Early brain swelling in acute hypoxia

David J. Dubowitz,1 Edward A. W. Dyer,2 Rebecca J. Theilmann,1 Richard B. Buxton,1 and Susan R. Hopkins1,2

1Centre for Functional MRI, Department of Radiology, and 2Division of Physiology, Department of Medicine, University of California, San Diego, California

Submitted 29 February 2008; accepted in final form 4 May 2009

Dubowitz DJ, Dyer EA, Theilmann RJ, Buxton RB, Hopkins SR. Early brain swelling in acute hypoxia. J Appl Physiol 107: 244–252, 2009. First published May 7, 2009; doi:10.1152/japplphysiol.90349.2008.—Acute mountain sickness (AMS) and high-altitude cerebral edema share common clinical characteristics, suggesting cerebral swelling may be an important factor in the pathophysiology of AMS. Hypoxia and hypocapnia associated with high altitude are known to exert strong effects on the control of the cerebral circulation, yet how these effects interact during acute hypoxia, and whether AMS-susceptible subjects may have a unique response, is still unclear. To test if self-identified AMS-susceptible individuals show altered brain swelling in response to acute hypoxia, we used quantitative arterial spin-labeling and volumetric MRI to measure cerebral blood flow and cerebrospinal fluid (CSF) volume changes during 40 min of acute hypoxia. We estimated changes in cerebral blood volume (CBV) (from changes in cerebral blood flow) and brain parenchyma swelling (from changes in CBV and CSF). Subjects with extensive high-altitude experience in two groups participated: self-identified AMS-susceptible (n = 6), who invariably experienced AMS at altitude, and self-identified AMS-resistant (n = 6), who almost never experienced symptoms. During 40-min hypoxia, intracranial CSF volume decreased significantly [−10.5 ml (SD 6.9), P < 0.001]. There were significant increases in CBV [+2.3 ml (SD 2.5), P < 0.005] and brain parenchyma volume [+8.2 ml (SD 6.4), P < 0.001]. However, there was no significant difference between self-identified AMS-susceptible and AMS-resistant groups for these acute-phase changes. In acute hypoxia, brain swelling occurs earlier than previously described, with significant shifts in intracranial CSF occurring as early as 40 min after exposure. These acute-phase changes are present in all individuals, irrespective of susceptibility to AMS.

magnetic resonance imaging; arterial spin labeling; cerebral blood flow; cerebral blood volume; cerebrospinal fluid volume

ACUTE MOUNTAIN SICKNESS (AMS) is a common condition that manifests with symptoms of headache, nausea, vomiting, anorexia, dizziness, lethargy, and fatigue within 6–12 h of rapid exposure to high altitude, as well as sleep disturbance (2). The development of symptoms is highly variable between individuals, and the combination of altitude, rate of ascent, extent of previous altitude acclimatization, as well as individual susceptibility all play a role (32). However, the pathophysiological mechanisms of AMS remain poorly understood. Although AMS is a benign and self-limiting condition, it shares some clinical characteristics with high-altitude cerebral edema (11). The current consensus is that AMS and high-altitude cerebral edema may be part of the same spectrum of illness, and that increased brain parenchyma volume (i.e., cerebral swelling) is likely an etiological factor in both.

Hypoxia and hypocapnia of high altitude exert strong effects on the control of the cerebral circulation. We investigated how these effects would play out acutely during hypoxia. We postulated that acute changes in cerebral hemodynamics, cerebral blood volume (CBV), and capillary hydrostatic pressure may result in brain swelling as an early response to acute hypoxia (before the onset of AMS symptoms) and would be evident as a corresponding decrease in the intracerebral cerebrospinal fluid (CSF) volume. How individuals respond to these short-term changes may determine who gets sick and who does not, and we postulated that differences in early cerebral swelling may be altered in self-identified susceptible subjects who consistently developed AMS on exposure to high altitude (AMS-susceptible) compared with subjects who rarely if ever reported symptoms (AMS-resistant).

The brain is buffered from the effects of parenchymal swelling, if CSF can be shifted out of the cranium to the high-compliance areas in the spine (20). Some authors have hypothesized that cerebral swelling, along with increased CBV and cerebral blood flow (CBF), may be part of the normal cerebral response to hypoxia and not specific to developing AMS per se (11, 16). Since the bony cranium is rigid, and the intracranial space is fixed, the sum of the volumes of all intracranial components is constant [the Munro-Kellie doctrine (19, 26)]. Thus, if normal intracranial pressure is to be maintained, any increase in cerebral parenchyma volume or blood volume must be balanced by a corresponding decrease in intracranial CSF volume (6). If the brain is not able to accomplish this shift of CSF out of the cranium, then intracranial pressure will rise, and, in the context of hypoxia, symptoms of AMS may ensue (30).

The purpose of this study was to test our hypotheses: 1) if brain swelling (as evidenced by shifts in intracranial CSF) is an early response to acute hypoxia, and 2) if there is a difference in how individuals adapt to early volume changes that may determine who develops AMS and who does not.

