

## Dynamic cerebral autoregulation during and following acute hypoxia: role of carbon dioxide

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**Querido JS, Ainslie PN, Foster GE, Henderson WR, Halliwill JR, Ayas NT, Sheel AW.** Dynamic cerebral autoregulation during and following acute hypoxia: role of carbon dioxide. *J Appl Physiol* 114: 1183–1190, 2013. First published March 7, 2013; doi:10.1152/jappphysiol.00024.2013.—Previous research has shown an inconsistent effect of hypoxia on dynamic cerebral autoregulation (dCA), which may be explained by concurrent CO<sub>2</sub> control. To test the hypothesis that hypoxic dCA is mediated by CO<sub>2</sub>, we assessed dCA (transcranial Doppler) during and following acute normobaric isocapnic and poikilocapnic hypoxic exposures. On 2 separate days, the squat-stand maneuver was used to determine dCA in healthy subjects ( $n = 8$ ; 3 women) in isocapnic and poikilocapnic hypoxia exposures (end-tidal oxygen pressure 50 Torr for 20 min). In isocapnic hypoxia, the amplitude of the cerebral blood flow response to increases and decreases in mean arterial blood pressure were elevated (i.e., increases in gain of +35 and +28%, respectively;  $P < 0.05$ ). However, dCA gain to increases in pressure was reduced compared with baseline (–32%,  $P < 0.05$ ) following the isocapnic hypoxia exposure. Similarly, intravenous bolus injections of sodium nitroprusside and phenylephrine in a separate group of subjects ( $n = 8$ ; 4 women) also demonstrated a reduction in dCA gain to hypertension following isocapnic hypoxia. In contrast, dCA gain with the squat-stand maneuver did not significantly change from baseline during or following poikilocapnic hypoxia ( $P > 0.05$ ). Our results demonstrate that dCA impairment in isocapnic hypoxia can be prevented with hypocapnia, and highlight the integrated nature of hypoxic cerebrovascular control, which is under strong CO<sub>2</sub> influence.

autonomic control; cerebral autoregulation; hypoxia; hypocapnia

CEREBRAL AUTOREGULATION (CA) refers to the physiological mechanisms that maintain blood flow constant during steady-state (static CA) and abrupt [dynamic CA (dCA)] changes in blood pressure. The mechanisms of CA are complex and incompletely understood, but likely rely on a combination and interaction of myogenic, neural, endothelial, and metabolic factors (1, 32). Although recently challenged in both healthy humans (18) and patients (13), the conventional model of static CA proposes that cerebral blood flow (CBF) is independent of steady-state changes in mean arterial pressure (MAP) between ~50 and 160 mmHg (17). In contrast, dCA responds to sudden changes in blood pressure and is frequently active throughout a typical day, such as during rapid adjustments in posture. Early methods of dCA assessment in humans often included an

abrupt decrease in blood pressure with rapid thigh-cuff deflation (1), whereas later assessments focused on transfer function analysis of spontaneous oscillations in blood pressure and CBF over both time and frequency domains (39). Although these methods have the advantage of being noninvasive and easily administered, they do not fully characterize dCA. Specifically, these methods do not differentiate the dCA response to hypotension and hypertension (i.e., hysteresis). Consistent with well-controlled animal studies (5), recent studies have reported the presence of hysteresis in humans (16, 34) and have revealed that dCA is more effective at buffering transient hypertension than hypotension.

A number of previous studies have examined the effect of hypoxia on dCA; however, the findings have been inconsistent and demonstrated that dCA is either maintained (2) or impaired (14, 22). Whereas hypoxia is a central component to these studies, the inconsistent findings between studies may lie in the simultaneous control (or lack thereof) in CO<sub>2</sub>, a potent regulator of CBF. Aaslid et al. (1) observed that, compared with normocapnic and hypercapnic backgrounds, dCA was improved following an abrupt decrease in MAP under hyperventilation-induced hypocapnia, findings that have recently been supported with transfer function analyses (33). Therefore, the hyperventilation-induced hypocapnia in poikilocapnic hypoxia may also counteract the impairment in dCA induced by hypoxia (22). The interpretation of the previous literature on the effects of hypoxia on dCA must be accompanied with the consideration of whether hypocapnia resulted from the hypoxia-induced hyperventilation, or whether isocapnia was maintained.

Although the mechanisms responsible for the impairment are unclear, the hypoxia-induced impairment in dCA may also persist in normoxia. For example, pathological models of hypoxia [e.g., obstructive sleep apnea (OSA)] demonstrate impairment in dCA in normoxia (21, 35). The nightly hypoxia exposure inherent of OSA has been implicated for many of the daytime cardiovascular and cerebrovascular impairments commonly associated with the syndrome. However, studies in patients with OSA are confounded by extraneous factors, such as night-time arousal, hypertension, and patient use of prescription medications. Furthermore, to our knowledge, no study has separately assessed the asymmetry of the cerebrovascular response against transient hypertension and hypotension during or following exposure to hypoxia. Accordingly, the purpose of this study was twofold. First, we tested the hypothesis that hypocapnia acts as a protective mechanism in hypoxia and blunts the impairment in dCA that has been reported in mild

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isocapnic hypoxia (22). Second, we tested the hypothesis that acute isocapnic hypoxia results in a persistent impairment in dCA following the hypoxic exposure.

