HIGHLIGHTED TOPIC | Hypoxia

Regional cerebral blood flow in humans at high altitude: gradual ascent and 2 wk at 5,050 m

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Willie CK, Smith KJ, Day TA, Ray LA, Lewis NC, Bakker A, Macleod DB, Ainslie PN. Regional cerebral blood flow in humans at high altitude: gradual ascent and 2 wk at 5,050 m. J Appl Physiol 116: 905–910, 2014. First published June 27, 2013; doi:10.1152/japplphysiol.00594.2013.—The interindividual variation in ventilatory acclimatization to high altitude is likely reflected in variability in the cerebrovascular responses to high altitude, particularly between brain regions displaying disparate hypoxic sensitivity. We assessed regional differences in cerebral blood flow (CBF) measured with Duplex ultrasound of the left internal carotid and vertebral arteries. End-tidal $\text{PCO}_2$, oxyhemoglobin saturation ($\text{SpO}_2$), blood pressure, and heart rate were measured during a trekking ascent to, and during the first 2 wk at, 5,050 m. Transcranial color-coded Duplex ultrasound (TCCD) was employed to measure flow and diameter of the middle cerebral artery (MCA). Measures were collected at 344 m (TCCD-baseline), 1,338 m (CBF-baseline), 3,440 m, and 4,371 m. Following arrival to 5,050 m, regional CBF was measured every 12 h during the first 3 days, once at 5–9 days, and once at 12–16 days. Total CBF was calculated as twice the sum of internal carotid and vertebral flow and increased steadily with ascent, reaching a maximum of 8.9 ± 1 ml/min/100 g (±3 ± 7% vs. 1,338 m; mean ± SE) at ~60 h after arrival at 5,050 m. These changes returned to +15 ± 12% after 12–16 days at 5,050 m and were related to changes in $\text{SpO}_2$ ($R^2 = 0.36$; $P < 0.0001$). TCCD-measured MCA flow paralleled the temporal changes in total CBF. Dilution of the MCA was sustained on days 2 (+12.6 ± 4.6%) and 8 (+12.9 ± 2.9%) after arrival at 5,050 m. We observed no significant differences in regional CBF at any time point. In conclusion, the variability in CBF during ascent and acclimatization is related to ventilatory acclimatization, as reflected in changes in $\text{SpO}_2$, cerebral blood flow; brain; altitude; acclimatization

Ascent to high altitude (HA) is a potent physiological stressor that manifests in acute and chronic ventilatory and acid-base adaptations that serve to elevate the partial pressure of arterial oxygen ($\text{PaO}_2$) and maintain pH, respectively. Cerebral blood flow (CBF) is intrinsically linked to ventilatory acclimatization by virtue of its sensitivity to $\text{PaO}_2$ and arterial carbon dioxide ($\text{PaCO}_2$). Changes in CBF vary proportionally to changes in $\text{PaCO}_2$ and inversely with reduced oxyhemoglobin saturation ($\text{SpO}_2$). Moreover, the change in CBF in response to altered arterial blood gases may influence the ventilatory response to the related blood-gas perturbations (38, 39). Laboratory studies at sea level have shown a ~3–4% decrease in CBF per millimeters of mercury reduction in $\text{PaCO}_2$, from eupneic $\text{PaCO}_2$ to ~20 Torr and a ~4–5% per mmHg increase in CBF above eupneic $\text{PaCO}_2$ (1, 28, 35). Isocapnic hypoxemia increases CBF ~3–4% per unit decrease in arterial oxygen saturation to maintain adequate oxygen delivery to the brain (2, 35). Yet each of these sensitivities is altered during the course of acclimatization (17, 26, 29) and differ between the internal carotid (ICA) and vertebral arteries (VA) at sea level (18, 28, 35). Flow through the ICA and VA are estimates of anterior and posterior (e.g., brain stem) perfusion, respectively.

Severinghaus et al. (31) first reported that total CBF increased by ~24% within the first 6–12 h of arrival at 3,810 m before decreasing to 13% above sea level values by 3–5 days; these data have been largely confirmed (6, 13, 15, 17). The time course of these changes varies within individuals and between studies and is likely reflective of the known intersubject variation in the ventilatory and cerebrovascular responses to hypoxia (11, 35), differing altitudes, ascent profiles, and measurement techniques utilized (see Fig. 1 for details).