We selected two groups of subjects who had extensive histories of high-altitude exposure: a self-identified AMS-susceptible group, who invariably reported developing symptoms on ascent above 3,000-m altitude, and a self-identified AMS-resistant group, who reported almost never developing symptoms of AMS, despite multiple rapid ascents. Using magnetic resonance imaging (MRI), we quantified changes in intracranial CSF volume during acute, normobaric hypoxia. We also quantified CBF from a separate measurement session and used this to derive the contribution of increased brain parenchyma volume and blood volume to changes in CSF volume.

METHODS

Subject selection. This study conformed to the standards of the Declaration of Helsinki and was approved by the Human Subjects

Address for reprint requests and other correspondence: D. J. Dubowitz, UCSD Centre for Functional MRI, 9500 Gilman Dr., MC 0677, La Jolla, CA 92093-0677 (e-mail: dubowitz@ucsd.edu).
Research Protection Program of the University of California San Diego. All subjects gave written, informed consent. Healthy, non-smoking, sea-level-resident subjects were recruited based on a history of repeated ascents above 2,500 m and self-reporting of susceptibility to symptoms of AMS. Subjects completed a questionnaire detailing their high-altitude exposure above 2,500 and 4,200 m, with particular emphasis on their symptoms relevant to AMS (headache, nausea, fatigue, dizziness, insomnia, confusion, ataxia, edema, malaise) (29), and filled out Lake Louise Consensus AMS scoring questionnaires based on their most recent ascents above 2,500 m. They also completed a health screening questionnaire for cardiac, pulmonary, or neurological disease. Subjects were selected based on frequency and severity of symptoms, as well as ability to undergo MRI examination. For at least 1 mo before the studies, all subjects slept below 1,800 m and did not sojourn above 2,400 m. Recruitment continued until 12 subjects, 6 in each group, were identified.

Experimental design. To quantify CSF volume changes with hypoxia, the subject lay supine in the magnetic resonance (MR) scanner wearing a close-fitting mask connected to a low-dead space, nonre-breathing valve (Hans Rudolph 8930/2700, Kansas City, MO). Baseline MRI data were obtained for 20 min while the subjects were breathing room air. The inspiratory port of the Hans Rudolph valve was then connected to a Douglas bag containing a gas mixture of 12.5% O2 in N2 (equivalent to a partial pressure of O2 of ~90 Torr or 3,800 m equivalent altitude) using a remotely controlled, pneumatically activated, three-way valve (Hans Rudolph 4285/8500, Hans Rudolph, Kansas City, MO) located on the inspired limb of the circuit.

MRI data were acquired during the subsequent 40 min of normobaric hypoxia. Heart rate and oxygen saturation were continuously monitored during MRI studies using an MRI-compatible physiological monitoring system (3150/3155 MRI patient monitor, In Vivo Research). Quantified CBF measurements were from a separate MRI examination with the same experimental setup, but a paradigm of 10-min normoxia, 30-min hypoxia followed by a return to 30-min normoxia.

MRI measurements. All MR images were acquired at 3 Tesla (Excite II, General Electric, Milwaukee, WI). Quantitative measurements of intracranial CSF volume used a high-resolution heavily T2-weighted three-dimensional fast spin echo (3D-FSE) sequence with echo time (TE) = 400 ms, repetition time (TR) = 3,000 ms, echo train length = 64, bandwidth 31.25 kHz, field of view 25 × 25 × 15 cm, matrix 256 × 256 × 124, ~1 × 1 × 1.2-mm resolution, 12 min). At 3 T, the transverse magnetization (T2) relaxation time of CSF is longer than 700 ms (17), whereas the T2 relaxation time of gray or white matter is ~74–92 ms (7). Thus using a T2-weighted image with a TE of 400 ms ensures that signal from the gray and white matter is almost completely absent (~98.7% relaxed), and the dominant source of signal in these images is from the CSF. A coregistered T1-weighted three-dimensional fast spoiled gradient recalled acquisition in steady state (3D-FSPGR) sequence was also acquired to generate an initial mask of the brain (TE = 3.1 ms, TR = 7.9 ms, inversion time = 450 ms, bandwidth 31.25 kHz, field of view = 25 × 25 × 15 cm, matrix 256 × 256 × 124, ~1 × 1 × 1.2-mm resolution, 8 min). This T1- and T2-weighted image pair (Fig. 1) was acquired during the initial period of normoxia and twice more at 20 and 40 min of hypoxia. CSF volume was quantified using the method described by Theilmann et al. (37). The three 3D-FSPGR T1-weighted data sets (normoxia, 20-min hypoxia, 40-min hypoxia) were coregistered to the initial normoxia data set using the FMRIB Linear Image Registration Tool (FLIRT, FSL library, FMRIB, Oxford, UK). The rotation matrix used to coregister each T1-weighted data set was then applied to each corresponding T2-weighted 3D-FSE pair (thus the degree of smoothing and interpolation was balanced for all three T2-weighted data sets). A single “brain mask” was defined for the three coregistered T1-weighted 3D-FSPGR data sets at the gray matter/CSF border using the FMRIB Brain Extraction Tool (FSL library, FMRIB, Oxford, UK). The brain mask was grown by 3 mm in all directions beyond the brain surface to encompass all intracranial CSF and applied to the 3D-FSE T2-weighted images. This intracranial mask was further optimized manually if needed to ensure only intracranial CSF was included, and additional extracranial high signal areas (e.g., paranasal sinuses, orbits) were appropriately masked out (Amira, Mercury Computer Systems). The foramen magnum was identified from bony landmarks (24) and used to define the caudal extent of the intracranial mask (Fig. 1). All CSF pixels from the T2-weighted 3D-FSE image within the intracranial mask were summed to generate the CSF volume at each time point. To correct for differences in absolute MR signal and image noise across subjects, the background noise was used to define a lower threshold for pixel values to remove low signal voxels. The noise level for this threshold was measured in the air immediately surrounding the scalp near the midline to avoid any possible ghosting artifacts. The intraventricular CSF was used as an internal reference to set the pixel value for a voxel containing 100% CSF. Voxels above the noise threshold were rescaled relative to 100% CSF and integrated across the intracranial space.

