Cerebrovascular Response to CO_2 Following 10 Days of Intermittent Hypoxia in Humans

Jordan S. Querido; Joseph F. Welch; Najib T. Ayas; A. William Sheel

INTRODUCTION: It has been demonstrated that the cerebrovascular response to hypoxia is blunted following 10 d of intermittent hypoxia (IH) in healthy humans. The purpose of this study was to test the hypothesis that IH reduces the cerebrovascular response to CO_2.

METHODS: Healthy male subjects ($N = 8$; 25 ± 2 yr) were exposed to 10 consecutive days of IH (12% O_2 for 5 min followed by 5 min of normoxia for 1 h/d). The cerebrovascular response to CO_2 was assessed prior to (PRE-IH) and following (POST-IH) the IH paradigm with transcranial Doppler ultrasound.

RESULTS: There was no change in eupnic measures during or following the IH paradigm; however, the ventilatory response to IH increased by the last exposure ($3.0 \pm 2.8\ L \cdot min^{-1}$). Cerebral blood flow velocity decreased and increased with hypocapnia and hypercapnia, respectively, but cerebrovascular sensitivity to CO_2 remained unchanged with IH (PRE-IH: 2.58 ± 0.50%/mmHg; POST-IH: 2.59 ± 0.74%/mmHg).

DISCUSSION: Our data indicates that 10 d of IH in healthy humans does not alter the cerebrovascular response to CO_2. Redundancy of cerebrovascular regulation mechanisms to CO_2 may work to counteract IH-induced dysregulation and protect cerebral tissue.

KEYWORDS: hypoxemia, cerebrovascular control, carbon dioxide.

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Exposure to intermittent hypoxia (IH) is considered to be unique from sustained hypoxia, whereby the inability to adapt to IH often leads to autonomic dysregulation.[18] For instance, isolated rodent vessels exhibit autonomic vasodilator dysregulation following IH.[19] Similarly, patients with obstructive sleep apnea demonstrate autonomic dysfunction in the ventilatory, cardiovascular, and cerebrovascular systems—an effect attributed to the IH inherent to the syndrome.[5] Healthy human models of IH show autonomic dysregulation that parallels pathology.[16,22] In a previous study, we found the cerebrovascular sensitivity to acute hypoxia was significantly blunted following 10 consecutive days of IH in healthy humans.[22] The mechanistic explanation for this impairment in hypoxic cerebrovascular regulation is still uncertain, but may be the result of an increase in IH-induced oxidative stress, leading to endothelial dysfunction.[13] Oxidative stress due to IH may increase reactive oxygen species, which in turn reduces the bioavailability of endothelial nitric oxide (NO)—a key regulator of cerebrovasculature in hypoxia.[13] Support for this hypothesis is demonstrated in studies that have prevented the typical IH-induced autonomic impairments with the administration of an antioxidant.[7,14]

Although multifactorial, cerebrovascular regulation is particularly sensitive to adjustments in arterial pressures of CO_2.[8] Similar to the cerebrovascular response to hypoxia, endothelial function plays a significant role in the cerebrovascular response to hypercapnia via a CO_2-NO pathway.[12,26] However, the effect of IH on the cerebrovascular response to CO_2 has shown conflicting results, being increased,[11] decreased,[20] or unchanged with IH.[10] Integrating and interpreting the available literature is difficult, given that differences in IH protocol (e.g., short- vs. long-duration hypoxia cycles) and subject characteristics

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(e.g., healthy humans vs. patients) could play a large role in the discrepancies between studies. Accordingly, the purpose of this study was to investigate cerebrovascular regulation to CO_2 following an IH protocol that we previously demonstrated elicited cerebral blood flow dysregulation.[22]

Our healthy human model of IH led to cerebrovascular dysregulation in acute hypoxia, a likely result of endothelial dysfunction from a reduced bioavailability of NO.[22] If NO plays a key role in the cerebrovascular response to CO_2, then it is expected that this identical IH paradigm would result in a blunted cerebral blood flow response to CO_2. Therefore, it is hypothesized that the cerebrovascular sensitivity to CO_2 is reduced following 10 consecutive days of IH in healthy humans.

