Pulmonary \( \text{O}_2 \) uptake and leg blood flow kinetics during moderate exercise are slowed by hyperventilation-induced hypocapnic alkalosis

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Chin LMK, Heigenhauser GJF, Paterson DH, Kowalchuk JM. Pulmonary \( \text{O}_2 \) uptake and leg blood flow kinetics during moderate exercise are slowed by hyperventilation-induced hypocapnic alkalosis. J Appl Physiol 108: 1641–1650, 2010. First published March 25, 2010; doi:10.1152/japplphysiol.01346.2009.—The effect of hyperventilation-induced hypocapnic alkalosis (Hypo) on the adjustment of pulmonary \( \text{O}_2 \) uptake (\( \dot{V}_{\text{O}_2p} \)) and leg femoral conduit artery (“bulk”) blood flow (LBF) during moderate-intensity exercise (Mod) was examined in eight young male adults. Subjects completed four to six repetitions of alternate-leg knee-extension exercise during normal breathing [Con; end-tidal partial pressure of \( \text{CO}_2 \) (\( \text{PETCO}_2 \)) ~40 mmHg] and sustained hyperventilation (Hypo; \( \text{PETCO}_2 \) ~20 mmHg). Increases in work rate were made instantaneously from baseline (3 W) to Mod (80% estimated lactate threshold). \( \dot{V}_{\text{O}_2p} \) was measured breath by breath by mass spectrometry and volume turbine, and LBF (calculated from mean femoral artery blood velocity and femoral artery diameter) was measured simultaneously by Doppler ultrasound. Concentration changes of deoxy (\( \Delta[Hb] \)), oxy (\( \Delta[\text{O}_2\text{Hb}] \)), and total hemoglobin-myoglobin (\( \Delta[HbTot] \)) of the vastus lateralis muscle were measured continuously by near-infrared spectroscopy (NIRS). The kinetics of \( \dot{V}_{\text{O}_2p} \), LBF, and \( \Delta[Hb] \) were modeled using a monoexponential equation by nonlinear regression. The time constants for the phase 2 \( \dot{V}_{\text{O}_2p} \) (Hypo, 49 ± 26 s; Con, 28 ± 8 s) and LBF (Hypo, 46 ± 16 s; Con, 23 ± 6 s) were greater (\( P < 0.05 \)) in Hypo compared with Con. However, the mean response time for the overall \( \Delta[Hb] \) response was not different between conditions (Hypo, 23 ± 5 s; Con, 24 ± 3 s), whereas the \( \Delta[Hb] \) amplitude was greater (\( P < 0.05 \)) in Hypo (8.05 ± 7.47 a.u.) compared with Con (6.69 ± 6.31 a.u.). Combined, these results suggest that hyperventilation-induced hypocapnic alkalosis is associated with slower convective (i.e., slowed femoral artery and microvascular blood flow) and diffusive (i.e., greater fractional \( \text{O}_2 \) extraction for a given \( \Delta[V_{\text{O}_2p}] \) \( \text{O}_2 \) delivery, which may contribute to the hyperventilation-induced slowing of \( \dot{V}_{\text{O}_2p} \) (and \( \text{O}_2 \) utilization) kinetics. Acute voluntary hyperventilation increases \( \text{CO}_2 \) output, thereby reducing the end-tidal (\( \text{PETCO}_2 \)) and arterial partial pressure of \( \text{CO}_2 \) (\( \text{PaCO}_2 \)) and lowering plasma and muscle [\( \text{H}^+ \)] (13, 26). As shown previously, hyperventilation-induced hypocapnic alkalosis results in a slower adjustment of pulmonary \( O_2 \) uptake (\( \dot{V}_{\text{O}_2p} \)) (13, 14, 33) and slower and greater breakdown of muscle phosphocreatine (PCr) (26) (both reflecting kinetics of muscle \( \text{O}_2 \) utilization) compared with a normal breathing condition during moderate-intensity exercise. The increase in the fundamental (phase 2) component of \( \dot{V}_{\text{O}_2p} \) during the transition to moderate-intensity exercise is not instantaneous but increases exponentially toward a new steady-state level. The adjustment of \( \dot{V}_{\text{O}_2p} \) (and muscle \( \text{O}_2 \) utilization) is believed to be limited by convective and diffusive delivery of \( \text{O}_2 \) (i.e., blood flow) to the active muscles, to activation of rate-limiting enzymes that provide oxidative substrate for mitochondrial oxidative phosphorylation, or to a combination of these factors (29, 54, 68). Whether the slower \( \dot{V}_{\text{O}_2p} \) adjustment during hyperventilation-induced hypocapnic alkalosis is related to either or both of these factors remains to be resolved.