Reliability for this CSF quantitation technique was established by rescanning during normoxia on two occasions. Five control subjects (11 and 12 from the present study and 3 additional subjects not preselected on any AMS susceptibility criteria) underwent the same measurements at baseline and 20 and 40 min while lying supine in the scanner and breathing room air throughout. To control for any influence of a supine position on CSF volume measurements, the 60-min studies (20-min normoxia/40-min hypoxia measurements) were compared with similar 60-min acquisition periods under normoxia alone. Data processing followed the same scheme detailed above.

The change in CBV (ΔCBV) during acute hypoxia was estimated from the fractional change in CBF (ΔCBF). All 12 subjects underwent quantitative measurement of CBF during a separate MRI measurement using an arterial spin labeling technique (41). The blood flow method and findings have previously been reported for 10 of these 12 subjects (8). Blood flow data were averaged into three bins: mean flow

![Fig. 1. T1- and T2-weighted image pair used to quantify cerebrospinal fluid (CSF) volume. A: whole brain, high-resolution, three-dimensional (3D) T1-weighted image is used to define a mask of intracranial CSF (bounded inferiorly at the foramen magnum). B: high-resolution 3D heavily T2-weighted image is dominated by signal from CSF (note: almost no residual signal from blood vessels, gray or white matter). The intracranial mask is applied to this image to quantify the intracranial volume of CSF.](https://jap.physiology.org/doi/10.1152/jappl.00151.2007)
RESULTS

Subject data. Twelve subjects (out of 35 who completed questionnaires) fulfilled the criteria for the two AMS groups and underwent MRI examination. Subject data are given in Table 1. Six were self-identified AMS-susceptible: four women and two men, mean age 35 yr (SD 10), with a mean AMS score during the most recent altitude visit of 13 (SD 4), headache score 2.6 (SD 0.5), and were symptomatic during 95% (SD 13) of prior ascents above 2,500-m altitude. An additional six were self-identified AMS-resistant: two women and four men, mean age 29 yr (SD 4), mean AMS score 1 (SD 2), headache score 0.2 (SD 0.4), and had symptoms of AMS during 2% (SD 4) of prior ascents above 2,500-m altitude. There was no significant difference in mean age between the two groups of subject (P = 0.2). Although the AMS-resistant group tended to have a greater number of trips to altitudes above 2,500 and 4,200 m and a greater highest elevation reached, these differences were of borderline significance (P = 0.06, P = 0.06, and P = 0.07, respectively). However, Lake Louise Consensus AMS scores and frequency of symptoms were highly significantly different between the two groups (P < 0.001 for both). Subject 2 (woman, AMS-susceptible) reported a past history of migraine headaches. All other subjects reported no cardiac, pulmonary, or neurological diseases (Table 1).

Reliability of CSF quantitation. Five subjects acted as controls to assess the reliability of the CSF volume quantitation: three men and two women, mean age was 32 yr (SD 8), mean interval between the two CSF volume measurements was 7 days (SD 5). The CSF volume from the two normoxic measurement sessions were highly correlated (r = 0.97, r² = 0.93). (Fig. 2). The normoxic control measurements to assess the effects of lying supine in the scanner showed a small (nonsignificant) reduction of −1.2 ml (SD 3.9) in CSF volume following 20 min of lying in the scanner that decreased to −0.1 ml.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>AMS Status</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Prior Highest Elevation Attained, m</th>
<th>Ascents &gt;2,500 m, no.</th>
<th>Ascents &gt;4,200 m, no.</th>
<th>Frequency of Symptom During Prior Altitude Visits, %</th>
<th>Mean Lake Louise Score for Prior Visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Susceptible</td>
<td>34</td>
<td>F</td>
<td>4,270</td>
<td>20+</td>
<td>10+</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>Susceptible</td>
<td>34</td>
<td>F</td>
<td>3,050</td>
<td>10+</td>
<td>0</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>Susceptible</td>
<td>32</td>
<td>M</td>
<td>4,270</td>
<td>20+</td>
<td>10+</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Susceptible</td>
<td>28</td>
<td>M</td>
<td>4,270</td>
<td>10+</td>
<td>10+</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Susceptible</td>
<td>27</td>
<td>F</td>
<td>4,270</td>
<td>20+</td>
<td>1</td>
<td>67</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>Susceptible</td>
<td>55</td>
<td>F</td>
<td>5,950</td>
<td>3</td>
<td>1</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>35±10</td>
<td></td>
<td>4,340±925</td>
<td>14±7</td>
<td>5±5</td>
<td>95±13</td>
<td>13±4</td>
</tr>
</tbody>
</table>