## EXPERIMENTAL PROCEDURES

### Subject Characteristics

All experimental procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia, which conforms to the Declaration of Helsinki. Healthy young subjects ( $n = 16$ ;  $29 \pm 5$  yr, 7 women) participated in this study after providing written, informed consent. One-half of the subjects participated in the squat-stand portion of the experiment, while the other one-half participated in the pharmacological intervention (see Experimental Protocols). All subjects were of normal height ( $175 \pm 8$  cm) and weight ( $69 \pm 10$  kg) and were free from any known respiratory, cardiovascular, or neural disease, and all were nonsmokers. For familiarization purposes, subjects attended a preliminary session in which they were accustomed with the experimental protocol. Subjects refrained from eating for a minimum of 2 h and caffeine and strenuous exercise for 24 h before testing.

### Experimental Protocols

**Squat-stand maneuver.** Subjects ( $n = 8$ ;  $29 \pm 4$  yr, 3 women) completed 2 experimental days in random order, which were separated by a minimum of 48 h and maximum of 7 days. Testing on the 2 experimental days was performed at the same time of day. On *day 1*, following instrumentation and before performing the squat-stand maneuver, the subject rested in a sitting position for a minimum of 15 min to obtain baseline cardiorespiratory measurements (baseline column in Table 1). The primary measure of dCA was obtained by having subjects perform the squat-stand maneuver as developed by Claassen et al. (6). This maneuver creates physiologically relevant changes in MAP through adjustments in posture and presents challenges to dCA that are typically experienced in daily life. From the original sitting position, subjects stood up and held this position for 5 s. Subjects then assumed a squatting position (knee angle  $\sim 45^\circ$ ), which was also held for 5 s. The 5-s stand followed by 5-s squat was repeated for 5 min, resulting in 30 full cycles (oscillations) in blood pressure. This squat-stand paradigm was used because it induces fluctuations that fall within the low-frequency range (0.07–0.20 Hz), where dCA is effective and under autonomic influence (33, 39). During the maneuver, the subject was instructed to breathe normally and avoid any strenuous breathing pattern (e.g., Valsalva maneuver); adherence was ensured by continuous monitoring of the respiratory trace. Following the 5-min squat-stand maneuver, subjects were exposed to 20 min of either isocapnic or poikilocapnic hypoxia. The

fraction of inspired oxygen was adjusted by a computer-controlled system (see *Physiological Measures*) to clamp the end-tidal pressure of oxygen ( $P_{ET_{O_2}}$ ) at 50 Torr. The squat-stand maneuver was repeated 15 min into the hypoxia exposure. All physiological measures were continuously recorded following the hypoxia exposure, and the squat-stand maneuver was repeated 5 min following the termination of hypoxia. *Day 2* was identical to *day 1*, except the hypoxia exposure differed in whether it was poikilocapnic or isocapnic (i.e., *day 1* isocapnic hypoxia, making *day 2* poikilocapnic hypoxia). Subjects were blinded to the nature of the hypoxic exposure between the 2 experimental days.

**Infusion of vasoactive drugs.** On a separate day and in a separate group of subjects ( $n = 8$ ;  $29 \pm 6$  yr; 4 women), a secondary measure of dCA was assessed during more gradual changes in blood pressure with the use of vasoactive drugs before and after 20 min of isocapnic hypoxia. Subjects remained semisupine throughout experimentation. Following instrumentation, a minimum of 15 min of data were collected, which served as a baseline measurement. Blood pressure manipulation was then performed with sequential intravenous bolus injections of sodium nitroprusside (100  $\mu$ g), followed by phenylephrine HCl (150  $\mu$ g) 60 s later, as per the modified Oxford technique (10, 27). Subjects were then exposed to isocapnic hypoxia, where oxyhemoglobin saturation ( $Sp_{O_2}$ ) was maintained at 80% for 20 min by titrating 100%  $N_2$  and  $CO_2$  to the inspire as needed. The modified Oxford technique was completed again  $\sim 5$  min following the hypoxic exposure while the subject breathed room air. The use of the modified Oxford technique to assess dCA has been validated against alternate methods (34) and enables investigation of a possible dCA asymmetry by separating the response to rising and falling pressures. Furthermore, the technique induces less frequent oscillations in blood pressure ( $< 0.01$  Hz) compared with the squat-stand maneuver (0.10 Hz).

### Physiological Measures

Ventilatory flow was continuously recorded by a pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO) connected to a facemask. From the flow signal, tidal volume and breathing frequency were determined; minute ventilation ( $\dot{V}_I$ ) was established from the product of tidal volume and breathing frequency. Ventilated  $O_2$  and  $CO_2$  were measured at the mouth with calibrated gas analyzers (models S-3A/I and CD-A, Applied Electrochemistry, Pittsburgh, PA). Beat-by-beat blood pressure and  $Sp_{O_2}$  were determined non-invasively with finger photoplethysmography (which was calibrated against automated blood pressure measurements from the arm) (Finometer, FMS, Arnhem, the Netherlands) and finger pulse oximetry (Nonin model 7500FO, Nonin Medical, Plymouth, MN), respectively. A 2-MHz pulsed-wave Doppler ultrasound (Neurovi-