Using near-infrared spectroscopy (NIRS), Chin et al. (13, 14) reported that the time course of muscle deoxygenation (\( \Delta[Hb] \); reflecting local muscle \( \text{O}_2 \) utilization-to-microvascular \( \text{O}_2 \) delivery ratio) at exercise onset was similar to that seen in the control condition, despite slower \( \dot{V}_{\text{O}_2p} \) kinetics and similar \( \dot{V}_{\text{O}_2p} \) amplitude, suggesting that the greater fractional \( \text{O}_2 \) extraction and lower muscle \( \text{O}_2 \) utilization early in the exercise transition were the consequences of an attenuated microvascular \( \text{O}_2 \) flow response in hyperventilation-induced hypocapnic alkalosis. Greater \( \text{O}_2 \) extraction would be expected to lower microvascular \( \text{PO}_2 \) and the capillary-to-muscle \( \text{PO}_2 \) gradient and to impair \( \text{O}_2 \) diffusion to the mitochondrial cytochrome c oxidase (assuming no change in muscle \( \text{O}_2 \) diffusion conductance). An attenuated convective and diffusive \( \text{O}_2 \) delivery could constrain the activation of mitochondrial oxidative phosphorylation and muscle \( \text{O}_2 \) utilization, as evidenced by the slower \( \dot{V}_{\text{O}_2p} \) kinetics.

To our knowledge, the effects of hyperventilation-induced hypocapnic alkalosis on blood flow and blood flow kinetics during exercise have not been examined in humans, although effects on resting blood flow have yielded conflicting findings. In animals mechanically ventilated to induce alkalosis, hind-limb blood flow was unchanged (41) or reduced (11, 31, 38, 40). In humans, forearm blood flow was shown to decrease in response to 6 min of voluntary hyperventilation (42), whereas increases in blood flow were measured in human forearm (12, 16, 57, 58) and calf (17) and inferred by decreased arteriovenous \( \text{O}_2 \) difference across the leg muscle (46) during bouts of hyperventilation lasting between 1 and 15 min.

Therefore, with confounding information regarding the response of blood flow during acute hypocapnic alkalosis at rest and limited knowledge available during exercise in humans, the purpose of this study was to examine the adjustments of leg femoral conduit artery (“bulk”) blood flow (by Doppler ultrasound) and microvascular perfusion (by NIRS) in relation to \( \dot{V}_{\text{O}_2p} \) during transitions to moderate-intensity exercise with normal breathing (control) and hyperventilation-induced hypocapnic alkalosis. It was hypothesized that in hyperventilation-induced hypocapnic alkalosis, compared with control, a slower...
adjacent of leg femoral conduit artery (and microvascular) blood flow would accompany the slower $V_{O2p}$ kinetics and greater and faster rate of muscle deoxygenation (and $O_2$ extraction).

**METHODS**

**Ethical Approval**

This protocol and all procedures were approved by The University of Western Ontario Ethics Committee for Research on Human Subjects, in accordance with the Declaration of Helsinki. Subjects were given thorough verbal and written explanation of the experimental protocol, including possible risks and discomforts associated with the testing procedure. Subjects provided written consent before volunteering to participate in this study.

**Subjects**

Eight young male subjects (age, $26 \pm 5$ yr; mean $\pm$ SD) participated in this study and were all nonsmokers and free of known respiratory, cardiovascular, and metabolic disease.