| 7           | Resistant   | 36     | M   | 6,700                             | 20+                  | 20+                  | 0                                      | 0                                       |
| 8           | Resistant   | 27     | F   | 5,640                             | 20+                  | 20+                  | 0                                      | 4                                       |
| 9           | Resistant   | 32     | F   | 6,400                             | 20+                  | 20+                  | 0                                      | 0                                       |
| 10          | Resistant   | 27     | M   | 4,270                             | 20+                  | 0                    | 0                                      | 2                                       |
| 11          | Resistant   | 26     | M   | 4,360                             | 20+                  | 10+                  | 10                                    | 2                                       |
| 12          | Resistant   | 25     | M   | 5,950                             | 20+                  | 10+                  | 0                                      | 0                                       |
| Mean ± SD  |            | 29±4   |      | 5,550±1,025                      | 20±0                 | 13±8                 | 2±4                                    | 1±2                                    |

P value                  | NS        | 0.07   | 0.06 | 0.06 | <0.001* | <0.001* |

12 subjects completed the study, divided into acute mountain sickness (AMS)-susceptible and -resistant groups. F, female; M, male. There was no significant difference in mean age between the two groups. The AMS-resistant group tended to have a greater number of trips to altitudes >2,500 and >4,200 m and a greater highest elevation reached, but these differences were of borderline significance (P = 0.06, P = 0.06, and P = 0.07, respectively). Lake Louise Consensus AMS scores and frequency of symptoms were highly significantly different between the 2 groups (P < 0.001 for both). P values are main effect for AMS group (*significant at P < 0.05).
ml (SD 6) by 40 min (P = 0.8, main effect for time, n = 5) (Fig. 3).

Oxygen saturation and heart rate. The changes in O2 saturation and heart rate over the course of the experiment are summarized in Table 2 (top). There was a significant decrease in arterial oxygen saturation during 40 min of hypoxia (P < 0.001), but no statistically significant difference between groups (P = 0.32), and no significant group by time interaction (P = 0.25). Heart rate significantly increased with hypoxia (P < 0.005), but was also not significantly different between groups (P = 0.3), and there was no significant group by time interaction (P = 0.3).

CSF volume in hypoxia. Intracranial CSF volume decreased significantly with hypoxia (P < 0.001), but this change was not significantly different between AMS groups (Table 2, Fig. 4). Following 20-min hypoxia, CSF volume decreased significantly for both AMS-susceptible and AMS-resistant groups (P < 0.05). CSF volume decreased further following 40-min hypoxia in both groups (P < 0.001, 40-min hypoxia compared with 20-min hypoxia and compared with normoxia).

CBF and CBV. Results are detailed in Table 2. CBF in the cerebrum was averaged within each of the three time points corresponding to the acquisition time for the CSF quantitation data: baseline blood flow, blood flow corresponding to 20-min hypoxia, and blood flow corresponding to 40-min hypoxia. Blood flow increased significantly with duration of hypoxia (P < 0.05), but was not significantly different between AMS groups.

ΔCBV for the same time periods were derived from each corresponding ΔCBF. CBV increased significantly with hypoxia (P < 0.01, main effect to duration of hypoxia). For 20-min hypoxia, CBV increased in both groups, although this failed to reach significance (P = 0.28, 20-min hypoxia compared with normoxia). For 40-min hypoxia, CBV increased significantly in both groups (P < 0.05, 40-min hypoxia compared with normoxia). The baseline whole brain volume (from which CBV0 was calculated) and ΔCBV were not significantly different between AMS groups.

Cerebral swelling. The contribution of brain parenchyma swelling to the shifts in CSF volume was calculated from the difference of CSF volume change and ΔCBV (Δbrain volume = ΔCSF − ΔCBV) for each subject at each time point (Fig. 4). There was a significant increase in calculated brain parenchyma volume with hypoxia (P < 0.001, main effect for duration of hypoxia). There were significant increases in brain parenchyma volume in both groups following 20-min hypoxia (P < 0.05, 20-min hypoxia compared with normoxia), with further significant increase in brain parenchyma volume in both groups after 40 min (P < 0.001, 40-min hypoxia compared with normoxia). The brain volume changes were not significantly different between AMS groups. Changes in blood flow, blood volume, CSF volume, and brain parenchyma volume are summarized in Table 2 and Fig. 4. Automated segmentation of the T1-weighted images (using SIENA) did not detect any change in overall brain volume.

DISCUSSION

The main finding of the present study is a ~5% reduction in CSF volume occurring very early in hypoxic exposure.