In summary, variability in ventilatory acclimatization likely reflects a spectrum of proportional contributions from central and peripheral chemoreceptor drives (11), variable rates of metabolic compensation for respiratory alkalosis, and variation in regional cerebrovascular reactivity to these collective changes. Whether this variation remains when CBF is considered regionally remains unknown. To provide insight into this question, we quantified regional CBF at three distinct sites, the middle cerebral artery (MCA) [using transcranial color-coded Duplex ultrasound (TCCD)] and the ICA and VA (using linear array vascular Duplex ultrasound), during an 8-day ascent to 5,050 m. We repeated ICA and VA measures every ~12 h during the first 3 days and following 5–9 days and 12–16 days at 5,050 m; TCCD measures were repeated after 2, 8, and 14 days at 5,050 m. Our goal was to assess if the variability in CBF during ascent and acclimatization was attributable to ventilatory adjustments (i.e., $\text{SpO}_2$) and/or regional differences in CBF. Based on greater posterior CBF sensitivity to hypoxia reported at sea level (18, 35), we hypothesized that changes in
Methods

Subjects and Protocols

Eight subjects were tested as part of protocol 1, and 13 tested for protocol 2; six subjects partook in both studies, making the total sample size 15 sea level residents [4 women; aged 28 ± 6 yr (mean ± SD), range: 20–38 yr; body mass index 23.9 ± 3.3 kg/m²]. The study was approved by the Clinical Ethical Review Board of the University of British Columbia. All volunteers provided written, informed consent. Participants were nonsmokers, had no previous history of cardiovascular, cerebrovascular, or respiratory diseases, and were not taking any cardiovascular medications. Before inclusion into the study, participants were screened by means of a 12-lead ECG stress test, transthoracic echocardiogram, pulmonary function testing, and full polysomnography; no subject exhibited any sleep disordered breathing at sea level. All participants were born and lived close to sea level (<1,000 m), and none had been to HA for >2 yr. This study was part of a larger research expedition conducted in April-June in 2012. As such, participants took part in a number of studies conducted during the 3 wk at the Ev-K2-CNR Pyramid Laboratory. The experimental question addressed in this paper was a priori driven, and the data included herein will not be duplicated in future reports. The recovery time between the various testing sessions was managed to prevent any potentially confounding results (e.g., >48 h between all drug and/or exercise intervention studies).

Ascent to HA

All participants spent 1 wk in Kathmandu (1,338 m) before flying to Lukla (2,860 m) to begin the trek to 5,050 m over 6–8 days. One acclimatization day was taken at 3,440 m, 3,860 m and 1–3 days at 4,371 m. Additionally, during the first 6–7 days of the trek to 5,050 m, participants were given low-dose acetazolamide (125 mg, oral) twice a day as an acute mountain sickness prophylactic (5, 21). Treatment of acetazolamide was discontinued on day 8 of the trek (i.e., 4,371 m) to allow sufficient time (e.g., >24 h) for the drug to clear participants’ system before the first data collection session at 5,050 m, as the half-life of acetazolamide is reported to be ~10 h (22), and this low dose is typically 90–100% passed through the system within 24 h of administration (20). This approach was utilized to ensure the safety of the experimental volunteers at 5,050 m.

Protocol 1

In eight subjects, baseline TCCD measurements (see below), mean arterial pressure, heart rate (HR), and SpO₂, and were collected in Kelowna (344 m), and repeated after 2, 8, and 14 days following arrival at 5,050 m (the Ev-K2-CNR Pyramid Laboratory; barometric pressure 413 ± 1 mmHg).

Protocol 2

In 13 participants, baseline measures were collected in Kathmandu (1,338 m), Nepal, 2 days before flying to Lukla (2,860 m). Measurements were repeated following at least 3 h rest the evening of arrival and during the first morning at 3,440 m (n = 13), 3 h following arrival to 4,371 m (n = 7), and again during the first three evenings and mornings at 5,050 m (n = 13). Measures were repeated again between days 5 and 9 (n = 6) and between days 12 and 16 at 5,050 m (n = 6).