Table 1. Effect of hypoxia on cardiorespiratory measures

	Isocapnic			Poikilocapnic		
	Baseline	Hypoxia	Posthypoxia	Baseline	Hypoxia	Posthypoxia
VT, liters	1.1 $\pm$ 0.3	1.5 $\pm$ 1.0	1.0 $\pm$ 0.3†	1.0 $\pm$ 0.3	1.3 $\pm$ 0.5*	1.0 $\pm$ 0.2†
F <sub>b</sub> , breaths/min	14.0 $\pm$ 4.0	13.6 $\pm$ 4.4	14.5 $\pm$ 4.9	12.9 $\pm$ 3.9	13.1 $\pm$ 4.3	14.1 $\pm$ 5.5
V <sub>I</sub> , l/min	13.7 $\pm$ 2.4	17.7 $\pm$ 2.3*	13.3 $\pm$ 3.7†	12.7 $\pm$ 3.1	16.2 $\pm$ 4.6*	12.9 $\pm$ 3.7†
P <sub>ET<sub>O<sub>2</sub></sub></sub> , Torr	101 $\pm$ 3	50 $\pm$ 1*	98 $\pm$ 4†	101 $\pm$ 4	50 $\pm$ 2*	100 $\pm$ 5†
P <sub>ET<sub>CO<sub>2</sub></sub></sub> , Torr	41 $\pm$ 3	40 $\pm$ 2	40 $\pm$ 4	40 $\pm$ 3	36 $\pm$ 4*	39 $\pm$ 5†
Sp <sub>O<sub>2</sub></sub> , %	96 $\pm$ 1	83 $\pm$ 2*	95 $\pm$ 1†	96 $\pm$ 1	83 $\pm$ 2*	95 $\pm$ 1†
HR, beats/min	65 $\pm$ 12	76 $\pm$ 10*	64 $\pm$ 12	64 $\pm$ 7	71 $\pm$ 9*	63 $\pm$ 9†
MAP, mmHg	89 $\pm$ 10	86 $\pm$ 10	91 $\pm$ 13	91 $\pm$ 5	91 $\pm$ 12	87 $\pm$ 12
MCAV <sub>mean</sub> , cm/s	64 $\pm$ 7	70 $\pm$ 10*	63 $\pm$ 8†	61 $\pm$ 9	60 $\pm$ 7	58 $\pm$ 10
CVR, mmHg·cm <sup>-1</sup> ·s <sup>-1</sup>	1.40 $\pm$ 0.17	1.26 $\pm$ 0.18*	1.47 $\pm$ 0.24†	1.50 $\pm$ 0.19	1.51 $\pm$ 0.13	1.50 $\pm$ 0.15

Values are means  $\pm$  SD. VT, tidal volume; F<sub>b</sub>, breathing frequency; V<sub>I</sub>, minute ventilation; P<sub>ET<sub>O<sub>2</sub></sub></sub>, end-tidal pressure of oxygen; P<sub>ET<sub>CO<sub>2</sub></sub></sub>, end-tidal pressure of carbon dioxide; Sp<sub>O<sub>2</sub></sub>, oxyhemoglobin saturation; HR, heart rate; MAP, mean arterial pressure; MCAV<sub>mean</sub>, mean middle cerebral artery blood velocity; CVR, cerebrovascular resistance. \*Significantly different from baseline, †significantly different from hypoxia:  $P < 0.05$ .

sion 500 M, Multigon Industries, Yonkers, NY) was used to measure middle cerebral artery blood velocity (MCAv). The Doppler probe was secured in place with a headband device (Marc 600, Spencer Technologies, Seattle, WA). To ensure a constant angle of insonation, placement of the Doppler probe was performed by the same investigator, and an outline of the subjects' ear, eye, mouth, and probe placement was traced on a transparency film to be used as a guide on the second experimental session. Heart rate was determined with standard three-lead electrocardiography. Isocapnic and poikilocapnic hypoxia exposures were accomplished with a custom-built, computer-controlled end-tidal forcing system. The system analyzes the expirate of each breath to generate the necessary inspirate for the subsequent breath. This was accomplished with solenoid valves, which provided appropriate volumes of 100% O<sub>2</sub>, CO<sub>2</sub>, and N<sub>2</sub> to the inspiratory circuit to maintain PETO<sub>2</sub> at 50 Torr, and end-tidal pressure of carbon dioxide (PETCO<sub>2</sub>) at resting levels (for isocapnic hypoxia) on a breath-by-breath basis. For isocapnic hypoxia, resting PETCO<sub>2</sub> levels were determined from a resting 10-min average.

