INTERMITTENT HYPOXIA: CARDIORESPIRATORY AND CEREBROVASCULAR CONSEQUENCES TO ACUTE HYPOXIA AND SUBMAXIMAL EXERCISE

by

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ABSTRACT

Intermittent hypoxia (IH) is broadly defined as repeatedly breathing decreased amounts of oxygen (hypoxia) interspersed with periods of room air breathing (normoxia). In animal, human diseased, and healthy human models, research has shown IH to negatively affect cerebrovascular vessel dilation. We have previously shown poikilocapnic (uncontrolled carbon dioxide (CO_2)) IH to blunt the vasodilatory response of a cerebral vessel during acute hypoxia. The purpose of this study was to measure the ventilatory, cardiovascular and cerebrovascular responses to: I) acute hypoxia and; II) to submaximal exercise following an isocapnic (controlled CO_2) IH protocol. Healthy males (n = 9) with normal pulmonary function underwent 10 consecutive days of isocapnic IH (oxyhaemoglobin saturation (SaO_2) = 80%, 1 hr/day). Ventilatory, cardiovascular, and cerebrovascular (transcranial Doppler) responses to acute isocapnic hypoxia (SaO_2 = 80%, 5 minutes) were measured before (PRE-IH) and after (POST-IH) IH. Also, ventilatory, cardiovascular, and cerebrovascular parameters were measured during a submaximal cycle exercise test (50, 100, 150 watts) PRE-IH and POST-IH. To further investigate cerebrovascular regulation during exercise, 5% CO_2 was added for two minutes of each exercise stage. Over the 10 days of IH, there was a significant increase in minute ventilation (V_E) during the IH bouts ($p<0.05$). IH did not significantly alter the ventilatory, cardiovascular, and cerebrovascular responses to acute hypoxia. However, there was a significant association ($r = 0.86$, $p<0.05$) between the change in the mean arterial blood pressure (MAP) and mean middle cerebral arterial blood flow velocity (MCAVm) responses to acute hypoxia. Exercise caused significant increases in V_E, MCAVm, and MAP ($p<0.05$), but there were no differences in measured variables between PRE-IH and POST-IH exercise trials ($p>0.05$). Similarly, hypercapnia caused significant increases in V_E and MCAVm ($p<0.05$), although the magnitude of the response did not change following IH. Our results suggest that the effect of IH on ventilatory, cardiovascular, and cerebrovascular regulation during acute hypoxia is

individualistic, and changes in the MAP response may strongly influence the changes in cerebral blood flow (CBF). Also, our results suggest that IH does not alter ventilatory, cardiovascular, or cerebrovascular regulation during submaximal exercise or responsiveness to hypercapnia.

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INTRODUCTION

Acute hypoxia causes abrupt increases in minute ventilation ($\dot{V}E$), mean arterial blood pressure (MAP), muscle sympathetic nerve activity (MSNA) , and cerebral blood flow (CBF) (7, 95). When hypoxia exposure becomes long-term or chronic, it initiates changes in other physiological parameters. Commonly experienced by mountaineers, residents of high-altitude, and athletes as part of an intensive high altitude training regimen, the effect of hypoxia also has many long-term ventilatory (54), cardiovascular (4), and haemotological (36) consequences. Indeed, the study of acute and chronic hypoxia has shown a diverse outcome to this type of environment; some of which can aid in athletic performance (72), others that can possibly be fatal (e.g. high altitude cerebral edema) (21).

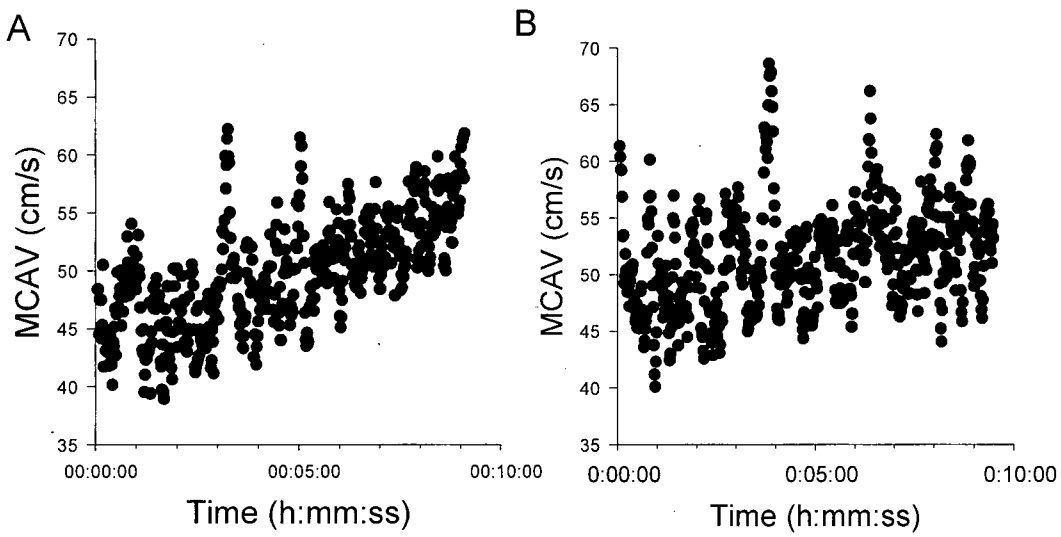
Hypoxia is particularly stressful on the brain, since cerebral tissue is intolerant of ischaemia; thus adequate CBF is essential. Traditionally, it was thought that CBF remained steady under all types of activity; however, there is clear evidence showing regional changes in CBF during different types of cerebral activity (e.g. mental stimulation, exercise) (58, 62). In general, CBF increases in response to hypoxia (48). This finding, although widespread, is affected by additional factors. Most notably, the confounding effect of carbon dioxide (CO_2) which is a strong determinant of the effect of hypoxia on CBF (7). When CO_2 is not controlled, poikilocapnic hypoxia has been shown to mask the increase in CBF, which is attributed to the hyperventilation induced hypocapnia (7). In fact, the responsiveness by cerebral vasculature to CO_2 is of such significance, that CO_2 is considered by many to be the major regulator of CBF (12). Similar to hypoxia, hypercapnia dilates cerebral vessels, resulting in an increase in CBF. An increase in CO_2 is thought to affect increases in cerebral vessel diameter by its simple diffusion through the blood brain barrier into the cerebrospinal fluid (CSF) (48). There, it dissociates into H^+ and HCO_3^-, after which H^+ produces a relaxant effect on cerebral vessels. In contrast, hypocapnia (67), and possibly hyperoxia (11) cause decreases in CBF by

vasoconstriction of cerebral vessels. Collectively, cerebral chemoregulation refers to the dynamic maintenance of oxygen delivery, and adequate CO_2 removal from cerebral tissue.

It is important to note that hypoxia is commonly experienced in short, repeated bouts (e.g. sleep apnea); rather than a continuous exposure (e.g. residence at high altitude). The physiological magnitude that hypoxia patterning possesses has recently been appreciated (33, 112). Further, not only is hypoxic patterning of prime importance, but a key contributing factor, CO_2, can influence the magnitude of the hypoxic response. In order to evaluate the effect of hypoxia, or IH, it is common to test the response to acute hypoxia. In general, IH causes increases in the ΔVe (33, 75, 78), ΔMAP (78), and ΔMSNA (95) response to acute hypoxia, while no change is observed in heart rate (HR) sensitivity (78). Recent research has shown rats that have been exposed to 10 days of repeated bouts of hypoxia (15s of 5% O_2 at 5 minute intervals, 9 episodes/hr, 8hr/day) to have an increased carotid body sensitivity to acute hypoxia, when compared to rats who experienced less iterations in their hypoxic exposure (112). Further, IH has been shown to increase hypoxic MSNA in the human model, attributed to a greater burst frequency of the peroneal nerve (95). These results are in line with data in rats which show IH to cause an increase in daytime MAP (31, 78), although the causal relationship between MSNA and limb arteries is undefined (117). These findings extend to vessel function. Phillips et al. (113) investigated the effects of IH (FiO_2 = 10% for one minute at four minute intervals, 12 h/day, 14 days) on isolated peripheral (gracilis artery) and cerebral (middle cerebral artery (MCA)) vessels in rats. Compared to control rats, the vasodilator response of both cerebral and peripheral vessels to a hypoxic bath was almost completely abolished. These findings have been substantiated in a diseased, human model. Sleep apnea is a condition defined by repetitive nightly apneas, thereby rendering the individual repeatedly hypoxic and hypercapnic. Sleep apnea patients are at a higher risk of many other ailments such as elevated daytime sympathetic nervous activity (20), hypertension (31), and stroke (26, 110). The typical IH that is experienced by this patient

population implies a key role of oxygen/reoxygenation for the increased risk of stroke (142). Foster et al. (32) found the CBF response to acute hypoxia to be reduced in sleep apnea patients when compared to healthy human controls. However, elimination of sleep apnea via continuous positive airway pressure normalized the CBF response in these patients (32). Also, we have shown data to support the key role of IH in the development of cerebral dysregulation in a healthy human model. Ten days of isocapnic IH attenuated cerebrovascular regulation, such that following IH there was a greater decrease in cerebral tissue oxygenation (near-infrared spectroscopy (NIRS)) for a given decrease in oxyhaemoglobin saturation (SaO_2) during acute hypoxia (33). More recently, we extended these results by measuring CBF. We found that 10 consecutive days of poikilocapnic hypoxia attenuated the increase in CBF (transcranial Doppler ultrasonography) to acute hypoxia (Figure 1).

Figure 1. Middle cerebral arterial blood flow velocity (MCAV) response to acute progressive hypoxia before (A) and after (B) 10 consecutive days of intermittent hypoxia (12% O_2 for 5 min, at 5 min intervals, for 1 hr/day) in one subject. Each dot represents MCAV during a cardiac cycle.



Collectively, the results from animal, human diseased, and healthy human models have suggested the role of reactive oxygen species (ROS) in the development of endothelial dysfunction. It has been proposed that IH causes oxidative stress which in turn increases the

production of ROS. Excess production of ROS has been suggested to decrease the bioavailability of nitric oxide (NO), an endothelium-derived relaxing factor (113, 141). However, cerebral dysregulation following IH is not a universal finding. Kolb et al. (84) found an increase in the CBF response to acute hypoxia following five nocturnal hypoxic exposures (FiO_2 ~13.8%). These results were recently supported by Ainslie et al. (2) who found the CBF response to acute poikilocapnic hypoxia to be augmented following 10-12 days of poikilocapnic IH (FiO_2 = 12% for 5 min at 5 min intervals, 90 min/day).

Vessel dilation to hypoxia is partly mediated by the vasodilatory action of NO. Blitzer and colleagues (13) measured the effect of N^G-monomethyl-L-arginine (L-NMMA), a NO synthase antagonist, on forearm blood flow during hypoxia (FiO_2 = 13.9%). The administration of L-NMMA during hypoxia caused an approximate 34% decrease in forearm blood flow (13). Additionally, NO plays a key role in the vasodilation of cerebral vessels to hypercapnia (132). With the use of the same NO blocker (L-NMMA), Iadecola (55) found the increase in CBF (rat brain) to hypercapnia (5% CO_2) to be decreased by 44%, in comparison to hypercapnia alone. In the human model, individuals with endothelial dysfunction (e.g. diabetes mellitus, hypertension) also have a blunted vessel reactivity to hypercapnia (5% CO_2), and infusion of a NO donor normalized the patients' reactivity. This study suggested the blunted CO_2 reactivity to be a consequence of an altered vascular endothelial production of NO (89).

In addition to hypoxia and hypercapnia, CBF responds to other stimuli, which includes exercise. Although early reports provided somewhat inconsistent conclusions (39, 81, 131, 154), more recent evidence points to a clear increase in CBF to exercise (34, 49, 53, 70, 71, 93, 101, 115-117, 144). The increase is intensity dependent (70, 101), with the magnitude of the change contingent on the vessel insonated (49). Increases in exercise intensity cause an increase in CBF up to a certain exercise intensity (~60%VO_{2max}), after which CBF decreases towards baseline values (sometimes decreasing below baseline values) with increasing exercise

intensity (49, 101). This is most likely attributed to a hyperventilation induced decrease in arterial partial pressure of CO_2 ($PaCO_2$) (93, 101). Exercise results in an increase in blood flow to working muscles and the brain, increasing in shear stress on vessel endothelium. This shear stress initiates the release of NO, which in turn instigates vessel dilation (130). Inhibition of NO during exercise by infusion of L-NMMA greatly reduces exercising blood flow, suggesting a key role in vessel dilation during exercise to the release of NO by the endothelium (38). Similar to the CBF response to hypoxia, the regulation of CBF during exercise is strongly contingent upon the accompanying contribution of CO_2. In fact, the typical increase in CBF during exercise can be completely abolished after correcting for the increase in end tidal partial pressure of carbon dioxide ($PETCO_2$) (16).

Although we have shown IH to cause cerebral dysregulation to acute hypoxia at rest, this was shown to occur in an IH protocol where CO_2 was uncontrolled. Currently, the contribution of CO_2 during IH, whether it be isocapnic or poikilocapnic, is uncertain. In addition, recent reports have created discrepancy in the literature regarding the effects of IH on cerebrovascular regulation during acute hypoxia. Also, it is not known if cerebrovascular dysregulation to acute hypoxia following IH persists under physiological conditions such as exercise. Most of the available literature suggests IH to cause vessel dysregulation, which is likely due to decreased bioavailability of NO, suggesting endothelial dysfunction. If these ideas can be extended to CBF during exercise, it may point to possible health risks for certain patient populations who undertake exercise programs, or healthy athletes who undergo IH as part of a high-altitude training regimen.

The purpose of this study was twofold. First, we sought to determine if 10 consecutive days of isocapnic IH would cause changes in the regulation of ventilatory, cardiovascular, and cerebrovascular variables to acute hypoxia. Second, we investigated the effect of the IH on the control of ventilatory, cardiovascular, and cerebrovascular variables during submaximal exercise.

Hypothesis

It was hypothesized that in comparison to baseline, 10 days of isocapnic IH would result in:

1) An increased V_E, and MAP response to acute hypoxia, and a blunted CBF response to acute hypoxia

2) A blunted CBF response to submaximal exercise

3) A reduced CBF response to hypercapnia at rest and exercise

METHODS

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. All testing procedures occurred in the Health and Integrative Physiology Lab on The University of British Columbia campus.

Subjects

A total of nine males consented to participate in the study. Females were excluded from participation, as oscillations in female sex hormones throughout the menstrual cycle influence CBF and ventilatory control in hypoxic conditions (86, 126). All subjects were non-smoking, healthy individuals, in the low risk category for exercise as defined by the American College of Sports Medicine (8).

Experimental Protocol

Each subject signed a consent form and filled out a Physical Activity Readiness Questionnaire, and underwent a familiarization session where all experimental procedures were completed. On a separate day, subject's response to acute hypoxia, submaximal exercise, and CO_2 reactivity was performed. Following the tests, subjects began the 10-day IH protocol. The day following the last IH session, subject's response to hypoxia, submaximal exercise and CO_2 reactivity were re-assessed as per Day 1.

Acute Hypoxic Response (AHR)

Subjects performed the AHR while in a semi-recumbent position, in a quiet room, with minimal auditory and/or visual stimuli. Following instrumentation, subjects had a 10-minute rest period to ensure they were in a resting state. Next, SaO_2 was decreased to 80% over an

approximate 2-3 minute time period by manual addition of N_2 to the inspiratory circuit and maintained for an additional 5 minutes. The test was maintained isocapnic (resting $P_{ET}CO_2$ values for that day) by the manual addition of 100% CO_2 as needed. The measured variables were averaged during the last minute of rest, and the last minute of hypoxia; the absolute difference between the two was taken to represent the AHR for a given physiological variable (e.g acute hypoxic response of ventilation = AHR_{V_E}).

Exercise Protocol

Identical exercise trials were performed in a normoxic environment on a semi-recumbent cycle ergometer (CombiCycle EX80, COMBI Corporation, Japan) one day prior to, as well as one day following the IH protocol. Subjects' feet were strapped to the pedals in order to maintain tight and constant contact as to minimize movement of the upper body. A five-minute rest period on the cycle ergometer preceded any exercise. An average of all measured variables was taken during the last 30 seconds of rest and was used to represent baseline. Following the 5-minute rest period, CO_2 reactivity was measured at rest (described below). Following the CO_2 reactivity at rest, subjects were given one minute to recover. Next, subjects began pedaling at a constant rate to a metronome set at 60 Hz, and at a work rate of 50 watts (W); this workload was maintained for six minutes. Subsequent workloads were performed at the same cadence at 100 W and 150 W; each lasting six minutes. During exercise, CO_2 reactivity was tested over the fourth and fifth minutes at each workload. Measurements continued in recovery for 5 minutes following the last exercise intensity. This protocol was used in order to invoke a measurable increase in CBF, while minimizing subjects' head movement at higher intensities which would compromise the measurement of CBF.

CO_2 Reactivity

In order to test ventilatory, cardiovascular and cerebral CO_2 reactivity, subjects were administered a hypercapnic gas mixture (5% CO_2, 21% O_2, and 74% N_2) via the inspired end of the non-rebreathing mouthpiece. Ventilatory, cardiovascular and cerebral CO_2 reactivity (e.g. ventilatory response to carbon dioxide = $V_{E_{CO_2}}$) were assessed following the 5-minute rest period, as well as during the fourth and fifth minute of each exercise intensity. Administration of the hypercapnic gas continued for a total of two minutes at each exercise intensity. The subjects were given a one-minute 'recovery' period following the administration of CO_2 before continuing on to the next stage.