Measurements

Subjects abstained from caffeine and alcohol for the duration of the study, but were permitted acetaminophen ad libitum. In supine position, subjects rested in a prewarmed sleeping bag for a minimum of 10 min before each measurement session. Efforts were made to ensure subjects were warm and calm before measures were taken. Evening measurements were collected between 1700 and 2200, at least 1 h after eating. Morning data were collected between 0400–0800, before eating. End-tidal partial pressure of CO₂ (PETCO₂; EMMA capnometer, Masimo, Sweden), SpO₂, HR (Nonin Onyx oximeter, Plymouth, MN), and mean arterial pressure (manual auscultation) were measured in triplicate at each point described above.

Metrics of CBF

In protocol 1, diameter and blood flow velocity in the MCA were measured using TCCD (GE Vivid-E-Ultrasound; 2.5 MHz probe; GE Healthcare). The MCA was identified using color Doppler in the same plane as the mesencephalon, with flow toward the probe. Diameter (by manual caliper placement) and velocity (EchoPac, GE Healthcare) were measured 1 cm distal to the ICA-MCA-anterior cerebral artery trifurcation and were averaged over 10 cardiac cycles. Blood flow through the MCA was calculated as the product of mean MCA cross-sectional area and mean blood velocity. One experienced sonographer (A. Bakker) collected all TCCD measures. Regional CBF was measured in protocol 2 and analyzed as previously described (35). In brief, left ICA and VA flows (Q˙ICA and Q˙VA) were determined using high-resolution duplex ultrasound (10-MHz multifrequency linear array probe; Terason 3300 ultrasound machine, Teratech, Burlington, MA). Continuous diameter and velocity recordings were obtained at least 2 cm distal to the carotid bifurcation for Q˙ICA, and between C6 and C7 for Q˙VA. Screen capture videos were recorded for subsequent offline analysis at 30 Hz using custom edge-tracking software, as detailed elsewhere (8). Two experienced sonographers (K. J. Smith and C. K. Willie) scanned the same subjects at each time point, and care was taken to obtain the same angle of insonation and vessel location within subjects. Reproducibility of diameter measurements using this software is better than manual methods, as it reduces observer error significantly and possesses an intraobserver coefficient of variation of 3%.
of variation of 6.7% (8). Global CBF was calculated as twice the sum of $Q_{\text{ICA}}$ and $Q_{\text{VA}}$.

**Statistics**

Normal distribution of variables were confirmed by Shapiro-Wilk test ($P < 0.05$). One-way ANOVA assessed differences with ascent/ time, and Dunnett’s post hoc test was used where appropriate to compare means to Kelowna (344 m; protocol 1) or Kathmandu (1,338 m; protocol 2). Repeated-measures t-tests were used to compare the relative change in ICA to VA flow at each point during the first 3 days at 5,050 m. Pearson’s correlation determined relationships between CBF and $\text{SpO}_2$. Interindividual coefficient of variation was calculated for the percent change in CBF values as $\sqrt{\text{SD}^2/\text{mean}}$ for all individuals at a given time point (see Discussion). No statistical differences were noted between sexes, and data were therefore pooled. Values are shown as means ± SE.

**RESULTS**

**Subjects**

All subjects were included in analysis. For protocol 2, it was not possible to collect data on four subjects in Kathmandu (1,338 m) due to time constraints and illness. Since these subjects were part of the University of British Columbia research team, data obtained in Kelowna (344 m) 1 mo prior as part of another study were available in these subjects and were substituted in these individuals only. Data that were available for both 344 m and 1,338 m for 9 of the 13 subjects showed that no variable significantly differed between the two altitudes. Likewise, the substituted values ($n = 4$) did not differ significantly from that of the other nine subjects at 1,338 m.

**Changes with Altitude**

**Protocol 1.** Figure 1 and Table 1 show the change in blood flow through the MCA that increased to a maximum of +42 ± 14% after 2 days at 5,050 m, ±8% after 8 days, and 19 ± 12% after 2 wk at 5,050 m. At 5,050 m, the diameter of the MCA significantly increased ($P < 0.05$) by +12.5 ± 4.6% and +12.9 ± 2.9% on days 2 and 8, respectively, and trended to remain increased on day 14 (+9.8 ± 3.6%).