#### Data and Statistical Analyses

All data were acquired with an analog-to-digital converter (Pow-erlab/16SP ML 795, ADInstruments, Colorado Springs, CO). Data were sampled at 1 kHz and stored on a computer for offline analyses. Beat-to-beat changes in blood pressure were aligned to the corresponding MCAv trace. For the squat-stand maneuver, MAP data were split into bins that spanned every oscillation in blood pressure from baseline (~30 oscillations per squat-stand trial). A signal average of each bin was then constructed to obtain a single average trace of all cycles during the maneuver. From this signal average, the peak (squat) and nadir (stand) response from baseline was determined for all cardiorespiratory measures. A measure of dCA was taken by plotting mean MCAv (MCAv<sub>mean</sub>) as a function of MAP during the squat-stand maneuvers, and the slope of the linear regression was taken to represent dCA gain (i.e., change in MCAv<sub>mean</sub> for a given change in MAP). Thus the dCA gain reports the gain from MAP to MCAv<sub>mean</sub>. The linear regressions were determined separately for the increases and decreases in MAP. Cerebrovascular resistance (CVR) was determined by dividing MAP by MCAv<sub>mean</sub>. Any difference in resting cardiorespiratory measure between test days was determined with a paired *t*-test. To obtain a measure of dCA from bolus injections of vasoactive drugs, CBF values were grouped into 1-mmHg blood pressure bins, and a Pearson product-moment correlation was performed between MAP and MCAv<sub>mean</sub> separately during periods of falling and rising blood pressure. The effect of hypoxia on all cardiorespiratory measures was compared with repeated-measures analysis of variance procedures. When a significant *F* ratio was detected, differences were further investigated with Tukey's post hoc analyses. The level of significance for

all statistical comparisons was set at  $P < 0.05$ . All data are presented as means  $\pm$  SD.

## RESULTS

### Exposure to Hypoxia

Cardiorespiratory measures in the sitting position at baseline, in hypoxia, and in the posthypoxia period are presented in Table 1. By design, the use of an end-tidal forcing system effectively controlled PETO<sub>2</sub> and PETCO<sub>2</sub> in hypoxia (Table 1). Specifically, PETO<sub>2</sub> was 50  $\pm$  1 Torr in isocapnic hypoxia and 50  $\pm$  2 Torr in poikilocapnic hypoxia. Isocapnia was maintained during the isocapnic hypoxia exposure (PETCO<sub>2</sub>: 40  $\pm$  2 Torr), whereas PETCO<sub>2</sub> was reduced in poikilocapnic hypoxia for all subjects (PETCO<sub>2</sub>: 36  $\pm$  4 Torr,  $P < 0.05$ ). In both hypoxia exposures, SpO<sub>2</sub> significantly decreased to ~83%, and this was accompanied with an increase in  $\dot{V}_I$  of almost 30%. There was a significant increase in heart rate in both hypoxic exposures, whereas there was no change in MAP. In isocapnic hypoxia, MCAv<sub>mean</sub> significantly increased; however, it was unchanged during poikilocapnic hypoxia. There was a significant reduction in CVR in isocapnic hypoxia ( $P < 0.05$ ), which was followed by an increase posthypoxia. However, the difference posthypoxia was not statistically significant compared with baseline ( $P > 0.05$ ). However, there was no change in CVR during the poikilocapnic hypoxia trial.

### Squat-Stand Maneuvers

During the repeated squat-stand maneuver, the maximum increase and decrease in MAP was +15 mmHg (during the squat) and -13 mmHg (during the stand), respectively. The mean cardio-respiratory measures during the squat-stand maneuvers are presented in Table 2. Figure 1 demonstrates the oscillations in certain cardio-respiratory measures during the squat-stand maneuver in one representative subject at baseline. As a group, the repeated squat-stand maneuvers caused an increase in  $\dot{V}_I$  and heart rate at baseline, in isocapnic and poikilocapnic hypoxia, and in the posthypoxia periods ( $P < 0.05$ ). In contrast, there was no effect on the average MAP during the maneuvers, despite the increased oscillation amplitude. There was a significant increase in MCAv<sub>mean</sub> during the squat-stands in isocapnic hypoxia (+7 cm/s from baseline), although the MCAv<sub>mean</sub> increase in poikilocapnic hypoxia was small and did not reach statistical significance ( $P > 0.05$ ).

Table 2. Cardiorespiratory measures during the squat-stand maneuver

	Isocapnic			Poikilocapnic		
	Baseline	Hypoxia	Posthypoxia	Baseline	Hypoxia	Posthypoxia
VT, liters	1.5 $\pm$ 0.7	1.9 $\pm$ 1.0	1.2 $\pm$ 0.3†	1.2 $\pm$ 0.3	1.7 $\pm$ 0.7	1.1 $\pm$ 0.1†
Fb, breaths/min	15.1 $\pm$ 4.2	15.3 $\pm$ 5.0	15.5 $\pm$ 4.2	15.7 $\pm$ 4.2	13.9 $\pm$ 4.4	15.9 $\pm$ 4.7
$\dot{V}_I$ , l/min	20.1 $\pm$ 2.2	25.4 $\pm$ 4.5*	17.6 $\pm$ 3.5†	17.5 $\pm$ 3.2	20.2 $\pm$ 3.9	17.8 $\pm$ 4.5
PETO <sub>2</sub> , Torr	99 $\pm$ 5	50 $\pm$ 1*	100 $\pm$ 5†	100 $\pm$ 5	49 $\pm$ 1*	99 $\pm$ 6†
PETCO <sub>2</sub> , Torr	41 $\pm$ 3	42 $\pm$ 3	39 $\pm$ 5	40 $\pm$ 5	38 $\pm$ 5*	39 $\pm$ 5
SpO <sub>2</sub> , %	96 $\pm$ 1	81 $\pm$ 2*	96 $\pm$ 1†	96 $\pm$ 1	81 $\pm$ 3*	96 $\pm$ 1†
HR, beats/min	79 $\pm$ 11	85 $\pm$ 11*	78 $\pm$ 9†	73 $\pm$ 8	82 $\pm$ 10*	77 $\pm$ 8
MAP, mmHg	89 $\pm$ 14	91 $\pm$ 15	91 $\pm$ 13	91 $\pm$ 10	90 $\pm$ 13	91 $\pm$ 11
MCAv <sub>mean</sub> , cm/s	62 $\pm$ 8	69 $\pm$ 6*	59 $\pm$ 8†	60 $\pm$ 11	62 $\pm$ 10	60 $\pm$ 11
CVR, mmHg·cm <sup>-1</sup> ·s <sup>-1</sup>	1.44 $\pm$ 0.14	1.32 $\pm$ 0.16*	1.54 $\pm$ 0.16*†	1.54 $\pm$ 0.21	1.47 $\pm$ 0.20	1.55 $\pm$ 0.21