Intermittent Hypoxia

Intermittent hypoxia consisted of a one hour daily exposure where SaO_2 measured with finger pulse oximetry (Criticare Systems Inc., 504 Series, Waukesha, WI) was maintained at 80%, for 10 consecutive days. Nitrogen was titrated into the inspired end of a non-rebreathing facemask in order to maintain SaO_2 at 80%. Isocapnia was maintained by the manual addition of 100% CO_2 as needed. Subjects completed the intermittent hypoxia sessions in a sitting position, and were free to use computers, watch movies, or read throughout the session. We have previously shown similar IH protocols to cause changes in ventilatory, cardiovascular, and cerebrovascular variables during acute hypoxia (33, 95).

Cerebral Blood Flow Velocity

Cerebral blood flow velocity (CBFV; an indication of cerebral blood flow) was measured throughout the pre- (PRE-IH) and post-testing (POST-IH) sessions with a 2 MHz pulsed Transcranial Doppler ultrasound (Neurovision, Multigon Industries Inc., Yonkers, N.Y.). CBFV was calculated from the maximal frequency of the Doppler shift, which is assumed to represent

the mean velocity in the centre of the vessel. The middle cerebral artery was insonated via the temporal window, directly superior to the zygomatic arch. The ultrasound probe was held in place with the use of a fixation head frame (Marc 600, Spencer Technologies, Seattle, WA) in order to maintain the position and angle of insonation throughout testing. Ultrasound gel (Aquasonic 100, Parker Laboratory Inc., Fairfield, New Jersey) was applied to the subjects' skin as well as the ultrasound probe before the experimenter located the segment of the middle cerebral artery to be insonated. The ultrasound probe was placed by the experimenter and adjusted by varying the angle and position of the ultrasound probe. Once a satisfactory signal was obtained, an outline of the subjects' ear, eye, mouth, and the placement of the probe was traced on a transparency film and used as a guideline for probe placement after IH (POST-IH). Ultrasound gel was then re-applied to the area of insonation and on the ultrasound probe, and the probe was placed in the ultrasound probe head fixation device. The experimenter re-located the area of the MCA insonated by decreasing the sample volume depth (originally at 6.0 cm) in incremental steps (0.1 cm), while varying the insonation angle at each step to obtain the best quality signal (121). The step-wise decrease in sample volume depth continued until the signal quality decreased. The lowest depth with an optimal Doppler signal was used for experimentation. An average of the last 30 seconds of the third minute at each exercise intensity was used to represent CBF for that exercise stage. Placement of the Doppler probe was conducted by the same investigator (J. S. Querido) for all tests, and the protocol used to find the MCA was identical to search techniques used by other laboratories (121).

Ventilatory Parameters

Inspiratory flow was recorded throughout experimentation and obtained using a heated pneumotach connected to the inspired side of a mouthpiece (AHR and exercise) and nasal breathing facemask (intermittent hypoxia). The mouthpiece replaced the mouth and nasal

breathing facemask during exercise in order to maintain a tighter seal around the mouth without obstructing placement of the Doppler probe. Inspiratory flow signals were integrated to determine volume and then multiplied by breathing frequency to obtain inspired minute ventilation. Inspired and expired fractions of oxygen (O_2) and CO_2 were continuously monitored (O_2: S-3A/I; CO_2: CD-3A, AEI Technologies Applied Electrochemistry, Pittsburgh, Pennsylvania).

Cardiovascular Parameters

Beat-by-beat systolic blood pressure (SBP), diastolic blood pressure (DBP), MAP, cardiac output (Q), as well as stroke volume (SV) were obtained throughout all experimentation by using finger pulse photoplethysmography (Finometer, Finapres Medical Systems, Arnhem, the Netherlands). Heart rate (HR) was calculated off-line from the arterial blood pressure trace. Prior to testing with the finger pulse photoplethysmograph, a return-to-flow systolic calibration was performed and the hydrostatic height sensor system was zeroed. The arm used to obtain blood pressure was placed on a platform at heart level in order to ensure the arm remained relaxed throughout testing.

Data and Statistical Analysis

All variables were sampled at 100 Hz using an analog-to-digital data acquisition system (Powerlab/16SP model ML795, ADIInstruments, Colorado Springs, CO) and analyzed with commercially available software (Chart V5.02, ADInstruments, Colorado Springs, CO). Paired t-tests were conducted to assess any changes is baseline physiological measures on the PRE-IH and POST-IH experimental days.

IH. A one-way repeated measures ANOVA was conducted for each variable over the 10 days of IH to asses the effect of IH on baseline variables. Also, a one-way repeated measures

ANOVA was conducted on measured variables during the IH sessions (averaged over the last 55 minutes of each session). Investigation into the change (from normoxic rest to IH) in all measured variables during the IH over the 10 days was also done through the use of a one-way repeated measures ANOVA. If the test showed statistical significance, a Dunnett test was conducted to further investigate where (on which day) the difference occurred.

AHR. The possible effect of IH on the AHR was investigated with a paired samples t-test. A Pearson product-moment was implemented to determine the relationship between selected variables during the PRE-IH AHR, POST-IH AHR, and the difference between PRE-IH to POST-IH.

Exercise. A one-way repeated measures ANOVA was used in the PRE-IH, as well as the POST-IH exercise trials to investigate the effect of exercise intensity on measured variables. When the statistical test revealed significance, a Tukey's post hoc analysis was performed to investigate where the difference occurred. 2 (PRE-IH/POST-IH) X 9 (exercise intensity) repeated measures ANOVA was utilized to investigate any effect of IH on exercising variables.

CO_2 reactivity. One-way repeated measures ANOVAs were used to investigate the effect of hypercapnia on measured variables during the PRE-IH, and POST-IH trials. In order to investigate the difference between room air and hypercapnia in the PRE-IH and POST-IH trials, a one-way repeated measures ANOVA was conducted at each exercise intensity.

All data are presented as mean ± SD. A level of significance of .05 was set for all statistical analyses.

RESULTS

Subject Characteristics

There was 100% compliance by subjects but one subject only completed 12 minutes of the second intermittent hypoxia session due to sensations of presyncope. Subjects' characteristics and spirometry values are presented in Table 1. All subjects were young, of normal height and weight. Seven of the nine subjects had BMI's within the normal range (18.5-24.9 kg/m^2), whereas two subjects had BMI's in the overweight range (25.0-29.9 kg/m^2). Nonetheless, none of the subjects BMI's would have been considered in the obese range.

Table 1. Subject characteristics. BMI = body mass index; FVC = forced vital capacity; $FEV_{1.0}$ = forced expiratory volume in 1 second

Age (yrs)	23.6 ± 2.4
Height (cm)	180.6 ± 6.6
Mass (kg)	77.2 ± 10.4
BMI (kg/m^2)	23.6 ± 2.0
FVC (L)	5.4 ± 0.9
$FEV_{1.0}$ (L)	4.5 ± 0.6
$FEV_{1.0}$/FVC (%)	83.5 ± 4.9
$FEV_{1.0}$/FVC (% predicted)	98.4 ± 5.7

Effect of IH on Baseline Physiological Measures

Between the two experimental days, there were no significant differences in resting physiological measures; furthermore, there were no significant differences between resting measures before the AHR and exercise tests (Table 2). Inevitably, there was minor day-to-day variability, although it was not in excess of what was expected or reported by others (2, 120). Evaluation of the impact of IH on baseline ventilation revealed that although PRE-IH ventilation (9.5 ± 1.7 L/min) was slightly higher than baseline ventilation POST-IH (8.4 ± 2.1 L/min), the difference did not reach statistical significance ($t(8) = 1.33$, $p>0.05$). Likewise, there was no difference in baseline $P_{ET}CO_2$ between PRE-IH and POST-IH ($t(8) = 0.05$, $p>0.05$). MCAVm

was not statistically different on experimental days ($t(8) = -1.45$, $p>0.05$), nor were differences detected in baseline MAP ($t(8) = -.07$, $p<0.05$), HR ($t(8) = .38$, $p>0.05$), SV ($t(8) = -.24$, $p>0.05$), or Q ($t(8) = .23$, $p>0.05$) between experimental days.

Table 2. Baseline ventilatory, cardiovascular, and cerebrovascular variables on PRE-IH and POST-IH test days. V_E = minute ventilation; $P_{ET}CO_2$ = end tidal partial pressure of carbon dioxide; MCAVm = mean middle cerebral arterial velocity; SV = stroke volume; HR = heart rate; Q = cardiac output; SaO_2 = oxyhaemoglobin saturation.

	PRE-IH	POST-IH	CV (%)
V_E (L/min)	9.5 ± 1.7	8.4 ± 3.3	19.4 ± 14.4
$P_{ET}CO_2$ (mmHg)	42.3 ± 2.2	42.3 ± 3.0	3.0 ± 1.8
MCAVm (cm/s)	64.2 ± 8.6	65.8 ± 10.4	2.7 ± 2.5
MAP (mmHg)	92.4 ± 5.8	92.6 ± 7.4	4.4 ± 2.3
SV (ml)	102.6 ± 18.9	103.7 ± 22.6	7.7 ± 6.9
HR (bpm)	61.2 ± 9.4	60.5 ± 10.8	5.7 ± 3.1
Q (L/min)	6.2 ± 1.2	6.2 ± 1.3	6.5 ± 5.4
SaO_2 (%)	97.8 ± 0.6	97.8 ± 0.7	0.3 ± 0.2

Intermittent Hypoxia

Intermittent hypoxia did not cause any significant change in baseline variables throughout the 10 days of IH (Table 3).

Table 3. Baseline variables throughout the 10 days of IH. V_E = minute ventilation; $P_{ET}CO_2$ = end tidal partial pressure of carbon dioxide; MAP = mean arterial blood pressure; SV = stroke volume; HR = heart rate; Q = cardiac output

	V_E	$P_{ET}CO_2$	MAP	SV	HR	Q
	L/min	mmHg	mmHg	ml	bpm	L/min
Day 1	11.1 ± 3.7	40.3 ± 1.7	94.2 ± 4.0	92.7 ± 22.1	67.4 ± 12.6	6.1 ± 1.4
Day 2	11.5 ± 1.8	40.8 ± 2.3	92.3 ± 7.9	91.0 ± 19.7	64.8 ± 11.8	5.9 ± 1.6
Day 3	12.7 ± 3.9	40.2 ± 2.9	94.3 ± 9.3	89.1 ± 16.1	72.4 ± 11.5	6.5 ± 1.6
Day 4	12.2 ± 1.7	39.7 ± 3.3	91.9 ± 9.2	94.1 ± 20.9	68.7 ± 8.8	6.4 ± 1.5
Day 5	13.6 ± 2.6	40.8 ± 2.9	95.5 ± 7.2	97.3 ± 22.4	71.3 ± 11.4	6.8 ± 1.7
Day 6	13.3 ± 2.4	38.9 ± 3.3	94.0 ± 7.3	92.4 ± 25.8	67.9 ± 9.0	6.3 ± 1.9
Day 7	13.4 ± 2.1	39.9 ± 2.2	95.5 ± 3.0	90.8 ± 17.1	72.1 ± 10.1	6.5 ± 1.5
Day 8	13.0 ± 2.1	39.9 ± 2.9	95.2 ± 6.2	91.4 ± 17.4	69.1 ± 11.4	6.3 ± 1.6
Day 9	11.4 ± 1.9	39.3 ± 2.3	92.3 ± 6.4	88.8 ± 17.4	66.1 ± 5.8	5.8 ± 1.1
Day 10	12.5 ± 3.5	38.5 ± 3.9	89.6 ± 7.4	94.5 ± 19.5	64.5 ± 9.4	6.1 ± 1.5
CV (%)	20.9 ± 7.8	4.5 ± 2.3	5.7 ± 1.8	12.2 ± 2.8	10.9 ± 2.5	16.3 ± 6.1

As a group, the average SaO_2 experienced throughout the intermittent hypoxia sessions (excluding the first five minutes as SaO_2 was gradually decreased) was 80.8 ± 1.6 % (Table 4). Most subjects watched movies during the IH, while the rest of the sessions subjects read. Subject's $P_{ET}CO_2$ during hypoxia was tightly regulated to the daily $P_{ET}CO_2$ obtained at rest. Decreasing subject's SaO_2 to 80% caused an increase in V_E; V_E progressively increased throughout the IH protocol (Figure 2). In contrast, the difference between rest and hypoxia did not change over the course of the IH in any other variable.

Figure 2. Increase in ventilation from rest during IH over 10 days. Data is presented as mean ± SE.

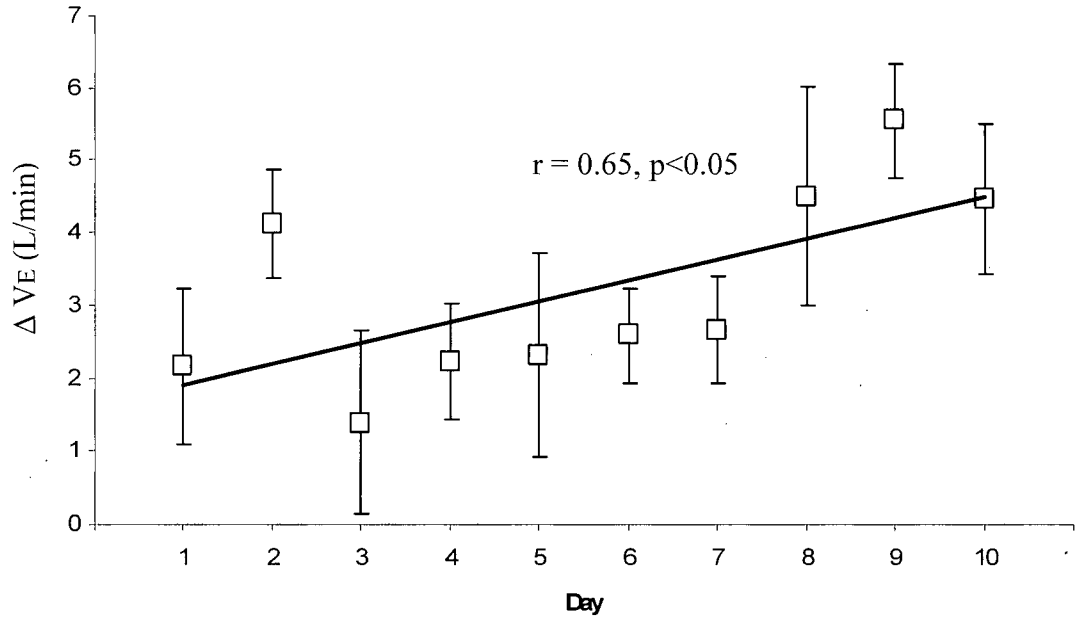


Table 4. Measured variables during the 10 days of IH (data is an average from the last 55 minutes of each IH session). V_E = minute ventilation; SaO_2 = oxyhaemoglobin saturation; $P_{ET}CO_2$ = end tidal partial pressure of carbon dioxide; FiO_2 = fraction of inspired oxygen; MAP = mean arterial blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; SV = stroke volume; Q = cardiac output.

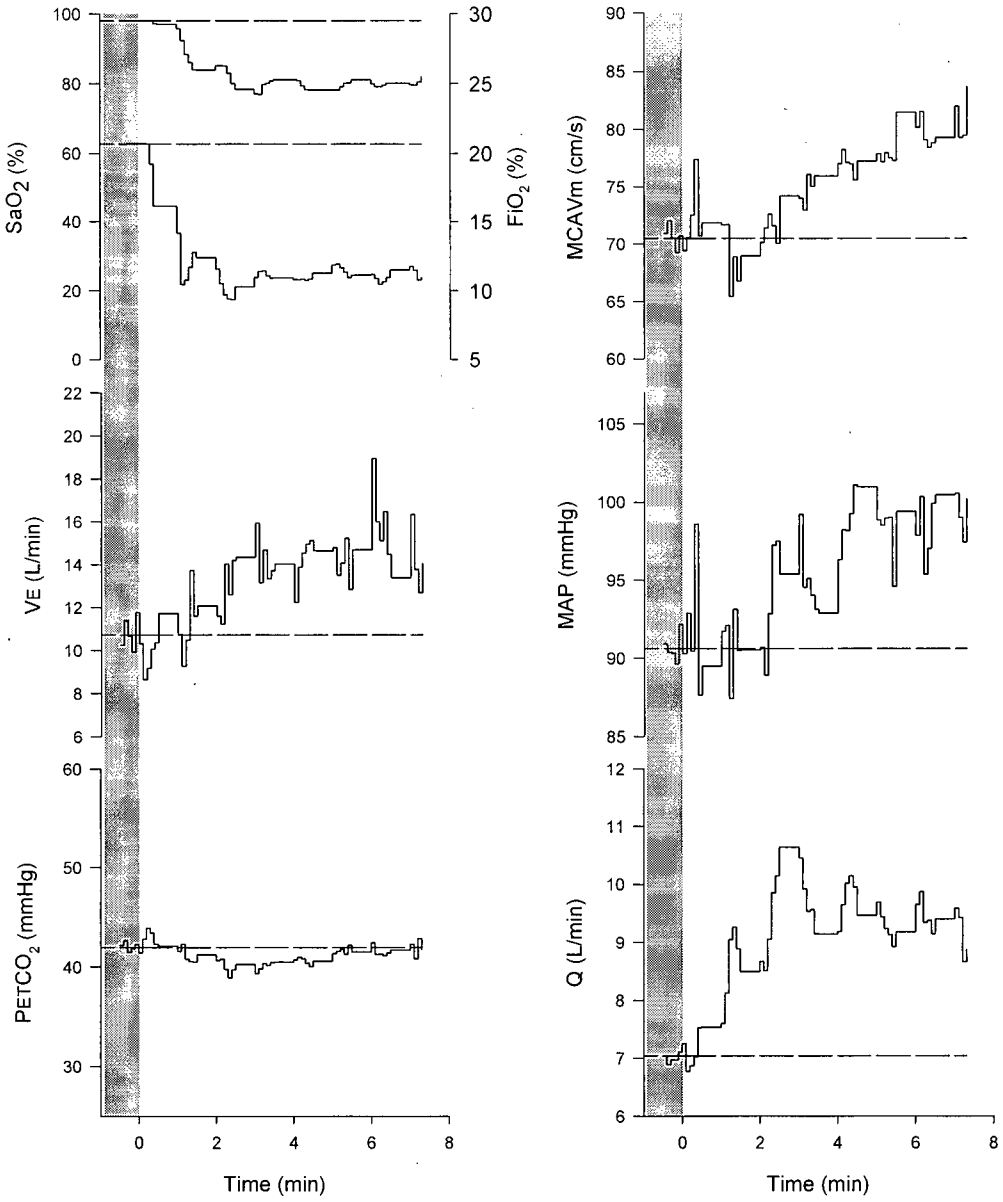
	V_E (L/min)	SaO_2 (%)	$P_{ET}CO_2$ (mmHg)	MAP (mmHg)	HR (bpm)	SV (ml)	Q (L/min)
Day 1	13.3 ± 1.8	79.9 ± 0.9	41.2 ± 1.1	99.6 ± 6.5	77.7 ± 14.6	96.8 ± 24.0	7.4 ± 2.0
Day 2	15.6 ± 2.1	81.0 ± 2.0	41.1 ± 1.8	98.7 ± 8.0	77.5 ± 11.3	96.5 ± 11.6	7.5 ± 1.4
Day 3	14.1 ± 1.9	81.8 ± 2.1	40.5 ± 2.6	101.3 ± 8.8	79.3 ± 12.4	97.6 ± 15.9	7.7 ± 1.6
Day 4	14.4 ± 2.4	80.7 ± 1.2	40.6 ± 2.3	101.6 ± 8.2	77.1 ± 9.8	102.8 ± 20.0	7.9 ± 1.6
Day 5	15.9 ± 4.6	81.1 ± 0.9	41.4 ± 2.5	100.8 ± 4.5	77.0 ± 10.7	96.3 ± 21.6	7.3 ± 1.7
Day 6	15.9 ± 3.3	80.4 ± 1.3	40.6 ± 2.5	100.4 ± 5.5	76.5 ± 7.6	99.8 ± 19.5	7.6 ± 1.5
Day 7	16.1 ± 2.0	80.9 ± 1.7	40.7 ± 2.0	101.9 ± 4.8	83.8 ± 9.8	99.4 ± 10.9	7.4 ± 3.0
Day 8	17.5 ± 3.6 *	80.8 ± 1.5	40.6 ± 2.4	101.2 ± 6.4	80.8 ± 11.4	95.1 ± 13.6	7.6 ± 1.3
Day 9	17.0 ± 2.0 *	81.1 ± 1.8	40.1 ± 2.4	100.8 ± 3.5	81.1 ± 18.0	98.3 ± 19.1	7.0 ± 3.2
Day 10	17.0 ± 2.0 *	81.1 ± 1.8	40.1 ± 2.4	100.8 ± 3.5	81.1 ± 18.0	98.3 ± 19.1	8.3 ± 1.7
CV (%)	16.2 ± 4.7	1.7 ± 0.5	3.2 ± 0.8	4.6 ± 2.7	9.4 ± 4.7	8.9 ± 3.4	20.1 ± 15.0

