Acetazolamide during acute hypoxia improves tissue oxygenation in the human brain

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Wang K, Smith ZM, Buxton RB, Swenson ER, Dubowitz DJ. Acetazolamide during acute hypoxia improves tissue oxygenation in the human brain. J Appl Physiol 119: 1494–1500, 2015. First published October 15, 2015; doi:10.1152/japplphysiol.00117.2015.—Low doses of the carbonic anhydrase inhibitor acetazolamide provides accelerated acclimatization to high-altitude hypoxia and prevention of cerebral and other symptoms of acute mountain sickness. We previously observed increases in cerebral O2 metabolism (CMRO2) during hypoxia. In this study, we investigate whether low-dose oral acetazolamide (250 mg) reduces this elevated CMRO2, and in turn might improve cerebral tissue oxygenation (PtiO2) during acute hypoxia. Six normal human subjects were exposed to 6 h of normobaric hypoxia with and without acetazolamide prophylaxis. We determined CMRO2 and cerebral blood flow (CBF) and cerebral venous O2 saturation. During normoxia, low-dose acetazolamide resulted in no significant change in CBF, CMRO2, or PtiO2. During hypoxia, we observed increases in CBF [48.5 (SD 12.4) (normoxia) to 65.5 (20.4) ml·100 ml−1·min−1 (hypoxia), P < 0.05] and CMRO2 [1.54 (0.19) to 1.79 (0.25) mmol·ml−1·min−1, P < 0.05] and a dramatic decline in PtiO2 [25.0 to 11.4 (2.7) mmHg, P < 0.05]. Acetazolamide prophylaxis mitigated these rises in CBF [53.7 (20.7) ml·100 ml−1·min−1 (hypoxia + acetazolamide)] and CMRO2 [1.41 (0.09) mmol·ml−1·min−1 (hypoxia + acetazolamide)] associated with acute hypoxia but also reduced O2 delivery [6.92 (1.45) (hypoxia) to 5.60 (1.14) mmol·min−1 (hypoxia + acetazolamide), P < 0.05]. The net effect was improved cerebral tissue PtiO2 during acute hypoxia [11.4 (2.7) (hypoxia) to 16.5 (3.0) mmHg (hypoxia + acetazolamide), P < 0.05]. In addition to its renal effect, low-dose acetazolamide is effective at the capillary endothelium, and we hypothesize that local interruption in cerebral CO2 excretion accounts for the improvements in CMRO2, and ultimately in cerebral tissue oxygenation during hypoxia. This study suggests a potentially pivotal role of cerebral CO2 and pH in modulating CMRO2 and ultimately in cerebral tissue oxygenation during hypoxia. Five normal human subjects were exposed to 6 h of normobaric hypoxia and five normal human subjects were exposed to 6 h of normobaric hypoxia.

METHODS

Subjects

Six healthy, nonsmoking, sea-level residents (5 females, 4 males) participated in the study. Ethical approval for these studies was granted by the Human Research Protection Program of the University of California, San Diego. Participants were informed of the experimental procedures and possible risks involved in the study, and written informed consent was obtained before participation.

Study Design

The impact of acetazolamide on CBF, CMRO2, and PtiO2, was evaluated during normoxia and following 6 h of normobaric hypoxia. Baseline MRI measurements were made during normoxia of steady-state cerebral blood flow (CBF) and steady-state transverse relaxation (T2) in superior sagittal sinus blood [from which we calculated venous oxygen saturation (SvO2)]. Additional measurements were made of hemoglobin (Hb) concentration, hematocrit (Hct), and pulse oxi-
calculated the partial pressure of tissue oxygen in the brain (PtiO2) (4). By incorporating these data into a diffusion model of O2 transport, we calculated the partial pressure of tissue oxygen in the brain (PtiO2) (4). The measurements were repeated following 6 h of acute normobaric hypoxia at SaO2 ≈ 85% (Fig. 1). These normoxic and hypoxic measurements were repeated on a subsequent day, following prophylaxis with oral acetazolamide. We chose the lowest clinically effective dose of acetazolamide for prophylaxis (250 mg; Refs. 1, 19, 24, 42) administered as a divided dose of 125 mg ~2 h before the baseline MRI scan and 125 mg ~3 h before the hypoxic MRI scan.

