Cerebral hypoperfusion during hypoxic exercise following two different hypoxic exposures: independence from changes in dynamic autoregulation and reactivity

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Ainslie PN, Hamlin M, Hellemans J, Rasmussen P, Ogoh S. Cerebral hypoperfusion during hypoxic exercise following two different hypoxic exposures: independence from changes in dynamic autoregulation and reactivity. Am J Physiol Regul Integr Comp Physiol 295: R1613–R1622, 2008. First published September 3, 2008; doi:10.1152/ajpregu.90420.2008.—We examined the effects of exposure to 10–12 days intermittent hypercapnia [IH: 5.5-min hypercapnia (inspired fraction of CO2 0.05)-to-normoxia for 90 min (n = 10)], intermittent hypoxia [IH: 5.5-min hypercapnia-to-normoxia for 90 min (n = 11)] or 12 days of continuous hypoxia [CH: 1,560 m (n = 7)], or both IH followed by CH on cardiorespiratory and cerebrovascular function during steady-state cycling exercise with and without hypoxia (inspired fraction of oxygen, 0.14). Cerebrovascular reactivity to CO2 was also monitored. During all procedures, ventilation, end-tidal oxia (inspired fraction of oxygen, 0.14). Cerebrovascular reactivity to CO2 was also monitored. Following any intervention, during hypoxic exercise, the apparent impairment in CA, reflected in lowered cerebral oxygenation during hypoxic exercise, potential mediately by the greater hypocapnia, rather than a compromise in CA or MCAv reactivity. Hypoxic exercise resulted in increased ventilation, hypocapnia, heart rate, and cardiac output when compared with normoxic exercise (P < 0.05); these responses were unchanged following IHC but were elevated following the IH and CH exposure (P < 0.05) with no between-intervention differences. Following IH and/or CH exposure, the greater hypocapnia during hypoxic exercise provoked a decrease in MCAv (P < 0.05 vs. preexposure) that was related to lowered cerebral oxygenation (r = 0.54; P < 0.05). Following any intervention, during hypoxic exercise, the apparent impairment in CA, reflected in lowered low-frequency phase between MCAv and BP, and MCAv-CO2 reactivity, were unaltered. Conversely, during hypoxic exercise following both IH and/or CH, there was less of a decrease in muscle oxygenation (P < 0.05 vs. preexposure). Thus IH or CH induces some adaptation at the muscle level and lowers MCAv and cerebral oxygenation during hypoxic exercise, potentially mediated by the greater hypocapnia, rather than a compromise in CA or MCAv reactivity.

hypoxia; exercise; intermittent and continuous hypoxia; cerebral blood flow

ELEVATIONS IN VENTILATORY SENSITIVITY to hypoxia following exposure to either continuous hypoxia [CH, e.g., high altitude (7, 35)] or intermittent hypoxia [IH (11, 20, 28, 42)] results in subsequent hypocapnia (i.e., reduction in end-tidal CO2). Because hypocapnia results in cerebral vasoconstriction (23, 32), elevations in ventilatory chemosensitivity may result in cerebral hyperperfusion. During normoxic exercise, ventilatory chemosensitivity is only one of multiple signals that integrate to increase ventilation (45); however, following exposure to IH, an enhanced chemosensitivity activation was evident during hypoxic exercise (21). Although cerebral perfusion was not monitored in that study (21), it seems reasonable to speculate that an enhanced ventilatory chemosensitivity to hypoxic exercise would result in subsequent hypocapnia and reductions in cerebral perfusion. Consistent with this notion, cerebral oxygenation has been reported to fall during submaximal exercise at high altitude (15). Moreover, following exposure to short-duration IH (5 min at 12% O2 separated by 5 min of normoxia for 1 h/day for 10 days), when compared with preexposure, there is a lowering (~2%) in cerebral tissue oxygenation (as monitored using near-infrared spectroscopy) in response to acute progressive isocapnic hypoxia (8). Although cerebral blood flow (CBF) was not monitored, it was proposed that the blunting vasodilatory response in cerebral tissue oxygenation may potentially be mediated by a lowered CBF or by a failure in the ability of the brain to maintain effective autoregulation (8). Broadly consistent with this view, exposure to high altitude (16, 43) and acute hypoxic exercise (3) may also impair dynamic cerebral autoregulation. Importantly, however, no studies have confirmed the independent influence of prior IH when compared with that of CH on the concurrent change in related cardiorespiratory and cerebrovascular function during hypoxic exercise. Collectively, the aforementioned studies highlight that exposure to IH or CH may alter normal cerebrovascular function; how such hypoxic exposures may lead to cerebrovascular alterations during hypoxic exercise is not known.