METHODS

Subjects

Healthy young male subjects ($N = 8$; 25 ± 2 yr) were recruited to participate in the study after providing written informed consent. All subjects (183 ± 6 cm; 77 ± 10 kg) were free of any known cardiorespiratory illness. Subjects underwent a preliminary session to familiarize themselves with the experimental setup and procedures. All procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia, which conforms to the Declaration of Helsinki.

Equipment

Breathing frequency, tidal volume, and minute ventilation (V_I) were determined from a pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO). Ventilated O_2 and CO_2 were measured at the mouth and analyzed using gas analyzers (models S-3A/I and CD-A, respectively, Applied Electrochemistry, Pittsburgh, PA). Beat-by-beat blood pressure (including mean arterial pressure) and oxyhemoglobin saturation (S_pO_2) were measured noninvasively at the finger with photoplethysmography (Finometer, FMS, Arnhem, Netherlands) and finger pulse oximetry (Model 3740, Ohmeda, Louisville, CO), respectively. Standard 3-lead electrocardiography was used to determine heart rate. Cerebral blood flow velocity was continuously measured at the proximal segment of the middle cerebral artery with a 2 MHz pulsed-wave transcranial Doppler ultrasound (Neurovision 500 M, Multigon Industries, Yonkers, NY). All data was collected using an analog to digital converter (PowerLab/16SP ML 795, ADInstruments, Colorado Springs, CO), sampled at 200 Hz and stored on a computer for offline analysis (Chart V5.02, ADInstruments).

Procedure

The cerebrovascular sensitivity to CO_2 was assessed on the day prior to (PRE-IH) and following (POST-IH) 10 consecutive days of IH. The middle cerebral artery was insonated through the right temporal window, above the zygomatic arch, to obtain backscattered Doppler signals. Optimal Doppler signals were achieved using previously described methods.[21] A transparency of each subject's facial features was taken to ensure consistency of probe placement during the two experimental sessions (PRE-IH and POST-IH). The probe was then secured using a headband device (Marc 600, Spencer Technologies, Seattle, WA) to provide a fixed angle of insonation. For each subject, the same depth and gain of the Doppler signal was used during PRE-IH and POST-IH testing. Placement of the Doppler probe was performed by the same investigator. Each experimental day began with a minimum of 10 min of resting eupnea to ensure stable baseline measurements. Subjects abstained from caffeine, alcohol, and exhaustive exercise for 24 h prior to the experimental tests. The IH protocol consisted of subjects breathing a hypoxic inspirate [fraction of inspired oxygen (F_IO_2) of 12%, corresponding to an approximate altitude of 13,123 ft (4000 m)] from a reservoir for 5 min followed by 5 min of room-air breathing; this cycle was repeated for 1 h each day (**Fig. 1**). Subjects wore a sealed facemask during hypoxic exposures and breathed through a mouthpiece during sensitivity

