Adenosine receptor dependent signaling is not obligatory for normobaric and hypobaric
hypoxia-induced cerebral vasodilation in humans

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ABSTRACT

Hypoxia increases cerebral blood flow (CBF) with the underlying signaling processes potentially including adenosine. A randomized, double-blinded, placebo controlled design, was implemented to determine if adenosine receptor antagonism (theophylline, 3.75 mg/Kg) would reduce the CBF response to normobaric and hypobaric hypoxia. In 12 participants the partial pressures of end-tidal oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$), ventilation (pneumotachography), blood pressure (finger-photoplethysmography), heart-rate (electrocardiogram), CBF (duplex ultrasound), and intra-cranial blood velocities (transcranial Doppler ultrasound) were measured during five-minute stages of isocapnic hypoxia at sea-level (98, 90, 80, & 70% $SaO_2$). Ventilation, $P_{ET}O_2$ and $P_{ET}CO_2$, blood pressure, heart-rate and CBF were also measured upon exposure (128±31 minutes following arrival) to high-altitude (3800m) and six-hours following theophylline administration. At sea-level, although the CBF response to hypoxia was unaltered pre/post placebo, it was reduced following theophylline (P<0.01); a finding explained by a lower $P_{ET}CO_2$ (P<0.01). Upon mathematical correction for $P_{ET}CO_2$, the CBF response to hypoxia was unaltered following theophylline. Cerebrovascular reactivity to hypoxia (i.e. response slope) was not different between trials, irrespective of $P_{ET}CO_2$. At high-altitude, theophylline (n=6) had no effect on CBF compared to placebo (n=6) when end-tidal gases were comparable (P>0.05). We conclude that adenosine receptor dependent signaling is not obligatory for cerebral hypoxic vasodilation in humans.

Key Words: Adenosine; Cerebral blood flow; High-altitude; Transcranial Doppler; Ultrasound
The signaling pathways that regulate human cerebral blood flow in hypoxia remain poorly understood. Using a randomized, double-blinded, and placebo controlled study design, we determined that adenosine receptor dependent signaling is not obligatory for the regulation of human cerebral blood flow at sea-level, these findings also extend to high-altitude.
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INTRODUCTION

Cerebral blood flow (CBF) increases in response to reductions in arterial oxygen content (CaO₂) (9, 24, 30, 56) in order to maintain cerebral oxygen delivery (CDO₂) in normobaric (3, 66) and hypobaric hypoxia (4, 26). This increase in CBF occurs at a partial pressure of arterial oxygen (PaO₂) of approximately 50-55 mmHg, where reductions in PaO₂ lead to appreciable reductions in CaO₂—i.e., upon descending from the flat portion of the oxygen dissociation curve. For example, hypoxia leads to an increase in blood velocity through the middle (MCA) and posterior (PCA) cerebral arteries (23, 45, 66), and an increase in blood flow through the internal carotid (ICA) and vertebral (VA) arteries thus increasing global CBF (gCBF) (33); however, this has been previously demonstrated to not occur until PaO₂ has been reduced to a level below 59mmHg (66). At high-altitude, CBF is similarly increased, but compared to isocapnic sea-level conditions the magnitude of vasodilation is contingent on individual variability of the hypoxic ventilatory response, and consequent hypocapnic vasoconstriction (4). While the regulation of hypoxic cerebral vasodilation is complex and undoubtedly involves a multitude of signaling pathways [reviewed in: (24)], several factors are commonly recognized as the likely key mediators of this response. These include, but are not limited to: nitric oxide (39), adenosine triphosphate (11), and adenosine (5, 70).

Adenosine is vasoactive in the cerebral vasculature through binding of A₂ₐ receptors (34, 38) secondary to endogenous production during hypoxia (5, 48, 68, 69) (Figure 1). Extensive animal data has highlighted a prominent role of adenosine in the regulation of hypoxic cerebral vascular vasodilation. For example, extravascular application of adenosine
Cerebral Blood Flow in Hypoxia dilates pial vessels in vivo (5, 40, 63), while adenosine receptor antagonism markedly blunts the vasodilatatory response to moderate and severe hypoxia in dogs (12) and rats (21, 40). However, whether adenosine plays a significant role in hypoxic cerebrovascular vasodilation in humans remains to be determined.

In humans during normoxic rest, adenosine receptor antagonism (intravenous aminophylline) reduces CBF (16, 36, 64). Similarly, CBF is lowered by aminophylline during normobaric hypoxia (7), but reactivity (i.e., the magnitude of the vasodilatatory response per unit reduction in saturation) to hypoxia does not appear altered (7, 41). However, due to methodological limitations of the xenon-133 technique (44) used by Bowton et al., 1988 (7) and indirect inference of changes in CBF by Nishimura et al., 1993 (41), the direct role of adenosine in hypoxic cerebral vasodilation in humans remains unclear in normobaric hypoxia. Moreover, the putative role of adenosine in cerebral vasodilation has not been investigated in hypobaric conditions where the magnitude of elevations in CBF can be markedly variable [reviewed in: (4, 24)]. Although posterior cerebrovascular reactivity to isocapnic hypoxia is reportedly greater than that of anterior cerebrovascular reactivity (66) it has yet to be investigated if regional differences in CBF regulation are attributable to adenosine.

Using a double blinded, randomized, placebo controlled and counter balanced design, the primary purpose of this study was to determine the effect of non-selective adenosine receptor antagonism with theophylline on the cerebral vascular response to normobaric isocapnic hypoxia and during exposure to hypobaric hypoxia. We hypothesized that: 1)
normobaric isocapnic hypoxia would increase gCBF and induce vasodilation of the ICA & VA, 2) that this response would be attenuated following theophylline, and 3) based upon previous studies (33, 45, 66) posterior cerebral vascular reactivity (i.e., VA flow) would be greater than that of the anterior circulation (i.e., ICA flow). To follow up our sea-level study, we examined the effect of theophylline on cerebral vasodilation during acute exposure to hypobaric hypoxia following a rapid ascent profile to 3800m. We further hypothesized that ICA and VA diameter would increase upon exposure to high-altitude, and that theophylline would again reduce the magnitude of observed vasodilation.

**MATERIALS AND METHODS**

**Participants**

Twelve healthy young individuals (age: 26.0±5.6 years; body mass index: 22.9±2.3 kg/m²; 2 female) were recruited to participate in both the sea-level and high-altitude component of this study. The sea-level portion of the study preceded the high-altitude experiment. All participants provided written informed consent prior to arrival at the laboratory for a familiarization session. Participants were screened to ensure that reliable ICA and VA ultrasound images could be acquired and then familiarized with the remaining experimental equipment and procedures. All participants were free of cardiovascular, respiratory & cerebrovascular disease, were non-diabetic, and were not taking any prescription drugs (other than oral contraceptives, n=1) at their time of participation, as determined by a screening questionnaire. This study was approved by the University of British Columbia Clinical Research Ethics Board and conformed to standards set by the Declaration of
Helsinki and the Canadian Government Tri-Council Policy Statement (TCPS2) for Integrity in Research.

Experimental measures

Collection and analysis of all cardiorespiratory and cerebrovascular variables for the entire study was completed while blinded to the specific protocols (e.g., placebo vs. theophylline).