Although animal studies indicate that it is the IH, rather than hypercapnia, which has the more marked influence (33), no attempts have been made to conduct comparable studies in humans. This is somewhat surprising given the high sensitivity of the cerebral circulation to CO2 (32). Therefore, the main aim of this investigation was to examine the effects of daily exposures to IH on cerebrovascular function at rest and during exercise under hypoxic and normoxia conditions. For the first time, to confirm the selective influence of IH, subjects were exposed to J) a continuous 12-day exposure to mild CH (1.560 m); 2) both IH and the 12-day exposure to mild CH; or 3) 12 days of intermittent hypcapnia. In contrast to exposure to intermittent hypcapnia, we hypothesized that hypoxia (both

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IH and CH) would elevate ventilation during hypoxic exercise, leading to a greater degree of hypocapnia and subsequently lowering MCAv and cerebral oxygenation. As has been previously reported during conditions of rest (1), we reasoned that a greater hypocapnia induced by an elevated ventilatory response to hypoxia during exercise would act to preserve dynamic cerebral autoregulation.

During exercise, there is an enhanced proportional distribution of cardiac output to the active muscle (~80%), whereas, in comparison, the amount of redistribution to the brain (~11%) is slight (12). Interestingly, following IH, there have been reports of subtle adaptations at the level of the muscle (i.e., changes in mitochondrial or oxygenation status of the muscle) (25, 36). Following exposure to IH (or CH), if elevations in chemosensitivity result in marked hypocapnia during hypoxic exercise, a redistribution of cardiac output from the hyperperfused brain, greater vasodilatation in the muscle, or both might underpin this reported improvement in muscle oxygenation following IH. Thus our secondary hypothesis was that, following exposure to IH or CH, there would be an improvement in muscle oxygenation when compared with that of the brain during steady-state hypoxic exercise.

METHODS

Subjects. This study was conducted, in part, in conjunction with an investigation designed to examine the effects of IH and CH on resting cardiorespiratory and cerebrovascular responses to acute poikilocapnic hypoxia (2). Twenty eight well-trained healthy individuals [16 men; 12 women aged 26 ± 5 (SD) yr; body mass index 24 ± 3 kg/m²; and maximal oxygen consumption 55 ± 4 ml·kg⁻¹·min⁻¹] volunteered for this study, which was approved by the University of Otago’s Human Ethics Committee and conformed to the standards set by the Declaration of Helsinki. Subjects were informed of the experimental procedures and possible risks involved in the study, and written informed consent was obtained. Subjects were not taking any medication, all were nonsmokers, and none had any history of cardiovascular, cerebrovascular, or respiratory disease. All subjects were sea level residents and had not spent time at altitude in the 6 mo before this study. Subjects maintained similar physical activity levels and diet during the study.

Experimental design. Subjects were instructed to abstain from exercise and alcohol 12 h before and not to eat a heavy meal or consume caffeine 4 h before experimental testing. A summary of the timing and experimental procedures performed during the study is outlined in Fig. 1. Subjects were randomly selected to: 1) complete 10–12 days of IH before ascending to mild altitude for a further 12 days (n = 11, 6 male, 5 female); 2) ascend to mild altitude only for the same 12 days (n = 7, 4 male, 3 female) and receive no IH before ascent; and 3) complete 11 days of intermittent hypercapnia (n = 10, 6 male, 4 female). Measurements were completed at baseline, immediately following the IH, following the 12 days at altitude and 12–13 days following altitude exposure (Fig. 1). Subjects were tested at the same time of day, and within 8 h of descending from altitude. In the intermittent hypercapnia condition, the subjects acted as a control group and did not ascend to altitude; therefore, measures were made at baseline, immediately following the intermittent hypercapnia intervention, and once again 12–13 days later.

Manipulation of end-tidal O₂ and CO₂. Baseline control measurements were obtained in normoxia [inspired fraction of oxygen (FI O₂) = 0.21] and at selected time points in normobaric hypoxia during rest and exercise. During rest, following 10 min of baseline measurements, in a randomized order, subjects received 20 min of hypoxia ( Fi O₂ = 0.12) followed by a 3–4 min normoxic recovery (2) or were exposed to 3 min of hypercapnia followed by hypocapnia. Hypercapnia was induced by switching the inspired gas from room air to 5% CO₂ (in 21% O₂ and N₂ balance) for 3 min. Following the hypercapnia, subjects were allowed to recover and were then instructed to increase their rate and depth of breathing to reduce end-tidal CO₂ (PET CO₂) to 20–22 mmHg. Verbal feedback was provided to assist subjects to reach and maintain the target levels of hyperventilation. The hypercapnic condition was always conducted first because prior hypocapnia (but not prior hypercapnia) may cause persistent cerebral vasoconstriction, thus influencing the normal middle cerebral artery blood flow velocity (MCAv)-CO₂ response to hypercapnia (13). Adequate time was permitted in between the hypoxic and CO₂ steps to ensure that end-tidal gases and blood pressure (BP) were returned to normal. Because the hypoxic conditions only caused very mild (~3 mmHg) and transient (<5 min) changes in PET CO₂ (2), it would seem unlikely that there would be any persistent cerebral vasconstriction. The subjects were rested in a supine position in a darkened room and were instructed to close their eyes and to relax to reduce external stimuli that could affect respiration.
CEREBROVASCULAR REGULATION DURING HYPOXIC EXERCISE