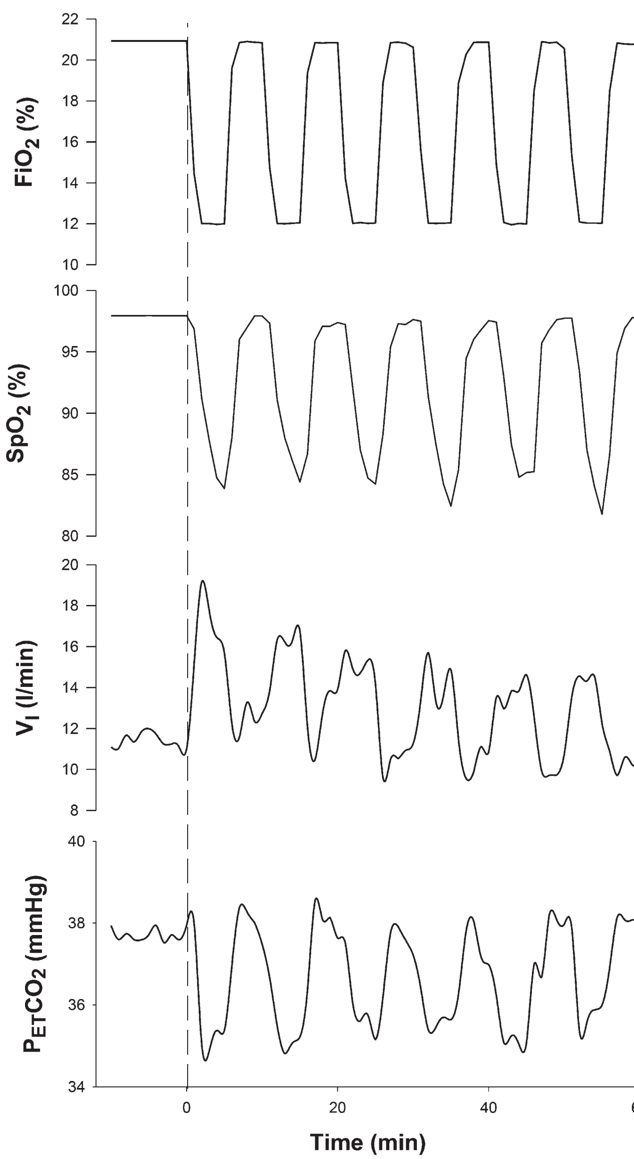

Fig. 1. Physiological measurements during an IH exposure in a representative subject.

testing. Ventilated gases were continuously monitored at the mouth to ensure there were no leaks in the inspiratory circuit and precise hypoxic control was attained. The cerebrovascular sensitivity to CO_2 was determined by the rebreathe method as described by Duffin et al.[3] Subjects were required to hyperventilate for 5 min in order to reduce end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) by ~15 mmHg. Following the hyperventilation period, subjects expired completely before taking three deep breaths from the rebreathing bag, consisting of 7% CO_2 and balance O_2. Subjects then continued to rebreathe from the bag at a self-paced rate. During the rebreathe portion of the test, O_2 pressure of the inspirate was maintained at 150 mmHg by a computer-controlled device. The test was terminated once $P_{ET}CO_2$ reached 60 mmHg.

Statistical Analysis

For each measured physiological variable, an average was determined at each $P_{ET}CO_2$ in 1-mmHg intervals. The slope of the linear regression between each variable and $P_{ET}CO_2$ was taken to represent the sensitivity to CO_2 (**Fig. 2**). For ventilatory measures, linear regression began at the ventilatory threshold. Student's paired *t*-tests were used to investigate the differences in physiological variables during eupnea between PRE-IH and POST-IH experimental sessions, as well as the differences in sensitivities to CO_2 (Statistica V7, Statsoft Inc., Tulsa, OK). Pearson product moment correlations were performed on selected dependent variables. The level of significance was set at $P < 0.05$ for all statistical comparisons. All data is presented as mean ± SD.

RESULTS

All subjects completed the entire experimental protocol. There was no effect of IH on eupnic measures during the 10-d IH protocol. Within an IH exposure, the hypoxic inspirate was well-controlled (mean $F_IO_2 = 12.15 \pm 0.3\%$, corresponding to a

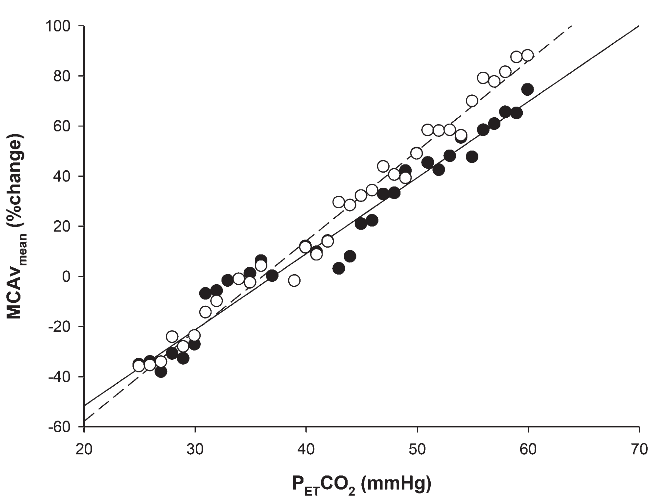

Fig. 2. Cerebrovascular responsiveness to CO_2 PRE-IH (black circles) and POST-IH (white circles) in a representative subject. MCA_{Vmean} = mean middle cerebral artery velocity.