Cardiorespiratory measures

Sea-level Study. All cardiorespiratory variables were sampled continuously throughout the protocol at 1KHz via an analogue-to-digital converter (Powerlab, 16/30; ADInstruments, Colorado Springs, CO). Heart rate (HR) was measured by a 3-lead electrocardiogram (ADI bioamp ML132), and beat-to-beat blood pressure by finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands). The Finometer reconstructed brachial waveform was used for the calculation of mean arterial pressure (MAP) after values were back calibrated to the average of three automated brachial blood pressure measurements made over five-minutes at rest (Tango+; SunTech, Morrisville, NC). Both the partial pressure of end-tidal CO2 ($P_{ETCO2}$) and O2 ($P_{ETO2}$) were sampled at the mouth and recorded by a calibrated gas analyzer (ML206, ADInstruments, Colorado, CO, USA), while respiratory flow and minute ventilation ($V_E$) were measured by a pneumotachograph (HR800L, HansRudolph, Shawnee, KS) connected in series to a bacteriological filter. Due to the time delay associated with pulse-oximeter based estimation of arterial oxyhemoglobin saturation ($SaO_2$), and assuming a constant $Pa-P_{ETO2}$ gradient throughout isocapnic hypoxia (61), $SaO_2$ was calculated from $P_{ETO2}$ using a previously
validated equation (54). This equation has been previously demonstrated to have a maximum error of 0.55% SaO$_2$ when pH is standardized (54). Given that the experimental protocol was conducted under isocapnic conditions (i.e., stable pH), this calculation should accurately represent changes in SaO$_2$. Measurements of CBF were conducted as described below (see “Cerebral vascular measures”). Average values for the last minute of each stage were recorded (see “Experimental Protocol”).

**High Altitude Study.** Cardiorespiratory variables were sampled continuously at 1KHz (Powerlab, 16/30; ADInstruments). Respiratory flow was measured by a pneumotachograph (HR 800L, HansRudolph, location), while P$_{ET}$O$_2$ and P$_{ET}$CO$_2$ were sampled continuously at the mouth by a calibrated gas analyzer (ML206, ADInstruments). Similar to the sea-level study, changes in SaO$_2$ were estimated from P$_{ET}$O$_2$ (54). The established changes in pH at 3800m (55) were used to correct for P$_{ET}$O$_2$ prior to SaO$_2$ calculation (54). An automated brachial blood pressure cuff was used to measure HR and blood pressure (HEM-775CAN, Omron Healthcare, Illinois). Measurements of CBF were conducted as described below (see “Cerebral vascular measures”).

**Dynamic end-tidal forcing**

The P$_{ET}$O$_2$ and P$_{ET}$CO$_2$ were controlled by a portable dynamic end-tidal forcing system. This system has been described in detail elsewhere (61, 62). End-tidal steady-state for each stage (see “experimental protocol”) was determined once values were within one-mmHg of the desired target point for at least three consecutive breaths. Our end-tidal forcing system
effectively controls end-tidal gases through wide ranges of $P_{ET}CO_2$ and $P_{ET}O_2$, independent of ventilation at low (61, 62), and high-altitude (61).

**Cerebral vascular Measures**

Blood velocity through the right MCA (MCAv) and left PCA (PCAv) was measured using a 2MHz transcranial Doppler ultrasound (TCD; Spencer Technologies, Seattle, WA). The TCD probes were attached to a specialized headband (model M600 bilateral head frame, Spencer Technologies), and secured in place. Insonation was achieved through the trans-temporal window using previously described location and standardization techniques (65). Based upon these guidelines a satisfactory PCAv signal could not be acquired in one participant, rendering a sample size of 11 for this metric.

Blood velocity and vessel diameter of the ICA and VA were measured using a 10MHz multi-frequency linear array duplex ultrasound (Terason T3200, Teratech, Burlington, MA). Arterial diameter was measured with B-mode imaging while pulse-wave mode was used to simultaneously measure peak blood velocity. Measures of ICA ($Q_{ICA}$) and VA ($Q_{VA}$) blood flow were made ipsilateral to the MCA and PCA, respectively. The ICA diameter and velocity were measured at least 1.5 cm distal to the common carotid bifurcation to eliminate recordings of turbulent and retrograde flow, while VA diameter and velocity were measured between C4-C5, C5-C6, or proximal to entry into the vertebral column. The location was determined on an individual basis in an attempt to select the most reproducible measures, with the same location repeated within participants and between
trials. For all experimental sessions, upon acquisition of the first ultrasound image there was no alteration of B-mode gain to avoid any artificial changes in arterial wall brightness/thickness.

Ultrasound recordings were screen captured and stored as video files for offline analysis. Concurrent measures of arterial diameter and peak blood velocity were acquired at 30Hz using customized edge detection and wall tracking software designed to mitigate observer bias (71). No less than 12 consecutive cardiac cycles were used to determine $Q_{ICA}$ or $Q_{VA}$.

Volumetric blood flow was calculated using the following formula:

$$Q_{ICA} or Q_{VA} = \frac{\text{Peak Envelope Velocity}}{2} \cdot [\pi(0.5 \cdot \text{Diameter})^2]$$

Of note, half of peak envelope velocity equals mean blood velocity through a vessel (13, 53). To account for MAP in our analysis of the CBF responses, cerebrovascular conductance (CVC) was subsequently calculated for both the ICA and VA (e.g., $Q_{ICA}/\text{MAP}$). Due to the high ventilations associated with hypoxia and associated neck movement (particularly during sea-level experimentation), acquisition of adequate quality images was not achieved in all participants. Therefore, the resulting sample sizes for $Q_{VA}$ and $gCBF$ at sea-level was 8 participants (out of 12), and at high-altitude was 11 participants (out of 12), while all ICA images ($n=12$) were used for both studies. We conducted our ultrasound scanning in accordance with published guidelines (59) and have previously reported a within and between day coefficient of variation for extra-cranial
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artery scanning of 1.5 and 4.4%, respectively (25).

Experimental protocols

The sea-level portion of this study was carried out in the Centre for Heart, Lung and Vascular Health at the University of British Columbia’s Okanagan Campus, Kelowna, British Columbia, Canada (344 m) one month prior to the high-altitude investigations. The high-altitude portion of this study was carried out upon arrival (day 1) to the Barcroft Station, located on White Mountain, CA, USA (3800 m).

Sea-level study

This study consisted of two laboratory visits: a placebo day and the drug intervention day, which were randomized, counter balanced, and double blinded. Participants attended the laboratory having abstained from exercise and alcohol for ≥24 hours and were fasted for a minimum of two hours. Given the use of an adenosine receptor antagonist in the protocol, participants were instructed to strictly abstain from methyl xanthine containing products (e.g., caffeine, chocolate) for ≥48 hours prior to each testing day. Upon arrival, participants lay supine and rested for 15-minutes, during which time they were instrumented with the experimental equipment.

Following instrumentation and five minutes of baseline measurements (HR, MAP, VE, MCAv, PCAv, QICA, QVA, PETO2, and PETCO2) the participant completed an isocapnic hypoxia test. Participants breathed simulated room air (e.g., PETO2 and PETCO2 were
approximately 100 mmHg and 40 mmHg, respectively) on the end-tidal forcing system for
a five-minute period prior to commencing three hypoxic stages. To achieve the target \( \text{SaO}_2 \)
values of 90%, 80%, and 70%, \( P_{\text{ETO}_2} \) was lowered in three sequential steps to 60 mmHg,
45 mmHg, and 37 mmHg, with each stage lasting five-minutes once steady state was
reached. Following the last stage participants returned to breathing room air. Participants
then orally ingested either 3.75 mg/kg of theophylline or a placebo pill (sugar pill matched
for capsule size, colour, and weight). Sustained release theophylline pills have been shown
to reach peak plasma concentration between five & seven hours post-ingestion with plasma
concentration stable during that time period (10, 60). Therefore, the post intervention test
took place six-hours following ingestion of theophylline or placebo. On the second day of
testing, participants received the opposite intervention as day one, but completed the same
hypoxic protocols. Testing days were on average separated by three days (range: two to
eight days).