Values are means  $\pm$  SD. \*Significantly different from baseline, †significantly different from hypoxia:  $P < 0.05$ .

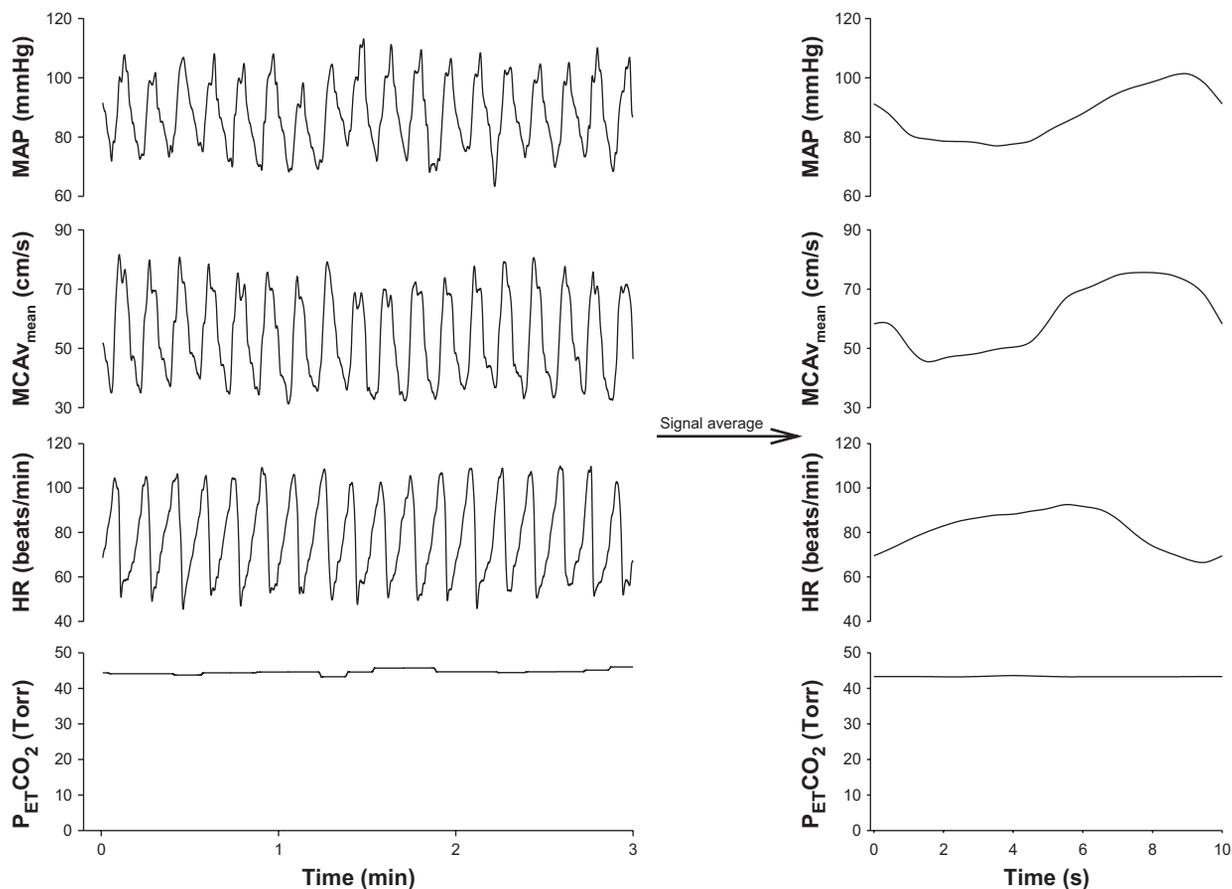


Fig. 1. Example recording from one representative subject during the squat-stand maneuver at baseline. MAP, mean arterial pressure;  $MCAV_{mean}$ , mean middle cerebral artery blood velocity; HR, heart rate;  $P_{ETCO_2}$ , end-tidal pressure of carbon dioxide.