*significantly different from Day 1

Acute Hypoxic Response

For each individual subject, both the PRE-IH and POST-IH AHR were performed at the same time of day. As a group, mean SaO_2 on both experimental days during the 5-minute AHR was 79.6 ± 1.6 %, which took an average of 3.1 ± 1.1 minutes to reach. An average FiO_2 of 11.4 ± 0.1% was required to evoke the appropriate drop in SaO_2. Figure 3 displays the typical response during a PRE-IH acute hypoxic response.

Figure 3. Ventilatory, cardiovascular, and cerebrovascular response to acute hypoxia. Dashed lines are baseline, shaded area represents rest prior to the AHR. SaO_2 = oxyhaemoglobin saturation; FiO_2 = fraction of inspired oxygen; VE = minute ventilation; $PETCO_2$ = end tidal partial pressure of carbon dioxide; MCAVm = mean middle cerebral arterial velocity; MAP = mean arterial blood pressure; Q = cardiac output.



PRE-IH, VE significantly increased during the AHR from 9.5 ± 1.7 L/min at rest to 15.5 ± 1.8 L/min during acute hypoxia ($t(8) = -7.7$, $p<0.05$) (Table 5); similarly, the POST-IH AHR caused significant increases in VE from rest (8.4 ± 2.1 L/min) to hypoxia (16.1 ± 3.3L/min). Significant increases in systolic middle cerebral arterial velocity (MCAVs) (Pre-IH: $t(8)=-2.38$, $p<0.05$; Post-IH: $t(8)=-2.67$, $p<0.05$), HR (Pre-IH: t(8)-8.04, $p<0.05$; Post-IH: $t(8)=-6.63$,

$p<0.05$), and Q (Pre-IH: $t(8)=-9.93$, $p<0.05$; Post-IH: $t(8)=-5.41$, $p<0.05$) were also noted. The PRE-IH AHR did not cause an increase in diastolic middle cerebral arterial velocity (MCAVd), although significant increases in MCAVd occurred Post-IH ($t(8)=-3.34$, $p<0.05$).

Table 5. Change in ventilatory, cardiovascular and cerebrovascular variables from rest in response to the AHR. V_E = ventilation; SaO_2 = oxyhaemoglobin saturation; $P_{ET}CO_2$ = end tidal partial pressure of carbon dioxide; FiO_2 = fraction of inspired oxygen; MCAVm = mean middle cerebral arterial velocity' MCAVs = systolic middle cerebral arterial velocity; MCAVd = diastolic middle cerebral arterial velocity; MAP = mean arterial blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; SV = stroke volume; HR = heart rate; Q = cardiac output

		Pre-IH	Post-IH
V_E (L/min)	**rest**	9.5 ± 1.7	8.4 ± 2.1
	80%	15.5 ± 1.8 *	16.1 ± 3.3 ^{t}
SaO_2 (%)	**rest**	97.8 ± 0.6	97.8 ± 0.7
	80%	79.8 ± 1.7	79.4 ± 1.6
$P_{ET}CO_2$ (mmHg)	**rest**	42.3 ± 2.2	42.3 ± 3.0
	80%	41.9 ± 1.8	41.7 ± 3.4
FiO_2 (%)	**rest**	20.7 ± 0.1	20.7 ± 0.2
	80%	11.4 ± 0.8 *	11.3 ± 1.0 ^{t}
MCAVm (cm/s)	**rest**	64.2 ± 8.6	65.8 ± 10.4
	80%	68.1 ± 14.9	69.5 ± 12.2 ^{t}
MCAVs (cm/s)	**rest**	105.9 ± 11.1	107.8 ± 15.1
	80%	113.5 ± 16.5 *	113.2 ± 16.1 ^{t}
MCAVd (cm/s)	**rest**	41.6 ± 6.7	43.3 ± 7.5
	80%	44.3 ± 10.7	46.6 ± 9.0 ^{t}
MAP (mmHg)	**rest**	92.4 ± 5.8	92.6 ± 7.4
	80%	94.1 ± 7.3	94.3 ± 7.4
SBP (mmHg)	**rest**	126.8 ± 7.0	127.7 ± 6.1
	80%	130.7 ± 6.6	131.8 ± 7.3
DBP (mmHg)	**rest**	74.3 ± 7.1	74.7 ± 9.3
	80%	75.3 ± 7.1	76.1 ± 9.0
SV (ml)	**rest**	102.6 ± 18.9	103.7 ± 22.6
	80%	102.1 ± 18.2	102.4 ± 24.5
HR (bpm)	**rest**	61.2 ± 9.4	60.5 ± 10.8
	80%	75.7 ± 11.3 *	73.2 ± 10.3 ^{t}
Q (L/min)	**rest**	6.2 ± 1.2	6.2 ± 1.3
	80%	7.6 ± 1.4 *	7.4 ± 1.8 ^{t}

*significantly different from PRE-IH rest
^{t} significantly different from POST-IH rest

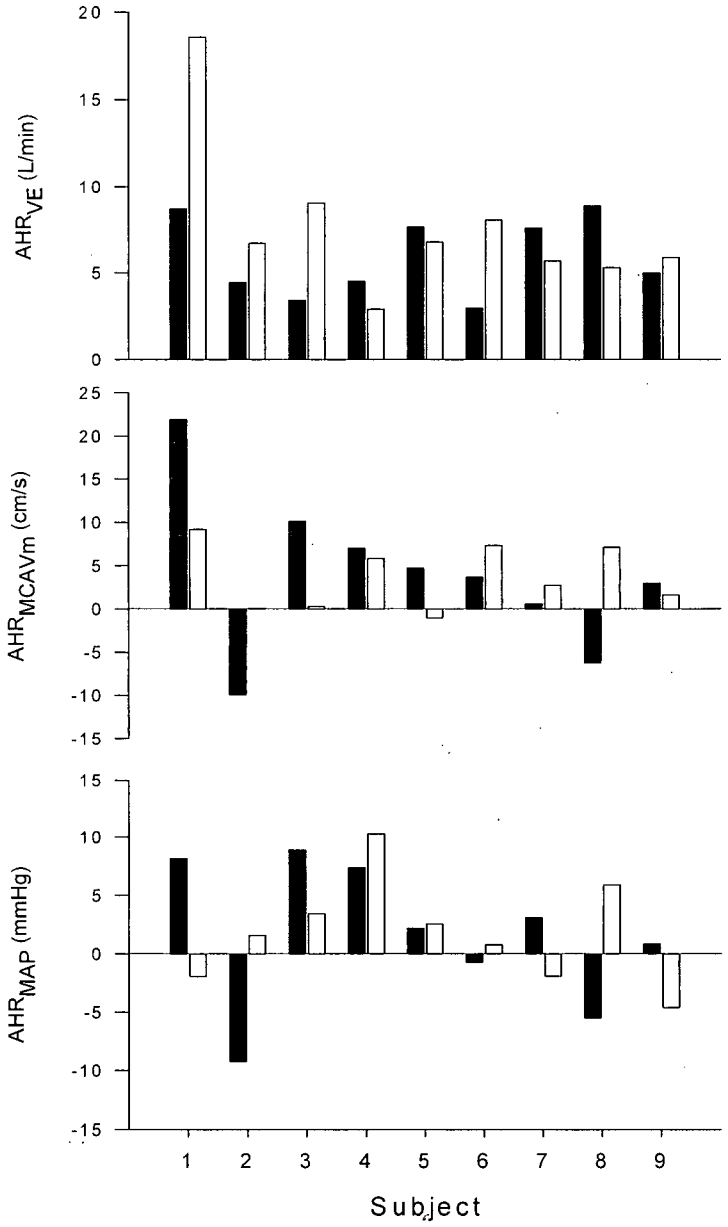
As a group, the AHR_{VE} increased POST-IH (7.6 ± 4.4 L/min,) when compared to PRE-IH (5.9 ± 2.3 L/min, $t(8)=-1.20$, $p<0.05$), although this did not reach statistical significance (Table

6). Individually, five of the nine subjects experienced an increased AHR_{VE} Post-IH; four of the nine subjects displayed a decrease (Figure 4). No significant difference in MCAVm was detected between the PRE-IH AHR (3.9 ± 9.2 cm/s) and POST-IH AHR (3.7 ± 3.7 cm/s, t(8)=.07, p>0.05); although five of the subjects experienced a decreased AHR_{MCAVm} response during the POST-IH AHR compared to the PRE-IH AHR. Similarly, there was no significant difference between the PRE-IH AHR_{MAP} (1.7 ± 6.2 mmHg) and the POST-IH AHR_{MAP} (1.8 ± 4.5, t(8)=-0.05, p>0.05); although, four of the nine subjects experienced a decrease in the POST-IH AHR_{MAP} compared to the PRE-IH AHR_{MAP}, whereas five of the subjects showed an increase.

Table 6. Change in the ventilatory, cardiovascular and cerebrovascular response to acute hypoxia between the PRE-IH and POST-IH AHR tests. V_E = ventilation; SaO_2 = oxyhaemoglobin saturation; $P_{ET}CO_2$ = end tidal partial pressure of carbon dioxide; FiO_2 = fraction of inspired oxygen; MCAVm = mean middle cerebral arterial velocity' MCAVs = systolic middle cerebral arterial velocity; MCAVd = diastolic middle cerebral arterial velocity; MAP = mean arterial blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; SV = stroke volume; HR = heart rate; Q = cardiac output

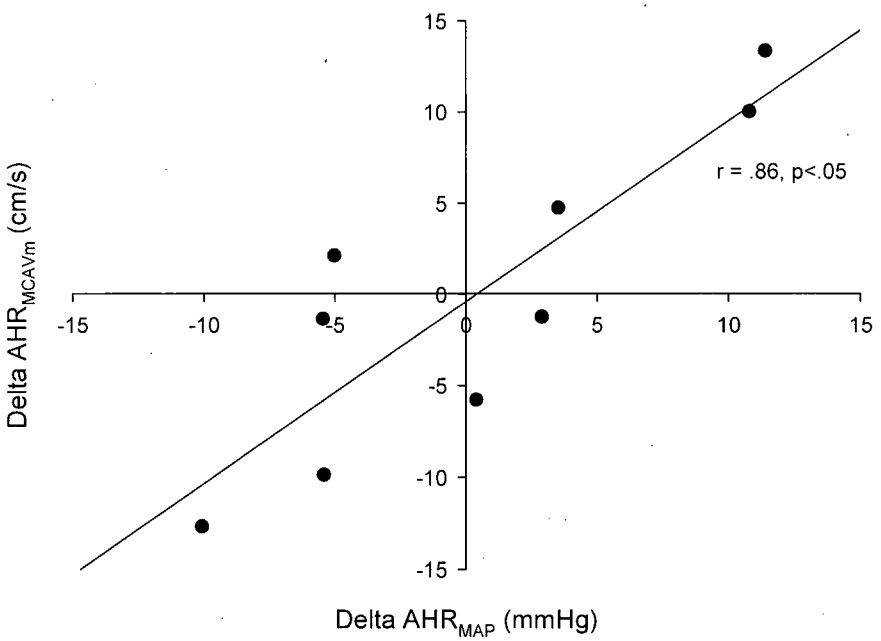
	Pre-IH			Post-IH			p
V_E (L/min)	5.9	±	2.3	7.6	±	4.4	0.27
SaO_2 (%)	-18.1	±	1.8	-18.3	±	1.7	0.73
$P_{ET}CO_2$ (mmHg)	-0.4	±	0.9	-0.8	±	1.4	0.43
FiO_2 (%)	-9.3	±	0.8	-9.3	±	1.1	0.96
MCAVm (cm/s)	3.9	±	9.2	3.7	±	3.7	0.95
MCAVs (cm/s)	7.5	±	9.5	5.4	±	6.1	0.48
MCAVd (cm/s)	2.7	±	8.0	3.3	±	2.9	0.83
MAP (mmHg)	1.7	±	6.2	1.8	±	4.5	0.96
SBP (mmHg)	3.8	±	8.7	4.1	±	6.5	0.94
DBP (mmHg)	1.0	±	4.9	1.4	±	4.3	0.83
SV (ml)	-0.5	±	5.6	-1.4	±	5.1	0.49
HR (bpm)	14.5	±	5.4	12.8	±	5.8	0.50
Q (L/min)	1.4	±	0.4	1.3	±	0.7	0.51

Figure 4. AHR_{VE}, AHR_{MCAVm}, and AHR_{MAP} PRE-IH and POST-IH. Filled bars = PRE-IH; open bars = POST-IH; AHR_{VE} = ventilatory response to acute hypoxia; AHR_{MCAVm} = mean middle cerebral arterial blood flow velocity response to acute hypoxia; AHR_{MAP} = mean arterial blood pressure response to acute hypoxia.



In the PRE-IH AHR, there was a strong, positive relationship between the AHR_{MAP} and AHR_{MCAVm} ($r(7)=4.8$, $p<0.05$); although this relationship did not reach statistical significance during the POST-IH AHR. Furthermore, investigation into the difference between PRE-IH AHR_{MCAVm} and POST-IH AHR_{MCAVm} to the difference between PRE-IH AHR_{MAP} and POST-IH AHR_{MAP} showed that a blunted AHR_{MAP} was strongly associated with a blunted AHR_{MCAVm} ($r(7)=4.4$, $p<.05$) (Figure 5).

Figure 5. Association between the change in AHR_{MAP} and AHR_{MCAVm} between PRE-IH and POST-IH.