Decreased CSF volume along with increased CBF were seen in all subjects and may thus be part of the normal cerebral response to hypoxia. Estimates of ΔCBV showed only moderate increases with hypoxia, from which we infer that CBV alone could not account for the decreased CSF volume, and brain parenchyma swelling may also be part of this normal response. Contrary to our hypothesis, the displacement of CSF in response to hypoxia was not significantly different in subjects who invariably self-reported symptoms of AMS compared with subject who almost never developed AMS.

One shortfall of this study in drawing conclusions about eventual AMS symptoms is that our measurements were made in the first 40 min of acute hypoxia, whereas symptoms of AMS at this level of hypoxia peak after 24–48 h of exposure. The time course of the stimulus was also more rapid than would usually be experienced during an actual ascent to altitude. However, the primary aim of this study was to examine if individuals who are susceptible or resistant to developing AMS demonstrate identifiable cerebral physiology characteristics during acute hypoxia, which imply cerebral vulnerability to the effects of more prolonged hypoxic exposure. This has been the case with subjects who get high-altitude pulmonary edema in which an altered pulmonary vascular response is evident within 10 min of exposure to acute hypoxia (12). Our
Table 2. Cardiorespiratory and cerebral physiology data

<table>
<thead>
<tr>
<th>Group</th>
<th>Normoxia</th>
<th>Hypoxia 20 min</th>
<th>Hypoxia 40 min</th>
<th>P (Hypoxia)</th>
<th>P (AMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpO₂, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>98 ± 1</td>
<td>87 ± 4</td>
<td>83 ± 5</td>
<td>&lt;0.001*</td>
<td>0.33</td>
</tr>
<tr>
<td>AMS-R</td>
<td>98 ± 1</td>
<td>87 ± 4</td>
<td>87 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>65 ± 7</td>
<td>76 ± 13</td>
<td>79 ± 15</td>
<td>&lt;0.005*</td>
<td>0.29</td>
</tr>
<tr>
<td>AMS-R</td>
<td>61 ± 14</td>
<td>70 ± 14</td>
<td>67 ± 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF volume, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>232.3 ± 62.4</td>
<td>227.4 ± 60.7</td>
<td>219.8 ± 62.3</td>
<td>&lt;0.001*</td>
<td>0.68</td>
</tr>
<tr>
<td>AMS-R</td>
<td>213.1 ± 80.1</td>
<td>210.2 ± 80.6</td>
<td>204.6 ± 75.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF, ml·100 g⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>43.8 ± 15.3</td>
<td>44.7 ± 16.5</td>
<td>47.8 ± 19.5</td>
<td>&lt;0.05*</td>
<td>0.59</td>
</tr>
<tr>
<td>AMS-R</td>
<td>47.8 ± 10.5</td>
<td>49.0 ± 8.9</td>
<td>52.8 ± 10.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain volume, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>1,416.9 ± 127.5</td>
<td>1,416.9 ± 127.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-R</td>
<td>1,562.5 ± 108.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔCBF, ml·100 g⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>0.8 ± 5.7</td>
<td>4.0 ± 6.5</td>
<td>&lt;0.05*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-R</td>
<td>1.2 ± 3.5</td>
<td>5.0 ± 5.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔCBV, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>0.5 ± 2.5</td>
<td>1.9 ± 2.4</td>
<td>&lt;0.005*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-R</td>
<td>0.9 ± 1.7</td>
<td>2.7 ± 2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔCSF volume, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>-4.9 ± 4.4</td>
<td>-12.5 ± 7.5</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-R</td>
<td>-2.9 ± 1.9</td>
<td>-8.5 ± 6.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔBrain volume, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-S</td>
<td>4.4 ± 3.5</td>
<td>10.6 ± 6.3</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS-R</td>
<td>2.0 ± 2.3</td>
<td>5.8 ± 6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD for AMS-susceptible (AMS-S) and AMS-resistant (AMS-R) groups for measurements during normoxia and after 20 min and 40 min of hypoxia. Shown are changes in O₂ saturation (spO₂) and heart rate and cerebrospinal fluid (CSF) volume and cerebral blood flow (CBF) measurements. Baseline brain volume was measured during normoxia (and used to calculate baseline cerebral blood volume for each subject). Measured changes are shown in CBF (ΔCBF) and CSF volume (ΔCSF volume), and derived changes in cerebral blood volume (ΔCBV) and brain volume (Δbrain volume) during hypoxia. P values are main effect for duration of hypoxia, and main effect for AMS group (*significant at P < 0.05). (Post hoc analysis between ΔCBV, ΔCSF volume, and Δbrain volume for different time points are in the RESULTS section.)

experimental design selected subjects into distinct AMS-susceptible and -resistant groups based on self-reporting histories. Subjects with an intermediate or indeterminate likelihood of getting AMS were excluded from the study. Thus, if there were baseline physiological differences in the brains of those who are at risk from AMS, we expect it to be evident in our subject population. Although subjects expressed no ambiguity when reporting their symptoms to us, we have not allowed for the possibility that subjects tend to generalize their symptoms when self-reporting. Most of the time the self-reports would be expected to correctly categorize subjects in AMS-susceptible and -resistant groups based on self-reporting histories. However, since contemporaneous clinical evaluations to substantiate subjects’ historical self-reports were not available, some variability in our groups is still expected. Although the acute increase in blood volume and brain swelling that we observed are universal and not predictive of a susceptibility to AMS, this may alter if hypoxia is sustained. Swelling and blood volume changes may regress in some individuals and progress in others. Therefore, it would be important to extend our measurements and to continue the exposure for a further 6 h and observe if the blood flow, blood volume, and brain swelling changes are sustained and which physiological changes correlate with actual AMS symptomatology.