Compared with the squat-stand maneuver performed at baseline, CVR decreased and increased during the squat-stands in isocapnic hypoxia and the posthypoxia periods, respectively (Table 2). In contrast, there was no significant change in CVR from baseline during the squat-stands in poikilocapnic hypoxia, or in the posthypoxia period ( $P > 0.05$ ). dCA gain (i.e., the change in  $MCAV_{mean}$  for a given change in MAP) to hypotension at baseline was  $0.76 \pm 0.25 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$ , and this was not different between test days (gain at baseline on poikilocapnic hypoxia day:  $0.73 \pm 0.20 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$ )

(Fig. 2). In hypertension, the baseline dCA gain was also similar between days (isocapnic hypoxia day:  $0.74 \pm 0.15 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$ ; poikilocapnic hypoxia day:  $0.81 \pm 0.19 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$ ;  $P > 0.05$ ). In isocapnic hypoxia, dCA gain in hypertension and hypotension increased ( $P < 0.05$ ); however, only the hypertension gain was significantly reduced from baseline in the posthypoxia period ( $0.51 \pm 0.14 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$ ,  $P < 0.05$ ). In contrast, hypertension and hypotension dCA gain did not change from baseline in poikilocapnic hypoxia, or following the exposure.

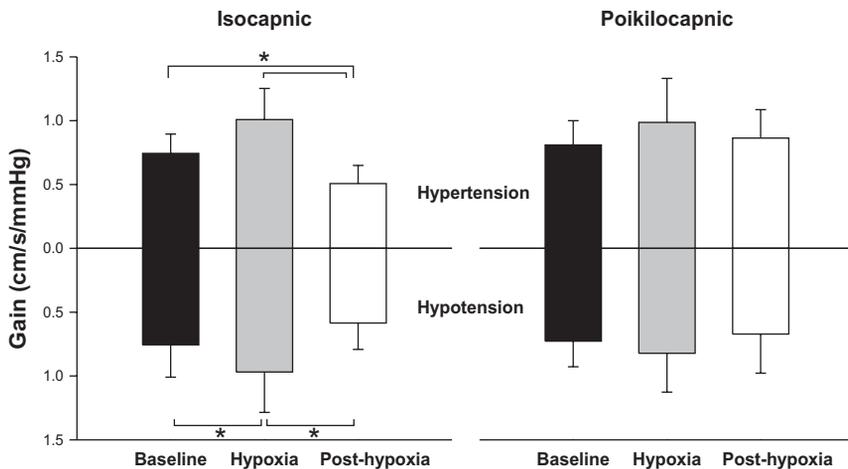


Fig. 2. The effect of isocapnic and poikilocapnic hypoxia on dynamic cerebral autoregulation (dCA) gain. Note that the dCA gain to hypotension is positive. The dCA bars in hypotension are extended below the zero line on the x-axis (dCA gain) simply for visual purposes. Values are means  $\pm$  SD. \* $P < 0.05$ .

### Cerebrovascular Regulation During Drugs Infusions

Within 5 min from the start of the hypoxic exposure,  $SpO_2$  was significantly decreased and maintained at  $82 \pm 3\%$  for the remainder of the exposure. Throughout the protocol,  $PETCO_2$  was held constant relative to baseline levels ( $P > 0.05$ ). During the drug infusions, there was no change in any ventilatory measure from baseline ( $P > 0.05$ ). Intravenous injection of sodium nitroprusside and phenylephrine resulted in a significant reduction ( $-20 \pm 8$  mmHg) and increase ( $+22 \pm 11$  mmHg) in MAP, respectively. The magnitude of the drug-induced changes in MAP did not change posthypoxia ( $P > 0.05$ ). Figure 3 shows the dCA gain during blood pressure perturbations for the group. At baseline, dCA gain to hypotension was  $0.55 \pm 0.11$   $cm \cdot s^{-1} \cdot mmHg^{-1}$  and was not significantly different in posthypoxia ( $0.48 \pm 0.13$   $cm \cdot s^{-1} \cdot mmHg^{-1}$ ,  $P < 0.05$ ). In response to increases in MAP, dCA gain was  $0.32 \pm 0.13$   $cm \cdot s^{-1} \cdot mmHg^{-1}$  at baseline; however, dCA gain to hypertension was significantly reduced from baseline in the posthypoxia period ( $-0.04 \pm 0.16$   $cm \cdot s^{-1} \cdot mmHg^{-1}$ ).

## DISCUSSION

### Main Findings

The primary new finding of the present study is that the impairment of dCA in isocapnic hypoxia is absent when hyperventilation-induced hypocapnia is permitted. Furthermore, measures of dCA demonstrated a reduction in the  $MCAV_{mean}$  response to hypertension following isocapnic hypoxia (i.e., the increase in  $MCAV_{mean}$  for a given increase in MAP was attenuated), changes that did not occur following the poikilocapnic hypoxic condition. These findings reinforce the integrated nature of cerebrovascular control to chemical stimuli and highlight the strong influence of  $CO_2$  on dCA.