\textit{High-altitude study}

The study participants were split into a placebo and theophylline group (n=6 for each).
Participants awoke at 5am and were transported by two vans from Palm Springs, CA (146
m) to the Barcroft Station, White Mountain Research Center, White Mountain, CA (3800
m) in approximately six-hours. One participant slept in Bishop, CA (1265 m) the night
prior to arrival and arrived at the same time as the other participants from Palm Springs.
Participants arrived at the laboratory having abstained from methyl-xanthine containing
products (e.g., chocolate & caffeine) for ≥48 hours, exercise and alcohol for ≥24 hours, and
were fasted for a minimum of 2 hours. Participants in the placebo and theophylline group
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were tested 125±32 (mean±standard deviation) and 128±31 minutes following arrival to 3800 m, respectively. Following initial testing, the placebo or theophylline capsule was ingested, and post testing for the placebo and theophylline groups occurred 315±13 and 300±0 minutes later, respectively. Participants and investigators were blinded to the specific intervention.

Upon initial arrival participants were tested in the supine position after 15-minutes of rest, during which they were instrumented with the experimental equipment. Resting measurements of HR, MAP, QICA, QVA, PETO2, and PETCO2 were collected for a five-minute period (ventilation was not assessed at this time point due to logistical issues). For the post intervention (i.e. placebo/drug) testing, participants again rested supine for 15-minutes prior to measurement. Measurements were collected over five-minutes of room air breathing. To account for any between group differences in ventilation due to theophylline as well as inherent variability in the hypoxic ventilatory response, participants were then controlled at the same PETO2 and PETCO2 using the portable end-tidal forcing system for 5-minutes prior to the second set of measurements. The clamped PETO2 and PETCO2 were determined from the mean values of all participants during the pre drug/placebo testing upon arrival (52 mmHg PETO2 & 33 mmHg PETCO2). This end-tidal clamping was initiated immediately following the post-intervention measure. Therefore, data were collected over three distinct time points: 1) two hours following arrival to the laboratory; 2) five-hours post drug/placebo ingestion (seven-hours after arrival), and; 3) post intervention with controlled PETCO2 and PETO2 (seven-hours after arrival). Prior to testing at the arrival time point, and post drug time point, as well as during the following morning, each participant
completed the Lake Louise AMS questionnaire (50) to assess any symptoms of AMS.

Calculations

Arterial oxygen content was estimated \( (eCaO_2) \) using the following equation:

\[
eCaO_2 (\text{ml. dl}^{-1}) = [\text{Hb}] \cdot 1.36 \cdot \frac{\text{SaO}_2(\%)}{100} + 0.003 \cdot P_{ETO_2}
\]

Where \([\text{Hb}]\) is the concentration of hemoglobin, which we have assumed to be 15.5 g/dL, 1.36 is the affinity of O\(_2\) for hemoglobin, \(\text{SaO}_2\) is arterial oxygen saturation calculated using the Severinghaus equation (54), and 0.003 is the solubility of oxygen dissolved in the blood. We replaced \(\text{PaO}_2\) with \(P_{ETO_2}\) for our estimation of \(CaO_2\).

Cerebral oxygen delivery \((CDO_2)\) was subsequently estimated \( (eCDO_2) \) by using \(eCaO_2\) in the following equation:

\[
eCDO_2 (\text{ml. min}^{-1}) = eCaO_2 \cdot \frac{gCBF}{100}
\]

In this study the end-tidal forcing baseline for each trial was determined individually pre- and post-intervention based on \(P_{ETO_2}\) and \(P_{ETCO_2}\) measured during the 5-minutes of resting room air breathing. While theophylline and/or time of day may have caused post-intervention end-tidal gases to deviate from their respective pre-intervention trials, it was determined \textit{a priori} to adopt this approach so that each trial began from a natural baseline.
Given interaction between O\textsubscript{2} and CO\textsubscript{2} on cerebral reactivity (37) we chose not to manipulate P\textsubscript{ET}CO\textsubscript{2} from resting values to normalize between pre- and post-intervention baselines. Therefore, following analysis of the respiratory data we noted a difference in P\textsubscript{ET}CO\textsubscript{2} in the theophylline trial. Specifically, P\textsubscript{ET}CO\textsubscript{2} was lower post-theophylline than pre-theophylline trial (see “results”), which necessitated mathematical corrections for the effect of P\textsubscript{ET}CO\textsubscript{2} on CBF. Therefore, we corrected the CBF variables for changes in P\textsubscript{ET}CO\textsubscript{2}. The following equations were used to correct for changes in P\textsubscript{ET}CO\textsubscript{2}:

\[
corrQ\textsubscript{ICA} = Q\textsubscript{ICA} + [P\textsubscript{ET}CO\textsubscript{2}(Pre) - P\textsubscript{ET}CO\textsubscript{2}(Post)] \cdot (0.084 \cdot bQ\textsubscript{ICA})
\]

Where for a given stage of hypoxia, corrQ\textsubscript{ICA} is the estimated Q\textsubscript{ICA} following correction for CO\textsubscript{2}, P\textsubscript{ET}CO\textsubscript{2}(Pre) is the P\textsubscript{ET}CO\textsubscript{2} of that stage pre-theophylline, P\textsubscript{ET}CO\textsubscript{2}(Post) is the P\textsubscript{ET}CO\textsubscript{2} of that stage post-theophylline, 0.084 is the reactivity of the ICA to a 1 mmHg change in P\textsubscript{ET}CO\textsubscript{2} (i.e., 8.4\%) (23), and bQ\textsubscript{ICA} is the baseline Q\textsubscript{ICA} prior to hypoxic exposure. The percent reactivity was applied to the baseline Q\textsubscript{ICA} so that the impact of CO\textsubscript{2} was not inflated by the increase of Q\textsubscript{ICA} during the hypoxic stages.

\[
corrQ\textsubscript{VA} = Q\textsubscript{ICA} + [P\textsubscript{ET}CO\textsubscript{2}(Pre) - P\textsubscript{ET}CO\textsubscript{2}(Post)] \cdot (0.076 \cdot bQ\textsubscript{VA})
\]

Where for a given stage of hypoxia, corrQ\textsubscript{VA} is the estimated Q\textsubscript{VA} following correction for CO\textsubscript{2}, P\textsubscript{ET}CO\textsubscript{2}(Pre) is the P\textsubscript{ET}CO\textsubscript{2} of that stage pre-theophylline, P\textsubscript{ET}CO\textsubscript{2}(Post) is the P\textsubscript{ET}CO\textsubscript{2}
of that stage post-theophylline, 0.076 is the reactivity of the VA to a 1 mmHg change in $P_{ETCO_2}$ (i.e., 7.6%) (23), and $bQ_{VA}$ is the baseline $Q_{VA}$ prior to hypoxic exposure.

$$corr_{CBF} = (corr_{Q_{ICA}} + corr_{Q_{VA}}) \cdot 2$$

Where $corr_{CBF}$ is the gCBF corrected for the change in $P_{ETCO_2}$ following theophylline.