Automated segmentation of the T1-weighted images with SIENA failed to detect any change in brain volume between normoxia and 40-min hypoxia [0.1% (SD 0.25), P = 0.15]. This finding is not altogether unexpected; SIENA calculates percent volume changes of the combined volume of gray matter + white matter + intrasulcal CSF. The CSF in the cerebral sulci is not well delineated on the T1-weighted images due to partial volume averaging with signal from adjacent gray matter. Thus, although SIENA (and similar automated algorithms) are very sensitive for detecting changes in the intraventricular volume, or volume loss (atrophy) in combined volume of brain tissues (36), they may underestimate shifts in the volume fraction between gray matter and the adjacent CSF within the small sulci.

To quantify CSF volume in the small extraventricular subarachnoid spaces, as well as within the ventricles and surrounding the cortical convexity, we used a very heavily T2-weighted high-resolution 3D MRI sequence to maximize our ability to detect CSF changes in isolation and hence to determine changes in brain parenchyma volume. Existing semiautomated methods to segment brain parenchyma volume or CSF from anatomic MRI (e.g., FAST, SIENA, FMRIB Software Library, Oxford, or Statistical Parametric Mapping, London, UK) do not work well with these heavily T2-weighted images, as most of the normal gray matter and white matter contrast is absent. Thus we needed to develop the alternate segmentation strategies outlined in the METHODS section. To ensure sensitivity to the small CSF spaces, we used high-resolution 3D imaging techniques. Our CSF-sensitive images highlighted changes in CSF volume that were not detected using SIENA. Since the CSF “blind spots” for SIENA are the small cerebral sulci, or the CSF around the convexity of the cortex, this suggests this as the location for the CSF volume changes we observed during acute hypoxia, rather than changes in ventricular volume.

We used the FMRIB brain extraction tool to define an initial “brain mask” of the intracranial boundary on a T1-weighted image. Since this algorithm is designed to find the CSF/gray matter boundary, rather than the CSF/skull boundary, small rotation differences between image data sets resulted in very different amounts of CSF being “edited” by the extraction algorithm. To eliminate this error, we coregistered all of the T1-weighted data sets to the initial normoxia data set and used this to define a single “brain mask”. To remove any bias...
Although other methods exist to quantify CSF volume (radionuclide cisternography, contrast-enhanced X-ray computed tomography cisternography, gadolinium-enhanced MRI cisternography), all are more invasive than the technique presented here, and none has emerged as a gold standard. Thus it has not been possible to do a formal validation of these CSF volume measurements in our subjects. Resting CSF volume in our 15 subjects ranged from 120 to 349 ml (mean 221 ml). We found CSF volumes are ~50 ml higher than other MRI studies, which we attribute to our high-resolution contiguous 3D imaging and increased sensitivity of our technique to extraventricular CSF, which other studies tend to underestimate. Our CSF measurement technique is highly reproducible for repeated measurements within the same individual 2–13 days apart (Pearson correlation coefficient, $r = 0.97$) (Fig. 2).

One potential confound of CSF quantitation using MRI is that measurements were made with the subjects recumbent, which in itself would be expected to have an effect on CSF dynamics. To control for this, we analyzed the changes during 40 min of hypoxia vs. 40 min of normoxia in a subset of five subjects (subjects were in the same recumbent posture for both measurements). While recumbent, increases in the venous pressure cause distension of the spinal epidural veins and a reduction in intraspinal CSF (20). Thus an initial increase in intracranial CSF is expected. We observed a decrease in intracranial CSF volume after 20 min (irrespective of inspired oxygen level, Fig. 3), which is likely a recovery from any initial reflex increase. Beyond 20 min, further changes in intracranial CSF volume are only seen in the presence of hypoxia and are absent in the control (normoxia) group. Thus these later changes are due to effects of hypoxia itself, independent of posture. The majority of control subjects showed a decrease in CSF volume during the initial 20 min of the MRI and no significant change in the subsequent 20 min (Fig. 3). However, one subject showed a sustained increase in CSF volume at 20 min and at 40 min, which may skew the mean result of the group. Even if this subject is removed, the overall observation is unchanged, and there is still no significant change in mean CSF volume during normoxia after the initial 20 min, so our conclusions are not affected by this one apparent outlier.

