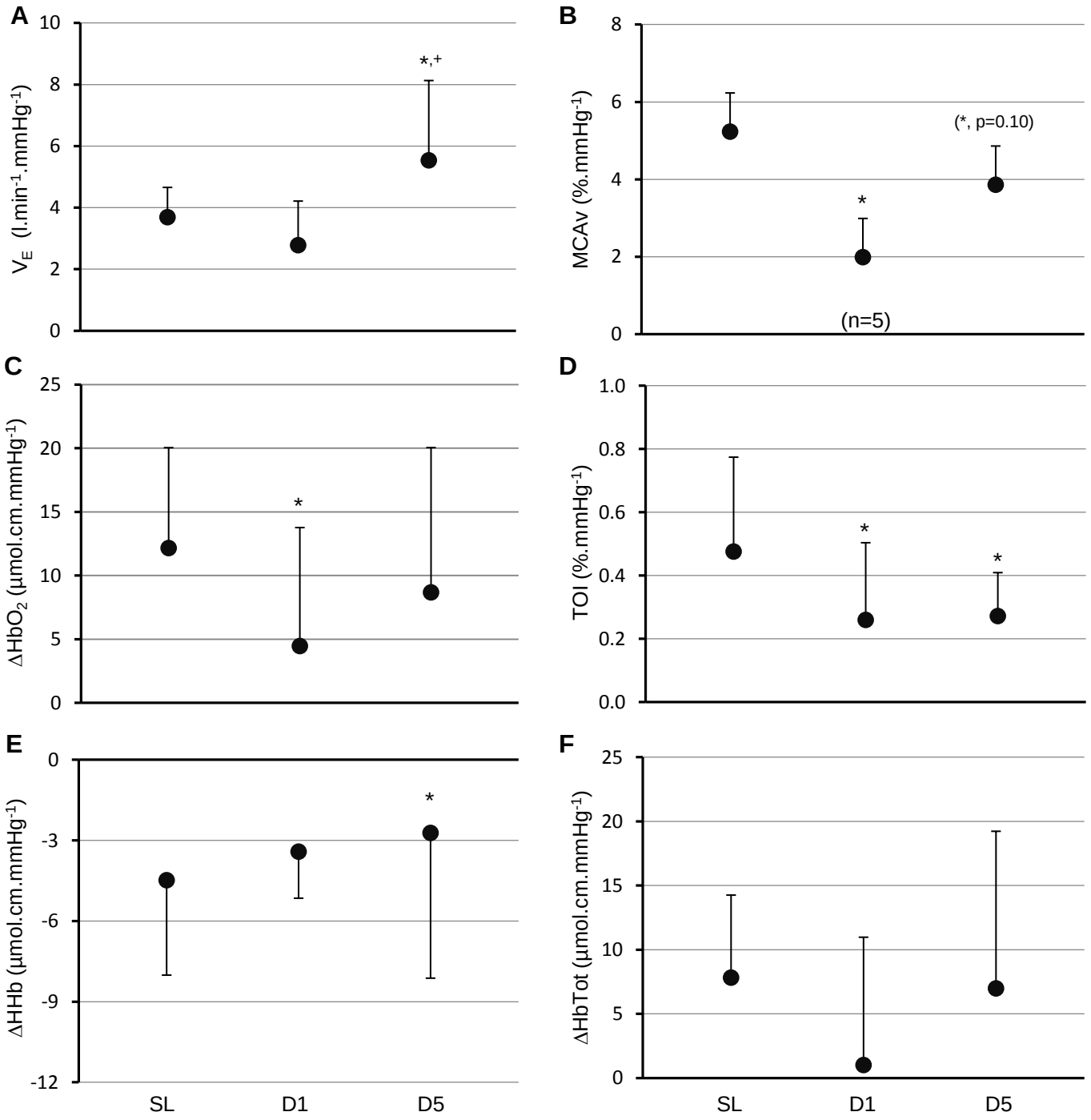
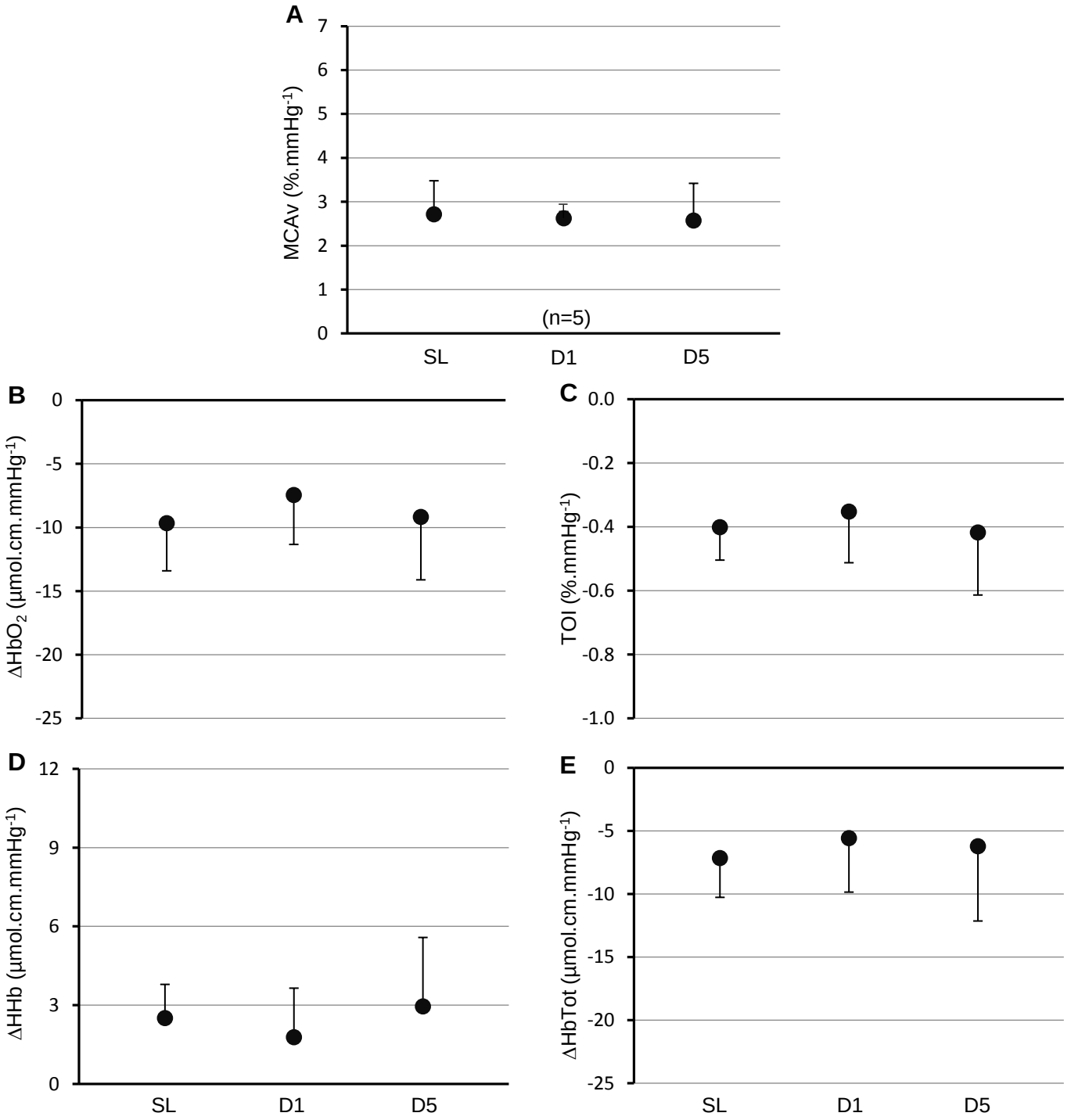
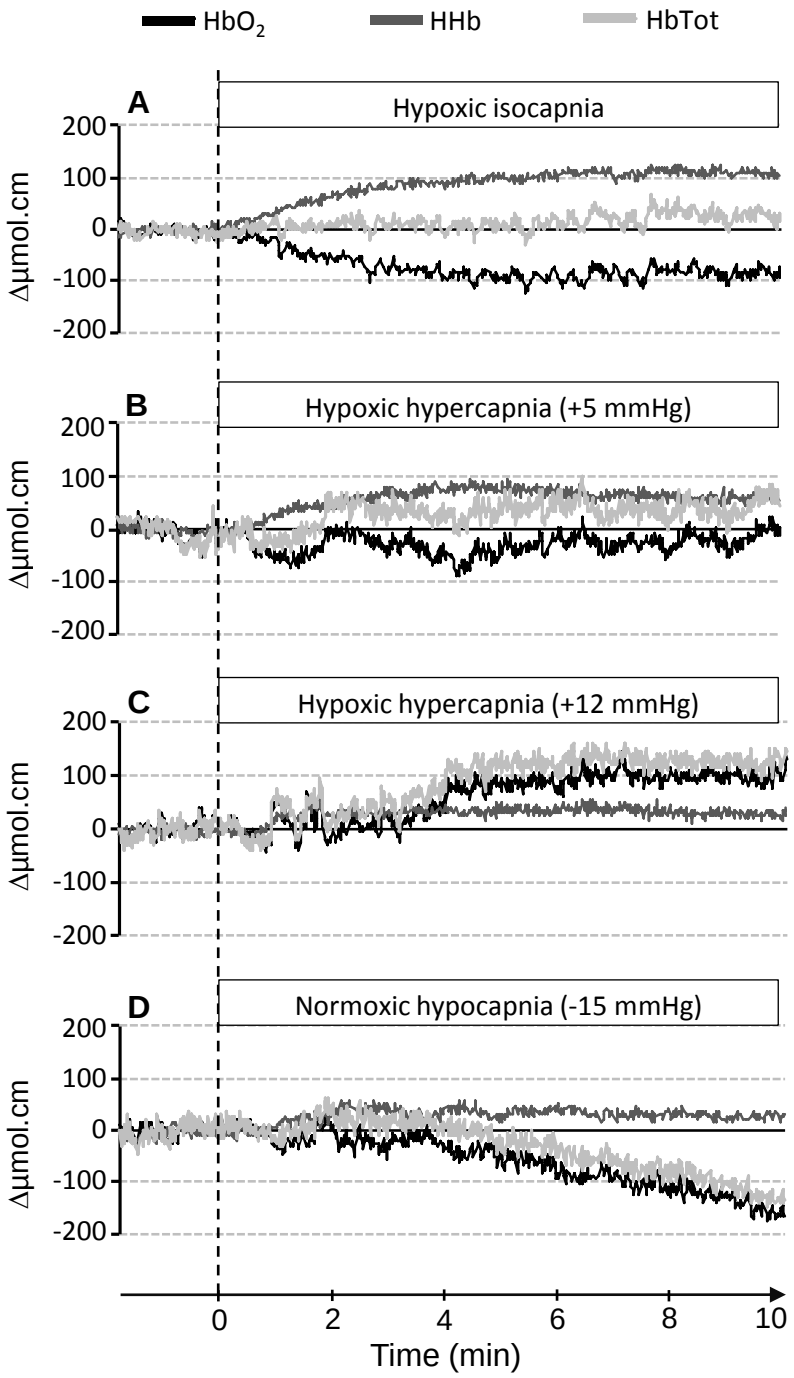
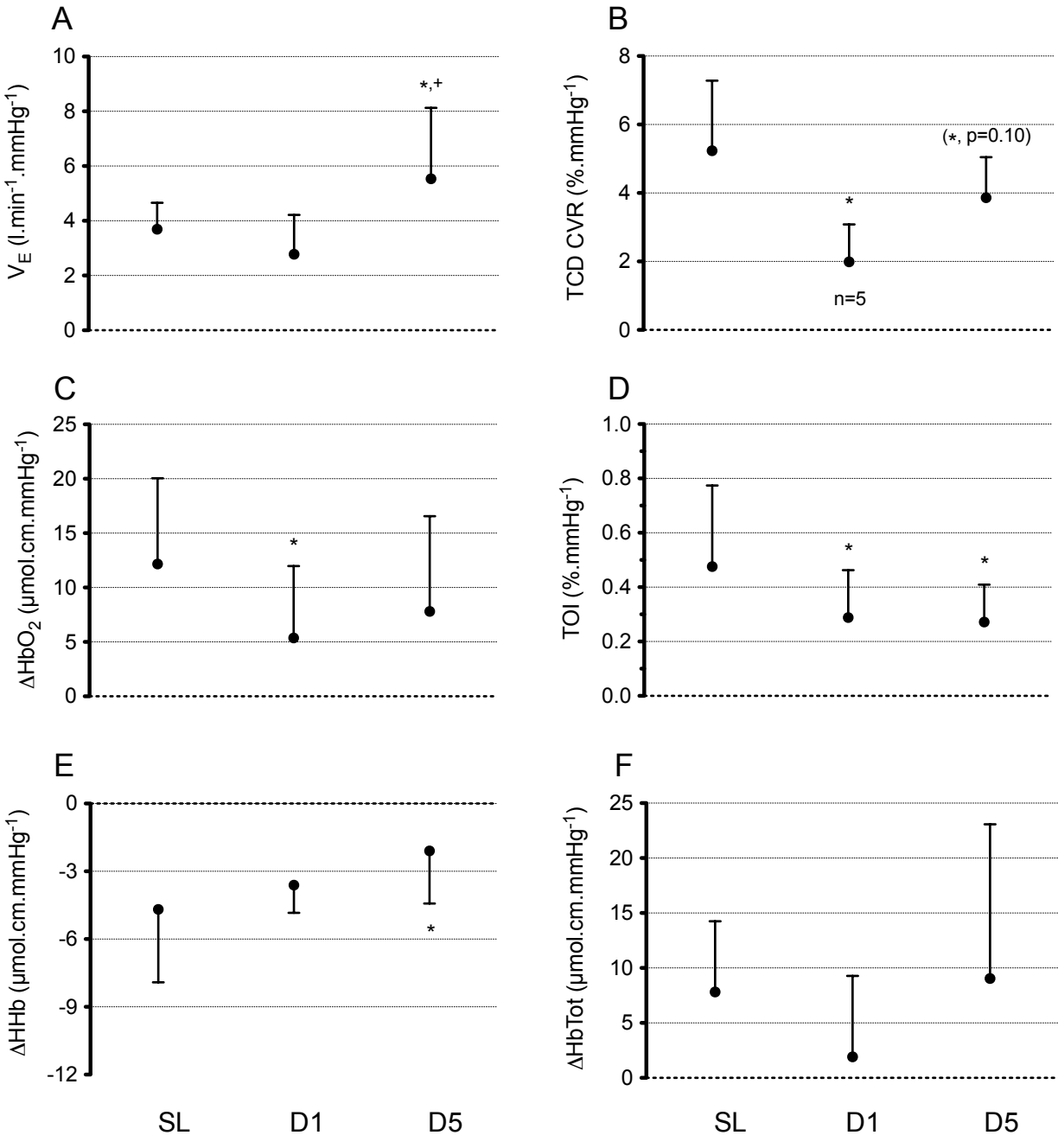
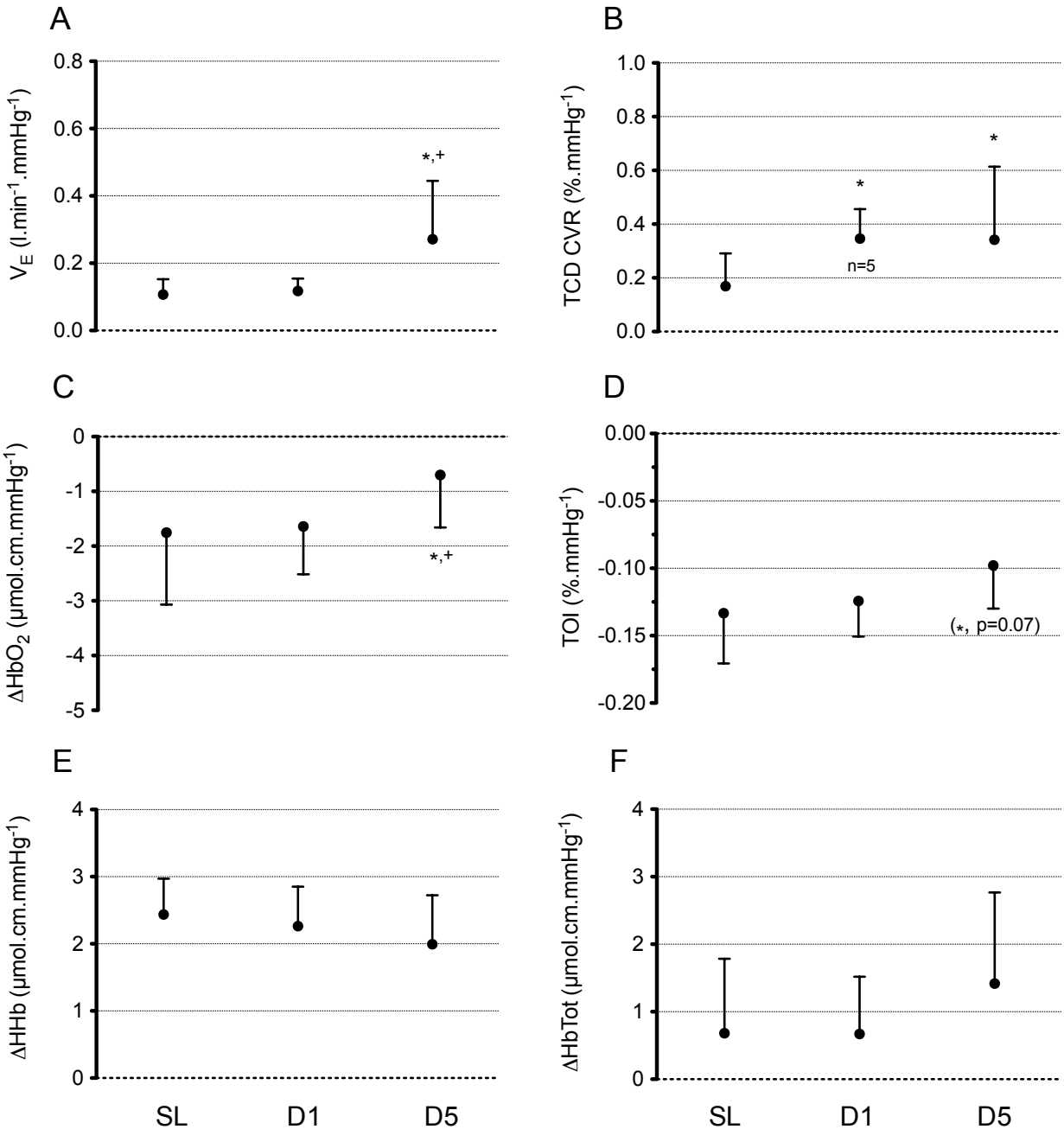

A
V_E (l.min^{-1}.mmHg^{-1})
*,+
B
MCAv (%.mmHg^{-1})
(*, p=0.10)
*
(n=5)
C