Acute Hypoxia Paradigm

Within the MRI scanner subjects breathed a premixed 90-Torr hypoxic mixture (12.5% O2, balance N2) via a close-fitting low-deadspace nonrebreathing mask (Hans Rudolph 7900/2600, Kansas City, MO). Thirty minutes were included before the MRI measurements for subjects to acclimate to the mask. For the 6-h hypoxic exposure outside the scanner, the close fitting masks were poorly tolerated (and precluded subjects from drinking or eating). Instead, subjects breathed nitrogen-enriched air via a nonrebreathing loose-fitting Venturi mask (Hudson, Temecula, CA), and gas flow rate was adjusted to maintain a stable SaO2 at ~85% (FiO2~0.12-0.13). Since we did not have ambulatory O2 monitoring to specifically target FIO2, we monitored SaO2 as a surrogate. The main motivation for this additional setup was to improve subject comfort and compliance. The lighter weight mask was better tolerated by subjects for prolonged periods and improved SaO2 stability during the hypoxic exposure.

Physiological Measurements

Arterial O2 saturation was continuously measured using a Nonin 3100 Wrist Pulse Oximeter (during the 6-h hypoxia exposure between MRI measurements) and a Nonin 8600FO MRI-compatible pulse oximeter (Nonin Medical, Plymouth, MN; during MRI measurement). MRI measurements) and a Nonin 8600FO MRI-compatible pulse oximeter (during the 6-h hypoxia exposure between MRI measurements) and a Nonin 8600FO MRI-compatible pulse oximeter (Nonin Medical, Plymouth, MN; during MRI measurement). Hematocrit was determined from direct measurements of packed cell height in a capillary tube following centrifuging. To ensure that the hypoxic and cardiorespiratory state remained consistent during the steady-state measurements, SaO2 and PETCO2 were compared during CBF and venous T2 MRI measurements. Imaging sequences were repeated if SaO2 deviated by more than ±2%, or PETCO2 deviated by ±2 Torr between these scans.

MRI Measurements

Cerebral blood flow. CBF was measured in gray matter in the cerebrum using a PICORE QUIPPS 2 arterial spin labeling (ASL) technique (TE = 9.1 ms, TR = 2.5 s, TI1 = 700 ms, TI2 = 1,500 ms, six 6-mm slices, 5 min). Additional images were collected to determine proton density of cerebrospinal fluid and coil sensitivity profile to allow absolute quantitation of CBF from the MRI signal (28, 44, 46).

Venous T2 relaxation. T2 was measured in the superior sagittal sinus using a TRUST (T2 relaxation under spin tagging) MRI technique (25) with single shot spiral readout (TE = 2.8 ms, TR = 8 s, TI = 1.2 s, 4 echoes at effective TE 0, 40, 80, and 160 ms, 10-mm slice, 80 mm tag, 4.5 min). Images were acquired just superior to the torcula. The T2 value was then used to determine venous O2 saturation.

Data Analysis

Cerebral blood flow: Raw ASL data for each subject were corrected for the expected changes in T1 relaxation of blood based on the measured SaO2 (33). Images were corrected for cardiorespiratory physiological noise (29) and field inhomogeneities (27). Resting CBF was averaged across the 5 min of data collection in cerebral gray matter using a mask generated from a separate high-resolution Fast Spoiled GRASS (FSPGR) T1-weighted 3D anatomical MRI (TE = 4.2 ms, TR = 10.1 ms, TI = 450 ms, bandwidth = 20.83 kHz, field of view = 25 × 25 × 16 cm, matrix 256 × 256 × 128, ~1 × 1 × 1.3 mm resolution, 5.5 min). Cerebral gray matter was automatically segmented in the FSPGR scan using FAST software (FMIRB Software Library, Oxford, UK).

Venous oxygen saturation (SvO2). Cerebral venous O2 saturation was derived from the T2 relaxation of venous blood in the superior sagittal sinus. This uses a simplified Luz-Meiboom model, with a quadratic dependence of T2 on O2 saturation and hematocrit, which provides a validated method for determining SvO2 (26, 48). Calibration scaling constants were determined from a prior normative group (35).

Cerebral oxygen delivery (DO2). Cerebral oxygen delivery was calculated based on CBF and O2 content of the arterial blood (Ca). We assumed negligible O2 dissolved in plasma and approximated the Ca by 1.36 × [Hb] × SaO2 (6).

Cerebral oxygen metabolism (CMR O2). Cerebral O2 metabolism was calculated using the Fick equation:

\[
\text{CMR O}_2 = \text{CBF} \cdot \text{OEF} \cdot \text{Ca}
\]  

where CBF is the cerebral blood flow, OEF is the O2 extraction fraction between arterial and venous blood, (Sao2-SvO2)/Sao2, and Ca is the O2 content of arterial blood.

Since the arterial O2 content is approximated by the product of Sao2, and the maximum O2 content of fully saturated blood, this can be rewritten as:

\[
\text{CMR O}_2 = \frac{\% \text{SaO}_2 - \% \text{SvO}_2}{100} \cdot \frac{\text{[Hb]}}{2}
\]

In this notation, Hb concentration is expressed in units of molar equivalents of oxygen carried per liter of blood when hemoglobin is fully saturated (meq/l) (6). CBF was calculated from the arterial spin labeled MRI measurements, Sao2 from a pulseoximeter, SvO2 from the sagittal sinus T2 MRI measures, and Hb concentration from a blood sample. CBF is in units of ml·100 ml−1·min−1, CMR O2 is in μmol·ml−1·min−1 (assuming 1 ml tissue/g, this is numerically equivalent to μmol·g−1·min−1), and SvO2 and SaO2 are in percentages.
Table 1. Primary outcome variables

<table>
<thead>
<tr>
<th></th>
<th>SaO2 %</th>
<th>SvO2 %</th>
<th>PtO2mmHg</th>
<th>CBF, ml·100 ml⁻¹·min⁻¹</th>
<th>DO2 mmol/min</th>
<th>OEF</th>
<th>CMRO2 µmol·ml⁻¹·min⁻¹</th>
<th>PtiO2 mmHg</th>
<th>SaO2-SvO2</th>
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<tbody>
<tr>
<td><strong>Normoxia</strong></td>
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<tr>
<td>no-Rx (n = 6)</td>
<td>97.6 (0.6)</td>
<td>57.8 (3.1)</td>
<td>38.6 (2.3)</td>
<td>48.5 (12.4)</td>
<td>6.36 (0.91)</td>
<td>0.41 (0.03)</td>
<td>1.54 (0.19)</td>
<td>25.0 (–)</td>
<td>39.8 (3.1)</td>
</tr>
<tr>
<td>CI</td>
<td>97.1–98.1</td>
<td>55.3–60.3</td>
<td>36.8–40.5</td>
<td>38.6–58.5</td>
<td>5.63–7.09</td>
<td>0.38–0.43</td>
<td>1.39–1.69</td>
<td>25.0–25.0</td>
<td>37.3–42.3</td>
</tr>
<tr>
<td>Rx-Az (n = 6)</td>
<td>97.7 (1.1)</td>
<td>58.8 (4.3)</td>
<td>38.1 (2.4)</td>
<td>49.7 (19.0)</td>
<td>6.26 (1.29)</td>
<td>0.40 (0.04)</td>
<td>1.48 (0.27)</td>
<td>25.7 (5.0)</td>
<td>38.9 (4.1)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>96.8–98.5</td>
<td>55.3–62.2</td>
<td>36.2–40.0</td>
<td>34.5–64.9</td>
<td>5.22–7.29</td>
<td>0.36–0.43</td>
<td>1.26–1.70</td>
<td>21.7–29.7</td>
<td>35.6–42.2</td>
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<tr>
<td>6-h Hypoxia</td>
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<tr>
<td>no-Rx (n = 6)</td>
<td>79.2 (4.4)</td>
<td>44.3 (4.0)</td>
<td>36.3 (3.9)</td>
<td>65.5 (20.4)</td>
<td>6.92 (1.45)</td>
<td>0.44 (0.05)</td>
<td>1.79 (0.25)</td>
<td>11.4 (2.7)</td>
<td>34.9 (4.8)</td>
</tr>
<tr>
<td>CI</td>
<td>75.6–82.7</td>
<td>41.1–47.5</td>
<td>33.2–39.5</td>
<td>49.2–81.7</td>
<td>5.76–8.08</td>
<td>0.40–0.48</td>
<td>1.60–1.99</td>
<td>9.3–13.6</td>
<td>31.0–38.7</td>
</tr>
<tr>
<td>Rx-Az (n = 6)</td>
<td>81.3 (5.5)</td>
<td>46.3 (6.4)</td>
<td>33.4 (4.7)</td>
<td>53.7 (20.7)</td>
<td>5.60 (1.14)</td>
<td>0.43 (0.07)</td>
<td>1.41 (0.09)</td>
<td>16.5 (3.0)</td>
<td>35.0 (5.5)</td>
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<tr>
<td>Mean (SD)</td>
<td>76.9–85.7</td>
<td>41.2–51.4</td>
<td>29.7–37.2</td>
<td>37.1–70.3</td>
<td>4.69–6.51</td>
<td>0.38–0.48</td>
<td>1.34–1.48</td>
<td>14.1–18.9</td>
<td>30.6–39.4</td>
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<tr>
<td><strong>Statistics (two-way ANOVA)</strong></td>
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<tr>
<td>P hypoxia</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.02</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.0001</td>
<td>0.04</td>
</tr>
<tr>
<td>P Rx</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.05</td>
<td>0.01</td>
<td>NS</td>
<td>0.02</td>
<td>0.04</td>
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<tr>
<td>P hypoxia × Rx</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.06</td>
<td>NS</td>
<td>NS</td>
<td>0.06</td>
<td>NS</td>
</tr>
</tbody>
</table>

Primary outcome variables are summarized as mean (SD) and 95% confidence intervals (CI) for each experimental session and are defined as follows: sao2%, arterial O2 saturation; SvO2, venous O2 saturation; PetCO2, end-tidal partial pressure of CO2; CBF, cerebral blood flow; DO2, O2 delivery; OEF, oxygen extraction fraction; CMRO2, cerebral oxygen metabolic rate, PtiO2, O2 partial pressure in cerebral tissues. The data were analyzed using two-way repeated measures ANOVA with 2 grouping variables, each at 2 levels: 1) normoxia vs. 6-h hypoxia; and 2) treatment with acetazolamide (Rx-Az) vs. no treatment (no-Rx). The P values for the main effect of hypoxia (P hypoxia), main effect of acetazolamide (P Rx), and hypoxia × acetazolamide interaction (P hypoxia × Rx) are reported only for statistical significant results (P < 0.05). Additional post hoc statistical analysis is included in the relevant figure legends. Note: PtiO2 for normoxia, no-Rx are reference values (4) so no SD are reported.

**Cerebral tissue oxygenation (PtiO2).** The calculation of cerebral tissue PtiO2 is based on the theoretical framework proposed by Buxton (4) but modified to account for an additional hemoglobin saturation <100% (i.e., hypoxic). This model allows us to calculate PtiO2 from CBF and CMRO2 given a baseline (normoxic) and challenge (hypoxic) state. For hypoxia experiments, the measured quantities for the model are fractional arterial hemoglobin saturation, Ya (= %SaO2/100) (from pulseoximeter measurements), fractional venous hemoglobin saturation, Yv (= %SvO2/100) (from TRUST MRI), and the ratio (f) of CBF during hypoxia to CBF in the baseline normoxic state (from ASL MRI measurements).

The mass balance equation for the ratio (r) of CMRO2 in the hypoxic state to CMRO2 in the baseline state is:

\[ r = \frac{Y_a - Y_v}{Y_{a,0} - Y_{v,0}} \]  

(3)

The mean capillary Po2 (PcapO2) is taken as the Po2 given by the Hill equation for a saturation halfway between arterial and venous:

\[ P_{capO2} = P_{50} \left( \frac{Y_a + Y_v}{2 - Y_a - Y_v} \right)^{h} \]  

(4)

A second expression for the metabolic rate ratio r, based on blood/tissue O2 diffusion is given in Eq. 5 in terms of mean capillary PcapO2 and tissue PtiO2 during hypoxia and during baseline normoxia (4).

\[ r = \frac{P_{capO2} - P_{tiO2}}{P_{capO2,0} - P_{tiO2,0}} \]  

(5)

*Equation 3* is used to calculate the corresponding CMR O2 ratio and *Eq. 4* is used to calculate the PcapO2 in normoxic and hypoxic condition. *Equation 5* is then used to calculate PtiO2.

There are several model parameters that must be assumed in addition to the measured parameters. These are baseline capillary Po2 (PcapO2), the Po2 for 50% saturation of hemoglobin (P50), and the Hill exponents (h). These assumed values are PcapO2 = 25 mmHg, P50 = 26 mmHg, and h = 2.8 (4).

**Statistical Analysis**

Data were analyzed with repeated measures ANOVA of our primary outcome variables (CMR O2, CBF, DO2, PetCO2, SaO2, SvO2, PtiO2), with two repeated measure groupings (hypoxic exposure, acetazolamide prophylaxis), each at two levels (normoxia, 6-h hypoxia, no acetazolamide, acetazolamide) (StatView 5.0.1; SAS Institute, Cary, NC). This paired approach allowed each subject to act as his or her own control, which provided greater statistical power to detect small changes. Post hoc power was calculated using G*Power (G*Power 3.1.3; Heinrich Heine University, Dusseldorf, Germany). Data are expressed as mean (SD). Changes were significant at P < 0.05 two tailed.

**RESULTS**

All six subjects completed the study. The results are summarized in Tables 1 and 2.

**Normoxia vs. Hypoxia**

To investigate the physiological changes between normoxia and hypoxia, measurements of SaO2, SvO2, SaO2-SvO2, OEF, PetCO2, CBF, CMRO2, and DO2 were first compared between normoxic vs. hypoxic conditions. Subjects showed decreased SaO2, SvO2, and increased ventilatory drive during hypoxia, with decreased PetCO2 (Fig. 2). Both CBF and CMRO2 were significantly increased during hypoxia. There was no significant change in DO2 during hypoxia. The net effect of this was a significant decline in estimated PtiO2 during hypoxia (Fig. 3).
Pair-wise difference (no-acetazolamide)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normoxia (n = 6)</th>
<th>Hypoxia (n = 6)</th>
<th>Mean (SD)</th>
<th>CI 95%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaO2, %</td>
<td>18.4(4.1)</td>
<td>16.9(12.8)</td>
<td>-16.9</td>
<td>9.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>SvO2, %</td>
<td>13.5(5.4)</td>
<td>8.6(13.7)</td>
<td>-5.9</td>
<td>8.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>PETCO2, mmHg</td>
<td>-2.8(7.5)</td>
<td>-2.3(12.3)</td>
<td>-0.5</td>
<td>4.2</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Effect of Acetazolamide in Normoxia and Hypoxia

To assess the effect of acetazolamide, data were analyzed with and without acetazolamide prophylaxis for normoxia and hypoxia conditions. In normoxia, acetazolamide had no significant effect on all physiological variables measured. In hypoxia, prophylactic acetazolamide had no significant effect on SaO2, SvO2, but there was a trend towards decreased PETCO2 (P = 0.089, post hoc t-test) (Fig. 2). Acetazolamide prevented the hypoxia-related decreases in both CMRO2 and CBF, which resulted in a decrease in DO2 (Fig. 3). The relative reduction in CMRO2 exceeded the reduction in DO2, thus improving cerebral PtiO2 during hypoxia (Fig. 3B).

DISCUSSION

Hypoxia stimulates ventilatory drive via its effect on the peripheral chemoreceptors. This stimulated increase in alveolar ventilation also reduces PACO2, (itself a potent ventilatory stimulant); thus the resulting hypocapnia and respiratory alkalosis limit the potential magnitude of any hypoxia-driven increase in ventilation and hence limit the potential to increase arterial oxygenation. The established view of the therapeutic effect of acetazolamide in hypoxia is a renally mediated metabolic acidosis with an enhancement in minute ventilation and oxygenation that would otherwise be limited by hypocapnia (22, 37).

Cerebral Hemodynamic and Metabolic Responses During Acute Hypoxia

In the current study, we observed that CMRO2 increased moderately (16%) during 6 h of hypoxia with concurrent increase in CBF. This is consistent with previous studies showing that CMRO2 and CBF increase during acute and sustained hypoxic hypoxia (34, 35, 47). In addition, we observed a significant decrease in tissue PtiO2 during hypoxia (to 54% of normoxia level), and no change in OEF or O2 delivery (DO2). This mirrors previous findings during prolonged (>10 h) hypoxia in human subjects and animal experiments (36, 45).
doses of acetazolamide (750 mg to 1 g) given intravenously with measurements of CBF made within 15–30 min at the height of very high plasma concentrations of the drug rather than a more clinically applicable lower orally administered dose used in the current study and conventionally for AMS prophylaxis (250 mg, i.e., 8.3 mg/kg). In fact, Grossmann and Koeberle (13) observed a linear dose-response in CBF which increased from 5 mg/kg up to 20 mg/kg iv of acetazolamide administration, but no significant CBF change with lower doses (5 mg/kg even given iv).

We also found that low-dose acetazolamide did not change CBF during normoxia, but it did decrease the otherwise elevated CBF during acute hypoxia. Selective inhibition of carbonic anhydrase subtypes in different tissues likely contributes to these results; The more abundant type II carbonic anhydrase, which is mainly located in erythrocytes (20), is not inhibited at low doses of acetazolamide, so it is still fully functional in the vasculature. Type IV carbonic anhydrase on the endothelium of cerebral capillary beds (11), is completely inhibited by even very low doses of acetazolamide, leading to cerebral tissue CO2 retention and increases the cerebrospinal fluid Pco2 by ~1–2 mmHg (21, 41). This is sufficient to drive up alveolar ventilation since central chemoreceptors are highly sensitive to CO2 changes in the cerebrospinal fluid (22), which then result in ventilatory hypocapnia (36). Since erythrocyte type II carbonic anhydrase is still active, direct vasodilation is not a primary effect with low-dose acetazolamide (13), and the overall result is a decrease in CBF due to the hypocapnic vasoconstriction.

Acetazolamide Effect on CMRO2

The sensitivity of CMRO2 to hypoxic hypoxia and to the effects of low-dose acetazolamide suggests CO2 and local tissue pH play an important role in regulating O2 metabolism. Tissue CO2 indirectly stimulates adenosine generation in the brain via a reduction in extracellular pH, which suppresses neuronal firing (7). Changes in pH from carbonic anhydrase inhibition have also been shown to reduce neuronal excitability (23). We hypothesize that the increased CMRO2 observed during hypoxia in the current study might also represent adenosine-mediated modulation in neuronal excitability. Hypoxia-induced hyperventilation will decrease PaCO2, and ultimately reduce Pco2 in cerebral tissues, thus reducing CO2-mediated neuronal suppression and increasing neural excitability and oxygen utilization. Low-dose acetazolamide selectively inhibits its cerebral endothelial carbonic anhydrase, which leads to an increase in cerebral tissue Pco2. This in turn will impact cerebral adenosine generation and limit CMRO2. This mechanism is supported by recent studies demonstrating that the
hypoxia-induced rise in CMRO₂ can be partially mitigated during isocapnic hypoxia by maintaining PETCO₂ at normoxia levels (34). Elevating PETCO₂ during hypoxia also mitigates many of the cerebral symptoms associated with acute mountain sickness (14, 17). The pH sensitivity of phosphofructo-1-kinase, an important rate-limiting step in glycolysis, offers an additional pathway for reducing O₂ consumption, (10).

**Acetazolamide Effect on PtiO₂**

Selective inhibition of carbonic anhydrase by low-dose acetazolamide also impacts cerebral tissue oxygenation. A secondary outcome of preventing the rise in CBF during hypoxia by low-dose acetazolamide is a decrease in O₂ delivery to the brain. On the face of it, this does not appear to be a positive effect of acetazolamide prophylaxis. However, the reduction in CMRO₂ by acetazolamide exceeds the reduction in O₂ delivery, which reduces the degree of cerebral tissue PtO₂ decline otherwise present in hypoxia. In a study examining cerebral oxygenation during exercise in trekkers at high altitude, Vuyk et al. (43) observed a reduction in cerebral oxygenation measured by near-infrared spectroscopy (NIRS). Following acetazolamide the cerebral oxygenation increased. The increase in cerebral oxygenation exceeded the expected increase from just improved arterial oxygenation due to ventilation (37, 43). This finding supports our proposed mechanism of acetazolamide altering both the delivery and utilization of oxygen in hypoxia.

**Measurement Limitation**

PETCO₂ might not accurately reflect the underlying PaCO₂ after acetazolamide. Consistent with this notion is a recent study at high altitude, which showed the increase in PaCO₂ (∼4 mmHg) is greater than that in PETCO₂ (∼1 mmHg) after acetazolamide administration (9). The current study did not include separate arterial blood gas measurements. Thus our measurement of PETCO₂ following acetazolamide may in fact underrepresent the actual changes in PaCO₂.

Another potential limitation in our experimental design is the timing of the acetazolamide dose. We gave this as a divided dose to maintain a more constant plasma concentration and limit side effects. One-hundred and twenty-five milligrams were taken 2 h before the first (normoxic) MRI. Six hours later, a second 125-mg dose was taken ∼3 h before the second (hypoxic) MRI. Oral acetazolamide has an absorption half-life of ∼1 h, so maximum concentration may take up to ∼4 h after ingestion (30). Thus the degree of carbonic anhydrase inhibition could be less complete during the first MRI scan. Due to logistical constraints, the ordering of the normoxia/hypoxia MRI studies was fixed with the normoxia measurements occurring first. Thus this difference in carbonic anhydrase inhibition primarily impacted the normoxia measurements. The conclusions of this study thus focus on the more prolonged acetazolamide effects during the hypoxia measurements.

Our conclusions from these studies are impacted by the assumptions underlying our modeling of PtO₂ (reviewed in Ref. 4). Our model for PtO₂ assumed that increases in cerebral perfusion do not involve additional capillary recruitment (12). The PtO₂ calculation also assumed a constant oxygen-hemoglobin dissociation curve (4); however, the acidosis due to acetazolamide could cause a significant right shift in the dissociation curve, and this in turn would increase the capillary PO₂ and the tissue PO₂. We observed ∼3 mmHg decline in PETCO₂ with acetazolamide after 6 h of hypoxia, which is within the expected range for this low-dose (selective carbonic anhydrase inhibition) regimen (31). We did not make independent measurements of arterial pH in this study, but estimates of the effect on pH from the two 125-mg acetazolamide doses in normal human subjects would be a decrease from 7.4 to 7.33 during the second (hypoxic) MR measurement (39). We calculated that this decrease in pH would actually result in a higher PtO₂ of 19.2 mmHg (15, 32). Thus assuming a constant oxygen dissociation curve does not affect our primary finding of an improvement in tissue PtO₂ during hypoxia with acetazolamide prophylaxis, although the true magnitude of this effect may in fact be ∼16% greater than we present here.

**Conclusion**

Within the limits of our models discussed above, the primary findings of this study are that low-dose oral acetazolamide, when given prophylactically, attenuates the rise of CMRO₂ during hypoxia and thus improves cerebral tissue oxygenation. This suggests a potentially important role played by cerebral CO₂ and pH in modulating CBF, CMRO₂, as well as PtO₂ during hypoxia.

This mode of action of acetazolamide in altering both cerebral O₂ delivery and O₂ utilization and hence preserving tissue oxygenation during hypoxia may be an important mechanism underlying the prophylactic effect of acetazolamide on acute mountain sickness and warrants further investigation.

**REFERENCES**