Absolute and relative (i.e., %) reactivity for gCBF, $Q_{ICA}$, ICAv, $Q_{VA}$, VAv, MCA, and PCAv was calculated using linear regression analysis.

**Statistical Analyses**

For the sea level testing, all cardiovascular, cerebrovascular and respiratory variables were analyzed within trial (i.e., placebo & theophylline) using two-way repeated measures ANOVAs. Post-hoc comparisons were made using a Bonferroni correction. Sphericity of data was confirmed using Mauchly’s test of sphericity. When the test of sphericity was not passed, the Greenhouse-Geisser correction was used. Data did not significantly differ from a normal distribution as determined by the Shapiro-Wilks test. A sub analysis was performed where the reactivity slopes from the pre placebo, post placebo, pre theophylline, and post theophylline trials were compared using a one-way repeated measures ANOVA. These reactivity slopes were calculated using linear regression (57, 66). Regional differences in vessel reactivity were analyzed pre and post theophylline using a mixed design ANOVA (between subject factor: vessel; within subject factor: trial). Differences in
confluent vessel blood flow (ICA & VA) and velocity (MCAv & PCAv) reactivity were analyzed using a mixed design ANOVA (between subject factor: vessel; within subject factor: trial). The relationship between MAP and CBF variables was assessed using a Pearson r correlation between the %ΔMAP and %ΔCBF variables for each experimental trial.

The high altitude data were analyzed using a mixed design ANOVA (between subject factor: drug; within subject factor: exposure time). Post-hoc comparisons were made using a Bonferroni correction. When the test of sphericity was not passed, the Greenhouse-Geisser correction was used. Data did not significantly differ from a normal distribution as determined by the Shapiro-Wilks test. All data were analyzed using SPSS (Version 22, IBM statistics) and are expressed as mean ± standard deviation with a priori statistical significance set at $P < 0.05$.

RESULTS

Sea level study

Placebo trials

Cardiovascular variables at all stages of hypoxia during placebo trials are presented in Table 1. Under placebo conditions HR and VE increased at all stages of hypoxia pre and post intervention while MAP increased at 90% and 70% hypoxia. A main effect of placebo on MAP was observed; here, MAP was higher post placebo ($P=0.03$).
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Cerebral vascular variables at all stages of hypoxia during placebo trials are presented in Table 2. $Q_{ICA}$, ICA CVC, and ICAv increased pre and post placebo at 80% and 70% SaO$_2$ while ICA diameter and MCAv increased at all stages of hypoxia. Hypoxia increased $Q_{VA}$, $V_A$, VA diameter and PCAv pre and post placebo at 80% and 70% SaO$_2$ while VA CVC increased at 70% SaO$_2$. Pre and post placebo, gCBF and gCBF CVC increased at 80% and 70% SaO$_2$. Hypoxia increased eCDO$_2$ at 70% SaO$_2$, while there was no effect of placebo on eCDO$_2$ (Table 3).

Theophylline trials

Cardiovascular variables at all stages of isocapnic hypoxia during theophylline trials are presented in Table 1. Hypoxia elevated HR and VE at all stages pre and post theophylline trials, while MAP was only elevated at 70% SaO$_2$ and was higher pre-theophylline (P=0.02). Following theophylline, VE was lower at all stages (main effect: P=0.03) concomitant to a lower $P_{ETCO_2}$ clamp (main effect: P<0.01).

Cerebral vascular variables at all stages of hypoxia during the theophylline trials are presented in Table 2. Pre and post theophylline $Q_{ICA}$, ICA CVC, ICA diameter, and MCAv increased during all stages of hypoxia while ICAv increased at 80% and 70% SaO$_2$. There was a main effect of theophylline on $Q_{ICA}$, ICA CVC, ICAv, and MCAv with all variables lower post theophylline (all, P<0.05); however, this coincided with the previously mentioned lower $P_{ETCO_2}$ (main effect: P<0.01). Following correction for $P_{ETCO_2}$, there was no main effect of theophylline on corr$Q_{ICA}$ (P=0.29; Table 3).
Pre and post theophylline, $Q_{VA}$ and $V_{Av}$ increased at 80% and 70% $Sao_2$ while $VA$ diameter and $VA$ CVC only increased at 70% $Sao_2$; $PCA_v$ also increased, during all stages of hypoxia both pre and post intervention. There was a main effect of theophylline on $Q_{VA}$, $VA$ CVC, $VA_v$ and $PCA_v$ with all variables lower post theophylline. Following correction for $PE_{TCO_2}$, however, there was no main effect of theophylline on $corrQ_{VA}$ ($P=0.85$; Table 3). Hypoxia increased $gCBF$ and $gCBF$ CVC across all stages pre and post theophylline while there was a main effect of theophylline to lower $gCBF$ ($P<0.01$) and $gCBF$ CVC ($P<0.01$). Following correction for $PE_{TCO_2}$, there was no main effect of theophylline on $corrCBF$ ($P=0.80$; Table 3). Hypoxia increased $eCDO_2$ at 70% $Sao_2$, while there was no effect of theophylline on $eCDO_2$.

**Cerebrovascular Reactivity Slopes to Hypoxia**

The $R^2$ values for each CBF variable in the pre-theophylline, theophylline, pre-placebo, and placebo trials are reported in Table 4. These analyses were conducted to discern if the main effect of theophylline on CBF variables was simply a product of differing $PE_{TCO_2}$ values between trials and to determine between vessel differences in reactivity. The response slopes for absolute changes of $MCA_v$ ($P=0.89$), $PCA_v$ ($P=0.504$), $Q_{ICA}$ ($P=0.348$), $ICA_v$ ($P=0.231$), $Q_{VA}$ ($P=0.890$), $VA_v$ ($P=0.950$), and $gCBF$ ($P=0.694$) were not different between the pre-placebo, post-placebo, pre-theophylline, and post-theophylline trials. The response slopes for percent changes of $MCA_v$ ($P=0.598$), $PCA_v$ ($P=0.753$), $Q_{ICA}$ ($P=0.707$), $ICA_v$ ($P=0.08$), $Q_{VA}$ ($P=0.860$), $VA_v$ ($P=0.989$), and $gCBF$ ($P=0.953$) were not different between the pre-placebo, post-placebo, pre-theophylline, and
post-theophylline trials (Figure 2). The percent changes in gCBF, QICA, and QVA at each stage of hypoxia for all trials are depicted in Figure 3.

**Between vessel comparisons**

There was no main effect of theophylline (P=0.45) or vessel (P=0.41) on the percent reactivity of the MCAv versus PCAv to hypoxia. Similarly, for QICA versus QVA there was no main effect of theophylline (P=0.97) or vessel (p=0.69). Volumetric reactivity through the ICA (QICA) was greater than velocity reactivity of the MCA (main effect of vessel: P<0.001), while QVA reactivity was greater than PCAv reactivity (main effect of vessel: P=0.008) (Figure 2).

**High-altitude study**

The resting room air baseline measurements for the sea-level placebo trial (pre-intervention) were used as the sea-level baseline for comparison to the high-altitude data. Cerebrovascular, hemodynamic and respiratory variables for the sea-level baseline and at high altitude are presented in Table 5. Compared to sea-level, VE, HR and MAP were increased (main effect, all P<0.05) at all high-altitude time points, while SaO2, PETO2, PETCO2 were reduced (main effect, all P<0.05) at all time points. Six hours post-intervention QVA was significantly lower in the theophylline trial compared to placebo (P=0.047), while there was a tendency for gCBF to be lower in the theophylline group (P=0.058). However, these differences were abolished when end-tidal gases were matched between groups and to the initial exposure time (Table 5). There was no elevation in QICA
at high-altitude as compared to sea-level baseline and eCDO₂ was not different from sea-
level to high-altitude or between groups.