**Preexperimental Protocol**

All tests were performed on a custom-built alternate-leg knee-extension ergometer as previously described (49). Briefly, subjects were in a seated upright position with their legs strapped to a padded "arm" that was attached to a Monark cycle ergometer (model 814E). Passive movement of the subject's legs was accomplished by having an assistant pedal the cycle ergometer, whereas active exercise involved having the subject alternately extend their legs over a 2-s duty cycle (i.e., 1-s contraction, 1-s relaxation), which resulted in a frequency of 30 extensions per minute (epm) per leg. Each subject completed an initial incremental exercise test to volitional fatigue to determine their estimated lactate threshold ($\hat{V}L$) with no systematic increase in the ventilatory equivalent for $V_{CO2}$ ($\dot{V}E/\dot{V}CO2$).

The peak $O_2$ uptake ($V_{O2peak}$), and moderate-intensity work rate (WR). Testing began with 2 min of passive movement of the subject's legs, followed by an increase in work rate of 6 W (load, 200 g) every min, starting at 18 W (load, 600 g). The test ended when subjects were unable to sustain the kicking frequency of 30 epm despite verbal encouragement.

The $\dot{V}E/\dot{V}CO2$ at which the following were observed to occur simultaneously: $V_{O2peak}$ and the V/Q (ventilation/perfusion) ratio increased out of proportion to the increase of $V_{O2peak}$, 3) the ventilatory equivalent for $V_{O2peak}$ ($\dot{V}E/\dot{V}O2peak$) began to rise with no systematic increase in the ventilatory equivalent for $VCO2$ ($\dot{V}E/\dot{V}CO2$), and 4) the end-tidal $O_2$ pressure ($PECO2$) began to rise with a plateau in the end-tidal $O2$ pressure ($PECO2$). The $V_{O2peak}$ was calculated from the average of the final 20-s $V_{O2}$ values of the exercise test. The WR that elicited $\sim 80\%$ of the $\dot{V}E/\dot{V}CO2$ (Mod) was also determined from the results of the incremental exercise test.

Before data collection began, subjects familiarized themselves with the hyperventilation maneuver by establishing the degree of hyperventilation needed to attain the target $PETCO2$, of $\sim 20$ mmHg from a normal $PETCO2$, of $\sim 40$ mmHg (13, 45). Breath-by-breath $PETCO2$ data were collected and displayed (PowerLab Chart version 4.2; ADInstruments, Colorado Springs, CO) on a computer screen to allow subjects to monitor and adjust their breathing frequency and/or tidal volume to maintain $PETCO2$, relatively constant, even during exercise, at $\sim 20$ mmHg.

**Exercise Protocol**

The protocol began with 5 min of normal breathing (preaccommodation), followed by an "accommodation period" of 20 min, when subjects either continued to breathe normally (Con; $PETCO2$, $\sim 40$ mmHg) or were instructed to voluntarily hyperventilate to induce a hypocapnic alkalosis (Hypo; $PETCO2$, $\sim 20$ mmHg). During Hypo trials, the duration of the accommodation period was sufficient to reduce and equilibrate body $CO2$ stores to the new lower level (10), and continued hyperventilation throughout the remainder of the exercise protocol maintained $PETCO2$, at the required level (i.e., $PETCO2$, was kept at $\sim 20$ mmHg until the protocol was terminated). The exercise component of the protocol began with 2 min of passive alternate-leg knee-extension (KE) exercise, where an assistant pedaled the cycle ergometer to move the subject's legs passively at 30 epm, followed by 6 min of active baseline KE exercise at 3 W (100 g), where the subject actively contracted at 30 epm. The inclusion of passive leg movement in this study minimized the mechanical effect at initiation of active exercise by the subject (49) and ensured a consistent pace was maintained between subjects at the onset of the exercise protocol (56). Step transitions in WR occurred from the active baseline exercise to a WR corresponding to $\sim 80\%$ of $\dot{V}L$ (Mod).

Each step lasted 6 min, and transitions were made instantaneously and without warning the subjects. The protocol was repeated four to six times per condition to improve the signal-to-noise ratio and, therefore, the confidence of the measured responses. Trials for each condition were randomized, and only a single trial was performed per visit, with each visit separated by at least 24 h.