**Protocol 2.** There was a significant effect with ascent to altitude for all variables (Table 2). Increases with ascent were noted for HR and CBF, whereas $\text{PETCO}_2$ and $\text{SpO}_2$ decreased with ascent to 5,050 m (Table 2; Fig. 2). $\text{PETCO}_2$ increased ~2–3 Torr from its nadir after the first night at 5,050 m. Results for post hoc multiple comparisons are shown in Table 2.

### Table 1. Cardiorespiratory and transcranial color-code Doppler ultrasound measures of the middle cerebral artery upon ascent to 5,050 m and during acclimatization

<table>
<thead>
<tr>
<th>Kelowna (344 m)</th>
<th>Ev-K2-CRN Research Pyramid (5,050 m)</th>
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<tbody>
<tr>
<td></td>
<td>Day 2</td>
</tr>
<tr>
<td><strong>HR, beats/min</strong></td>
<td>58 ± 3</td>
</tr>
<tr>
<td><strong>MAP, mmHg</strong></td>
<td>90 ± 2</td>
</tr>
<tr>
<td><strong>SpO₂, %</strong></td>
<td>99 ± 0.3</td>
</tr>
<tr>
<td><strong>Diameter, mm</strong></td>
<td>4.2 ± 0.2</td>
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<tr>
<td><strong>Velocity, cm/s</strong></td>
<td>72 ± 4</td>
</tr>
<tr>
<td><strong>Flow, ml/min</strong></td>
<td>650 ± 68</td>
</tr>
</tbody>
</table>

Values are means ± SE based on $n = 8$ subjects at each time point. HR, heart rate; MAP, mean arterial pressure; SpO₂, percent saturation of hemoglobin. *$P < 0.05$ vs. Kelowna (344 m).
To our knowledge, this study is the sixth in the literature to assess the time course of CBF changes with exposure to HA, but the first to make multiple measurements per day during the first 3 days after arrival and during partial preacclimatization to 5,050 m. Figure 1 demonstrates that, following ~60 h at altitude (6, 13, 17, 31), CBF begins to fall from its zenith, returning to near sea level values by 2 wk. Importantly, our data show the largest CBF increase to date, despite our gradual ascent profile (vs. the other studies, which employed a rapid ascent by car or tram to altitude), and that this maximum occurred at ~60 h at 5,050 m. This is in contrast to the other studies that showed a less pronounced peak in CBF that more promptly began to decrease. This is likely a result of the higher temporal resolution employed here (every 12 h for the first 3 days) and greater altitude and degree of hypoxemia.

Whether cerebral metabolism changes with acclimatization remains unknown. The studies of Severinghaus et al. (31) and Jensen et al. (15) used the Kety-Schmidt and Xe\textsuperscript{133} methods (respectively) of CBF estimation, which assume constant brain metabolic rate; thus, if brain metabolism increases during acclimatization, these methods could underestimate CBF. Of interest is the more modest CBF (as indexed by MCA blood velocity) increase we observed previously following an identical ascent profile (17). Wilson et al. (36) recently reported an ~24% dilation of the MCA at 6,400 m, and we suggested the same with extreme hypoxemia (\(\text{P}_{\text{aO}} = 43\) Torr) (35). Both studies are consistent with our findings of a sustained ~9–12% dilation of the MCA at 5,050 m. Thus, relative to the present data, the attenuated CBF increase previously reported (as estimated by transcranial Doppler ultrasound that assumes constant diameter of the insonated vessel) (17) is likely explained by dilatation of the MCA.