In the placebo group, Lake Louise AMS scores did not change from initial exposure
(2.4±1.5) to six hours post intervention (4.4±3.1) or the following morning (2.6±0.9)
(P=0.585). Similarly, in the theophylline group there was no difference in AMS scores
from initial exposure (2.8±2.5) to six hours post intervention (2.3±3.0) or the following
morning (2.8±3.2) (P=0.585). There was no main effect of theophylline on AMS scores
compared to placebo (P=0.723).

DISCUSSION

At sea-level and under the conditions of isocapnic hypoxia the novel findings of this study
are; 1) hypoxic vasodilation of the ICA, while confirming earlier reports of hypoxic
vasodilation of the VA (66); 2) the vasomotor response of these extra-cranial arteries and
overall gCBF is unaffected by non-selective adenosine receptor antagonism during all
stages of isocapnic hypoxia, and 3) anterior (QICA) and posterior (QVA) cerebrovascular
reactivity to isocapnic hypoxia were not different. At high-altitude under the conditions of
poikilocapnic hypoxia, adenosine receptor antagonism reduced QVA compared to the
placebo group, however, this difference was abolished when end-tidal gases were matched
between groups. Collectively, these results indicate adenosine receptor dependent signaling
is not obligatory for hypoxic cerebral vasodilation under the conditions of normobaric and
hypobaric hypoxia.
Mechanisms of hypoxic cerebral vasodilation

Hypoxia increases production of adenosine in cerebral tissue (31, 69), which, in animal models, is reflected in cerebral arteriolar vasodilation (5). However, the present study provides evidence that, in humans, adenosine receptor dependent signaling is not an obligatory regulator of CBF during normobaric or hypobaric hypoxia. This is consistent with previous studies that have utilized pharmacological adenosine receptor antagonism under less controlled conditions (7, 41). Our current findings are further consistent with studies utilizing pharmacological inhibition of cyclic adenosine monophosphate (cAMP) prior to a hypoxic exposure (14, 19, 23, 47). Given that cAMP inhibition with indomethacin (17, 28) does not affect reactivity of cerebral vessels to hypoxia, and adenosine induces vasodilation largely through increasing cAMP levels (42, 52) downstream of A2A receptor binding (34, 38), it is perhaps not surprising that adenosine receptor antagonism had no effect on CBF in the current study. Nevertheless, adenosine has also been reported to induce cerebral vasodilation through increasing inward rectifying potassium channel conductance (20). While inward rectifying potassium channels have been shown to regulate skeletal muscle blood flow, it remains to be investigated if adenosine regulates hypoxic cerebral vasodilation in humans via action on inward rectifying potassium channels.

This study and others indicating an insignificant role of cAMP in hypoxic cerebral vasodilation (14, 19, 23, 47), lead to the speculation that signaling pathways relying on cyclic guanosine monophosphate (cGMP) must be the primary regulators of CBF during hypoxia. This is consistent with the notion that red blood cell mediated release of s-
Cerebral Blood Flow in Hypoxia

nitrosohemoglobin and adenosine triphosphate in addition to nitrite reductase activity, which all lead to up-regulation of cGMP, are the primary regulators of hypoxic cerebral vasodilation (11, 24, 27). Furthermore, given recent evidence that shear stress contributes to vasodilation of cerebral conduit arteries [e.g. ICA; (8)], this mechanism may also contribute to hypoxic cerebral vasodilation. These latter possibilities remain to be established.

In addition to hypoxia induced upregulation of specific signaling pathways, the potential for other physiological factors to influence gCBF and the diameter of the ICA and VA remains. For example, MAP directly influences CBF (35, 43) but increases in MAP do not seem to affect the %change in MCAv for a given CO₂ stimulus. Indeed, a pressor response of <10 mmHg or >10 mmHg increase in MAP does not lead to a difference in %MCAv reactivity, however, conductance is affected given the difference in denominator for calculating CVC (49). Similarly, changes in MAP were not related to changes in CBF variables in previous investigation under hypoxic conditions (66). While there was a significant relationship between MAP and CBF variables pre theophylline and placebo (but not post either intervention; Figure 4), the presence of ICA vasodilation (Table 2) prior to an increased MAP (Table 1) in all trials (irrespective of correlation with MAP) infers active, not passive, changes in vessel caliber and a relatively small role of MAP in our diameter responses. Nonetheless, direct pressure passive effects on CBF (35), and the potential for pressure mediated vessel distension can not be discounted as contributors to the integrative regulation of CBF during hypoxia.
As per previous studies utilizing drug interventions, this study highlights the necessity of using a placebo time control trial for proper interpretation (46). Much like a previous study by Peebles and colleagues if we had not had a placebo trial it would have lead to the erroneous conclusion that theophylline, while not affecting overall reactivity, diminishes the influences of MAP on CBF in hypoxia. However, given the similar lack of correlation between MAP and CBF variables following placebo (Figure 4), it appears that this may be a result of repeated within day exposure to hypoxia, or a time of day effect as the pre versus post trials were separated by six hours. However, given that CBF reactivity to hypoxia is not different, in the presence or absence of a correlation with MAP, suggests indirectly that MAP did not appreciably influence CBF in the present study i.e., correlation does not reflect causation. This finding is noteworthy as reductions in resting MAP or the MAP response during hypercapnia results in a blunting of CBF reactivity to CO₂ (2, 18); thus, it seems plausible that the influence of MAP on CBF differs depending on the prevailing blood gas stimulus.

Cerebral blood flow and normobaric hypoxia

At sea-level, there is conflicting evidence regarding hypoxic vasodilation of the extra-cranial cerebral arteries (ICA & VA) (33, 45, 66). Our study supports previous work (33, 58, 66) demonstrating vasodilation of the VA during hypoxia, although this has not been consistently reported (45). Differences in methodological and analytical factors may explain these differences. For example, in the study by Ogoh et al., manual measurements of vessel diameter were used to detect hypoxic vasodilation, whereas automated edge-detection software was used in the current study and our previous work (33, 66). Our
automated edge-detection software, which has been validated using phantom models (71), possess a lower intra-observer error than manual caliper measurements of arterial diameter (59, 71). Moreover, we observed hypoxic vasodilation of the ICA, the occurrence of which there is similarly both support for (33) and against (45, 58, 66). This study is the first to report ICA vasodilation during normobaric isocapnic hypoxia.