Another potential confound of CSF quantitation with MRI is that the signal in CSF immediately adjacent to vessels will be decreased during hypoxia due to the increased concentration of deoxyhemoglobin (the Blood Oxygenation Level Dependent effect, or BOLD effect), simulating a decrease in CSF volume. During hypoxia at 12.5% inspired oxygen, there is no change in cerebral metabolic rate of O$_2$ (33) or oxygen delivery (8); thus, assuming a resting venous saturation of 60%, decreasing arterial saturation to 87% changes the saturation in venous blood by 8 percentage points. By comparison, the change in venous saturation in visual cortex during functional MRI is 13 percentage points (21). At 3 Tesla for TE = 80 ms, this 13 percentage point change in venous saturation results in a MRI signal change of ~2% due to the spin echo BOLD effect (18). For TE = 400 that we used during our CSF measurements, the BOLD signal change for a 8 percentage point change in venous saturation due to hypoxia would be a ~6% decrease in the MRI signal. The majority of this effect is intravascular, and only about one-third of this signal change affects the extravascular compartment (18). In addition, for the CSF signal to be

introduced by using a single data set as the base image for image registration, only the T1-weighted images were coregistered. The three T2-weighted images were subsequently rotated into alignment using the rotation matrix from their corresponding (mutually coregistered) T1-weighted pair. This ensured that all three T2-weighted data sets underwent the same degree of smoothing and interpolation.
impacted at all, it must be immediately perivascular (so there is little or no influence on intrasulcal and intraventricular CSF, \sim 50\% of the CSF). Thus a BOLD effect contributes only \sim 1\% to the CSF signal change (\sim 5-fold smaller than the actual CSF signal changes we observed).

The arterial spin labeling technique used to quantify CBF during normoxia is well established and has been shown by ourselves and others to be both a valid (38, 40, 43) and reliable (22, 28, 42) measure of CBF. Although primarily used to quantify blood flow in cerebral gray matter, our laboratory has previously shown that arterial spin labeling can also quantify CBF in white matter (and thus in whole brain) (8). Estimates of whole brain blood volume change were derived from changes in whole brain blood flow.

Our estimate of \Delta CBV with hypoxia relied on a number of assumptions. We assumed that the baseline blood volume, CBV\textsubscript{0}, is related only to brain volume (i.e., constant blood volume fraction in all subjects). We have not addressed the possibility that the absolute baseline blood volume may vary with subject group (AMS-susceptible or AMS-resistant), independently of brain volume. However, since we used a repeated-measures design, each subject acted as their own control, and the changes in blood volume with hypoxia are not affected by this assumption. Furthermore, the power relationship with exponent 0.38 between fractional change in blood flow and fractional change in blood volume used here ([CBV/CBV\textsubscript{0}] = [CBF/CBF\textsubscript{0}]\textsuperscript{0.38}), where CBF\textsubscript{0} is baseline CBF, was originally derived from a hypercapnic stimulus (10). Most authors report similar exponent values for this CBF-CBV power relationship. During hypoxia, Shockley and LaManna (34) showed a comparable relationship in rats. In calculating the \Delta CBV, we have assumed that this hemodynamic relationship between flow and volume also holds true for a hypoxic challenge in humans. Using an exponent of 0.38 (10), the \Delta CBV after 40-min hypoxia was estimated to be 1.9 and 2.7 ml in self-identified AMS-susceptible and AMS-resistant subjects, respectively (Table 2). This does not account for all of the measured change in CSF volume during 40-min hypoxia, from which we conclude that brain parenchyma swelled by 10.6 ml (AMS-susceptible) and 5.8 ml (AMS-resistant). Other authors have reported exponent values ranging from 0.29 (13) to 1.0 (i.e., a linear relationship) (31). If the \Delta CBV after 40-min hypoxia were instead calculated using an exponent of 0.29, the estimated \Delta CBV is 1.4 ml (AMS-susceptible) and 2.0 ml (AMS-resistant), and brain parenchyma swelling is 11.0 ml (AMS-susceptible) and 6.5 ml (AMS-resistant). With an exponent of 1.0, the mean \Delta CBV is estimated at 5.2 ml (AMS-susceptible) and 7.4 ml (AMS-resistant), and brain parenchyma swelling is 7.2 ml (AMS-susceptible) and 1.1 ml (AMS-resistant). For this entire range of values, our findings of early brain parenchyma swelling, with no significant difference between AMS-susceptible and AMS-resistant groups, are unchanged. The calculation of the \Delta CBV from the \Delta CBF is thus relatively insensitive to the exact choice of exponent.

In this study, we estimated a mean increase in CBV of \sim 4\% in response to acute hypoxia, based on a measured increase in global CBF of 10\%. This CBF increase is comparable to several prior studies in the literature. Berre et al. (4) demonstrated a 20–23\% increase in middle cerebral artery velocity, and Mintun et al. (25) reported a 8.7\% increase in CBF with PET during acute hypoxia. Buck et al. (5) reported no change in quantified global CBF after acute (20 min) exposure to 3,000 m using \textsuperscript{15}O-H\textsubscript{2}O PET, but a 36\% increase at 4,500 m (i.e., a greater hypoxic stimulus than the present study). Using a \textsuperscript{133}Xe technique, Jensen et al. (16) reported a 24\% increase in global CBF at 3,475 m after 24 h. Other reports are more varied, differing by technique as well as duration and altitude of exposure. CBF has also been reported to show no change following acute hypoxia using single photon emission computed tomography radionuclide imaging (27) and middle cerebral artery velocity (39). Other studies report very variable hemodynamics, with no change in acute basilar artery velocity for hypoxia as low as 8% O\textsubscript{2}, but a 29% velocity decrease in climbers at 7% O\textsubscript{2}, (presumably AMS-resistant) and a 28% increase in controls (15). Other studies at 4,559 m (a more hypoxic stimulus than we used) have also shown varied responses ranging from 36% decrease to 45% increase in middle cerebral artery velocity following 3 h of acute hypoxia (3). Although universally referred to as a measure of “CBF”, these methods are not measuring exactly the same physiological process. These fundamental differences in the experimental design and in the hemodynamic parameters being measured make direct comparison of current and prior measures difficult.