P_aO_2 of 87 mmHg), resulting in a decrease and increase in S_pO_2 and V_I, respectively (Fig. 1).

Specifically, on the final day of IH exposure, the hypoxia resulted in a S_pO_2 of 84.7 ± 2.6% and a subsequent V_I of 15.6 ± 3.0 $L \cdot min^{-1}$. The V_I response to hypoxia significantly increased from the first to last day of IH exposures [3.0 ± 2.8 $L \cdot min^{-1}$ increase; $t(7) = -2.47$, $P = 0.046$]. There was no change in any physiological measure during eupnea between PRE-IH and POST-IH experimental sessions (**Table I**).

The cerebrovascular sensitivity to CO_2 was 2.58 ± 0.50%/mmHg PRE-IH and did not change with IH [POST-IH: 2.59 ± 0.74%/mmHg; $t(7) = -0.03$, $P = 0.97$; **Fig. 3**]. Similarly, there was no effect of IH on the CO_2 sensitivity of any other ventilatory or cardiovascular measures ($P > 0.05$).

DISCUSSION

We assessed the cerebrovascular response to CO_2 in healthy humans following 10 consecutive days of IH. Contrary to our previous report that demonstrated a reduced cerebrovascular response to acute hypoxia following an identical IH paradigm, cerebrovascular reactivity to CO_2 was unchanged, although there were adjustments in the ventilatory response to IH.

IH is a stimulus that leads to multiple adjustments in ventilatory, cardiovascular, neural, and cerebrovascular control.[1,6] Animal, healthy human, and patient models of IH have demonstrated blunted or augmented autonomic responsiveness depending on the autonomic system being investigated.[5,19,22] Although the physiological effects of IH are well-described, the available literature does show some inconsistencies. For example, improvements and impairments in cerebrovascular reactivity have been shown following IH,[11,22] leading some to suggest a therapeutic effect of IH rather than pathological.[24] A central caveat to integrating the results of the literature is the disparity between IH paradigms employed, an important consideration given that a distinct IH paradigm mediates the physiological response.[18] In particular, the severity of the hypoxic exposures and the number of hypoxic iterations (i.e., hypoxia-reoxygenation cycles) appear to be important in eliciting a pathological rather than therapeutic response.[18] The present study used an IH paradigm previously demonstrated

Table I. Physiological Measurements During Eupnea in the PRE-IH and POST-IH Experimental Sessions.

	PRE-IH	POST-IH
Fb (breath/min)	13.9 ± 4.4	13.6 ± 5.5
V_T (L)	0.94 ± 0.21	0.97 ± 0.24
V_I ($L \cdot min^{-1}$)	12.4 ± 3.4	12.3 ± 3.4
$P_{ET}CO_2$ (mmHg)	39 ± 2	38 ± 2
HR (bpm)	59 ± 8	59 ± 8
MAP (mmHg)	92 ± 5	94 ± 5
MCA_{Vmean} ($cm \cdot s^{-1}$)	62 ± 9	60 ± 11

IH = intermittent hypoxia; Fb = breathing frequency; V_T = tidal volume; V_I = inspired minute ventilation; $P_{ET}CO_2$ = end-tidal partial pressure of CO_2; HR = heart rate; MAP = mean arterial pressure; MCA_{Vmean} = mean blood flow velocity of the middle cerebral artery.