Contrary to previous studies investigating cerebral vascular reactivity to isocapnic hypoxia (66), we report no difference in anterior vs. posterior relative (%) reactivity. As approximately 25-40% of the increase in $Q_{ICA}$ and $Q_{VA}$ may be attributable to vasodilation (Figure 2), the regional differences observed by Willie et al., 2012 may be due to a failure to detect ICA vasodilation in the face of VA vasodilation (66). In addition to the small sample size (n=7 for ICA), and a prolonged stage of hypoxia (15-minutes), a technical consideration is the quality of the ultrasound used by Willie et al., 2012 (Terason t3000) versus that used in the present study (Terason t3200). For example, advances in spatial resolution of other imaging techniques (i.e., MRI) have produced new insight into vascular control during alterations in arterial blood gases [reviewed in: (22)]. We feel technical, not physiological, differences between studies may have precluded the ability to detect small, yet significant, changes in ICA diameter and lead to between study differences. Further, vasodilation of the ICA is consistent with downstream increases in MCA diameter that have been demonstrated with both MRI (51, 67) and transcranial colour coded ultrasound (26, 67), indicating vasodilation to hypoxia throughout the cerebrovascular tree. Despite no differences between posterior and anterior reactivity, it is important to note there still exists a heterogeneous reactivity among specific brain regions (6). While increases in CBF act to
Cerebral Blood Flow in Hypoxia

Despite a lower CBF during hypoxia in the theophylline trial, our data still provides evidence that cerebral vasodilation is unaltered by adenosine receptor antagonism. The lower CBF can be attributed to the lower $P_{ET\text{CO}_2}$ throughout the theophylline trial, as evidenced by the lack of difference between trials once CBF is mathematically corrected for the difference in $P_{ET\text{CO}_2}$ using standard volumetric reactivity values. Further, indicating unaltered vascular responsiveness, the reactivity slopes of $Q_{ICA}$, $Q_{VA}$, and gCBF were not different pre versus post theophylline. While our experimental design allowed for the quantification of extra-cranial cerebral artery vasodilation, which was unaltered (Table 2), the unaltered gCBF provides indirect evidence that adenosine receptor dependent signaling also has no effect on downstream resistance vessels (e.g., pial vessels). The lack of effect of theophylline is highlighted by the tight overlap of CBF during hypoxia pre- versus post-theophylline (Figure 3).

**Cerebral blood flow and hypobaric hypoxia**

Measurements of CBF at high-altitude have consistently demonstrated that CBF increases to an extent that compensates for the reductions in $CaO_2$ (or $SaO_2$) to maintain CDO$_2$ [reviewed in: (4, 24)]. Our calculations provide indirect evidence of adequate maintenance of CDO$_2$ and further demonstrate that maintenance of CDO$_2$ at high-altitude is not dependent on adenosine receptor dependent signaling (Table 3). Given that hemoglobin
concentration is likely unaltered (55) or even slightly increased (58) within 8 hours following rapid ascent to high-altitude, an unaltered CBF and SaO₂ in the face of adenosine receptor antagonism provides compelling evidence, albeit inferential, that CDO₂ is commensurately unaltered. Despite no main effect of O₂ (i.e., high-altitude) on QICA, contrary to previous study showing increased QICA upon ascent to high-altitude (58), the maintenance of eCDO₂ (i.e., global delivery) indicates adequate responsiveness of the cerebral vasculature as a whole to the hypoxic stimulus. No change in QICA may simply reflect the lower hypoxic stimulus (3800m, SaO₂ ~87%) in the present study versus that of Subudhi et al., (5260m, SaO₂ ~76%). In keeping, QICA was only modestly elevated at 90%SaO₂ in the sea level theophylline trials and did not significantly increase until SaO₂ dropped to 80% in the sea-level placebo trials (Table 2); therefore, it is perhaps not surprising that QICA was not statistically elevated at high-altitude where the relatively mild hypoxic stimulus (87% SaO₂) is occurring simultaneous to moderate hypocapnia (PETCO₂ ≈ 32-34mmHg). The latter response, which acts to constrict the cerebral vessels, likely balanced with the vasodilatory influence of the mild hypoxic stimulus to produce no overall change in diameter. However, given the small sample size (n=6) in each group for the high-altitude data, it is important to interpret these data judiciously and consider them more exploratory than confirmatory. Power calculations indicate that given the change in CBF observed a sample size of n=12 in each group would be necessary to observe a significant difference with a power of 0.80. Therefore, further study using a larger sample size is needed to make firm conclusions regarding adenosine receptor antagonism and CBF regulation at high-altitude.
Acute Mountain Sickness

Theophylline has been demonstrated to reduce acute mountain sickness (AMS) symptoms at 3454m (3) and 4559m (8), which was speculated to be due to a reduction in gCBF (3). However, given the speculative role of gCBF in the pathogenesis of AMS (1, 13) we aimed to concurrently assessed AMS and CBF following theophylline treatment at high-altitude (3800m). Contrary to previous study (3, 8) we demonstrate no effect of theophylline on AMS scores when end-tidal gases were matched; however, this (end-tidal clamping) is not reflective of normal physiology upon sojourn to high-altitude. Indeed, CBF did not increase following theophylline, despite seemingly, although not statistically, increasing (+20%) in the placebo trial, under poikilocapnic (natural) conditions. Therefore, as there was no difference when end-tidals were matched, it seems that theophylline may act indirectly via hyperventilation-induced hypocapnia (not adenosine receptor antagonism) to reduce CBF. While AMS was not statistically different between the placebo and theophylline group, it was 48% lower (concomitant to a tendency for lower gCBF). These differences in CBF and AMS following theophylline, if confirmed in a larger sample size, may be meaningful despite the lack of significance (note, n=6 in each group). For example, at a power of 0.8, a sample size of n=12 would be required for each group to detect a statistical difference when CBF increases to the magnitude observed in the present study. Therefore, although not through a direct effect on the vasculature, theophylline may indeed be prophylactic in treating AMS by causing hyperventilation-induced hypocapnia and consequent reductions in CBF via vasoconstriction. This finding provides preliminary data that CBF is potentially implicated in previously observed theophylline induced reductions in AMS (3). However, the effect of theophylline on AMS may simply also be attributable to other factors such as
an elevated resting SaO$_2$ (3). Collectively, given the small sample size for both AMS and CBF it is difficult to draw firm conclusions from the current study. Further research is needed to determine if CBF regulation and/or improved oxygenation due to hyperventilation is related to potential benefits of theophylline on AMS severity.

Conclusion

We demonstrated that the ICA and VA dilate during isocapnic hypoxia at sea-level and that this vascular response to hypoxia is unaltered by adenosine receptor dependent signaling. Contrary to previous studies we demonstrate that anterior and posterior reactivity, indexed by $Q_{ICA}$ and $Q_{VA}$ respectively, does not differ during hypoxia. We extend these findings to highlight that theophylline does not affect regional reactivity in a differential manner. Following rapid ascent to high-altitude, CBF is unaltered by theophylline. Collectively, our sea-level and high-altitude data indicate that adenosine receptor dependent signaling is not obligatory for cerebral vasodilation during hypoxia.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS
All authors contributed to data collection, critically assessed the manuscript for scientific content and approved the final manuscript. Study Conception: RLH, PNA; Data Analysis: RLH; Data interpretation: RLH, PNA, CH; Drafting manuscript: RLH, PNA

CONFLICT OF INTEREST

The authors declare no competing interests, financial or otherwise.
REFERENCES


Cerebral Blood Flow in Hypoxia


30. **Kety SS, Schmidt CF**. The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young


Cerebral Blood Flow in Hypoxia


59. Thomas KN, Lewis NCS, Hill BG, Ainslie PN. Technical recommendations for the


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FIGURES

Figure 1. Adenosine signaling pathways in hypoxia. Adenosine may lead to vasodilation (i.e., smooth muscle cell relaxation) by binding to adenosine A2A receptors, or by increasing inward rectifying potassium (KIR) channel conductance. A2A receptor binding activates → (cAMP), leading to up-regulation of cAMP dependent protein kinase and subsequent inhibition → of myosin light chain kinase (1). By inhibiting this response, downstream phosphorylation of myosin light chain and its consequent contribution to contraction does not occur, resulting in smooth muscle cell relaxation, and/or vasodilation (29). Theophylline is a non-selective adenosine receptor antagonist, and therefore, does not allow for insight into how adenosines action on KIR channels may influence CBF regulation in hypoxia. cAMP, cyclic adenosine monophosphate; KIR, inward rectifying potassium channels.