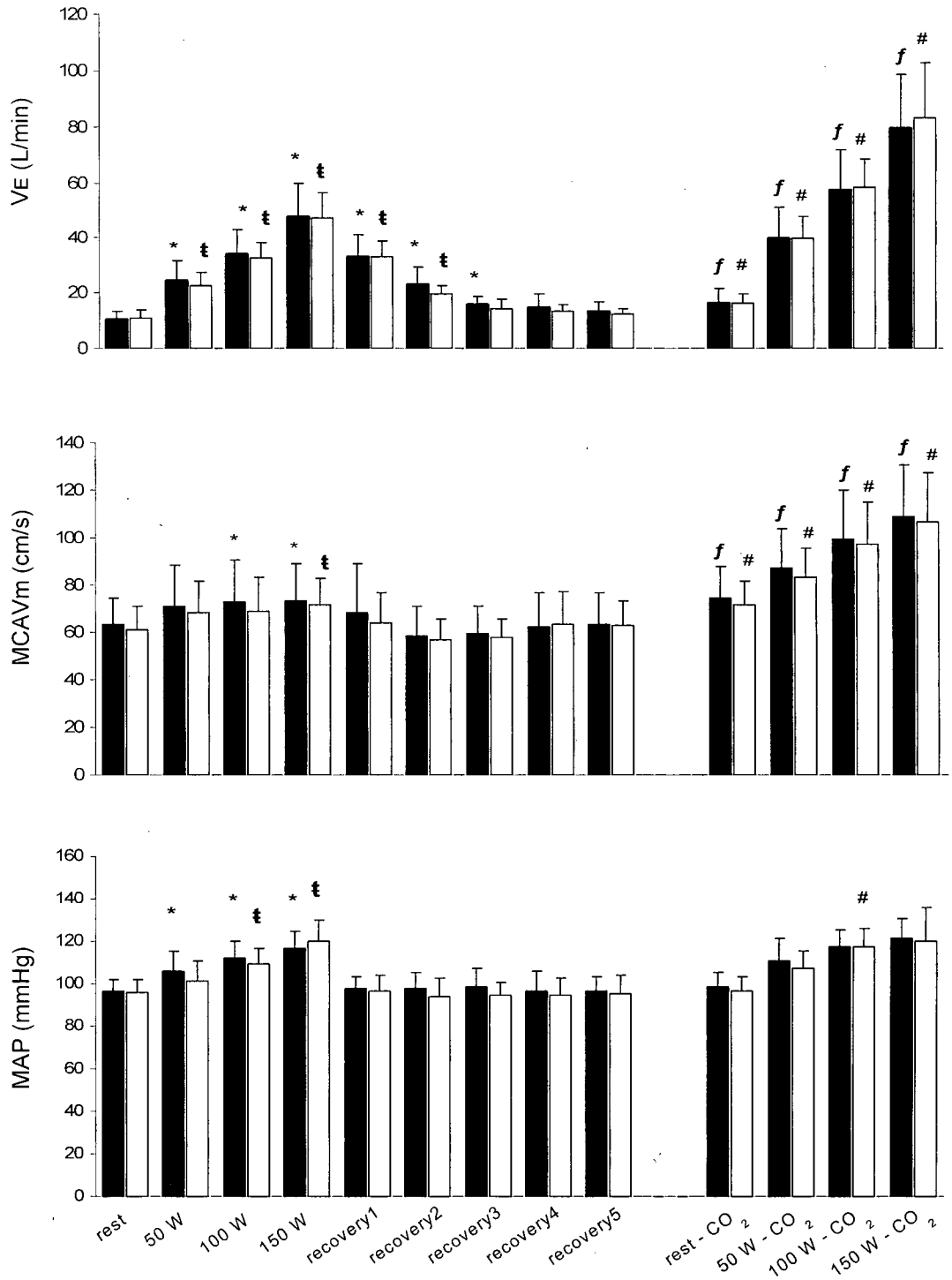
Exercise

PRE-IH, significant increases in V_E occurred in response to the different exercise intensities ($F(8,64)=78.2$, $p<0.05$) (Figure 6); V_E at 50 W (24.6 ± 7.1 L/min), 100 W (34.0 ± 9.1 L/min), 150 W (48.0 ± 11.7 L/min), Recovery1 (32.9 ± 8.1 L/min), and Recovery2 (23.0 ± 6.1 L/min) were significantly elevated from rest (Table 7). Similarly, V_E increased from rest during the POST-IH exercise trial ($F(8,64)=127.8$, $p<0.05$). The increases from rest occurred at each exercise intensity, and continued for 2 minutes into recovery. There was no difference in PRE-IH V_E and POST-IH V_E during the exercise tests ($F(8,128)=0.42$, $p>0.05$).

During the PRE-IH exercise trial, MCAVm significantly increased from rest at 100 W (72.6 ± 18.0 cm/s) and 150 W (73.5 ± 15.3 cm/s, $F(8,64)=8.6$, $p<0.05$) (Figure 6). In contrast, the POST-IH exercise trial only caused a significant increase in MCAVm from rest at 150 W (71.7 ± 11.1 cm/s, $F(8,64)=7.6$, $p<0.05$). IH did not result in any change in MCAVm at any exercise intensity ($F(8,128)=0.32$, $p>0.05$).

MAP during the PRE-IH exercise trial increased from rest at 50 W (106.0 ± 9.4 mmHg), 100 W (111.9 ± 8.0 mmHg), and 150 W (116.5 ± 8.0 mmHg, $F(8,56)=36.7$, $p<0.05$). POST-IH, MAP only increased from rest at 100 W (109.4 ± 7.5 mmHg), and 150 W (120.0 ± 10.6 mmHg, $F(8,56)=22.9$, $p<0.05$). MAP was not different between the PRE-IH and POST-IH exercise trials ($p>0.05$) (Figure 6).

Figure 6. Ventilation (V_E), mean middle cerebral arterial velocity (MCAVm), and mean arterial blood pressure (MAP) in response to exercise and carbon dioxide (5% CO_2).



*significantly different from PRE-IH rest
t significantly different from POST-IH rest
f significantly different from PRE-IH room air condition of same intensity
significantly different from POST-IH room air condition of same intensity

Table 7. Ventilatory, and cerebrovascular variables during exercise PRE-IH and POST-IH. V_E = ventilation; $P_{ET}CO_2$ = end tidal partial pressure of carbon dioxide; VO_2 = oxygen consumption; MCAVm = mean middle cerebral arterial velocity; MCAVs = systolic middle cerebral arterial velocity; MCAVd = diastolic middle cerebral arterial velocity

		V_E (L/min)	$P_{ET}CO_2$ (mmHg)	VO_2 (ml/kg/min)	MCAVm (cm/s)	MCAVs (cm/s)	MCAVd (cm/s)
rest	**pre**	10.5 ± 2.7	42.3 ± 2.1	5.2 ± 2.0	63.3 ± 11.1	105.8 ± 14.7	40.2 ± 7.9
	post	11.0 ± 3.2	40.8 ± 2.7	4.2 ± 1.0	61.3 ± 9.9	101.9 ± 13.7	39.0 ± 7.4
50 W	**pre**	24.6 ± 7.1 *	42.7 ± 2.5	15.4 ± 3.6 *	70.9 ± 17.4	125.5 ± 23.3 *	40.9 ± 11.9
	post	22.3 ± 4.9 †	42.3 ± 1.9	14.5 ± 3.7 †	68.6 ± 13.1	120.7 ± 19.1 †	39.7 ± 8.9
100 W	**pre**	34.0 ± 9.1 *	43.6 ± 1.7	19.2 ± 4.2 *	72.6 ± 17.7 *	132.9 ± 28.4 *	40.2 ± 9.8
	post	32.3 ± 6.1 †	42.8 ± 2.7	20.3 ± 7.3 †	69.0 ± 14.2	125.4 ± 20.1 †	39.3 ± 9.8
150 W	**pre**	48.0 ± 11.7 *	42.9 ± 1.9	29.4 ± 8.7 *	73.5 ± 15.3 *	135.2 ± 26.3 *	38.7 ± 8.1
	post	47.1 ± 9.2 †	41.8 ± 2.5	28.1 ± 9.8 †	71.7 ± 11.1 †	133.3 ± 20.1 †	37.4 ± 7.0
Recovery1	**pre**	32.9 ± 8.1 *	44.1 ± 5.1		68.5 ± 20.3	129.2 ± 34.8 *	39.4 ± 13.4
	post	32.9 ± 6.0 †	42.4 ± 4.5		64.0 ± 12.8	124.4 ± 24.6 †	35.6 ± 8.6
Recovery2	**pre**	23.0 ± 6.1 *	38.2 ± 2.5 *		58.4 ± 12.5	109.6 ± 24.6	33.9 ± 7.0
	post	19.5 ± 2.8 †	37.9 ± 3.2 †		56.4 ± 9.0	107.7 ± 19.4	32.7 ± 5.6 †
Recovery3	**pre**	15.6 ± 3.0 *	38.2 ± 3.2 *		59.3 ± 11.9	105.1 ± 22.0	36.6 ± 6.4
	post	14.4 ± 3.1	37.8 ± 2.3 †		57.7 ± 7.9	103.4 ± 17.4	35.6 ± 4.9
Recovery4	**pre**	15.0 ± 4.5	38.6 ± 1.8 *		62.5 ± 14.0	105.8 ± 22.3	40.2 ± 10.3
	post	13.5 ± 2.3	38.9 ± 2.5		63.1 ± 13.9	106.6 ± 24.7	40.6 ± 8.6
Recovery5	**pre**	13.4 ± 3.5	39.5 ± 1.7 *		63.3 ± 13.3	105.3 ± 20.5	41.4 ± 9.6
	post	12.4 ± 1.8	39.3 ± 2.1		62.7 ± 10.7	103.9 ± 19.9	40.8 ± 6.7

* significantly different from PRE-IH rest
† significantly different from POST-IH rest

Table 8. Cardiovascular variables during exercise PRE-IH and POST-IH. MAP = mean arterial pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; SV = stroke volume; HR = heart rate; Q = cardiac output

		MAP (mmHg)	SBP (mmHg)	DBP (mmHg)	SV (ml)	HR (bpm)	Q (L/min)
rest	**pre**	96.6 ± 5.6	133.5 ± 7.6	77.4 ± 6.5	104.2 ± 25.2	60.9 ± 10.5	6.3 ± 1.9
	post	95.7 ± 6.3	133.7 ± 10.9	75.8 ± 6.2	108.9 ± 27.4	56.9 ± 6.5	6.2 ± 1.7
50 W	**pre**	106.0 ± 9.4 *	151.0 ± 14.4 *	82.9 ± 8.2	119.5 ± 29.8	91.6 ± 10.9 *	10.9 ± 2.9 *
	post	101.6 ± 9.2	146.6 ± 13.5	78.3 ± 7.7	125.6 ± 30.1 †	86.4 ± 7.3 †	10.8 ± 2.5 †
100 W	**pre**	111.9 ± 8.0 *	160.5 ± 10.0 *	86.8 ± 8.0 *	122.9 ± 28.9 *	110.9 ± 13.5 *	13.5 ± 3.1 *
	post	109.3 ± 7.5 †	159.1 ± 8.5 †	83.4 ± 6.6 †	132.0 ± 32.4 †	107.4 ± 10.0 †	14.0 ± 3.1 †
150 W	**pre**	116.5 ± 8.0 *	173.6 ± 5.3 *	87.6 ± 9.5 *	131.2 ± 23.6 *	129.9 ± 14.8 *	16.9 ± 2.7 *
	post	119.7 ± 10.6 †	180.8 ± 10.1 †	88.4 ± 8.6 †	136.5 ± 29.5 †	132.1 ± 15.4 †	17.7 ± 2.7 †
Recovery1	**pre**	98.1 ± 5.3	137.9 ± 4.5	77.6 ± 6.7	113.3 ± 17.4	102.3 ± 23.3 *	11.4 ± 2.2 *
	post	96.7 ± 7.1	138.5 ± 12.0	75.0 ± 6.0	118.4 ± 21.1	106.8 ± 24.7 †	12.3 ± 2.0 †
Recovery2	**pre**	98.0 ± 7.2	134.5 ± 6.9	78.1 ± 7.9	106.8 ± 17.3	89.6 ± 20.6 *	9.4 ± 1.7 *
	post	94.1 ± 8.3	132.3 ± 12.2	73.8 ± 7.5	111.5 ± 21.7	91.4 ± 23.8 †	9.8 ± 1.5 †
Recovery3	**pre**	98.9 ± 8.1	134.3 ± 9.1	79.6 ± 7.9	102.4 ± 17.8	84.1 ± 18.3 *	8.4 ± 1.5 *
	post	94.5 ± 6.4	130.6 ± 10.4	75.1 ± 5.3	106.7 ± 24.0	87.7 ± 22.1 †	9.0 ± 1.4 †
Recovery4	**pre**	96.6 ± 9.3	129.7 ± 10.3	78.2 ± 9.4	99.6 ± 17.1	79.4 ± 19.1 *	7.7 ± 1.6
	post	94.9 ± 7.8	130.7 ± 12.3	75.4 ± 7.1	105.8 ± 22.6	84.4 ± 19.6 †	8.6 ± 1.1 †
Recovery5	**pre**	96.8 ± 6.6	129.8 ± 5.7	78.4 ± 7.2	99.0 ± 16.7	80.1 ± 17.3 *	7.8 ± 1.4
	post	95.1 ± 8.7	130.2 ± 13.2	75.8 ± 7.8	103.5 ± 23.0	83.2 ± 19.5 †	8.3 ± 1.2 †

* significantly different from PRE-IH rest
† significantly different from POST-IH rest

CO_2 Reactivity

Increases in exercise intensity brought about significant increases in $V_{E_{CO_2}}$ PRE-IH ($F(3,24)=143.83$, $p<0.05$); V_E at each exercise intensity was different from rest, as well as to one another ($p<0.05$). Furthermore, hypercapnia resulted in a significant increase in V_E when compared to V_E at the same exercise intensity while breathing room air ($p<0.05$) (Figure 6). Similarly, hypercapnia resulted in an increase in V_E POST-IH at each exercise intensity ($F(3,24)=95.76$, $p<0.05$) which was significantly increased from $V_{E_{CO_2}}$ POST-IH at rest ($p<0.05$) (Table 10). Hypercapnia caused a significant increase in V_E from room air ($p<0.05$) breathing at all exercise intensities during the POST-IH exercise trial. There was no significant difference in $V_{E_{CO_2}}$ at any exercise intensity between PRE-IH and POST-IH trials.

In the hypercapnic condition, increases in exercise intensity were accompanied by significant increases in MCAVm (PRE-IH: $F(3,24)=70.6$, $p<0.05$; POST-IH: $F(3,24)=47.2$, $p<0.05$). In both the PRE-IH and POST-IH exercise trials, hypercapnia resulted in an increase in MCAVm at each exercise intensity ($p<0.05$) (Table 9). No significant differences were detected in $MCAVm_{CO_2}$ at any exercise intensity between PRE-IH and POST-IH.

A significant increase in MAP_{CO_2} from rest occurred during the PRE-IH and POST-IH at every exercise intensity (PRE-IH: $F(3,21)=30.2$, $p<0.05$; POST-IH: $F(3,21)=19.7$, $p<0.05$). There was no significant effect of IH on MAP_{CO_2} (between PRE-IH and POST-IH exercise trials) ($F(3,42)=.41$, $p>0.05$). Also, MAP_{CO_2} only increased from room air breathing during the POST-IH at 100 W ($F(3,21)=7.2$, $p<0.05$). MAP_{CO_2} failed to show any significantly increase from MAP during room air breathing at any other exercise intensities.

Although the addition of CO_2 caused a significant increase in other cardiovascular variables, there was no difference between the PRE-IH and POST-IH CO_2 reactivity trials (Table 9).

Table 9. Ventilatory, cardiovascular, and cerebrovascular variables during hypercapnic (5% carbon dioxide) rest and exercise. $P_{ET}CO_2$ = end tidal partial pressure of carbon dioxide; MCAVm = mean middle cerebral arterial velocity; MCAVs = systolic middle cerebral arterial velocity; MAP = mean arterial blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; SV = stroke volume; HR = heart rate; Q = cardiac output

		Ventilation (L/min)	$P_{ET}CO_2$ (mmHg)	MCAVm (cm/s)	MCAVs (cm/s)	MCAVd (cm/s)
rest CO_2	**pre**	16.3 ± 5.1 *f*	48.1 ± 2.0, *f*	74.6 ± 13.1 *f*	119.9 ± 16.2, *f*	50.0 ± 9.4, *f*
	post	16.1 ± 3.3 #	46.7 ± 2.1 #	71.5 ± 10.3 #	113.2 ± 15.0 #	49.4 ± 7.9 #
50 CO_2	**pre**	39.8 ± 11.5 *, *f*	52.2 ± 2.5 *, *f*	87.1 ± 16.7 *, *f*	146.2 ± 23.9 *, *f*	54.4 ± 12.2, *f*
	post	39.8 ± 8.0 t, #	50.8 ± 2.4 t, #	83.1 ± 12.3 t, #	138.9 ± 18.2 t, #	51.9 ± 8.2 #
100 CO_2	**pre**	57.2 ± 14.4 *, *f*	54.8 ± 1.9 *, *f*	99.3 ± 20.5 *, *f*	163.7 ± 28.5 *, *f*	62.9 ± 15.2 *, *f*
	post	58.2 ± 10.3 t, #	53.2 ± 2.5 t, #	97.0 ± 18.2 t, #	159.0 ± 24.8 t, #	61.2 ± 13.6 t, #
150 CO_2	**pre**	79.5 ± 18.8 *, *f*	56.2 ± 2.7 *, *f*	108.8 ± 22.0 *, *f*	171.5 ± 28.0 *, *f*	70.1 ± 15.8 *, *f*
	post	83.0 ± 19.7 t, #	53.7 ± 3.6 t, #	106.5 ± 20.9 t, #	170.1 ± 26.7 t, #	67.0 ± 16.8 t, #

		MAP (mmHg)	SBP (mmHg)	DBP (mmHg)	SV (ml)	HR (bpm)	Q (L/min)
rest CO_2	**pre**	98.9 ± 6.8	137.0 ± 8.9	79.1 ± 7.1	106.0 ± 25.9	62.4 ± 10.6	6.6 ± 2.0
	post	96.8 ± 6.2	133.8 ± 8.8	77.2 ± 6.3	105.4 ± 25.2	62.0 ± 8.7	6.4 ± 1.5
50 CO_2	**pre**	110.8 ± 10.3 *	156.8 ± 16.8 *	86.4 ± 8.6 *	117.3 ± 31.1 *	93.2 ± 11.6 *	10.8 ± 3.0 *
	post	107.3 ± 7.9 t	153.5 ± 11.1 t	82.6 ± 6.9	125.1 ± 28.9 t	90.9 ± 5.1 t	11.3 ± 2.5 t
100 CO_2	**pre**	117.4 ± 8.1 *	167.8 ± 10.2 *	90.6 ± 8.2 *	123.0 ± 31.0 *	114.3 ± 12.9 *	13.9 ± 3.1 *
	post	117.3 ± 8.8 t, #	171.2 ± 9.2 t, #	88.5 ± 7.4 t	131.5 ± 35.5 t	112.5 ± 10.2 t	14.6 ± 3.4 t
150 CO_2	**pre**	121.4 ± 9.1 *	178.3 ± 9.4 *	90.6 ± 9.7 *	131.7 ± 25.1 *	133.8 ± 14.4 *	17.4 ± 2.6 *
	post	120.3 ± 15.5 t	180.6 ± 14.5 t	88.0 ± 11.5 t	138.1 ± 30.9 t	136.5 ± 16.5 t	18.6 ± 2.9 t

* significantly different from PRE-IH CO_2 rest
t significantly different from POST-IH CO_2 rest
f significantly different from PRE-IH room air breathing of same intensity
significantly different from POST-IH room air breathing of same intensity

DISCUSSION

Main Findings

The purpose of this study was to investigate the effects of IH on ventilatory, cardiovascular, and cerebrovascular regulation during acute hypoxia at rest, and during submaximal exercise. This study reports several main findings: 1) the results are in agreement with several lines of evidence that show IH to cause no change in baseline ventilatory, cardiovascular, and cerebrovascular variables; 2) the effect of IH on cerebrovascular regulation during acute hypoxia was blunted in certain subjects; 3) there was an association between the changes in cardiovascular and cerebrovascular regulation following IH; 4) IH did not change ventilatory, cardiovascular, or cerebrovascular regulation during submaximal exercise; 5) ventilatory, cardiovascular, and cerebrovascular regulation was maintained during hypercapnia following IH both at rest and submaximal exercise.

Effect of IH on Ventilatory Regulation

Arterial partial pressure of oxygen is continuously monitored by peripheral chemoreceptors (carotid bodies). Accordingly, the carotid body has been implicated as playing a key role in the ventilatory changes following IH (111). In the rat model, Peng et al. (111) investigated carotid body sensory activity to repeated bouts of hypoxia (12% O_2). They found a reversible increase in baseline (normoxia) sensory activity as the repeated bouts of hypoxia progressed; this phenomenon is known as long-term facilitation (LTF) (111). In contrast, rats that were exposed to sustained hypoxia did not exhibit LTF. The same group also found IH to cause an increased carotid body sensitivity to acute hypoxia of the same intensity (112), which is termed progressive augmentation (123). Similarly, this increase in sensitivity was transient, where increases in carotid body sensitivity following IH (15 s of 5% O_2 at 5 min intervals, 9 bouts/hour, 8 h/day, 10 days) is eliminated following 10 days of normoxia (112). It is important

to mention that the patterning and severity of hypoxia was a central component to the change in carotid body sensitivity.

Although there was a trend for a change in the AHR_{VE} following IH (PRE-IH: 5.9 ± 2.3 L/min; POST-IH: 7.6 ± 4.4 L/min), the change did not reach statistical significance. Many lines of evidence have shown IH to cause an increase in the AHR_{VE} (5, 33, 75, 77, 78, 95). Katayama et al. (75) measured the AHR_{VE} in six subjects following seven days of simulated altitude (4,500 m, 1hr/day). IH caused an increase in the AHR_{VE} which was maintained one week following IH (75). Also, our lab has shown different protocols of IH (e.g. short duration IH and long duration IH) in a lab setting to cause significant increases in the AHR_{VE} (33, 95). Foster et al. (33) found a significant increase in the AHR_{VE} following 10 days of two different IH protocols (short duration: 5 min 12% O_2 at 5 min intervals for one hour; long duration: 30 min 12% O_2). Although the IH protocols were not identical to the current study, an increase in the AHR_{VE} was expected nonetheless. The ventilatory response to acute hypoxia has been reported to be quite variable (29, 52, 147, 152), which could help explain the discrepancy in our results. Given this, other studies have failed to show an increase in the AHR_{VE} following hypoxia exposure (35, 76, 98). Even though Foster and colleagues (33) showed a significant increase in the AHR_{VE} following IH, not all subjects reached their greatest increase immediately following IH. Rather, the AHR_{VE} in certain subjects reached their peak before, as well several days following IH. It is possible that the design of the current study did not allow for detection of each subject's greatest increase in AHR_{VE}. Thus, it is conceivable that the time course of progressive augmentation is individualistic, and testing the AHR_{VE} at a single time point following IH could miss this effect. Most analogous to the current study, Lusina et al. (95) found a 49% increase in the AHR_{VE} following 10 days of isocapnic IH. However, differences in the AHRVE protocols could partially explain the conflicting results between the current study and that of Lusina et al. (95)

Although the data does not display a clear increase in AHR_{VE}, we do consider it probable that our protocol was severe enough to have caused changes in chemosensitivity. To support this statement, it is important to consider ventilation during the IH sessions. Figure 2 shows an increase in V_E (from rest) over the 10 days of IH. This shows a significant increase (0.29 L/min · day^{-1}) in V_E as the IH protocol progressed. Therefore, this data alone demonstrates progressive augmentation. Anecdotally, many of the subjects noted that they did not notice the hypoxia during the IH sessions, whereas they found breathing during the AHR_{VE} somewhat difficult due to the 'thin air'. Perhaps this could imply that behavioural factors masked any progressive augmentation that did occur. If we consider that the behavioural aspect of hypoxic ventilation was eliminated or minimized during the IH, then the current study would support research that has shown IH to cause an increase in the AHR_{VE}. Although LTF was discussed in the animal model, the existence of LTF in the human model is much less observed (123). Our data clearly shows no LTF effect, and is consistent with many other human studies (33, 68, 100).

It has been suggested that IH is an effective way to preaclimatize before high-altitude climbing, for example IH has been suggested to improve mitochondrial respiration (136). Katayama et al. (72) found 3 weeks of IH (4,500 m simulated altitude for 90 min, 3 days/week) to cause a decrease in oxygen consumption during submaximal exercise, although there was no change in V_E during submaximal exercise following IH. The same group supported these findings with the use of a more intense IH protocol (~12% O_2, 3 hrs/day, 14 days) (73), suggesting IH to produce an increased submaximal exercise efficiency (72). Our data show no change in submaximal V_E or VO_2 following IH (Table 7). Explanation for the lack of consistency between our results and the results of others, may be due to the severity of hypoxia experienced. Studies from Katayama et al. (72, 73) which showed a reduced VO_2 following IH implemented an IH (1.5-3 hr/day, 14-21 days) which was much more severe then that of the current study (1 hr/day, 10 days). Further, the same group has also shown no change in

submaximal V_E and VO_2 during submaximal exercise following IH (4,500 m simulated altitude, 1 hr/day, 7 days) (77). This is consistent with our data. Therefore, it is difficult to draw any definitive conclusions about ventilatory parameters during submaximal normoxic exercise following IH.

Although we found a trend for an increase in the AHR_{V_E}, there was no change in $V_{E_{CO_2}}$ following IH at rest or during submaximal exercise (Table 9). This is consistent with many other studies (33, 74, 76). Foster et al (33) found no change in the ventilatory response to CO_2 following two different protocols of IH (short duration: 5 min 12% O_2 at 5 min intervals for one hour; long duration: 30 min 12% O_2). Similarly, Katayama and colleagues found no change in the ventilatory response to CO_2 following IH with the use of a hypobaric chamber (4,500 m simulated altitude) (74, 76). In contrast, Ainslie et al. (6) demonstrated an increase (48%) in the $V_{E_{CO_2}}$ following five nights of simulated altitude (hypoxic tent; $FiO_2 \sim 13.8\%$), which then normalized to baseline five days following the last night of hypoxia. The authors of that study suggest that their atypical results are due to the severity of IH. To substantiate this claim, Schoene et al. (133) had subjects simulate a 40 day ascent of Mt. Everest (hypobaric chamber). An increase in the $V_{E_{CO_2}}$ was found at 305 Torr, but not at 428 Torr. Also, the majority of studies that have shown no change in the $V_{E_{CO_2}}$ have utilized an isocapnic IH protocol. It has been suggested that IH will only cause in increase in the $V_{E_{CO_2}}$ under poikilocapnic hypoxia; thereby producing respiratory alkalosis (6). Collectively, it appears that the current study did not show a change in the $V_{E_{CO_2}}$ due to the severity of the IH protocol and the use of an isocapnic IH protocol.

Effect of IH on Cardiovascular Regulation

The increase in MAP in response to hypoxia is a multifaceted response, which includes influences from increases in HR and possibly sympathetic influences (129). There is also strong evidence for the influence from activation of arterial chemoreceptors (47, 94). Similar to the AHR_{VE}, an increase in the AHR_{MAP} has been shown to occur following extended hypoxia exposure, although no change occurs in the AHR_{HR}. Insalaco et al. (63) found the AHR_{MAP} to be increased following one day at altitude (5,050 m), which was elevated further following 24 days at altitude. Also, many investigators have shown an increase in the AHR_{MAP} following IH. Katayama et al. (78) detected an increase in both the systolic and diastolic blood pressure response to acute hypoxia following seven days of IH (4,500 m simulated altitude, 1 hr/day), while no change was detected in the HR response to acute hypoxia. In addition, our lab has shown a trend for an increase in the systolic and diastolic blood pressure responses to acute hypoxia following IH (33). In contrast, a study by Lusina et al. (95) found no change in the blood pressure response to acute hypoxia following isocapnic IH (SaO_2 = 80%, 1 hr/day, 10 days). As of yet, our lab has failed to show a change in the AHR_{HR} following IH, which is consistent with other findings (63, 78). Although this is not the first study to show no change in the AHR_{HR} following IH, the reasoning for this is still uncertain. It has been suggested that the regulation could be the result of the coordination of multiple systems, which include carotid and aortic chemoreceptor reflexes, lung inflation receptors, and baroreflexes (78). Clearly, more research is needed to elucidate this issue. In addition, there was no effect of IH on the AHR_{SV} and AHR_{Q}. The AHR_{SV} and AHR_{Q} appear to be quite variable, and the effect of IH on the AHR_{SV} and AHR_{Q} has not been highly documented. Similar to the Lusina et al. (95) study, the current study did not show any effect of IH on the AHR_{MAP}, AHR_{SBP}, AHR_{DBP}, or AHR_{HR}. There are multiple explanations for the lack of increase in blood pressure response to acute hypoxia. First, the protocol used to test the blood pressure responses to acute hypoxia may have not been sensitive

enough to detect blood pressure changes. In fact, the acute hypoxic test did not cause a significant increase in MAP, SBP, or DBP. Similarly, Imadojemu et al. (59) found no increase in MAP during five minutes of 10% O_2, an FiO_2 similar to that achieved in the current study (~11% O_2). Comparable results were observed by Van Mil et al. (146) where 20 minutes of hypoxia (SaO_2 = 80%) caused no change to MAP. Second, the IH protocol employed in the current study may not have been severe enough to cause an increase in carotid body sensitivity, thereby increasing blood pressure sensitivity. To support this, Fletcher et al. (30) found IH to cause an increase in resting rat blood pressure; however, the increase was abolished in rats which had undergone carotid body denervation, implying a requisite role of the carotid body for changes in MAP. Although the current study shows a trend for an increase in the AHR_{VE}, it did not reach significance. Therefore, it appears that the IH protocol used in this study was not severe enough to cause the increase in carotid body sensitivity explaining the lack of increase in the AHR_{MAP}, AHR_{SBP}, AHR_{DBP}, and AHR_{VE}. Lastly, it is possible that a short duration-type of IH protocol is required to evoke an increase in the AHR_{MAP}. In the rat model, carotid body sensitivity has been shown to increase after a short duration IH protocol (15 s of 5% O_2 + 5 min of 21% O_2, 9 episodes/h, 8 h/day, 10 days), and not following a long duration IH protocol (4 h of hypobaric hypoxia (0.4 atm/day) + 20 h of normoxia, 10 days) (112).

During rhythmic muscular activity, measurable increases in MAP occur; due to an increase in SBP. Wang et al. (148) measured the MAP response to a graded exercise test (bicycle) before and after either 'moderate' (15% O_2) or 'severe' (12% O_2) IH (1 hr/day, 5 days/week, 4 weeks). A greater increase in MAP was shown to occur in the group who underwent the severe IH protocol. In contrast, the current study did not show any change in the blood pressure response to submaximal exercise following IH; although the exercise intensity was demanding enough to cause an increase in blood pressure. Rationale for the difference between the current study and that by Wang et al. (148) is twofold. First, the IH protocol used by

Wang et al. (148) (4 weeks) was much longer then that of the current study (10 days). They found a blunted hyperaemic response on the subjects in the severe IH group, but not in the moderate IH group. The authors suggested this was due to an impaired NO response, since there was a blunted reactive hyperaemia response and dilation to acetyclcholine; however, neither of the groups demonstrated a change in the dilatory response to sodium nitroprusside. It is possible that their study was severe enough to attenuate the shear-stress induced vasodilation during exercise, due to decreased bioavailability of NO (148). Second, in the severe IH group, the increase in the MAP response to exercise occurred at an exercise intensity of/or greater than 160 W. The highest exercise intensity that we used was 150 W. It is possible that our protocol was not intense enough to show any effect of IH on blood pressure during exercise.

In general, our data show no increase in any cardiovascular variable in response to hypercapnia. The implementation of a hypercapnic gas was to test cerebrovascular regulation, rather than cardiovascular regulation. Our data is consistent with previous literature. Serebrovskaya (135) found no change in the blood pressure response to a rebreathing hypercapnic protocol. In fact, a slight decrease in SV was observed, and a small increase in Q was observed once $P_{ET}CO_2$ reached high values (~58 mmHg). The increase in Q was attributed to an increase in HR. Our data shows an increase in MAP (due to an increase in SBP) at one time point (POST-IH, 100 W) in response to hypercapnia. The highest $P_{ET}CO_2$ experienced by our subjects was under hypercapnic conditions at 150 W, where they reached ~56 mmHg. This was accomplished with the administration of 5% CO_2. Even though CO_2 can cause an increase in cardiovascular variables (e.g. SBP, DBP, MAP, HR), it is most often the result of intense hypercapnia (>7% CO_2) (134). It is possible that the intensity of CO_2 was not great enough to cause changes in cardiovascular variables.

Effect of IH on Cerebrovascular Regulation

Hypoxia, a potent vasodilator, initiates an increase in CBF (12). The response is multifaceted, which includes NO, adenosine, K^+, and prostaglandins (48, 128, 146). Van Mil et al. (146) measured the change in CBF during hypoxia with and without infusion of L-NMMA. In the normoxic condition, no change was observed in CBF following infusion of L-NMMA, whereas in the hypoxic condition CBF significantly increased, after which it decreased following L-NMMA infusion. The authors suggested NO to be a key regulator of cerebral vessel dilation to hypoxia (146). In contrast, IH has been shown to alter the typical response of NO to hypoxia. Phillips et al. (113) found a decrease in the vasodilatory response of cerebral (middle cerebral) and peripheral (gracilis) arteries to acute hypoxia, which was attributed to an IH-induced decreased bioavailability of NO. Similar results are found in a human disease model, where sleep apnea sufferers have a reduced CBF response to acute hypoxia; however the response is normalized following four to six weeks of continuous positive airway pressure (CPAP) therapy (32). Lastly, in a healthy human model, our lab has shown 10 days of IH to cause cerebral dysregulation to acute hypoxia, measured both via near-infrared spectroscopy (33) and transcranial Doppler (unpublished). Lavie et al. (91) found sleep apnea patients to have significantly lowered levels of circulating NO in comparison to healthy controls. Further, nine months of CPAP therapy significantly increased circulating NO and L-arginine (91). IH (hypoxia/reoxygenation) can produce ROS and an inflammatory response (22), which leads to oxidative stress (90). Similar to the animal model, in the human disease model (sleep apnea), the development of ROS and oxidative stress causes dysregulation of NO which results in a blunted forearm vessel dilation to acetylcholine but not to sodium nitroprusside (exogenous NO donor) (79). This suggests that sleep apnea patients have a normal smooth muscle response, although an impaired endothelium-dependent dilation. Given this, we expected our subjects to have a blunted CBF response to acute hypoxia following IH; however, this was not necessarily the case. As a

group, the POST-IH AHR_{MCAVm} (3.7 ± 3.7 cm/s) was almost identical to the PRE-IH AHR_{MCAVm} (3.9 ± 9.2 cm/s). Individually, five subjects displayed a blunted AHR_{MCAVm} following IH, whereas four subjects showed an increase (Figure 4). The variability in the change in AHR_{MCAVm} warrants discussion. The rationale for maintaining the IH sessions at 80% was to expose each individual to the same absolute hypoxic stimulus. If we were to set the IH sessions at a desired FiO_2, which is most often used in this type of study design, the hypoxic stimulus would differ between individuals. Since the ventilatory response to hypoxia is variable between individuals, standardizing the FiO_2 would most likely result in a different SaO_2 between individuals. However, we still observed variability in the AHR_{MCAVm} in response to IH. In fact, this is consistent to what we have previously shown in our lab. Foster et al. (33) found a blunted cerebral oxygenation sensitivity (NIRS) following 10 days of isocapnic IH. However, it is important to note that two different protocols of IH were employed in this study; a short duration (5 min 12% O_2 at 5 minute intervals, 1 hr/day, 10 days) and a long duration protocol (30 min 12% O_2, 10 days). The blunted cerebral oxygenation response was primarily driven by the short duration group; specifically, eight of the nine subjects in the short duration group showed a blunted response whereas only five of the nine in the long duration group showed the blunted response. The protocol in the current study is most similar to the long duration group in the Foster et al. (33) study. Similar to these results, we also found that five of the nine subjects in the current study to show a blunted AHR_{MCAVm}. As discussed earlier, the patterning of the IH may have played a key role in the AHR_{MCAVm}. In a preliminary study, we found the AHR_{MCAVm} to be blunted following a short duration IH protocol which mimicked the short duration protocol in the Foster et al. (33) study. Also, if we consider animal work, it is possible that our protocol did not continue long enough to cause consistent vessel dysregulation. Phillips et al. (113) found a blunted vessel dilatory response to acute hypoxia in rat cerebral arteries following 14 days of IH (FiO_2 = 10% for 1 min at 4-min intervals, 12 h/day). Similar lines of evidence in the animal (30,

31) and human (148) models required ~28-35 days of IH to show vascular dysfunction. Therefore, although ROS production can increase quickly in response to hypoxia (141), the length of time needed for ROS to cause a measurable effect on the bioavailability of NO may be longer.

In contrast, Kolb et al. (84) found an increase in the CBF response to acute hypoxia following five poikilocapnic hypoxic nights (FiO_2 ~ 13.8%, 8 hrs/day). Additionally, Jensen et al. (66) showed an enhanced CBF response to acute isocapnic hypoxia following 5 days of altitude acclimatization (3,810 m). However, since the hypoxia exposure was at altitude, CO_2 was not controlled. Also, these two studies resemble more of a long duration hypoxia exposure which results in different ventilatory, cardiovascular and cerebrovascular adjustments to short duration exposures.

More recently, Ainslie et al. (2) found an increase in the CBF response to acute poikilocapnic hypoxia following 10-12 days of IH (5 minutes at a SaO_2 of 78-88% at five minute intervals, 90 mins/day). Methodological considerations for this study reveal problems for comparison to the current study. First, their sample included both males and females. Progesterone and estrogen vary across the menstrual cycle (~2-8 mmHg) which has direct ventilatory and cerebrovascular consequences (43, 86). Considering the length of their protocol, female sex hormones would have likely affected the results. Most importantly, the protocol to measure the response to hypoxia was poikilocapnic. The CBF response to acute hypoxia when CO_2 is not controlled is quite variable. In fact, the change in CBF to poikilocapnic hypoxia (4,559m simulated altitude) in one study has been shown to range from a 3% decrease to a 20% increase, and the variability increases with time in hypoxia (10). Comparison of the current study's results to that of Ainslie et al. (2) is problematic due to these reasons.

Although most research has attributed alterations in CBF following IH to changes in the cerebral vessels' ability to vasodilate, it is important to also consider perfusion pressure. Our

results showed an association (r = 0.86) between changes in AHR_{MCAVm} and AHR_{MAP}; therefore, those individuals who showed a blunted AHR_{MAP} POST-IH compared to PRE-IH were likely to show a blunted AHR_{MCAVm}. This points to a key role of perfusion pressure in CBF changes. Ainslie et al. (2) found an increase in both the MAP and CBF response to acute hypoxia following IH. Collectively, this could suggest that the changes observed in the CBF sensitivity were at least partially mediated by the changes in MAP sensitivity. Typically, cerebral autoregulation is effective in maintaining CBF constant to changes in arterial pressure. This system responds quickly, where changes in CBF in response to changes in MAP usually last only a few seconds (1). However, cerebral autoregulation appears to be impaired in hypoxia and high-altitude residents (65). Iwasaki et al. (64) found cerebral autoregulation to be impaired at an FiO_2 of 15%, but not at 17% or 19%. Similarly, Ainslie et al. (7) found the CBF and MAP sensitivities to acute hypoxia were related during hypercapnic and isocapnic hypoxia, but not during poikilocapnic hypoxia. In contrast, Ainslie et al. (3) recently reported cerebral autoregulation to be maintained during acute hypoxia (FiO_2 = 12%, 20 minutes). Also, administration of hyperoxia can improve autoregulation in high-altitude dwellers (65). It is apparent that evidence regarding the effect of hypoxia on cerebral autoregulation is far from conclusive, and more work is needed.

Exercise increases flow to vascular beds, which includes the brain (19, 45, 56, 60, 71, 93, 105, 107, 115-117). It is thought that the increase in flow in vessels of working muscles and the brain results in an increase in shear stress, thereby releasing NO from the endothelium (38, 102). In the animal model, the increase in flow has shown vasodilation (in situ), which is abolished by removal of the endothelium (140). In the current study, MCAVm significantly increased from rest at 150 W in both the PRE-IH and POST-IH exercise trials; there was no effect of IH on CBF during submaximal exercise. This is somewhat expected since there was no change in the AHR_{MCAVm}. However, the subjects that showed a decrease in the AHR_{MCAVm} following IH, did not necessarily show a change in MCAVm during exercise. We would expect that subjects who

had a blunted AHR_{MCAVm} following IH would have a decreased bioavailability of NO, thus decreasing the MCAVm during exercise. Even though NO has been implicated as a major cause for vessel dilation from increased shear stress (23), the increase in flow during exercise can be due to other reasons, including the release of metabolites, myogenic properties of the vessels, increase in metabolism, autonomic neural control, and mechanical factors (99). There is a possibility that other mechanisms made up for the vasodilation during exercise that was originally caused by the release of NO, or the contribution of NO to exercise-induced vasodilation is not as important as thought by some (23, 38). Even though the study design does not provide information regarding the mechanism of CBF regulation during exercise, it appears that cerebrovascular regulation is maintained during submaximal cycle exercise following IH.

In the rat model, NO has been shown to significantly contribute to cerebral vessel dilation during hypercapnia (55). Similarly, patients with endothelial dysfunction (e.g. diabetes mellitus and/or hypertension) show a reduced cerebral CO_2 reactivity, which is improved with infusion of L-arginine or sodium nitroprusside (89, 153). Therefore, we would expect our subjects to have a decreased CO_2 reactivity following IH. However, there was no difference in $MCAVm_{CO_2}$ between PRE-IH and POST-IH at rest or during exercise. Also, there was no association between the changes in AHR_{MCAVm} and $MCAVm_{CO_2}$. As discussed earlier, there is a possibility that our protocol was not severe enough to cause a change in the bioavailability of NO. Also, the regulation of CBF in hypercapnia is not entirely dependent upon NO. It has been found that CO_2 and pH have a direct effect on vessel tone; although the mechanisms of action are unclear (12, 48, 87). Therefore, even if our IH protocol was severe enough to cause a decreased bioavailability of NO, there is the possibility that the $MCAVm_{CO_2}$ would remain unchanged. We also designed the current study on the assumption that IH would cause endothelial dysfunction, thereby impairing endothelial-derived NO. It is important to note that NO is not produced solely by the endothelium. In fact, Toda et al. (145) found the cerebral vessel (dog basilar and middle

cerebral arteries) response to hypercapnia was unchanged after endothelial denudation. However, application of the findings to the current study is problematic since the study by Toda et al. (145) used much higher levels of CO_2 (5-15%). In contrast to the current study and previous literature, Kolb et al. (84) found IH (~13.8% O_2, 8 hr/day, 5 nights) to increase the CBF response to acute hypercapnia. Unfortunately, this is the only study to report this finding and, to our knowledge, physiological rationale for this change has not been documented.

Summary of Results in Context of Hypotheses

This study was designed in order to specifically test three hypotheses. It was hypothesized that in comparison to baseline, IH would result in:

(1) An increased $\dot{V}_E$, and MAP response to acute hypoxia, and a blunted CBF response to acute hypoxia. The results of this study show high variability in the change in the $\dot{V}_E$ response to acute hypoxia following IH. As a result, there was no significant increase in $\dot{V}_E$ chemosensitivity following IH, which does not support our hypothesis. Measuring $\dot{V}_E$ chemosensitivity simply once following IH may be insensitive to changes due to large intra- and inter-individual variability. Nonetheless, we are confident the IH protocol did alter $\dot{V}_E$ chemosensitivity, as supported by the progressive increase in $\dot{V}_E$ in response to hypoxia throughout the 10 days of IH.

The data also did not support our hypothesis for an increased MAP response to acute hypoxia following IH. Research has suggested a key role of the carotid body for an increase in MAP during hypoxia (94). Although our study did not directly measure carotid body sensitivity, the non-significant change in MAP chemosensitivity suggests that the IH was not severe enough or did not contain adequate hypoxia iterations to cause an increase in carotid body sensitivity. This was supported by no change in $\dot{V}_E$ chemosensitivity measured on PRE-IH and POST-IH tests.

There is evidence to show IH to increase ROS which decreases the bioavailability of NO, thereby reducing cerebral vessel dilation to hypoxia (113). However, contrary to our hypothesis, IH did not alter cerebrovascular chemosensitivity. Although we have reason to believe the IH did increase ROS (141), the physiological relevance is unclear; other compensatory mechanisms (e.g. adenosine) may have counteracted the maladaptive effect of IH. Despite that, the change in cerebrovascular chemosensitivity was highly related to the change in MAP chemosensitivity, which suggests a key role of perfusion pressure in CBF changes.

The change in the $\dot{V}_E$, MAP and CBF response to hypoxia is highly susceptible to the length, severity, and pattern of the IH protocol, which could have contributed to data which did not support our hypothesis. Although difficult due to logistical reasons, it would be beneficial to measure the $\dot{V}_E$, MAP and CBF responses to hypoxia throughout a long-term (months to years) IH study in humans to obtain the time-course of the cardiorespiratory and cerebrovascular chemosensitivity changes.

(2) A blunted CBF response to submaximal exercise. The data does not support the hypothesis, since IH did not alter CBF during exercise. CBF increased in response to exercise intensity, however, the magnitude of change was similar between the PRE-IH and POST-IH exercise tests. Although some have implicated NO as a key variable for exercise-induced vasodilation (38), it has also been suggested that the contribution of NO is overemphasized (28). If IH did in fact decrease available NO, it is possible that metabolic, neural, and mechanical mechanisms compensated to ensure adequate CBF during exercise.

(3) A reduced CBF response to hypercapnia at rest and exercise. Eliminating the contribution of NO during hypercapnia in a rat model reduces the typical increase in CBF (55). Similar results are found in humans with endothelial dysfunction (89). In the current study, CBF during hypercapnia was similar on PRE-IH and POST-IH tests, which does not support our hypothesis. Although the complete mechanism for hypercapnic vasodilation is unclear, CO_2 and

pH can have direct effects on cerebral vessel tone (48). Therefore, although we did not directly measure endothelial function, CBF during hypercapnia at rest may have been preserved following IH due to the direct effect of CO_2 and pH. In terms of the maintenance of CBF during exercise with the addition of hypercapnia, the regulation of CBF during exercise is a multifaceted system and endothelial dysfunction may play a smaller role during exercise due to other compensatory factors (e.g. cerebral metabolism). The lack of support for our hypotheses prompts serious consideration into the pattern of IH, as well as the mechanisms thought to be responsible for the physiological changes. The length of the protocol appears to dictate whether IH produces beneficial or harmful effects, and it is ill-advised to assume that the point where IH changes between helpful and harmful is the same for all physiological systems. Also, the mechanistic reasoning for the physiological changes needs extensive investigation, since it is a true integrative response. Unfortunately, the design of the current study only allows for speculation as to the mechanisms responsible.

Limitations

The study of the physiological responses to hypoxia by non-invasive means requires assumptions to be made about the measurement techniques. In the current study, MCAV was measured via TCD and taken to represent CBF. This measurement technique provides beat-by-beat measurement of changes in the vessel's blood velocity. It is calculated from the maximal frequency of the Doppler shift, which is assumed to represent the mean flow in the centre of the vessel. Interpretation of an increase in CBFV as a reflection of an increase in flow is also dependent upon the assumption of a constant diameter of the insonated vessel. Our protocol included the use of hypoxia and hypercapnia. Although hypoxia and hypercapnia induce increases in flow via vasodilation, it is likely that changes in CBF were not due to changes in diameter of the MCA. With the use of the TCD power (a measure of the amount of red blood

cells) Poulin et al. (121) found no change in the diameter of the MCA during hypoxia, or hypercapnia. These findings have been supported with the use of magnetic resonance imaging (137). TCD is often implemented for investigations into beat-to-beat changes to cerebral perfusion, especially during exercise. It should be noted that changes in flow can also be modified depending on the vessel insonated. Hellstrom et al. (49) measured the changes in the internal carotid artery (ICA), common carotid artery (CCA), and middle cerebral artery (MCA) during increasing workloads. The ICA and MCA both experienced increased blood flow at the onset of exercise, which decreased at the highest workload. In contrast, the CCA increased at every workload; it did not experience the decrease at the higher work rates as the ICA and MCA did. This suggests that conclusions drawn with the use of this technique may be limited (37, 122). Contrary to information suggesting that flow is distributed evenly between major arteries, this study by Hellstrom et al. (49) suggest that flow to the brain is not evenly distributed during exercise. However, the current study utilized the MCA for insonation in order to compare results with other literature. Similar to other methods, TCD requires a high-quality signal which can be compromised by head movement, especially during exercise; although the use of a custom-made headband device can help minimize artifacts produced by small head movements (122).

Throughout the acute hypoxic response and IH, we measured arterial oxyhaemoglobin saturation via pulse oximetry. Ideally, measurement of arterial gas concentrations would have been performed. Pulse oximetry does not account for changes in temperature or pH, which can affect oxygen-haemoglobin binding. However, we do not expect the subjects to have experienced drastic changes in temperature or pH, since the testing environment was maintained at room temperature and isocapnia was maintained during the acute hypoxic tests.

The AHR_{VE} can be a variable measure (29, 52, 147, 152), therefore the measurement at two time points may not have been ideal; however, similar study designs have previously shown changes in the AHR_{VE} (75, 78, 95). Our lab has shown repeated AHR_{VE} measurements (five

times) to yield a mean individual coefficient of variation of 27% (83), which is similar to what others have reported (152). In addition, the testing environment was kept tranquil in order to keep the subject calm, thus minimizing behavioural influences on $\dot{V}_E$.

The current study was based on the assumption that IH would increase ROS, decreasing the bioavailability of NO, thereby impairing the vasodilatory response to hypoxia and exercise. Steiner et al. (141) discovered graded hypoxia (15% O_2, 10% O_2, 7.5% O_2) to cause significant increases (123%, 147%, 167% of control, respectively) in DHR fluorescence, a marker of ROS, in rat vessels. During the IH in the current study, FiO_2 was ~ 11%; therefore it is probable that our IH protocol did in fact cause an increase in ROS. Unfortunately, we did not directly measure ROS in our subjects, and there is a possibility that an increase in ROS did not necessarily impair NO function; or our measurement techniques were not sensitive enough to detect the effect.

A final limitation concerns the recruitment for the study. Subjects consisted of healthy, college-aged males. Therefore, the findings can only be extended to this population. Although much of the study design was based on a clinical model (sleep apnea), comparison is difficult since sleep apnea patients most often have confounding ailments (e.g. obesity). Also, we did not include a control/sham group that underwent 10 consecutive days of breathing room air. Therefore, the possibility of a placebo effect does exist. Due to the logistics of the study design, a sham group is rare in IH studies. Given this, the conclusion of studies which have included a control group are unchanged (72, 77, 78).

Conclusions

The results from this study indicate that our IH protocol did not cause any form of ventilatory, cardiovascular, or cerebrovascular long-term facilitation, which is consistent with many other lines of evidence. Contrary to other studies, the current study did not show an increase in the ventilatory or cardiovascular sensitivities to acute hypoxia following IH. Rather,

there was a progressive increase in V_E during IH over the 10 days, indicating that our study did alter ventilatory chemosensitivity. Our data also suggests that cerebrovascular regulation to acute hypoxia following IH may be individualistic. There was an association between the AHR_{MAP} and AHR_{MCAVm} in response to IH. This suggests that perfusion pressure at least partially mediates the changes in AHR_{MCAVm} following IH. To our knowledge, this is the first study to investigate the effect of IH on CBF during exercise. The results from this study indicate that ventilatory, cardiovascular and cerebrovascular regulation is unchanged during submaximal exercise. Further research is needed to investigate the effect of IH length on the cerebrovascular changes to acute hypoxia and exercise.

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APPENDIX A - Review of Literature

The average human brain weighs 1.4 kg (128), receiving approximately 750 ml/min of blood at rest which accounts for 15% of total body cardiac output (87). Adequate cerebral blood flow is attained by a sound arterial system. The brain is primarily supplied by four arteries: two vertebral and two carotid arteries. The four arteries converge to from the Circle of Willis, an arterial system that allows multiple pathways to supply cerebral tissue, which protects against cerebral ischaemia if one artery becomes blocked. The Circle of Willis diverges to provide perfusion of different parts of the cortex via the anterior, posterior and middle cerebral arteries.

Originally thought to remain constant (48), cerebral blood flow is in fact a dynamic system that can experience sudden drastic changes in flow. However, variations regularly occur in response to metabolic, chemical, and pressure influences. Two important homeostatic systems are in place to ensure cerebral tissue is well protected: cerebral autoregulation and cerebral chemoregulation. Cerebral autoregulation maintains a steady cerebral blood flow during hypotension by decreasing resistance, and protects smaller vessels by constriction of feeder arteries during hypertension. Cerebral chemoregulation allows for constant metabolism by increasing flow during hypoxia, and hypercapnia.

Given that cerebral tissue is intolerant of ischaemia, an increase in activation is accompanied with an increase in VO_2, which is accomplished by increasing blood flow. In this sense, an increase in metabolism is in some way matched with an increase in flow (88). Linkis et al. (93) measured increases in middle cerebral and anterior cerebral artery velocities during right hand contractions, right foot movements, and cycling. The greatest increase in flow was seen in the left middle cerebral artery, and the left anterior cerebral artery during the right hand contraction and right foot movements, respectively. Also, the mean flow velocity in the left and right middle cerebral artery and anterior cerebral artery increased to the same extent during cycling. This suggests that the increase in flow is regional to the arteries which supply the

cortical representation of the exercising extremity (93). The hypothesis that increased brain activation is matched with an increase in flow has been supported by others (71).

Regulation of Cerebral Blood Flow

As discussed, a variety of influencing factors can lead to changes in brain perfusion; carbon dioxide possibly being the most important (12, 61). Carbon dioxide affects cerebral vessels similarly to how it affects other vascular beds: as a potent vasodilator, where hypercapnia elicits an increase in cerebral perfusion. Additionally, hypoxia elicits cerebral vessel dilation; however, the vasodilating effect of hypoxia is less potent to that of hypercapnia. Conversely, hypocapnia (67), and possibly hyperoxia (11), cause a reduction in CBF. Of the two main chemical stimuli for changes in CBF, CO_2 appears to produce the greatest effects. Ainslie and Poulin (7) measured the acute hypoxic cerebral blood flow response at rest with a hypocapnic, isocapnic, and poikilocapnia background. In comparison to the isocapnic condition, the poikilocapnic condition resulted in $\dot{V}E$ that elicited a decrease in CBF. It was suggested that poikilocapnia permitted a ventilation induced hypocapnic vasoconstriction (7). Therefore, it was demonstrated that the dilating effects of hypoxia can be attenuated, or even abolished by the constricting effects of hypocapnia.

Although the main regulating factor of CBF has been suggested to be extracellular pH, this may not be the only factor controlling cerebral vascular resistance (12). The blood brain barrier readily diffuses CO_2, where it dissociates to form H^+ and HCO_3^-. It is suggested that the H^+ produces the relaxant effect of cerebral vasculature. Therefore, CO_2 *per se* would not be the direct regulating factor. Further to this, arterial H^+ would be a poor indication of cerebral vessel dilation, as H^+ slowly crosses the blood brain barrier (88, 128). The time course of the response of cerebral vessels to a change in pH is quick, where changes in diameter can be observed within

10 s (41). The vasoactive response to CO_2 is quite significant, with a 30.5% · kPa^{-1} change in diameter (125). During exercise, CO_2 reactivity increased to 40.6% · kPa^{-1} (125).

Cerebral autoregulation is a homeostatic mechanism which retains cerebral blood flow stable in face of changes in arterial pressure. Myogenic properties of cerebral vessels respond to increases in arterial (perfusion) pressure by vasoconstricting and vasodilating during times of sudden hypertension and hypotension, respectively (85). This is an efficient system which maintains a steady cerebral blood flow from arterial pressures of ~50-175 mmHg (87, 128). To extend this, Ogoh et al. (2005) measured the effect of increased and decreased cardiac output on cerebral blood flow (106). Decreases and increases in cardiac output at rest by lower body negative pressure and infusion of albumin, respectively, showed a significant linear relationship between middle cerebral arterial blood flow velocity and cardiac output (106).

Cerebral Blood Flow Response to Hypoxia

The relationship between arterial oxygen pressure (PaO_2) and CBF is curvilinear (15, 48, 128). Maximal vessel dilation in response to hypoxia does not occur at the onset of inspiring hypoxic gas, but rather six minutes after the initiation of hypoxia (139). It is important to note that cerebral autoregulation is abolished once arterial oxygen saturation decreases below 60% (42). However, cerebral autoregulation has also been shown to be impaired at a less severe level of hypoxia (SaO_2 ~80%) (7). Furthermore, hyperoxia has been shown to cause a slight decrease in CBF (80, 92).

As mentioned previously, an accompanying hyperventilation induced hypocapnia, can mask any changes in CBF during hypoxia. Huang et al. (53) found no significant difference between internal carotid artery blood flow velocity at rest during normoxia and acute hypoxia; attributing the lack of change to concomitant hypocapnia (53). In contrast, others have shown acute hypoxia to increase CBF when CO_2 is uncontrolled, overriding the constricting effects of

hypocapnia (15, 80). Also, when isocapnia is maintained, acute hypoxia causes an increase in CBF (7, 48, 119, 128). The mechanisms for cerebral vessel dilation to hypoxia are not fully understood; however, a likely cause stems from hypoxia induced release of adenosine, a strong vasodilator, NO, K^+, and prostaglandins (48, 128, 146). Although hypoxia may cause acidosis, changes in vessel diameter are observed before changes in pH, attributing the change in diameter during hypoxia to a factor other than pH (51).

More recently, evidence for the effect of NO on cerebral vessel dilation has gained attention. Human vascular tone is strongly mediated by endothelial derived NO. As such, inhibition of nitric oxide synthesis by infusion of N^G-monomethyl-$_L$-arginine (L-NMMA) during hypoxia, results in reduced forearm blood flow (13). NO induces vasodilation by working through guanalyl cyclase resulting in relaxation of smooth muscle (13). In addition, the relaxant effect of NO has been suggested to mediate cerebral dilation during acute hypoxia (146).

When hypoxia exposure is lengthened and becomes chronic, CBF tends to slowly return to pre-exposure levels. Severinghaus et al. (138) found CBF to increase from rest by 24% and 13% after 6-12 hours and 3-5 days at altitude, respectively (138). The changes in CBF occurred even though arterial CO_2 pressure ($PaCO_2$) remained lower than sea level. To extend this, the increase in CBF during chronic hypoxia has been shown to be abolished after three weeks of exposure (151). Possible explanations for the re-normalization of CBF are an increased in oxygen carrying capacity, restoration of tissue oxygen tension, and increased capillary density (151).

The hypoxic protocol, whether it be sustained or intermittent, appears to be important in the physiological changes that result, where episodes of hypoxia interspersed with re-oxygenation incur a greater magnitude in the physiological response, which will be discussed below (33, 112). Prolonged hypoxia may differ in the CBF response to acute hypoxia, due to changes that occur in the cerebrospinal fluid (CSF). Acute exposure will decrease $PaCO_2$, with a

similar decrease in CSF PCO_2, therefore increasing CSF pH. However, prolonged hypocapnia which accompanies prolonged hypoxia eventually normalizes CSF pH, thereby stabilizing CBF to pre-hypoxia values (138)

Intermittent Hypoxia and Cerebral Blood Flow

Intermittent hypoxia (IH) refers to transient episodes of hypoxia. These environments are voluntarily experienced by mountaineers and athletes as a training regimen, and free-divers. Also, involuntary recurrent hypoxia is experienced in pathophysiological conditions such as sleep apnea, chronic obstructive pulmonary disease, and apneas in infants. Depending on the condition, hypoxic episodes can range from seconds to days (124).

IH is used in the laboratory as a model of these preceding conditions in order to understand the pathophysiological changes inherent to the condition. Research has shown that IH causes many different physiological changes, many of which are detrimental to health. Some examples include elevated sympathetic nervous activity (95), hypertension (31), and stroke (26, 110). Obstructive sleep apnea, where cessation of breathing occurs during sleep as a consequence of an obstructed airflow, is an important health concern with high mortality (44). The increased incidence of stroke in patients with sleep apnea was suggested to be a consequence of the episodic hypoxia and not the concurrent hypertension or obesity, which often accompanies obstructive sleep apnea (79). Kato et al. (79) tested endothelial function in obstructive sleep apnea patients and matched controls. The results show that those patients with obstructive sleep apnea had a severely blunted response to acetylcholine, an endothelium-dependent vasodilator, when compared to matched controls (79). The authors suggested the mechanism to be attributed to a defect in the biosynthesis of nitric oxide from L-arginine (79). Also, Foster et al. (32) measured the CBF response to acute hypoxia in sleep apnea patients before and after CPAP therapy. Although the sleep apnea patients showed an impaired CBF

response, following CPAP therapy the CBF response was similar to those of control subjects. In the rat model, Phillips et al. (113) measured the vasodilator response to acute hypoxia in cerebral and limb arteries, following IH. Experimental male rats were exposed to IH (10% fraction of inspired oxygen for 1 min at 4 min intervals, 12 h/day) for 14 days. After the intermittent hypoxic protocol, the middle cerebral and gracilis arteries were removed in order to test vasodilator response to reduced oxygen. Both the middle cerebral and gracilis arteries' vasodilator response to acute hypoxia were almost completely abolished following IH (113). It was suggested that the mechanism for the reduced response to acute hypoxia was most likely due to the endothelium dependent relaxation rather than a decreased sensitivity to nitric oxide.

Recently, our laboratory tested the sensitivity of cerebral oxygenation in the human model following IH. Human subjects underwent either a long-duration (30 min 12% O_2) or short-duration IH protocol (5 min 12% O_2 at 5 min intervals), for 10 days. Cerebral sensitivity (Δ cerebral tissue oxygenation/Δ SaO_2) was significantly lower by the end of the IH protocol in both groups; however, the response reached greater magnitude in the short-duration group. These findings support the study by Phillips et al. (113) and suggest cerebral vascular dysregulation following IH (33). The Foster et al. (33) study measured cerebral oxygenation via near-infrared spectroscopy. Near-infrared spectroscopy measures differences in chromofore concentrations which is an indication of oxyhaemoglobin and deoxyhaemoglobin (143); however, it does not provide an actual indication of flow. Therefore, cerebral perfusion could not be assessed. We recently followed this study by measuring the effects of IH on cerebral blood flow velocity, a better indication of cerebral perfusion. The cerebral blood flow velocity response to acute hypoxia was measured in eight subjects who underwent 10 consecutive days of IH (12% O_2 for 5 min at 5 min intervals, 1 h/day). Results showed a blunted cerebral blood flow response to acute hypoxia. This would suggest cerebral dysregulation, and upon review of the literature it is

cautiously suggested to be due to a defect in the vasodilating effect of NO. Taken with the available literature, IH appears to cause a blunted cerebral vasodilator response to acute hypoxia.

In contrast, Kolb et al. (84) found an increase in the cerebral response to acute hypoxia following IH. Differences in methodologies must be considered, as this study defined IH as a fraction of inspired oxygen of ~13.8%, 8 h/night, for 5 nights. As discussed earlier, the cycling between normoxia and hypoxia may be the cause for many of the differences in physiological variables. In addition, the total amount of hypoxia experienced differs between the Foster et al. (33) and Kolb et al. (84) studies (e.g. 40 hrs (84) vs. 5 hrs (33)). Further to the increase in cerebral sensitivity to acute oxygen, Kolb et al. (84) found an increase in the cerebral sensitivity to acute hyperoxic hypercapnia, following the intermittent hypoxic protocols. In fact, cerebral sensitivities to oxygen and carbon dioxide, as well as acute mountain sickness scores, were comparable to results previously published with chronic sustained hypoxia. This might suggest that although Kolb and his colleagues (84) defined their protocol as IH, it may be more suitable to in fact consider their protocol as continuous. Recently, Ainslie et al. (2) found an increase in the CBF response to acute poikilocapnic hypoxia following 10-12 days of IH (12% O_2 at 5 min intervals, 90 min/day). Although there are methodological shortcomings of their study (see Discussion), they suggest that IH may aid in pre-acclimatization.

Placidi et al. (114) measured cerebrovascular sensitivity to a breath-hold, in sleep apnea patients and healthy controls. With the use of a 30 second breath-hold, cerebrovascular reactivity to CO_2 was measured in the morning and afternoon. The breath-hold index (obtained by dividing the percent increase in CBF during the breath-hold by the duration of the breath-hold) was significantly lower in the patient group compared to the control group (114). This finding suggests cerebral dysregulation to CO_2 in individuals who experience IH, and further contests the results from Kolb et al. (84). Further, both patient and control groups experienced an increase in the breath-hold index in the afternoon compared to the morning trial. Authors of the study

suggest that this is due to overnight relative hypercapnia which causes transient hyposensitivity of cerebrovascular chemoreceptors (114). It should be noted that arterial oxyhaemoglobin saturation was not measured during the breath-hold; therefore, changes in CBF to possible oxyhaemoglobin desaturation could not be assessed. Similar to the proposed mechanism to cerebral dysregulation to acute hypoxia following IH, NO appears to be involved in endothelial dysfunction during hypercapnia. This hypothesis was tested in patients with endothelial dysfunction, as well as controls (89). Hypercapnia was induced with the inhalation of 5% CO_2, 95% O_2 for 6 min both with and without infusion of sodium nitroprusside. In comparison to the control group, the patient group showed a compromised cerebral chemoregulation; furthermore, the difference between the control and patient group was abolished in the sodium nitroprusside trial. Vasoreactivity remained unaffected to sodium nitroprusside infusion in the patient group, proposing that endothelial dysfunction blunts nitric oxide release during carbon dioxide stress (89).

Although IH has been shown to cause increased baseline MAP in the animal model (41), this is not consistent for humans (78, 95). Katayama et al. (78) exposed 14 subjects to a simulated altitude of 4500 m, for 1 hr/day, for seven days. Despite the fact that systolic and diastolic blood pressure sensitivities to acute hypoxia increased, resting MAP was unchanged. Although clinical populations (e.g. sleep apnea) most often have higher resting blood pressures (9), some have suggested the mechanistic explanation does not appear to be attributed to the recurrent hypoxia episodes (108). In contrast, Xie et al. (150) exposed subjects to 20 min of isocapnic hypoxia and 20 min of normoxic hypercapnia while continued measurement of muscle sympathetic nerve activity was conducted at the peroneal nerve. Upon withdrawal of the isocapnic hypoxia, sympathetic activity remained elevated, whereas sympathetic activity returned to baseline soon after cessation of the normoxic hypercapnic stimulus. This implies the importance of hypoxia in the sustained elevation of sympathetic activity, and may contribute to

the elevated resting blood pressures observed in sleep apnea patients (150). These results may suggest that alterations in blood pressure are not observed in most human studies due to the length of the protocol.

Cerebral Blood Flow during Exercise

Although there is evidence for no change (131, 154), or a decrease (39, 81) in CBF during exercise, there is consistent support for an increase in CBF during exercise (19, 25, 34, 37, 45, 46, 49, 53, 56-58, 70, 71, 93, 96, 101, 105, 107, 115-118, 122, 144). Early investigations failed to show an increase in CBF during exercise (81, 131); however, more recent work has shown a significant increase (19, 45, 56, 60, 71, 93, 105, 107, 115-117) that is intensity dependent (70, 101) and contingent on the artery being insonated (49). Increases in CBF are observed up to an exercise intensity of ~60%VO_{2max}, after which the exercise induced hyperventilation causes a decrease in CBF (93), sometimes below baseline values (49, 101). The initial increase in CBF in response to exercise has been suggested to be due to the increase in metabolism. Herholz et al. (50) measured the CBF response to cycling at 25 and 100 watts. They showed a greater increase in CBF during cycling at 100 watts when compared to cycling at 25 watts; attributing the increase to increased neuronal activity and brain metabolism. This has further been suggested by Linkis et al. (93), who measured the increase in the middle cerebral artery and anterior carotid artery blood flow velocity during right hand contractions, right foot movements, and cycling. The middle cerebral artery and anterior carotid artery showed the greatest increases in flow during right hand and right foot contractions, respectively. Further to this, the mean flow velocity increased similarly in the right and left middle and anterior carotid arteries during cycling. This suggests that it is those arteries that supply the cortical representation of the exercising limb, which experience the greatest increase in flow (93). However, cerebral autoregulation has been suggested to be disturbed upon cessation of exercise

of dynamic (49, 82) and static exercise (27). Both increases and decreases have been observed during the post-exercise period; however, the meaningfulness of the change is unclear (82).

Regulation of Cerebral Blood Flow during Exercise

Similar to the abundant support for CO_2 to be a major contributor to CBF at rest, CO_2 also appears to play a major role in regulating CBF during exercise (49, 61, 101, 104, 105, 125). Many of these studies are correlative in nature, where an increase in ventilation, and thus a decrease in end tidal PCO_2 ($P_{ET}CO_2$) is accompanied with a decrease in CBF (49, 69). Moderate intensity cycle exercise has been shown to increase middle cerebral arterial blood flow velocity by 21%; however, once correcting for $P_{ET}CO_2$, the increase in blood flow velocity was eliminated (16).

Although there is strong support for $PaCO_2$ to be a major player in the regulation of CBF during exercise, some suggest that it has been overemphasized (25, 46, 106). Heckmann et al. (46) measured $PaCO_2$ during cycling at 75-100 W for three minutes. Middle cerebral arterial blood flow velocity increased before any increase in $PaCO_2$ was noted, suggesting that the increase in blood flow, at least in the early stages of exercise, is strongly influenced by other factors than CO_2 (46). Similarly, cycling at three different intensities resulted in increases in MCAV, however, end tidal PCO_2 did not change (70).

Increased cardiac output to working muscles, decreased cognitive function (17), possible arterial hypoxemia (24, 127), and decreased oxyhaemoglobin binding due to a lowered pH during exercise might suggest that the brain is compromised during exercise. Furthermore, increases in CBF appear to only occur up to a of 50-60% VO_{2max}, and further increases in exercise intensity are actually accompanied with decreases in CBF (49, 101, 144). Given this information, it may suggest that the brain is compromised during high-intensity exercise; however, PaO_2 is most often unchanged in the general population during exercise (18),

suggesting that it does not commonly contribute to the regulation of CBF during exercise. Also, tissue oxygen extraction increases at high intensity exercise (40) and the increase in CBF surpasses the oxygen demand (56) implying that the brain is well protected during exercise (40). It should be noted that oxygen supplementation during high intensity exercise can blunt cerebral desaturation, and possibly improve performance in high-level athletes (103). However, this study consisted of elite rowers who are more likely to experience exercise induced arterial hypoxemia; therefore it is intuitive that oxygen supplementation by these athletes would attenuate cerebral desaturation. For this reason, generalization of the results to the general population is problematic.

The increase in MCAV during exercise has further been attributed to MAP (56, 97, 104, 116). Exercise at 30% and 60% VO_{2max} produces similar increases in MCAV and MAP, although $PaCO_2$ remains unchanged from rest (56). This increase in MCAV may be a result of an inability for cerebral autoregulation to dampen the drastic increases in perfusion pressure. When exercise involves the use of a large muscle mass, the contribution of cardiac output becomes more important to the regulation of CBF. Ide et al. (57) tested this hypothesis by pharmacologically inhibiting the increase in cardiac output during hand contractions and cycle ergometry. It was found that inhibiting the increase in cardiac output only decreased MCAV during cycling, and not during hand contractions (57). The authors of that study attributed the decrease in MCAV to competition between the working muscle mass and cerebral tissue. The importance of cardiac output to CBF appears to be attributed to stroke volume, as heart rate does not affect MCAV during exercise (14). However, increases in CBF have been observed without a concurrent increase in cardiac output (39). Furthermore, at maximal exercise, MCAV quickly peaks, then quickly declines, whereas cardiac output remains elevated (40). This suggests other factors aside from cardiac output that contribute to CBF regulation during maximal exercise (40). Determining the relative contribution of each regulating factor to CBF is difficult due to

contradictory evidence, however, given this, cerebral autoregulation appears to maintain protection of cerebral tissue in healthy individuals (19).