Our measures may also underestimate the dynamic range of blood flow changes in early hypoxia. For this study, measurements were made under poikilocapnic hypoxic conditions. In this setting, the potential for increased CBF stimulated by hypoxia may be inhibited by the hypoxic ventilatory response and increased alveolar ventilation (1). Hyperventilation causes reduced arterial P\textsubscript{CO\textsubscript{2}}, which, in turn, has a CBF-lowering effect at lower P\textsubscript{CO\textsubscript{2}} values. While an isocapnic hypoxic stimulus would help elucidate the potential magnitude of the CBF responses to hypoxia alone, any influences of the acute hypocapnia on the cerebral response would be negated. The aim of this study was to look for altered cerebral physiology that may indicate a selective vulnerability to developing AMS, and thus we chose a poikilocapnic hypoxic stimulus to more closely mirror the actual physiological stimulus of acute altitude exposure. In addition, the T1 of blood is shortened during hypoxia (35), which tends to reduce the apparent blood flow using arterial spin labeling MRI. This results in a \sim 6\% underestimate in the \Delta CBF, and a \sim 2\% underestimate in \Delta CBV during hypoxia. The effect is small and does not impact our overall results (i.e., of early brain parenchyma swelling during acute hypoxia).

Alternate methods to measure changes in blood volume that are not based on CBF do exist, but are also limited; VASO provides a time-varying MR signal based on changes in vascular space occupancy (23). However, obtaining quantitative data remains challenging, and assumptions about baseline blood volume fraction would still be needed. Additional methods based on dynamic susceptibility contrast also provide estimates of relative blood volume change, but attaining the necessary temporal resolution is challenging (9). Ideally, the CBF and CSF measurements would be acquired simultaneously, but, as subjects were in the MRI scanner for over 1 h concurrently, but, as subjects were in the MRI scanner for over 1 h, the CBF and CSF measurements would be acquired simultaneously, but, as subjects were in the MRI scanner for over 1 h, the CSF measurements, including the CBV measurements during the same session would have extended the study beyond the endurance of even the most tolerant subjects. CBV (derived from CBF measurements) and CSF measurements were thus acquired on separate days by necessity. To avoid acclimatization effects from the prior measurements, or other hypoxic
exposure, subjects remained at sea level between the two measurement sessions. Mean interval between measurements was 31 days (SD 26).

Another potential issue is whether the present study has sufficient power to justify the statistical conclusions reached regarding the differences between self-identified AMS-susceptible and AMS-resistant subjects. One approach is to calculate a priori the number of subjects that would be required to detect a statistically significant difference in future studies (if, in fact, it were present in the population), based on the observed changes in CSF volume in the present study. Assuming a two-tailed test, \( P < 0.05 \), and a power of 0.8, 61 subjects per group would be required based on the observed differences in the change in CSF volume between groups. This suggests that the true biological difference between groups is likely very small. Additionally, the changes in CSF volume were numerically greater (although statistically not significant) in AMS-susceptible subjects than AMS-resistant subjects, further strengthening our statistical conclusions.

In conclusion, our observations during acute hypoxia demonstrate that decreases in CSF volume, increases in CBV, and increases in brain parenchyma volume occur as very early responses to hypoxia (within 40 min).

ACKNOWLEDGMENTS

The authors are grateful to Khaled Restom for assistance with methods for physiological monitoring during the MRI, and to Thomas Liu and Yashar Smith for valuable discussion on the capabilities and application of his FSL physiological monitoring during the MRI, and to Thomas Liu and Yashar Smith for valuable discussion on the capabilities and application of his FSL.

Present address of E. A. W. Dyer: Department of Medicine, Oregon Health & Sciences University, Portland, OR 97239.

GRANTS

This work was supported by National Institutes of Health Grants R01-NS059334 (D. J. Dubowitz), R01-HL081171 (S. R. Hopkins), and R01-GRANTS & Sciences University, Portland, OR 97239.

REFERENCES


19. Kellie G. An account of the appearances observed in the dissection of two of three individuals presumed to have perished in the storm of the 3rd, and whose bodies were discovered in the vicinity of Leith on the morning of the 4th of November 1821 with some reflections on the pathology of the brain. In: Trans Med Chir Sci Edinb 1: 84–169, 1824.


J Appl Physiol • VOL 107 • JULY 2009 • www.jap.org