Figure 2. Reactivity slopes of extra- and intra-cranial blood vessels in all experimental trials at sea-level. The percent increase in CBF per percent drop in SaO2 is depicted for each vessel insonated, during all trials. Volumetric reactivity (gCBF, QICA, QVA) was not different within or between trials, while MCAv and PCAv reactivity were lower than QICA (main effect: P<0.001) and QVA (main effect: P=0.008) reactivity respectively in all trials. The percent reactivity of ICAv and VAv are superimposed onto their respective flow reactivity columns to highlight the contribution of velocity versus diameter changes in determining overall flow reactivity. gCBF, global cerebral blood flow; QICA, internal carotid artery blood flow; QVA, vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; ICAv, internal carotid artery blood velocity; VAv, vertebral artery blood velocity. * indicates significant difference between confluent vessel velocity and volumetric reactivity (e.g., QICA vs MCAv), P<0.05 after correction for multiple comparisons. n=12 unless otherwise specified.

Figure 3. Global and regional cerebral blood flow during isocapnic hypoxia prior to and following placebo and theophylline interventions. The three panels on the left depict CBF (gCBF, QICA, QVA) responses pre (●) and post (○) placebo, while the three panels on the right depict the CBF responses pre (■) and post (□) theophylline. Data are presented as the percent change of each variable from baseline. There was no effect of theophylline treatment on the %changes in CBF from baseline. * indicates a post-hoc derived significant change from baseline (P<0.05) following a main effect of SaO2 on CBF variables (P<0.05). n=12 unless otherwise specified.

Figure 4. The relationships between mean arterial pressure (MAP) and cerebral blood flow (CBF) variables in the theophylline and placebo trials. The two left panels represent the changes in MAP during a reduction in arterial oxygen saturation during the theophylline (upper panel) and placebo (lower panel) trials. The upper row of correlations depict the relationship between the percent change in MAP (%ΔMAP) and the percent change in CBF variables (%ΔFlow or Velocity) pre (●) and post (□) theophylline, while the
bottom row shows the same data, but for the pre (●) and post (○) placebo trials. *indicates a significant difference from baseline, P<0.05; ‡ indicates significant difference between drug and placebo, P<0.05. n=12 unless otherwise specified.
### Table 1. Cardiorespiratory variables during sea level hypoxia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Theophylline</th>
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</thead>
<tbody>
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<td></td>
<td>Baseline</td>
<td>90%</td>
</tr>
<tr>
<td>$P_{ET}O_2$</td>
<td>Pre</td>
<td>93.5±7.1</td>
</tr>
<tr>
<td>(mmHg)</td>
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<tr>
<td>$P_{ET}CO_2$</td>
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<td>40.4±3.0</td>
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<td>(mmHg)</td>
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<td>VE</td>
<td>Pre</td>
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<td>(L · min⁻¹)</td>
<td>Post</td>
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<tr>
<td>MAP</td>
<td>Pre</td>
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<tr>
<td>(mmHg)</td>
<td>Post</td>
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<td>HR</td>
<td>Pre</td>
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<tr>
<td>(beats · min⁻¹)</td>
<td>Post</td>
<td>62.4±15.1</td>
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Bolded Pre or Post indicates main effect of the intervention, with the bolded trial significantly greater. *signifies a significant difference from baseline. ‡ indicates significant interaction between pre and post. n=12 for all measurements.
### Table 2. Cerebrovascular variables during sea level hypoxia.

<table>
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<tr>
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<tr>
<td>( P_{1/2} )</td>
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<td>13</td>
<td>12</td>
<td>11</td>
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<tr>
<td>( P_{T} )</td>
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**Placebo**

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<td>( \text{O}_{2} )</td>
<td>( \text{mmHg} )</td>
<td>( \text{mmHg} )</td>
<td>( \text{mmHg} )</td>
<td>( \text{mmHg} )</td>
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**Theophylline**

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<td>( \text{mmHg} )</td>
<td>( \text{mmHg} )</td>
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<td>( P_{T} )</td>
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**Notes:**

- Bolded **Pre** or **Post** indicates main effect of the intervention, with the bolded trial significantly greater.
- *signifies a significant difference from baseline.
- ‡ indicates significant difference between pre and post. \( n=12 \) unless otherwise specified.
Table 3. Theoretical calculations of arterial oxygen content, cerebral oxygen delivery, and CBF parameters corrected for CO2.  
For both the placebo and theophylline trial, CaO2 and CDO2 were estimated (eCaO2 & eCDO2, respectively). Due to the difference in P_{ET}CO2 between the pre and post theophylline trials, Q_{ICA}, Q_{VCA}, and gCBF post theophylline (corrQ_{ICA}, corrQ_{VCA} & corrCBF, respectively) were all corrected for differences in P_{ET}CO2 (explained in methods section “calculations”).

<table>
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<tr>
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<td>Baseline</td>
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<tr>
<td></td>
<td>Pre vs. Post: ( P=0.275; O_2: P&lt;0.001; ) Interaction: ( P=0.808 )</td>
<td>Pre vs. Post: ( P=0.527; O_2: P&lt;0.001; ) Interaction: ( P=0.294 )</td>
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<tr>
<td>eCaO2 ((\text{mL} \cdot \text{dL}^{-1})) Pre</td>
<td>20.77±0.14</td>
<td>19.21±0.12*</td>
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<tr>
<td>Post</td>
<td>20.81±0.09</td>
<td>19.20±0.18*</td>
</tr>
<tr>
<td>eCDO2 ((\text{mL} \cdot \text{min}^{-1})) Pre</td>
<td>152.2±36.1</td>
<td>145.0±35.6</td>
</tr>
<tr>
<td>Post</td>
<td>148.3±38.4</td>
<td>139.9±53.8</td>
</tr>
<tr>
<td>Q_{ICA} (n=12) Pre</td>
<td>273.2±38.9</td>
<td>309.8±54.5*</td>
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<tr>
<td>corrQ_{ICA} Post</td>
<td>280.3±42.6</td>
<td>296.3±46.1*</td>
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<td>Q_{VCA} Pre</td>
<td>111.8±30.1</td>
<td>121.7±28.6</td>
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Bolded Pre or Post indicates main effect of the intervention, with the bolded trial significantly greater. *signifies a significant difference from baseline. ‡ indicates significant interaction between pre and post. n=8 unless otherwise specified.
Table 4. R-squared values for the linear regression of cerebrovascular reactivity.