**Data Collection**

**Gas exchange.** Inspired and expired airflow and volumes were measured throughout the exercise protocol by a low dead space (90 ml) bidirectional volume turbine (VMM-110; Alpha Technologies, Laguna Hills, CA) that was calibrated before each test using a syringe of known volume (3.0 liters; Hans Rudolph, Kansas City, MO). Respired gases were sampled continuously (1 ml/s) at the mouth and analyzed by the fractional concentrations of $O2$, $CO2$, and $N2$ by mass spectrometry (AMIS 2000; Innovision, Lindvedvej, Denmark) following calibration with precision-analyzed gas mixtures. Inspired and expired volumes were time-aligned with changes in gas concentrations by measuring the time delay for a bolus of gas to travel through a capillary line from the turbine transducer and be detected by the mass spectrometer. The algorithms of Beaver et al. (8) were used to calculate breath-by-breath alveolar gas exchange.

**Leg blood flow and heart rate.** Measures of femoral artery mean blood velocity ($MBV$) were measured at rest using pulsed-wave Doppler ultrasound (Vingmed System FiVe; GE Medical Systems, Horten, Norway). Data were collected continuously using a 7.5-MHz probe with an isoinsonation angle of 45° positioned at $\sim 2-3$ cm distal to the inguinal ligament and proximal to the femoral artery bifurcation. This location was selected to avoid blood flow interference from the inguinal and surrounding regions, as well as to minimize the turbulence from the femoral bifurcation (49, 56). $MBV$ was obtained from each trial by integrating the total area under the $MBV$ profile and averaging over the 2-s duty cycle. Trials in the same condition for a given subject were averaged together to yield a single $MBV$ profile for each subject in Con and Hypo trials. Although femoral artery (FA) diameters were not expected to change from rest and at any point during moderate-intensity exercise in Con [as demonstrated previously by Koga et al. (39) and MacDonald et al. (47), MacPhee et al. (49), Radegran (55), and Radegran and Saltin (56)], hypocapnic alkalosis has been shown to reduce the diameter of peripheral microvessels in rabbits (40). As such, trials were recorded and stored on VHS tapes for further determination of FA diameters throughout the experimental protocol. Measures of diastolic FA diameters were made in triplicate using on-screen calipers included with the Doppler ultrasound and were averaged together to produce a single diameter value that at any given time point. During Con trials, diameter measures were taken before the accommodation period, at the end of the accommodation period, during passive exercise, and during steady-state baseline and Mod. During the Hypo trials, diameter measures were obtained as follows: at least twice during the
preaccommodation period; every 2 min during the accommodation period, passive exercise, and baseline; every 10 s during the first 2 min of the Mod transition; and every 30 s during the last 4 min of Mod. The FA diameter data were analyzed in a manner similar to MacDonald et al. (48), where averages for each subject by condition were fit with either a linear (for Con) or an exponential regression (for Hypo) to yield a single averaged response across time. With the averaged MBV data and the FA diameter data obtained from the regression equation, calculation of LBF was made as follows: LBF (l/min) = MBV (cm/s) × πr² (mm²) × 0.0006, where r is the radius of the FA.

Beat-by-beat heart rate (HR) was monitored and recorded continuously using an electrocardiogram (LifePulse; HME, South Mimms, UK) with a three-lead arrangement and stored on a separate computer for further analysis (PowerLab Chart version 4.2).

Near-infrared spectroscopy. Changes in local muscle oxy (\([O_2Hb]\), deoxy (\([HHb]\), and total hemoglobin-myoglobin concentrations (\([HHb_{Tot}]\)) were continuously measured by NIRS (NIRO 300; Hamamatsu Photonics, Hamamatsu, Japan). Optodes were housed in an optically dense rubber holder to ensure the specific distance between the optodes was fixed at 5 cm and then placed on the skin above the vastus lateralis muscle midway between the lateral epicondyle and greater trochanter of the femur of the right leg. A black vinyl sheet was taped to the skin surface to cover the optode assembly and to minimize the loss of near-infrared transmitted light from the region of interrogation and reduce the intrusion of extraneous light. To further secure the position of the optodes during the exercise protocol, an elastic bandage was wrapped around the leg to prevent any movement of the optode assembly while still permitting freedom of movement. The NIRS signals were monitored until a steady baseline was established, at which time the signals were set to zero.