Exercise. Following a 3 - to 4-min warm up, subjects then exercised on a cycle ergometer (Lode, Groningen, Netherlands) in the upright position at 60–70% of their maximal oxygen uptake. Following 5–6 min of steady-state exercise, the FiO₂ was immediately reduced to 0.14 [arterial O₂ saturation (SaO₂) ~80%] for 4–5 min followed by a 2- to 3-min normoxic recovery. The identical work load and position on the bike (seat and handlebar height and angle and cycling shoes) were used on each occasion.

Intermittent hypoxic intervention. Subjects in the IH group carried out 10–12 days of IH at sea level, in which they intermittently breathed hypoxic air delivered through a facemask [GO2Altitude Hypoxic Training System (Hypoxicator); Biomedtech, Victoria, Australia] for 90 min 10–12 times over 15 days. In four additional subjects, before and after 10 days of IH (as outline above), arterial pressure was measured with a radial arterial catheter (Abbott Critical Care System) together with the noninvasive Finapres method to corroborate changes in arterial pressure and to measure arterial partial pressure of CO₂ (PaCO₂). IH was administered in a ratio of 5:5 (minutes of hypoxic air to minutes of ambient air), and the sessions took place at least 1–2 h before or after exercise training. The temporal and quantitative details of this IH protocol represent the standard intermittent hypoxic treatment suggested for athletes according to the manufacturer’s instructions and is the protocol most frequently used by competitive athletes (19). During the first 2–3 days, a target SaO₂ of 86–90% was maintained before reaching a SaO₂ of 75–82% for the remaining sessions. The rationale for this protocol is purported to provide a hypoxic stimulus severe enough to induce partial acclimatization (19). The progressive decrease in FiO₂ over the IH trial is to provide a maximal tolerable hypoxic stress by the end of the IH session but allow progressive acclimatization to minimize symptoms and improve tolerance. Peripheral O₂ saturation was measured within the last 5-min “hypoxic” bout using a standard fingertip pulse oximetry clip (Sport-Stat, Nonin Medical, Minneapolis, MN), and the values were also recorded during the last minute of each hypoxic and normoxic step. Subjects ceased IH immediately before ascending to altitude.

Altitude exposure. Subjects in both groups stayed at mild altitude (Snow Farm, Wanaka, 1,560 m) for a period of 12 days and maintained similar physical activity levels and diets.

Intermittent hypercapnia intervention. In a comparable pattern and timing to the IH intervention (described in Intermittent hypoxic intervention), intermittent hypercapnia was administered in a ratio of 5:5 [minutes of hypercapnic [inspired fraction of CO₂ (FiCO₂), 0.05] air to minutes of ambient air].

Measurements of respiratory gas exchange. Subjects breathed through a leak-free respiratory mask (Hans-Rudolph 8980, Kansas City, MO) attached to a one-way nonbreathing valve (Hans-Rudolph 2700). Expiratory flow was measured using a heated pneumotach (Hans-Rudolph HR800). SaO₂ was measured using pulse oximetry at the finger (model ML320; ADInstruments, Colorado Springs, CO). PetCO₂ and end-tidal O₂ were sampled from the leak-free mask and measured by a gas analyzer (model CD-3A; AEI Technologies, Pittsburgh, PA). Ventilation (flow, tidal volume, frequency) and gas values were displayed in real time during testing (PowerLab; ADInstruments, Springs, CO). Expiratory volume was calculated using the integrated flow signal and the frequency of breathing. Because PetCO₂ significantly overestimates PaCO₂ during exercise (18), PetCO₂ values were adjusted for changes in tidal volume to ensure better reflection of PaCO₂ (18).

Measurements of CBF velocity and arterial BP. CBF velocity in the right middle cerebral artery (MCAv) was measured using a 2 MHz pulsed Doppler ultrasound system (DLW Doppler, Sterling, VA) using search techniques described elsewhere (2). Beat-to-beat arterial BP was monitored using finger photoplethysmography (Finometer; TPD Biomedical Instrumentation).

For comparative purposes, arterial pressure was measured with a radial arterial catheter in seven subjects before and after the IH (n = 4) and intermittent hypercapnia (n = 3) interventions. Heart rate, stroke volume, and cardiac output were calculated from the BP waveform using the Model Flow method, incorporating age, sex, height, and weight (BeatScope 1.0 software; TNO TPD; Biomedical Instruments); this method provides a reliable estimate of changes in cardiac output during exercise in healthy young humans (41). It is not clear, however, if absolute measurements of cardiac output by the Model Flow method are valid during conditions of exercise and hypoxia; thus, emphasis of these data is placed on the relative, rather than the absolute, changes. All data were acquired continuously at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML795; ADInstruments) interfaced with a computer and stored for subsequent analysis using commercially available software (Chart version 5.02; ADInstruments).

Cerebral and muscle oxygenation. Cerebral and muscle oxygenation were monitored using a commercially available near-infrared spectroscopy (NIRS) system (NIRO-200; Hamamatsu Photonics KK, Hamamatsu, Japan). A probe holder containing an emission probe and detection probe was attached at the right side of the forehead with a distance of 5 cm between the probes. The methodology of this system has been described previously (34). For assessment of muscle oxygenation, optodes were positioned on the middle portion of the right vastus lateralis muscle at the mid-thigh level and parallel with the long axis of the muscle. Similar to the brain, the optodes were housed in an optically dense plastic holder secured on the skin with tape to minimize extraneous light. In the brain and muscle, local oxygenation was measured simultaneously every 1 s throughout the experiment and expressed as the magnitude of the change from the initial value. All NIRS data were time-aligned and simultaneously collected at 200 Hz along with the other aforementioned variables.

Dynamic cerebral autoregulation. Three-minute steady-state data segments were used for transfer function analysis to identify an index of dynamic cerebral autoregulation. The mean arterial pressure (MAP) at the level of the MCA took into consideration the vertical distance from the fourth intercostal space in the midclavicular line (heart level) to the transcranial Doppler probe (i.e., hydrostatic pressure = vertical distance × 0.77 mmHg/cm). The beat-to-beat data of mean BP and MCAv were then linearly interpolated and resampled at 2 Hz for spectral analysis. The spectra of mean BP and MCAv were calculated with a Fast-Fourier transformation algorithm, and the transfer function between these two variables was calculated with a cross-spectral method to assess dynamic CBF autoregulation, as described in detail elsewhere (48). For these calculations, 3 min of steady-state mean BP and MCAv were used during the last 3 min of normoxic exercise and the last 3 min of each level of hypoxic exercise. Mean value of transfer function gain, phase, and coherence function were calculated in the very low (VLF, 0.02–0.07 Hz), low (LF, 0.07–0.20 Hz), and high (HF, 0.20–0.35 Hz) frequency ranges to reflect different patterns of the dynamic pressure-velocity relationship (48). Rapid BP fluctuations in the HF range, such as those induced by respiratory frequency, are transferred to MCAv, whereas BP fluctuations in the LF range are independent of the respiratory frequency and dampened by autoregulatory mechanisms. Furthermore, the VLF range of both flow and velocity variability appears to reflect multiple physiological mechanisms (48). Coherence function, between BP and MCAv, is used to assess the linear relation and reliability of the transfer function gain and phase. Transfer function gain estimates are used as an “index” of the ability of the cerebrovascular bed to buffer changes in MCAv induced by transient changes in BP in the different frequencies (48). The phase was used to estimate the temporal relationship between BP and MCAv (48). Dynamic cerebral autoregulation decreases the transmission effect of pressure on velocity; therefore, an increased transfer function gain or decreased transfer function phase between pressure and velocity can be interpreted as an increased effect of transmission, which suggests that dynamic cerebral autoregulation is impaired.
Data and statistical analyses. Because there were no statistical differences between men and women, data from the two groups were combined for statistical analysis. At rest and during exercise, the ventilatory sensitivity to poikilocapnic hypoxia was calculated using the change in ventilation by the change in SaO₂ (i.e., \( \Delta V_e / \Delta SaO_2 \)). Peak ventilation and its time of occurrence were calculated as 1-min averages, centered on the visually identified time of maximal ventilation. To calculate the absolute and percent (%) change from baseline, data were averaged over the 3-min period of baseline immediately preceding any changes in SaO₂. All data were analyzed using the SPSS social statistics package (version 13). A Shapiro-Wilks test was applied to each dependent variable to mathematically assess distribution normality. Parametric and nonparametric equivalents of a two-way mixed ANOVA with one between (state: rest vs. exercise) and one within (normoxia vs. hypoxia) factor were incorporated to examine the effects of time and state on selected variables. Significance for all two-tailed tests was established at an α-level of \( P < 0.05 \), and data are expressed as a means ± SD. Effect size estimates for the major hemodynamic dependent variables were derived from these between-day tests in the control condition using conventional \( \alpha \) (0.05) and \( \beta \) (0.20) values. On the basis of a one-way ANOVA model, a study sample of six was adequate to detect meaningful physiological changes in the main hemodynamic variables, defined as any value outside the 68% limits of agreement (±1 SD) for between-day repeated differences without intervention.

RESULTS

Subject compliance. All of the subjects completed 10–12 days of exposure to IH and intermittent hypercapnia. Over the first 3 days of IH, when averaged over the last minute of hypoxia of each 5-min period, SaO₂ was maintained at an average of 88 ± 2% before being reduced to 78 ± 3% for the remaining days of exposure. Out of the 11 subjects in the IH group, 7 subjects continued on to ascend to mild altitude for 10–12 days. Of these participants, and those in the altitude-only group (\( n = 7 \)), all of them completed testing immediately postaltitude. Six participants from the IH group and five participants from the altitude-only group completed the testing session 12 days following the continuous hypoxic exposure.

Cardiorespiratory and cerebrovascular responses to acute hypoxic exercise following intermittent and/or continuous hypoxic exposure. During hypoxic exercise, following IH and continuous hypoxic exposure, ventilation and consequently SaO₂ were elevated (\( ~2–4\% ; \ P < 0.05 \)), and PCO₂ was reduced, which resulted in a lowered MCAv (\( P < 0.05 \) vs. preexposure; Fig. 2). The elevation in peak ventilatory sensitivity at rest to hypoxia following both exposures was related
to the elevation during hypoxic exercise ($r = 0.56-79; P < 0.05$; Fig. 3). Whereas heart rate and BP were elevated at this time point, cardiac output was only elevated in the CH group; elevations in cardiac output following IH did not quite reach significance ($P = 0.07$; Fig. 4). When data from both groups were pooled, following the hypoxic interventions, cardiac output was elevated during hypoxic exercise ($P < 0.05$). During hypoxic exercise, the apparent impairment in cerebral autoregulation, reflected in the lower low-frequency phase between MCAv and mean BP, was unaltered following IH and/or CH exposure (Fig. 5). There were no other changes in transfer function indexes in the VLF or HF range, or in any frequency range for the MAP and MCAv variability (data not shown). Following the two hypoxic interventions, there were no differences in dynamic cerebral autoregulation or the cardiorespiratory or cerebrovascular responses during normoxic exercise.

Cerebral and muscle oxygenation following IH and/or CH exposure. Following IH and/or CH exposure, the greater hypocapnia during hypoxic exercise provoked a decrease in MCAv ($P < 0.05$ vs. preexposure; Fig. 2) that was related to a reduced cerebral oxygenation ($r = 0.54; P < 0.05$). Conversely, in the muscle during hypoxic exercise, following both hypoxic interventions, there was less of a decrease in local oxygenation ($P < 0.05$ vs. preexposure; Fig. 6).

Cardiorespiratory and cerebrovascular responses to acute hypocapnia and hypocapnia exercise following hypoxic or hypercapnic exposures. There was no change in the sensitivities of either ventilation, MCAv, or cerebral or muscle oxygenation to hypocapnia following any of the three interventions.

Intermittent hypcapnic intervention. In contrast to IH or CH, exposure to intermittent hypcapnia did not alter any of the cardiorespiratory or cerebrovascular responses to acute hypoxia at rest or during exercise ($P > 0.05$; data not shown).

Reliability of noninvasive measurements of BP and PetCO2. For comparative purposes, arterial pressure was measured with a radial arterial catheter in six subjects before and after the IH ($n = 4$) and intermittent hypcapnia ($n = 3$) interventions. In these subjects, absolute differences of 5 mmHg ($P < 0.05$) were found between the measurements of radial and finger arterial pressure. However, the magnitude of elevations in arterial pressure monitored by the Finometer and direct intra-arterial line were comparable during exercise with (MAP: 19% by Finapres, 20% by catheter) and were further elevated during hypoxic exercise following (MAP: 24% by Finapres, 26% by catheter) IH, whereas it was unchanged following intermittent hypercapnia when compared with preexposure. These data confirm the validity of using finger photoplethysmography to measure changes in arterial pressure during the conditions imposed in the present study. In addition, during hypoxic exercise both before and following IH, the declines in $P_{aCO_2}$ with the corrected $P_{ETCO_2}$ values to better reflect $P_{aCO_2}$, were very comparable ($P > 0.05$).

**DISCUSSION**

The three main novel findings of this study are: 1) the elevation in hypoxic ventilatory response (rather than a compromise in cerebral autoregulation or cerebrovascular reactivity to CO2), following either IH or continuous exposure, resulted in greater hypocapnia and consequential reduction in MCAv and cerebral oxygenation; 2) exposure to IH and/or CH resulted in less of a decrease in muscle oxygenation during hypoxic exercise, indicating a potential physiological adaptation at the muscle tissue level during hypoxic exercise; 3) intermittent hypercapnia did not provoke any cardiorespiratory or cerebral changes at rest or during exercise, confirming the requirement of hypoxia rather than changes in hyperpnoea per se. Collectively these findings indicate that the consecutive and ongoing deoxygenation followed by reoxygenation that typically occurs during IH is not a necessary component to elicit changes in cardiorespiratory and cerebrovascular function during hypoxic exercise; rather, the changes seem to be due to the exposure of hypoxia per se.

Cardiorespiratory and cerebrovascular function following intermittent and/or continuous hypoxic exposure: rest, normoxic, and hypoxic exercise. Consistent with previous reports, following exposure to IH of similar duration (8, 22, 27), baseline cardiorespiratory and cerebrovascular variables were unchanged. The lack of change in cardiovascular measurements (BP, heart rate, cardiac output) at rest is consistent with recent reports (10, 27), indicating an absence of change to autonomic control. During hypoxic but not normoxic exercise, following both IH and CH, there was a greater elevation in

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**Fig. 3.** Relationships between the peak change in the ventilatory response to hypoxia (HVR) at rest and during exercise following the IH and continuous hypoxic (CH) interventions. These data indicate that the peak changes in the ventilatory response to hypoxia at rest are closely related to the changes during hypoxic exercise following the IH and/or the CH exposure. *P ≤ 0.05. There were no relationships with the 12-day time point (for clarity, these data are not shown), indicating that the related changes were returned to preexposures during this time. Likewise, there were no changes in the hypoxic ventilatory response following the intermittent hypcapnia intervention.
MAP. This finding is broadly consistent with a recent report (44) that detailed an elevation in MAP during normoxic exercise following 4 wk of IH (12% O₂, 1 h/day, 5 days/wk). That study speculated that the IH suppressed the vascular antioxidative capacity and the availability of nitric oxide, leading to an increased MAP (44). Another possibility is that reexposure to acute hypoxia following relief of an IH or CH stimulus may evoke a transient elevation in sympathoexcitation and subsequent elevations in MAP, potentially mediated by a heightened peripheral chemosensory activity (27). The potential mechanisms involved in such sympathoexcitation have been elegantly reviewed (27). Previous reports indicate that dynamic cerebral autoregulation is impaired by exhaustive exercise (29) and during moderate-intensity exercise in acute hypoxia (3), despite a hyperventilation-induced reduction in PaCO₂ and likely exercise-induced elevations in sympathetic activation (3, 29). Such effects contrast with those that occur at rest, in which reductions in PaCO₂ and elevations in sympathetic activation act to enhance cerebral autoregulation by widening the static cerebral autoregulation curve and causing a rightward shift, respectively (31). Thus hypocapnia and sympathetic activation seem to have differential influences on dynamic cerebral autoregulation depending on whether induced at rest or as a consequence of exercise. The findings of the present study, however, suggest that prior exposure to IH or CH subsequent elevations in ventilatory chemosensitivity and related hypocapnia do not alter further dynamic cerebral autoregulation during hypoxic exercise.

**Effects of an enhanced hypoxic ventilatory response on exercise gas exchange.** Elevations in ventilatory chemosensitivity to hypoxia following hypoxic exposure are consistent with previous reports (8, 11, 20, 26-28, 42). The lack of influence of this elevation in ventilatory chemosensitivity during normoxic exercise has been reported (9, 21). Consistent with our findings, Katayama et al. (22) has shown that increasing ventilatory chemosensitivity following exposure to IH was related to an improved SaO₂ during hypoxic exercise. A noteworthy observation from our study was that, following IH/CH exposure, the elevation in ventilatory chemosensitivity to hypoxia at rest is related to the associated change during hypoxic exercise (Fig. 3). Although clearly not establishing cause and effect, this finding indicates that there is, at least some, translation of resting hypoxic peripheral chemosensitivity to the apparent elevation in hypoxic sensitivity during hypoxic exercise.

Fig. 4. Change in heart rate (A), cardiac output index (B), and mean arterial blood pressure (C) during normoxic and hypoxic exercise before IH, following IH and altitude exposure and a 12-day recovery (IH and altitude group; left; n = 7) and before and following altitude exposure and a 12-day recovery (altitude-only group; right; n = 7). Values are means ± SD, expressed as absolute or relative (i.e., percent) change from baseline. *P < 0.05, different compared with normoxic exercise (*) and different compared with baseline and 12-day posthypoxic exercise (†). There were no between-condition differences in ventilation, PCO₂, or MCAv at any point during normoxic exercise. There were no changes in any of the monitored variables following the intermittent hypercapnia intervention; for clarity, these data are not shown.
Differential alterations in cerebral and muscle oxygenation following intermittent and/or continuous hypoxic exposure. Using simultaneously measured oxygenation in the brain and muscle, our results show that, following IH or CH, there are differential alterations in cerebral and muscle oxygenation during steady-state hypoxic exercise. The stronger influence of PaCO2 on the brain, when compared with the muscle, is consistent with previous reports (34). During exercise, the smaller redistribution of cardiac output to the brain, compared with that of the muscle (12), in addition to the action of dynamic cerebral autoregulation in buffering marked elevations in arterial BP, might underlie why there are only slight (10 –20%) elevations in global CBF during moderate-intensity (i.e., 60 –70% VO2 max) exercise (reviewed in Ref. 38). Under maximal exercise, however, there is compelling evidence that global CBF and oxygenation are subsequently reduced because of marked hyperventilation-related hypocapnia (reviewed in Ref. 38). In the current study, since there was a greater degree of hypocapnia during hypoxic exercise following the IH and/or CH hypoxic exposure and because cerebral perfusion is highly sensitive to reductions in Pco2, MCAv and cerebral oxygenation were reduced. We consider the following three possibilities that might underlie this selective reduction in MCAv during hypoxic exercise following the IH and/or CH hypoxic exposure: 1) IH and/or CH changes in the MCAv response to CO2 during hypoxic exercise; 2) there is a threshold of MCAv response to hypocapnia during exercise that was reached by the elevations in chemoreflex activation to hypoxia, and related decline in arterial Pco2; and 3) because changes in cardiac output independent of MAP can affect MCAv (14), another possibility is that a greater redistribution of cardiac output to the muscle during hypoxia might further compound the hypocapnia-induced lowering of MCAv. Because of our findings of an unchanged cerebrovascular reactivity to CO2, at least at rest, and a greater degree hypocapnia during exercise, we favor the latter two explanations as the most likely to underlie our findings.

The apparent improvement in muscle oxygenation for the two hypoxic exposures, indicating some subtle adaptations at the level of the muscle to hypoxia during exercise, is consistent with another report during maximal exercise (25). Recent studies indicate that IH of a comparable duration and intensity to that used in the current study can alter intrinsic properties of mitochondrial function (i.e., substrate preference) without any change in maximal oxygen uptake (36). The underlying mechanisms by which an enhancement in mitochondrial function may translate to an improvement in muscle oxygenation are not known. Because there was a tendency for cardiac output to be elevated during hypoxic exercise following both interventions, another possibility is that redistribution of this cardiac output, coupled with more vasodilatation to the working muscle, might help improve its relative oxygenation. It should be acknowl-

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Fig. 5. Changes in group-averaged low-frequency (0.07–0.02 Hz) transfer function phase (A), gain (B), and coherence (C) between MAP and MCAv during normoxic (hatched bars) and hypoxic (filled bars) exercise following intermittent and CH. Values are means ± SD, P ≤ 0.05, different compared with normoxic rest (*) and different compared with normoxic exercise (†).
edged, however, that our study examined the cardiorespiratory and cerebrovascular alterations following two paradigms of hypoxia and not during maximal exercise; as such, no extrapolation can be made to the controversial benefits of IH as a means to improve performance (46).

IH vs. CH. Over the last decade, there has been an exponential increase in experimental studies in animals and, more recently, in humans investigating the effects of IH on peripheral (i.e., muscle/systemic) and central (i.e., brain) vascular function. The chronic (i.e., years) repetitive and brief “apneas” associated with obstructive sleep apnea cause hypoxia, hypercapnia, and asphyxia, all of which contribute to sympathoexcitation (6), which seem critical to evoke adverse vascular changes. It should be acknowledged that comparable hypoxia-to-reoxygenation cycles do not occur during the “typical” IH protocols used for athletic training [e.g., 5:5-min hypoxia-to-normoxia for 60–90 min/day (2, 8), or 30 min to 3 h continued hypoxia/day (10)] or by researchers as interventions to examine the potential consequences on systemic and cerebrovascular function (8, 10, 27, 44). For example, during a typical 5:5-min hypoxia-to-normoxia session for 60 min or during a 1-h hypoxic exposure, the episodes of hypoxia to normoxia will be 6/h and 1/h, respectively; this represents very different conditions to >50 apneic events/h in a patient with moderate to severe obstructive sleep apnea. Therefore, there can be limited extrapolation between the hypoxic events associated with obstructive sleep apnea and the typical IH paradigms used. To the best of our knowledge, however, this is the first investigation to consider detailed cerebrovascular changes following both IH and intermittent hypercapnia as well as CH during exercise. In relation to the current findings, and the typical IH paradigm used, our data indicate that the reoxygenation of IH or hypercapnia is not a necessary component to eliciting changes in cardiorespiratory and cerebrovascular function during hypoxic exercise; rather, it is simply due to the exposure of hypoxia per se.

Methodological considerations. Although we used Doppler ultrasound to measure flow velocity, rather than blood flow, in the MCA, the majority of research suggests that MCAv is a reliable index of CBF. Recent data from our laboratory indicate that changes in MCAv during related changes in arterial blood gases are closely related to the changes in global CBF, as estimated from direct measurements based on the Fick principle (32). The advantages and limitations of NIRS have been well described (34). The NIRS monitors oxygenation in tissue (17) and confirms the capillary-oxygen-level-dependent related increase in oxygenation observed by functional magnetic resonance imaging during neural activation (40). We further acknowledge that NIRS measures only local (i.e., to one depth) oxygenation and that discreet regions of the brain and/or muscle may respond differently during conditions of exercise. In the human brain, however, NIRS-determined capillary oxygenation is functionally related to the balance between arterial and internal jugular venous oxygen saturation (34). Cerebral NIRS has been shown to track changes in jugular venous bulb saturation in healthy volunteers under conditions of isocapnic hypoxia (24) and has also been validated compared with positron emission tomography scanning (37), with $^{133}$Xe washout methods (39), and with internal carotid artery stump pressures (47). Moreover, a recent report indicates that de-
creases in cerebral tissue oxygen index, as assessed using NIRS, of $-13\%$ (4) is required to reach a “threshold” of cerebral ischemia. The aforementioned physiological and pathophysiolog-ical experimental studies support the validity of using NIRS; however, to the authors’ knowledge, similar information has not been reported that has related muscle oxygenation to arterial-venous oxygen saturation. For the first time following exposure to different hypoxic interventions, we combined transcranial Doppler to assess MCAv and NIRS for the monitoring of local cerebral oxygenation, in addition to activation-dependent information, at rest and during exercise with and without hypoxia. Although the novel combination of NIRS and MCAv provides complementary information for the evaluation of cerebral hemodynamic function, it should be noted that, because of the marked differences in regional cerebral perfusion during exercise (5) and the additional influence of hypoxia, the changes in cerebral perfusion and oxygenation assessments may not be purely explained by the chemoreflex-induced hypocapnia and/or cardiac output redistribution. Despite these limitations, it seems unlikely that they would explain the findings of the current study, which were selective changes from baseline following the IH or CH interventions that recovered 12 days after the interventions and were absent following the intermittent hypocapnia intervention.

We used transfer-function analysis to assess dynamic cerebral autoregulation, rather than earlier-described methods that have used ‘static’ methods (30). It should be noted that quantification of cerebral autoregulation with static methods obscures the fact that most of the challenges to cerebral perfusion originate from rapid shifts (i.e., within seconds) in cerebral perfusion. The use of dynamic autoregulation has been validated extensively to show that it is a highly sensitive and reliable index of a threatened cerebral circulation. Indeed, comparable changes in transfer function analysis (especially in the low-frequency phase) have been shown to be sensitive to pathophysiological changes in patients with carotid artery disease, stroke, severe head injury, subarachnoid hemorrhage, and other conditions (reviewed in Ref. 30). However, it should be noted that a constraint of assessing dynamic cerebral autoregulation is that it cannot provide information on the position of the autoregulatory curve or describe the range of arterial BP over which autoregulation might be affected. It should also be noted, however, that this pressure range has not been clearly assessed in conscious humans and a “gold standard” method for the quantification of cerebral autoregulation has not been identified for human experimentation.

**Perspectives and Significance**

Prior exposure to either IH or CH can lower MCAv and cerebral oxygenation during hypoxic exercise, potentially mediated by the greater hypocapnia, rather than a compromise in cerebral autoregulation or alterations in cerebrovascular reactivity to CO2. Despite this potential compromise in cerebral perfusion, exposure to IH and/or CH may induce some physiological adaptations at the muscle tissue level that lead to enhanced oxygenation.

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**REFERENCES**


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