Exercise and Nitric Oxide

Nitric oxide (NO), a key biological messenger for the vasodilation of vessels, should be considered as a possible mechanistic explanation to vascular dysfunction in response to IH. Produced in endothelial cells from L-arginine, NO diffuses locally to smooth muscle to bring about vessel relaxation (109). The increase in flow during exercise, increases shear stress of vessels walls, which in turn initiates vasodilation through the release of NO (130). Inhibition of NO by administration of L-NMMA leads to a reduced arm blood flow during exercise; attributing at least 26% of vascular resistance during exercise to NO production (38). Determining the relative contribution of NO to increased blood flow (smooth muscle relaxation) during exercise is arduous as vascular relaxation can be caused by various physiological mechanisms, such as withdrawal of autonomic control, release of metabolites, and mechanical factors. However, cerebral autoregulation does appear to be regulated by NO. White et al. (149) found cerebral autoregulation to be significantly delayed after infusion of L-NMMA, suggesting that depressing NO reduces the speed of cerebral autoregulation (149). Therefore, the possibility exists that IH may decrease the bioavailability of NO for cerebral circulation during exercise.

APPENDIX B - Individual Raw Data

Table 10. Individual subjects' anthropometric, pulmonary function, and transcranial Doppler ultrasound insonation depth characteristics. BMI = body mass index

Subject	Age	Height	Mass	BMI	FVC	$FEV_{1.0}$	$FEV_{1.0}$/FVC	$FEV_{1.0}$/FVC	MCAV depth
	(yrs)	(cm)	(kg)	(kg/m^2)	(L)	(L)	(%)	(% predicted)	(cm)
1	25	166	60	21.8	4.25	3.3	77.6	91.2	5.3
2	22	184	84	24.8	5.93	4.85	81.8	95.7	5.0
3	23	181	74	22.6	5.28	4.55	86.2	100.6	5.0
4	26	186	72	20.8	5.27	4.63	87.9	106.7	5.1
5	19	181	76	23.2	5.79	4.9	84.6	98.7	5.0
6	24	184	84	24.8	6.31	4.94	78.3	91.6	5.0
7	24	182	84	25.4	5.79	4.82	83.2	97.2	5.0
8	22	174	67	22.1	3.78	3.5	92.6	107.3	5.3
9	27	187	94	26.9	6.35	5.05	79.5	96.8	5.2

Table 11. Individual subject baseline ventilation (V_E), mean arterial blood pressure (MAP), and mean middle cerebral arterial blood flow velocity (MCAVm) prior to the acute hypoxic response and exercise, PRE-IH and POST-IH. AHR = baseline ventilation prior to AHR; Exercise = baseline ventilation prior to exercise; PRE-IH = experimental day before intermittent hypoxia; POST-IH = experimental day after intermittent hypoxia.

		V_E (L/min)		MAP (mmHg)		MCAVm (cm/s)	
		PRE-IH	POST-IH	PRE-IH	POST-IH	PRE-IH	POST-IH
1	AHR	8.3	4.4	89.3	100.1	72.6	80.0
	Exercise	4.3	4.4	98.0	100.8	81.7	74.4
2	AHR	11.2	9.8	97.2	87.4	53.3	53.7
	Exercise	14.1	11.3	90.4	87.9	48.3	58.2
3	AHR	10.7	7.3	90.6	97.0	70.3	72.0
	Exercise	10.9	16.3	89.7	93.9	68.9	56.1
4	AHR	9.3	10.4	89.4	89.8	58.5	57.2
	Exercise	10.8	8.7	93.0	97.2	58.7	56.9
5	AHR	6.0	9.9	93.0	87.5	61.4	67.3
	Exercise	9.9	11.3	92.8	95.9	62.2	52.4
6	AHR	10.6	10.4	100.2	105.3	62.5	62.9
	Exercise	11.7	11.0	101.6		52.8	58.7
7	AHR	10.7	9.7	100.4	95.9	78.1	77.7
	Exercise	11.3	12.0	106.5	105.9	76.4	78.8
8	AHR	8.5	6.4	87.8	82.3	68.0	70.9
	Exercise	10.1	11.1	97.3	87.1	66.3	66.8
9	AHR	10.7	7.7	83.7	87.7	53.3	50.6
	Exercise	11.6	11.2	99.6	97.0	54.7	49.6

Table 12. The effect of acute hypoxia on ventilatory, cardiovascular and cerebrovascular parameters before and after intermittent hypoxia in each subject. FiO_2 = fraction of inspired oxygen; SaO_2 = oxyhaemoglobin saturation; V_E = minute ventilation; MCAVm = mean middle cerebral arterial blood flow velocity; MAP = mean arterial blood pressure; HR = heart rate; Q = cardiac output; PRE-IH = experimental day before intermittent hypoxia; POST-IH = experimental day after intermittent hypoxia.

	FiO_2 (%)		SaO_2 (%)		V_E (L/min)		MCAVm (cm/s)		MAP (mmHg)		HR (bpm)		Q (L/min)	
Subject	PRE-IH	POST-IH	PRE-IH	POST-IH	PRE-IH	POST-IH	PRE-IH	POST-IH	PRE-IH	POST-IH	PRE-IH	POST-IH	PRE-IH	POST-IH
1	-8.4	-8.1	-20.8	-17.7	8.7	18.6	21.9	9.2	8.1	-2.0	25.4	6.9	1.6	0.0
2	-8.9	-10.7	-18.3	-18.6	4.4	6.7	-9.9	0.1	-9.2	1.6	12.3	13.4	1.4	1.5
3	-9.4	-11.5	-17.7	-16.4	3.4	9.0	10.1	0.3	8.9	3.4	19.8	23.1	2.2	2.2
4	-10.8	-8.8	-18.1	-16.7	4.5	2.9	7.0	5.8	7.4	10.3	13.2	6.8	1.0	0.5
5	-10.0	-9.3	-17.6	-21.4	7.6	6.7	4.7	-1.1	2.2	2.6	10.4	11.2	1.0	1.0
6	-9.6	-8.4	-19.6	-20.2	3.0	8.0	3.7	7.3	-0.8	0.8	16.4	11.4	1.9	1.5
7	-9.3	-9.6	-19.2	-19.1	7.6	5.7	0.6	2.7	3.1	-1.9	10.8	17.1	1.6	2.0
8	-8.8	-9.0	-14.8	-18.8	8.9	5.3	-6.3	7.1	-5.5	5.9	14.5	18.1	1.0	1.4
9	-8.6	-8.6	-16.5	-16.3	5.0	5.9	3.0	1.6	0.8	-4.6	7.6	6.7	1.2	1.4

Table 13. Effect of exercise intensity on ventilation (V_E) before (PRE-IH) and after (POST-IH) intermittent hypoxia. Values are in L/min.

Subject		50 W	100 W	150 W	recovery1	recovery2	recovery3	recovery4	recovery5
1	PRE-IH	9.5	13.7	22.9	20.0	16.1	12.0	7.2	5.8
	POST-IH	10.0	17.4	24.5	22.3	15.3	8.9	9.0	8.3
2	PRE-IH	27.7	43.2	54.9	28.2	27.8	15.1	15.5	15.1
	POST-IH	25.1	29.3	43.0	28.1	16.3	14.3	16.0	13.4
3	PRE-IH	30.1	39.2	64.8	45.3	26.1	19.2	20.5	15.7
	POST-IH	25.1	34.4	50.5	38.7	20.4	15.8	12.2	13.1
4	PRE-IH	25.0	30.9	49.4	36.1	20.2	16.1	13.0	11.9
	POST-IH	24.0	36.0	51.1	37.1	21.7	17.2	14.9	11.5
5	PRE-IH	17.4	27.1	38.3	24.1	14.0	11.4	9.2	10.5
	POST-IH	21.8	34.1	48.3	28.2	18.6	12.5	13.2	13.3
6	PRE-IH	26.4	39.6	53.8	42.8	32.1	19.7	17.8	16.1
	POST-IH	26.2	34.8	55.2	40.3	20.1	19.7	14.6	14.3
7	PRE-IH	24.0	33.8	49.3	34.4	19.8	16.2	16.0	14.6
	POST-IH	23.2	32.6	53.1	30.8	17.9	13.1	12.5	12.4
8	PRE-IH	33.1	39.7	48.1	32.2	28.9	13.2	19.5	14.8
	POST-IH	24.0	34.2	50.4	34.1	24.1	12.8	12.5	11.4
9	PRE-IH	27.8	38.4	50.6	33.1	22.2	17.4	15.9	16.4
	POST-IH	21.7	38.3	48.0	36.8	21.3	15.5	16.7	13.6

Table 14. Effect of exercise intensity on mean arterial blood pressure (MAP) before (PRE-IH) and after (POST-IH) intermittent hypoxia. Values are in mmHg.

Subject		50 W	100 W	150 W	recovery1	recovery2	recovery3	recovery4	recovery5
1	PRE-IH	111.5	121.6						
	POST-IH	103.7	124.7	139.3	109.0	100.8	95.9	104.5	106.9
2	PRE-IH	97.7	107.8	114.0	92.5	91.7	90.8	87.5	86.3
	POST-IH	95.2	103.9	109.3	103.2	99.7	97.6	94.0	95.8
3	PRE-IH	97.4	105.4	114.4	98.8	98.2	101.0	93.8	94.8
	POST-IH	108.2	113.0	116.6	98.6	99.2	100.0	95.5	97.6
4	PRE-IH	105.0	114.5	117.9	96.6	97.4	100.4	102.1	99.6
	POST-IH	111.2	106.8	116.8	93.4	91.4	92.4	93.6	94.1
5	PRE-IH	96.1	99.3	107.4	92.8	93.7	93.3	92.7	92.5
	POST-IH	94.2	104.5	115.6	95.6	97.7	96.3	96.9	96.1
6	PRE-IH	115.4	115.7	128.3	104.9	109.9	109.1	107.1	105.5
	POST-IH								
7	PRE-IH	117.3	123.4	127.9	106.2	105.5	108.8	108.7	104.4
	POST-IH	113.1	113.2	129.2	96.8	99.5	100.5	102.6	102.3
8	PRE-IH	96.1	105.2	107.4	93.4	87.9	86.8	82.0	91.7
	POST-IH	86.8	101.0	123.5	86.8	77.1	80.4	78.5	77.5
9	PRE-IH	117.2	114.3	114.6	99.9	99.2	101.1	99.2	99.5
	POST-IH	100.4	107.7	107.5	90.2	87.3	93.2	93.5	90.7

Table 15. Effect of exercise intensity on mean middle cerebral arterial blood flow velocity (MCAVm) before (PRE-IH) and after (POST-IH) intermittent hypoxia. Values are in cm/s.

subject		50 W	100 W	150 W	recovery1	recovery2	recovery3	recovery4	recovery5
1	PRE-IH	106.0	107.9	103.7	103.8	80.3	77.0	91.6	89.8
	POST-IH	94.2	97.5	93.3	83.0	58.2	69.0	92.2	84.0
2	PRE-IH	51.2	49.6	56.4	47.1	43.4	45.1	51.8	49.3
	POST-IH	67.2	65.5	61.4	54.6	53.9	53.5	53.7	56.6
3	PRE-IH	77.1	83.0	81.1	74.5	67.7	66.9	68.4	70.0
	POST-IH	73.8	71.2	76.3	65.9	65.7	62.7	63.7	66.4
4	PRE-IH	59.2	61.2	63.1	56.6	49.6	53.2	54.8	57.8
	POST-IH	56.7	57.7	61.4	59.3	46.9	49.5	50.0	52.1
5	PRE-IH	81.6	77.1	79.5	57.3	62.9	71.2	68.5	70.4
	POST-IH	55.5	52.9	74.9	59.0	62.5	63.8	68.4	66.0
6	PRE-IH	58.6	58.8	59.6	53.4	43.0	43.5	46.2	45.5
	POST-IH	64.2	61.6	63.3	52.3	49.9	53.0	52.8	54.9
7	PRE-IH	82.9	78.3	79.7	73.9	66.0	64.6	71.3	66.4
	POST-IH	80.5	83.4	79.7	80.1	70.9	67.3	73.6	70.2
8	PRE-IH	58.0	79.1	79.3	96.9	62.2	62.7	58.8	64.5
	POST-IH	70.7	73.5	74.6	74.5	56.5	49.2	64.1	64.3
9	PRE-IH	63.6	58.4	59.3	53.2	50.7	49.7	51.0	56.0
	POST-IH	54.4	58.0	59.9	46.8	43.3	51.4	49.6	49.7

Table 16. The ventilatory response to hypercapnia (5% CO_2) at rest and during exercise before (PRE-IH) and after (POST-IH) intermittent hypoxia. Values are in L/min.

subject		rest	50 W	100 W	150 W
1	PRE-IH	6.6	14.9	27.8	39.0
	POST-IH	7.9	20.7	33.4	46.2
2	PRE-IH	20.2	52.1	77.7	94.1
	POST-IH	18.9	39.7	54.7	72.2
3	PRE-IH	22.9	49.8	71.8	97.9
	POST-IH	16.2	44.3	61.5	85.3
4	PRE-IH	16.2	34.7	49.9	79.8
	POST-IH	18.4	37.2	59.7	116.5
5	PRE-IH	12.2	33.4	49.6	68.8
	POST-IH	16.9	47.7	63.8	82.1
6	PRE-IH	22.1	42.5	59.4	92.1
	POST-IH	16.0	40.6	63.0	94.3
7	PRE-IH	16.8	46.7	62.6	94.5
	POST-IH	15.6	41.6	65.3	92.3
8	PRE-IH	14.9	36.4	56.3	68.0
	POST-IH	18.5	39.3	55.0	67.2
9	PRE-IH	15.2	47.9	60.0	80.9
	POST-IH	16.5	47.3	67.6	90.5

Table 17. The mean arterial blood pressure (MAP) response to hypercapnia (5% CO_2) at rest and during exercise before (PRE-IH) and after (POST-IH) intermittent hypoxia. Values are in mmHg.

subject		rest	50 W	100 W	150 W
1	PRE-IH	101.3	114.4	129.5	
	POST-IH	101.9	112.7	134.0	153.7
2	PRE-IH	91.0	103.0	111.7	116.6
	POST-IH	88.0	100.9	106.4	114.5
3	PRE-IH	95.2	104.5	110.8	122.0
	POST-IH	102.1	116.8	123.0	119.6
4	PRE-IH	92.4	109.0	113.0	118.6
	POST-IH	98.9	113.6	114.7	112.9
5	PRE-IH	92.5	97.8	105.9	110.6
	POST-IH	91.1	100.9	109.3	114.7
6	PRE-IH	107.1	122.0	125.4	134.0
	POST-IH				
7	PRE-IH	108.5	123.3	122.1	133.5
	POST-IH	104.8	114.0	122.2	129.8
8	PRE-IH	96.9	100.1	113.7	125.0
	POST-IH	90.7	95.4	113.5	115.0
9	PRE-IH	105.1	123.3	124.1	110.7
	POST-IH	96.7	104.3	115.6	102.1

Table 18. The mean middle cerebral arterial blood flow velocity (MCAVm) response to hypercapnia (5% CO_2) at rest and during exercise before (PRE-IH) and after (POST-IH) intermittent hypoxia. Values are in cm/s.

subject		rest	50 W	100 W	150 W
1	PRE-IH	100.8	125.4	146.7	157.9
	POST-IH	83.1	109.0	133.3	150.2
2	PRE-IH	57.9	66.3	75.7	84.5
	POST-IH	63.0	77.8	82.4	92.5
3	PRE-IH	78.4	84.3	97.3	107.1
	POST-IH	75.1	87.9	94.4	103.7
4	PRE-IH	63.5	75.5	83.5	94.2
	POST-IH	63.9	78.2	86.6	91.7
5	PRE-IH	74.4	90.0	100.9	113.6
	POST-IH	66.9	75.4	94.5	112.4
6	PRE-IH	64.5	78.5	88.1	97.3
	POST-IH	63.6	70.4	77.0	88.2
7	PRE-IH	85.8	96.4	107.7	115.8
	POST-IH	88.7	94.3	115.6	124.3
8	PRE-IH	77.8	82.9	103.9	118.4
	POST-IH	79.1	83.1	105.6	110.1
9	PRE-IH	68.3	84.4	89.6	90.2
	POST-IH	59.8	71.9	83.9	85.4

APPENDIX C – UBC Research Ethics Board's Certificate of Approval

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: RENEWAL WITH AMENDMENTS TO THE STUDY

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC CREB NUMBER:
William Sheel		H05-70517

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
UBC	Point Grey Site

Other locations where the research will be conducted:
N/A

CO-INVESTIGATOR(S):
Meaghan MacNutt
Jordan Querido

SPONSORING AGENCIES:
Natural Science Engineering Research Council - "Cerebral and Cardio-Ventilatory Responses to Short Duration Intermittent Hypoxia"

PROJECT TITLE:
Cerebral and Cardio-Ventilatory Responses to Exercise Following Intermittent Hypoxia

The current UBC CREB approval for this study expires: January 12, 2008

AMENDMENT(S):

Document Name	Version	Date
Protocol:		
Protocol version 1	version 1	November 24, 2006
Consent Forms:		
Consent form version 3	version 3	January 1, 2007
Advertisements:		
Recruit flyer version 1	Version 1	November 24, 2006
Questionnaire, Questionnaire Cover Letter, Tests:		
PAR-Q	N/A	January 1, 2002

AMENDMENT APPROVAL DATE:
January 12, 2007

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in