As highlighted by previous reports (18, 35), the variability in CBF with hypoxia was substantial. Indeed, the intersubject coefficient of variation for the percent increase in CBF during the first night at 3,440 m was 640%. This coefficient of variation in \(\Delta\text{CBF}\) decreased to 48% during the third night at 5,050 m when absolute global CBF, and the relative change from 1,338 m in global CBF, were greatest. The increase in CBF with hypoxia presumably serves to maintain cerebral oxygen delivery (34, 37), as reflected in a strong correlation between \(\text{SpO}_2\) and CBF, that accounts for ~40% of the
statistical variability ($R^2 = 0.36$). The remaining variance in individual CBF with altitude/hypoxia exposure is perhaps not surprising, given the known variability in the hypoxic ventilatory response (11), speed of acclimatization (4, 24), and cerebrovascular response to both CO$_2$ and hypoxia (17, 35). However, two aspects of the present data indicate oxygen delivery to the brain is critical for successful acclimatization to HA: 1) the strong relationship between CBF and SpO$_2$; and 2) that the increase in CBF during ascent (i.e., at 3,440 m and 4,371 m) showed marked variability, whereas at 5,050 m the increase in CBF was homogeneous between subjects.

Regional CBF Changes at HA

Recent reports indicate greater reactivity to hypoxia in the brain stem than cortex (18, 28, 35), and greater gray matter relative to white matter hypoxic blood flow sensitivity (7). In contrast, we found no significant difference between $Q_{VA}$ and $Q_{ICA}$ at any point. These findings are consistent with those of Huang et al. (13), who measured VA and ICA velocities (as an estimate of flow) at 4,300 m. While it is possible that the intersubject variability in our data reduced the precision needed to observe the relatively small differences between brain regions, there were no statistical differences at any point at 5,050 m when the between-subjects coefficient of variation was least and the mean difference greatest. Indeed, at these time points, to achieve a power of 0.8 and $\alpha = 0.05$, a sample size $\geq 70$ would be required (3), which is clearly not practical in a field study of this nature. Moreover, based on previously reported data from our laboratory (35) collected by the same two sonographers and equipment, we were able to demonstrate regional CBF differences during severe step changes in P_{ACO}_2 and P_{AO}_2. While advanced imaging modalities (not yet logistically possible in a HA field study) would likely yield lower variability, the high intersubject variability observed in these data is likely representative of normal variation in the human response to a multifaceted stress, such as altitude.

Acetazolamide, CBF, and Ventilatory Acclimatization

Acetazolamide is a carbonic anhydrase inhibitor that increases ventilation and arterial oxygen saturation through enhanced renal excretion of bicarbonate, resulting in metabolic acidosis (34). CBF is reciprocally affected by acidosis-induced cerebrovascular dilation on the one hand (10, 16), but hyper-ventilation-induced decreases in P_{ACO}_2 that cause cerebral vessel constriction on the other (16, 35). Thus the use of acetazolamide in the present study somewhat obscures the interpretation of these data. To our knowledge, this is the first study to document the altered ventilatory acclimatization pattern at 5,050 m following cessation of acetazolamide. Figure 2 shows that, at 5,050 m, $P_{T\text{CO}_2}$ increased from its nadir at PM1 (evening 1). This is not consistent with the steady increase in ventilation and P_{AO}_2 and consequent reduction in $P_{T\text{CO}_2}$ typically seen with acclimatization (19). Termination of acetazolamide may have resulted in a transient hypoventilation, as acetazolamide-induced metabolic acidosis was withdrawn. Oral acetazolamide per se does not produce altered CBF at sea level (12), but, because we did not measure or manipulate base excess, it is difficult to determine the potential influence of acid-base balance on prevailing CBF at HA.

Conclusion

Following gradual ascent to 5,050 m over 5–9 days, CBF continued to increase over the first $\sim$60 h at 5,050 m to $\pm$53 ± 7.6% of low-altitude values; it then began to decrease to approximately $\pm$20% of low-altitude values after 2 wk at 5,050 m. The sustained MCA dilation observed for the duration of exposure to 5,050 m indicates intracerebral vessel dilation facilitates increased brain oxygen delivery and should be taken into consideration in future studies utilizing conventional TCD at HA. We (35) have previously reported VA dilation with 70% SpO$_2$ (P_{AO}_2 $\sim$36 Torr). That no dilation of the neck arteries was observed at 5,050 m indicates this vessel may only dilate under conditions of extreme isocapnic hypoxia with minimum acid-base compensation and suggests greater relative hypoxic sensitivity of the intracerebral arteries. Finally, there is tremendous interindividual variability in both the CBF increase with ascent, and CBF decrease with acclimatization; SpO$_2$ appears to explain $\sim$40% of the statistical variation in CBF.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES