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<th>Pre-placebo</th>
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<td>MCAv</td>
<td>0.97±0.03</td>
<td>0.90±0.10</td>
<td>0.95±0.03</td>
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<tr>
<td>PCAv</td>
<td>0.95±0.05</td>
<td>0.89±0.08</td>
<td>0.92±0.05</td>
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<tr>
<td>QICA</td>
<td>0.92±0.08</td>
<td>0.94±0.05</td>
<td>0.93±0.05</td>
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<td>ICAv</td>
<td>0.88±0.16</td>
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<tr>
<td>QVA</td>
<td>0.84±0.10</td>
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<td>0.90±0.16</td>
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<td>VAv</td>
<td>0.84±0.08 (n=8)</td>
<td>0.86±0.17 (n=8)</td>
<td>0.74±0.10 (n=8)</td>
<td>0.88±0.22 (n=8)</td>
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<td>gCBF</td>
<td>0.90±0.08 (n=8)</td>
<td>0.93±0.08 (n=8)</td>
<td>0.91±0.05 (n=8)</td>
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n=12 unless otherwise specified.
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<th>High-Altitude (3800m)</th>
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<tr>
<td></td>
<td>Kelowna</td>
<td>Initial exposure (2hrs)</td>
<td>5 hours post drug (7hrs)</td>
<td>5 hours post drug clamped (7hrs)</td>
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<tr>
<td></td>
<td></td>
<td>Drug vs. Placebo: P=0.062; O₂: P&lt;0.001; Interaction: P=0.247</td>
<td>Drug vs. Placebo: P=0.124; O₂: P&lt;0.001; Interaction: P=0.671</td>
<td>Drug vs. Placebo: P=0.368; O₂: P&lt;0.001; Interaction: P=0.724</td>
</tr>
<tr>
<td><strong>SaO₂</strong> (% oxyhemoglobin)</td>
<td>Drug 97.5±0.4 89.2±1.1*</td>
<td>87.3±0.7*</td>
<td>88.0±0.4*</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 97.4±0.3</td>
<td>87.7±1.7*</td>
<td>88.0±2.3*</td>
<td>87.9±0.7*</td>
</tr>
<tr>
<td><strong>P_{ETO₂}</strong> (mmHg)</td>
<td>Drug 95.3±3.3 54.4±1.2*</td>
<td>51.0±3.6*</td>
<td>54.5±1.3*</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
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<td>Placebo 95.3±3.3</td>
<td>54.4±1.2*</td>
<td>51.0±3.6*</td>
<td>54.5±1.3*</td>
</tr>
<tr>
<td><strong>P_{ETCO₂}</strong> (mmHg)</td>
<td>Drug 39.7±4.1 32.6±1.4*</td>
<td>34.7±2.5*</td>
<td>33.0±0.5*</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 41.4±2.2</td>
<td>33.1±1.5*</td>
<td>35.5±2.0*</td>
<td>33.0±0.4*</td>
</tr>
<tr>
<td><strong>V̇E</strong> (L · min⁻¹)</td>
<td>Drug 56.2±14.1 62.5±11.4</td>
<td>72.5±8.7*</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo 65.4±11.7</td>
<td>74.5±23.3</td>
<td>79.8±19.9</td>
<td>85.4±25.2*</td>
</tr>
<tr>
<td><strong>MAP</strong> (mmHg)</td>
<td>Drug 78.0±3.8 87.6±2.0*</td>
<td>91.9±7.1*</td>
<td>86.9±8.8*</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 76.7±5.0</td>
<td>87.6±2.0*</td>
<td>91.9±7.1*</td>
<td>86.9±8.8*</td>
</tr>
<tr>
<td><strong>HR</strong> (beats · min⁻¹)</td>
<td>Drug 10.7±2.5 13.3±1.5*</td>
<td>18.5±2.6*</td>
<td>21.4±5.5*</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 10.7±2.5</td>
<td>13.3±1.5*</td>
<td>18.5±2.6*</td>
<td>21.4±5.5*</td>
</tr>
<tr>
<td><strong>gCBF</strong> (mL · min⁻¹)</td>
<td>Drug 682.5±96.6 (n=5) 778.0±80.6 (n=5)</td>
<td>706.9±67.7 (n=5)</td>
<td>693.9±56.4 (n=5)</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 748.7±108.5</td>
<td>850.7±94.0</td>
<td>898.3±126.7</td>
<td>822.8±111.9</td>
</tr>
<tr>
<td><strong>Q_{ICA}</strong> (mL · min⁻¹)</td>
<td>Drug 271.1±70.5 272.7±33.8</td>
<td>255.6±31.5</td>
<td>260.7±47.9</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 256.1±28.2</td>
<td>288.1±44.5</td>
<td>297.4±54.1</td>
<td>281.1±36.9</td>
</tr>
<tr>
<td><strong>ICA diameter</strong> (mm)</td>
<td>Drug 45.1±6.8 43.8±3.4</td>
<td>42.2±6.2</td>
<td>42.7±7.0</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 48.4±6.4</td>
<td>48.1±7.7</td>
<td>55.4±12.0</td>
<td>50.5±10.4</td>
</tr>
<tr>
<td><strong>Q_{VA}</strong> (mL · min⁻¹)</td>
<td>Drug 5.02±0.60 5.14±0.43</td>
<td>5.08±0.42</td>
<td>5.09±0.44</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 4.74±0.42</td>
<td>5.05±0.33</td>
<td>4.80±0.47</td>
<td>4.88±0.26</td>
</tr>
<tr>
<td><strong>VA diameter</strong> (mm)</td>
<td>Drug 96.4±25.6 (n=5) 121.0±31.9 (n=5)</td>
<td>98.5±25.9 (n=5)</td>
<td>102.1±28.4 (n=5)</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 118.2±28.4</td>
<td>137.2±27.4</td>
<td>151.8±29.6</td>
<td>130.4±31.6</td>
</tr>
<tr>
<td><strong>eCDO₂</strong> (mL · min⁻¹)</td>
<td>Drug 137.9±16.7 143.8±14.1</td>
<td>127.0±12.0</td>
<td>125.9±9.9</td>
<td>Drug vs. Placebo: P=0.069; O₂: P&lt;0.001; Interaction: P=0.636</td>
</tr>
<tr>
<td></td>
<td>Placebo 150.9±21.7</td>
<td>153.6±17.0</td>
<td>158.5±22.0</td>
<td>149.0±20.9</td>
</tr>
</tbody>
</table>

**Bolded Drug** or **Placebo** indicates main effect of the intervention, with the bolded trial significantly greater, P<0.05.

*signifies a significant difference from baseline, P<0.05. ‡ indicates significant difference between drug and placebo, corrected for multiple comparisons, P<0.05. n=6 unless otherwise specified.
cAMP Dependent Protein Kinase
Myosin Light Chain Kinase (phosphorylated)
Phosphorylation
Relaxation
Smooth muscle cell membrane

Theophylline

Adenosine

Hyperpolarization

A<sub>2A</sub> K<sub>IR</sub>

↑cAMP

↑cAMP Dependent Protein Kinase

Phosphorylation

Myosin Light Chain Kinase (phosphorylated)

Relaxation
Pre: $r^2 = 0.41; P<0.01$
Post: $r^2 = 0.03; P=0.42$

Pre: $r^2 = 0.39; P<0.01$
Post: $r^2 = 0.02; P=0.37$

Pre: $r^2 = 0.43; P<0.01$
Post: $r^2 = 0.05; P=0.18$

Pre: $r^2 = 0.46; P<0.01$
Post: $r^2 = 0.05; P=0.32$

Pre: $r^2 = 0.30; P=0.01$
Post: $r^2 = 0.12; P=0.09$

Pre: $r^2 = 0.36; P<0.01$
Post: $r^2 = 0.06; P=0.27$

Pre: $r^2 = 0.48; P<0.01$
Post: $r^2 = 0.10; P=0.06$

Pre: $r^2 = 0.38; P<0.01$
Post: $r^2 = 0.03; P=0.33$

Pre: $r^2 = 0.31; P<0.01$
Post: $r^2 = 0.36; P<0.01$

Pre: $r^2 = 0.40; P<0.01$
Post: $r^2 = 0.02; P=0.44$

(n=8)