ΔHbO_2 (µmol.cm.mmHg^{-1})
*
D
TOI (%.mmHg^{-1})
*
*
E
ΔHHb (µmol.cm.mmHg^{-1})
*
F
$\Delta HbTot$ (µmol.cm.mmHg^{-1})
SL
D1
D5
SL
D1
D5

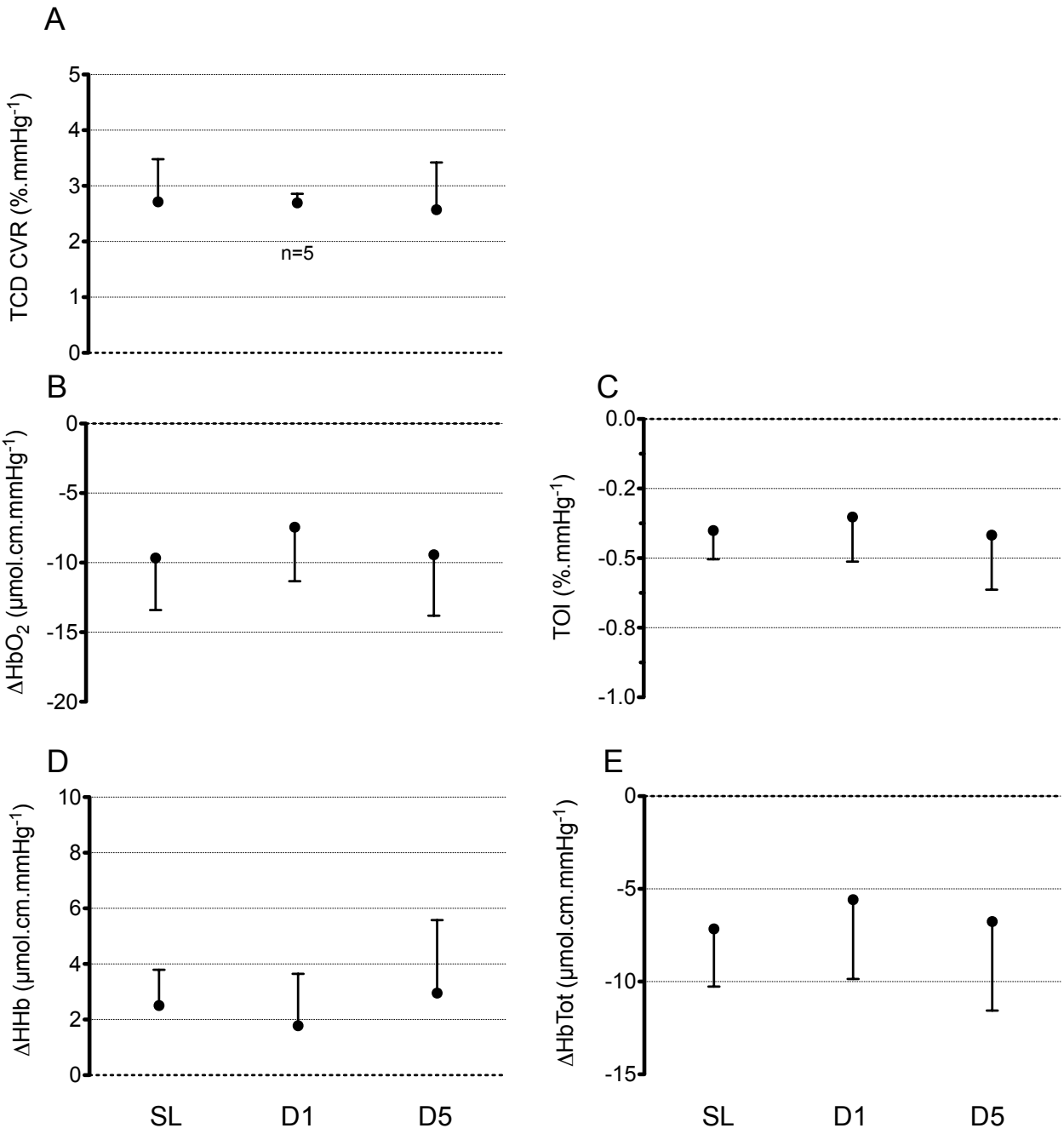
Fig. 5


A
MCAv (%.mmHg-1)
7
6
5
4
3
2
1
0
(n=5)
SL
D1
D5
B
ΔHbO2 (µmol.cm.mmHg-1)
0
-5
-10
-15
-20
-25
C