### CA in Hypoxia

In the present study, the squat-stand maneuver performed in hypoxia provided a measure of dCA to conditions of both hypotension and hypertension. Compared with baseline, dCA gain increased in isocapnic hypoxia, leading to cerebral blood velocity that was much more pressure passive. Acute exposure to isocapnic hypoxia leads to dilation of small pial cerebral vessels (12), also accompanied with an increase in peripheral

vasoconstrictor sympathetic outflow (19, 25, 38). Previous investigations have implicated a strong neural influence on dCA, given that eliminating neural control of cerebral vessels with a ganglion blockade impairs indexes of dCA (3, 40). Also, reducing cerebral vessel tone with hypercapnia reduces the effectiveness of dCA (1, 33), suggesting that adequate cerebral vessel tone is required for effective dCA. Thus, although the hypoxia-induced sympathoexcitation in isocapnic hypoxia may work to improve dCA in the present study, the concurrent hypoxia-induced dilation of cerebral arterioles may have counteracted the sympathetically mediated constriction. Overall, this would lead to an inability to buffer sudden changes in cerebral perfusion, resulting in an increase in dCA gain. The hypocapnia that accompanies poikilocapnic hypoxia leads to a chemical stimulus that causes cerebral vessel constriction. This hypocapnia-induced arteriole constriction in poikilocapnic hypoxia could have played a role in maintaining cerebral vessel tone and prevented the impairment in dCA observed in isocapnic hypoxia.

Consistent with the present study, Ogoh et al. (22) found dCA impairment in mild isocapnic hypoxia, but not in poikilocapnic hypoxia. However, important methodological differences between the work of Ogoh et al. and the present study makes comparison difficult. First, in the study by Ogoh et al., hyperventilation in poikilocapnic hypoxia led to an increase in  $PETO_2$ , which was significantly different from isocapnic hypoxia. Consequently,  $SpO_2$  was also significantly elevated in poikilocapnic hypoxia compared with isocapnic hypoxia. Previous research has shown the impairment in hypoxic dCA is dependent on the severity of the hypoxic exposure (14); therefore, comparing dCA between different levels of hypoxia ( $PETO_2$  and  $SpO_2$ ) is problematic. In the present study,  $PETO_2$  was tightly controlled in both hypoxic exposures with the use of a computer-controlled end-tidal forcing system that adjusted gas concentrations of the inspirate from breath-by-breath feedback. As a result, subjects in our study were exposed to identical levels of hypoxia between the two testing sessions. Second, Ogoh et al. (22) assessed dCA with the rapid thigh-cuff deflation method, a technique that produces a sudden reduction in MAP. Although this technique has been used in previous studies (1), assessing dCA based on only hypotension (or hypertension) does not fully characterize dCA (34). In contrast, the present study used a protocol that includes both hypertensive and hypotensive challenges and permits separating the two

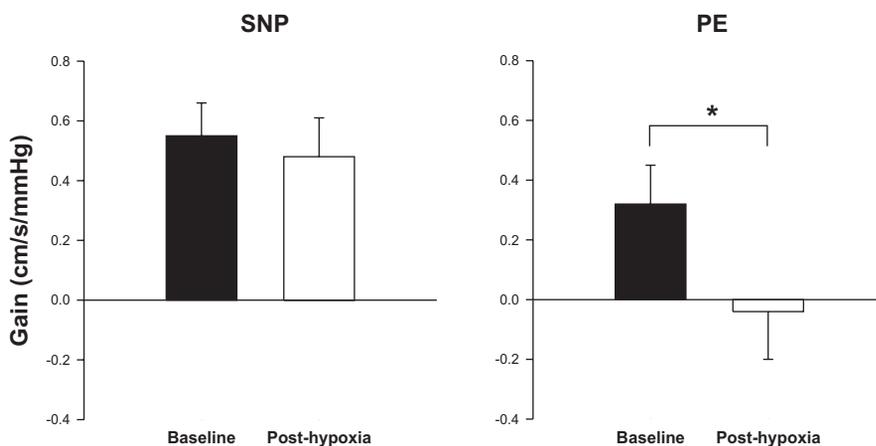


Fig. 3. Group mean data of relative changes in  $MCAV_{mean}$  with changes in MAP during bolus injections of sodium nitroprusside (SNP) and phenylephrine (PE). Values are means  $\pm$  SD. \* $P < 0.05$ .

responses. Another methodological benefit in the present study was the assessment of dCA following the hypoxia exposures, which has demonstrated a “rebound” of dCA to hypertension that exceeds baseline following isocapnic hypoxia, but no effect following poikilocapnic hypoxia.

### CA Following Acute Hypoxia

Pathological models of hypoxia suggest the hypoxia-induced impairment in dCA outlasts the hypoxia stimulus. In particular, patients with OSA demonstrate a coupling of  $MCA_{v_{mean}}$  and MAP during hypoxic apneic events while sleeping (4). The coupling between  $MCA_{v_{mean}}$  and MAP persists during the day, while the patient is eupneic and normoxic (21). Accordingly, it was anticipated that dCA would be impaired following the isocapnic hypoxia exposure in the present study. However, the opposite was found: a pressure-passive system at baseline (impairment) and improvement posthypoxia. The reason for the inconsistency in the present results from those in pathological models is unknown, but differences in our acute hypoxia exposure protocols to that of the long-term intermittent hypoxic/hypercapnic paradigms of OSA could partly explain the discrepancy (7, 23, 31).