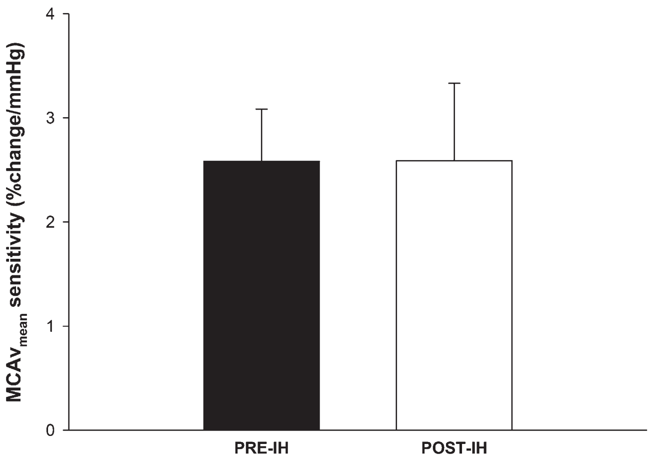

Fig. 3. The effect of IH on cerebrovascular responsiveness to CO_2. Error bars represent SD. MCA_{Vmean} = mean middle cerebral artery velocity.

to cause cerebrovascular dysfunction in acute hypoxia. However, our results demonstrated no effect of IH on cerebrovascular response to CO_2, suggesting that diverse mechanisms mediate cerebrovascular control in hypoxia and CO_2.

The cerebrovasculature is particularly sensitive to changes in arterial pressure of CO_2, with increases and decreases leading to cerebral vessel dilation and constriction, respectively.[8] Previous investigators have demonstrated the importance of NO in mediating the cerebrovascular response to CO_2.[26] Given the reduced bioavailability of NO following IH,[4] we expected a blunted cerebrovascular response to CO_2 following our IH paradigm. Surprisingly, our data shows no effect of IH on cerebrovascular responsiveness to CO_2; however, we are not the first to show this.[10,27] An explanation for the absence of an effect of IH is uncertain, but the multifaceted and redundant nature of cerebrovascular control may play a role. For instance, it is theorized that CO_2 directly influences cerebrovascular tone independently of any intermediates (e.g., NO).[23] It is possible that an impairment in cerebrovascular chemoregulation via NO unavailability could have been compensated for by a direct CO_2 influence on the cerebral vessels. In this sense, previous studies that demonstrated a blunted cerebrovascular responsiveness to CO_2 in patients with obstructive sleep apnea may be explained by a desensitization of cerebral CO_2 chemosensors from the apnea-induced periods of hypercapnia.[2,20] Although the poikilocapnic IH in the current study may have reduced the bioavailability of NO, a direct local effect of CO_2 on cerebral vessels could have maintained proper autonomic function, thereby negating the influence of NO on cerebrovascular response to hypoxia.[9] This implies a critical role of CO_2 on the physiological consequences of IH. Data from breath-hold divers, on the other hand, have shown normal cerebrovascular responsiveness to CO_2;[10] thus, an isolated intermittent hypo-/hypercapnia paradigm may provide further insights into the mechanisms of cerebrovascular control to CO_2.

There are inherent limitations in the current study. Transcranial Doppler ultrasonography provides an estimation of cerebral blood flow with the assumption that the insonated vessel maintains a fixed diameter. Previous reports have demonstrated no change in cross-sectional area of the middle cerebral artery during CO_2 challenges.[21,25] As a result, we consider our transcranial Doppler measurement to represent real changes in cerebral blood flow due to cerebral vessel dilation and constriction of small resistance vessels downstream of the area of insonation. In addition, extending our results to other models of IH must be done with caution. As previously mentioned, the specific IH paradigm combined with confounding factors can mediate the physiological response. While not the focus of the current study, the effect of prior IH exposure on subjective measures of performance and cognition in acute hypoxia has been the topic of previous investigations.[15,17] Further research investigating this possible effect could prove useful for practically relevant models of hypoxia, such as aviation and high-altitude.

In summary, we previously demonstrated cerebrovascular dysregulation in acute hypoxia following 10 d of IH. In the current study, we used an identical IH paradigm and found no effect on the cerebrovascular response to CO_2, although ventilatory responsiveness to hypoxia increased. Our data suggest a possible redundancy in cerebrovascular control during CO_2 challenges, which may act to protect the cerebrovasculature from IH-induced impairments.

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The authors declare that they have no conflict of interest.

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