TOI (%.mmHg-1)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
D
ΔHHb (µmol.cm.mmHg-1)
12
9
6
3
0
SL
D1
D5
E
ΔHbTot (µmol.cm.mmHg-1)
0
-5
-10
-15
-20
-25
SL
D1
D5


HbO2
HHb
HbTot
A
Hypoxic isocapnia
B
Hypoxic hypercapnia (+5 mmHg)
C
Hypoxic hypercapnia (+12 mmHg)
D
Normoxic hypocapnia (-15 mmHg)
Δμmol.cm
200
100
0
-100
-200
0
2
4
6
8
10
Time (min)


A
V_E (l.min^{-1}.mmHg^{-1})
10
8
6
4
2
0
*,+
B
TCD CVR (%.mmHg^{-1})
8
6
4
2
0
*
n=5
(*, p=0.10)
C
ΔHbO$_2$ (µmol.cm.mmHg^{-1})
25
20
15
10
5
0
*
D
TOI (%.mmHg^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
*
*
E
ΔHHb (µmol.cm.mmHg^{-1})
0
-3
-6
-9
-12
*
F
ΔHbTot (µmol.cm.mmHg^{-1})
25
20
15
10
5
0
SL
D1
D5
SL
D1
D5


A
B
C
D
E
F
V_E (l.min^{-1}.mmHg^{-1})
TCD CVR (%.mmHg^{-1})
ΔHbO_2 (µmol.cm.mmHg^{-1})
TOI (%.mmHg^{-1})
ΔHHb (µmol.cm.mmHg^{-1})
ΔHbTot (µmol.cm.mmHg^{-1})
0.8
0.6
0.4
0.2
0.0
1.0
0
-1
-2
-3
-4
-5
0.00
-0.05
-0.10
-0.15
-0.20
4
3
2
1
*,+
*
n=5
(*, p=0.07)
SL
D1
D5


A
TCD CVR (%.mmHg-1)
5
4
3
2
1
0
n=5
B
ΔHbO2 (µmol.cm.mmHg-1)
0
-5
-10
-15
-20
C
TOI (%.mmHg-1)
0.0
-0.2
-0.5
-0.8
-1.0
D
ΔHHb (µmol.cm.mmHg-1)
10
8
6
4
2
0
E
ΔHbTot (µmol.cm.mmHg-1)
0
-5
-10
-15
SL
D1
D5
SL
D1
D5