One of the new findings of the present study highlights the importance of  $CO_2$  control in acute hypoxia studies. That is, there was no change in dCA following poikilocapnic hypoxia, which is in contrast to the reduced gain following isocapnic hypoxia. To our knowledge, this is the first study to focus on dCA following an acute hypoxia exposure of different  $CO_2$  backgrounds. Although this study was not designed to specifically investigate the mechanism for the effect of different  $CO_2$  backgrounds, the balance between hypoxia-induced vasodilators and neural influences may play a role in dCA. Specifically, following isocapnic hypoxia, sympathetic activity remains elevated (25, 38), which could be responsible for the reduction in dCA gain given that autonomic neural control of the cerebral circulation has been suggested to be tonically active and plays an important role in buffering sudden change in perfusion (40). Not only is muscle sympathetic nerve activity elevated following isocapnic hypoxia, but the sensitivity of vasomotor outflow is also increased (albeit to chemical stimuli) (20). Taken together, the hypoxia-induced increase in sympathetic outflow that outlasts the isocapnic hypoxia exposure could provide a protective mechanism for cerebral vessels to more effectively buffer sudden increases in perfusion. Thus, when the hypoxia exposure is terminated, along with the local vasodilatory influence, sympathoexcitation persists and could represent a mechanism for the improvement in dCA demonstrated in the present study. In contrast, others have found muscle sympathetic nerve activity and forearm vascular resistance to return to baseline following poikilocapnic hypoxia (31). Thus hypocapnia, as a sympathoinhibitor (8), may counteract the persistent sympathoexcitation from hypoxia. It is important to note that peripheral measures of vascular function may not represent autonomic control in the cerebrovasculature; however, the results do provide insight into the possible mechanisms responsible for the findings in the present study.

### Methodological Considerations

A transcranial Doppler was used to measure beat-by-beat changes in CBF  $MCA_v$ . A limitation of this method in

estimating CBF is that any change in MCA diameter may lead to a change in velocity, but not in flow. However, the technique has been validated during exposures to a range of  $O_2$  and  $CO_2$  pressures and simulated orthostasis (9, 24, 29, 36, 37). It is possible that infusion of the vasoactive drugs had a direct effect on the diameter of the MCA. However, previous studies have demonstrated that intra-arterial infusion of nitroprusside or phenylephrine does not cause any relevant change in MCA diameter (9, 15). It has been suggested that the integrity of the blood-brain barrier eliminates a possible influence of these drugs on cerebral vessels of different sizes (9, 15). Therefore, it is likely that the observed changes (or lack thereof) in  $MCA_{v_{mean}}$  measured via the transcranial Doppler represented real changes in CBF. Nonetheless, the present experimental design did not include direct measures of cerebral vessel diameter, and a direct effect of drug infusion on the cerebral vessels remains a possibility. Furthermore, the transcranial Doppler measured CBF velocity only in the MCA, which did not allow investigation into possible regional differences in cerebral perfusion. Previous studies have shown certain cerebral vessels are more susceptible to changes in posture (11), large alterations in arterial blood gases (37), and moderate-to-high-intensity exercise (28, 30). Thus our results only pertain to dCA in the MCA, and further work is needed to determine whether other cerebral vessels, such as those in the posterior cerebral circulation, respond in a similar manner. Lastly, the regulation of CBF is multifactorial; thus subsequent investigations that are focused on other mediators of CBF, such as cardiac output, metabolites, and cerebral neural outflow, would be worthwhile.

We used hypoxia protocols that have been shown to cause significant adjustments in certain cardiorespiratory measures both during and following the exposure (19, 25, 26, 38). While dCA was assessed at one time point following the termination of the hypoxia exposures (i.e., 5 min), future research focusing on the time course of the physiological adjustments posthypoxia would provide a greater understanding of the long-term effects of acute hypoxia. Furthermore, although we consider the data from the modified Oxford technique complement the squat-stand maneuver data by assessing dCA during more gradual fluctuations in blood pressure, subsequent research with additional serial measurements using the modified Oxford technique that includes a poikilocapnic hypoxia component would be useful.

### Conclusion

In the present study, dCA in isocapnic hypoxia was impaired, whereas there was no change in poikilocapnic hypoxia compared with baseline. The maintenance of dCA in poikilocapnic hypoxia is likely due, at least in part, to the hyperventilation-induced hypocapnia, which increases cerebral vessel tone and counteracts the hypoxia-induced dilation. Furthermore, while dCA gain to hypertension is reduced following isocapnic hypoxia, there is no effect following poikilocapnic hypoxia. This study highlights the integrated nature of cerebrovascular control and suggests a potential protective effect of hypocapnia on dCA in and following acute hypoxia.

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## DISCLOSURES

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

## AUTHOR CONTRIBUTIONS

Author contributions: J.S.Q., P.N.A., N.T.A., and A.W.S. conception and design of research; J.S.Q., G.E.F., and W.R.H. performed experiments; J.S.Q. analyzed data; J.S.Q., P.N.A., G.E.F., W.R.H., J.R.H., N.T.A., and A.W.S. interpreted results of experiments; J.S.Q. prepared figures; J.S.Q. drafted manuscript; J.S.Q., P.N.A., G.E.F., W.R.H., J.R.H., N.T.A., and A.W.S. edited and revised manuscript; J.S.Q., P.N.A., G.E.F., W.R.H., J.R.H., N.T.A., and A.W.S. approved final version of manuscript.

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