A detailed explanation of the principle and theory of NIRS as used in the present study is described by Elwell (23). Briefly, four laser diodes produce different wavelengths (775, 810, 850, and 910 nm) that are pulsed in rapid succession and transmitted through fiber optic bundles to the tissue of interest. The transmitted light returns through a separate fiber optic bundle to a photomultiplier tube, where the light intensities are coupled with the relevant specific extinction coefficient and optical path length to give rise to changes (Δ) in \([O_2Hb]\), \([HHb]\), and \([HHb_{Tot}]\) relative to steady-state resting values. Given the uncertainty of the optical path length in the vastus lateralis at rest and during exercise, NIRS data are presented as arbitrary units (a.u.). Changes in light intensities were monitored and recorded continuously at 2 Hz, and the raw attenuation signals were transferred and stored on a computer for later analysis.

Analysis of Data

\(\dot{V}O_2p\), MBV, and HR data for each individual trial were initially filtered for erroneous data points that lay outside four standard deviations of the local mean, because they do not conform to a Gaussian distribution as described by Lamarra et al. (44). Data were then interpolated on a second-by-second basis and time-aligned to correspond to the onset of the Mod transition (time 0). The data for each repetition within a condition were further ensemble-averaged for each subject and time-averaged into 10-s bins to yield a single response profile. Second-by-second LBF (calculated from the averaged interpolated MBV and femoral artery radius) were averaged into 10-s time bins.

The on-transient responses for \(\dot{V}O_2p\), MBV, LBF, and HR were modeled using a monoeponential of the form

\[ Y(t) = Y_{(B)L} + A \exp(-t - (TD)^\gamma) \]

where \(Y_{(B)L}\) represents \(\dot{V}O_2p\), MBV, LBF, or HR as a function of time \(t\) throughout the exercise transient; \(Y_{(B)L}\) is the baseline of \(Y\) during steady-state baseline exercise before the step increase in WR; Amp is the amplitude of the increase in \(Y\) above the baseline value; \(\gamma\) is the time constant (i.e., time taken to reach 63% of the steady-state response); and TD is the time delay.

The fit for \(\dot{V}O_2p\) began at the phase 1-phase 2 transition [as previously described by Rossiter et al. (59) and Gurd et al. (30)], to the end of the exercise transition (i.e., 360 s). Fitting for MBV, LBF, and HR began at the first data point after the start of Mod, through to the end of the Mod bout.

The NIRS-derived \(\Delta[O_2Hb]\), \(\Delta[HHb]\), and \(\Delta[HHb_{Tot}]\) data were time-aligned to the onset of Mod (time 0) and ensemble-averaged into 5-s bins to yield a single response for each subject for both Con and Hypo trials. The time delay for the \(\Delta[HHb]\) response (i.e., TD-\(\Delta[HHb]\)) at the onset of Mod was determined using second-by-second data for each transition and defined as the first increasing point that consistently remained above the nadir of the signal. This was performed on every trial for all subjects and averaged to yield one TD-\(\Delta[HHb]\) value for Con and Hypo trials for each subject. To determine the time course for muscle deoxygenation, the \(\Delta[HHb]\) data were modeled using an exponential function as in Eq. 1 from the TD-\(\Delta[HHb]\) to 90 s of the WR step; extending the HHb fitting window beyond 90 s to 360 s did not affect the parameter estimations for \(\Delta[HHb]\) kinetics. Visual inspection of the NIRS-derived \(\Delta[HHb]\) profile and minimal variation of residuals around the Y-axis (Y = 0) suggest this fitting procedure provides a reasonable estimate of the time course of muscle deoxygenation during the period corresponding to the phase 2 \(\dot{V}O_2p\) response. The overall time course for muscle deoxygenation is shown as the mean response time (MRT = TD-\(\Delta[HHb]\) + \(\tau\)\(\Delta[HHb]\)). Analysis of the \(\Delta[O_2Hb]\) and \(\Delta[HHb_{Tot}]\) signals were restricted to steady-state baseline and end-exercise values, since these signals do not exhibit an exponential-like response.

Statistical Analysis

The kinetic parameter estimates for \(\dot{V}O_2p\), MBV, LBF, HR, and \(\Delta[HHb]\) were analyzed using one-way analysis of variance (ANOVA) for repeated measures. Comparisons for all variables across time were analyzed by two-way ANOVA for repeated measures with the main effects of condition and time. A significant F-ratio was analyzed using Tukey’s post hoc analysis with statistical significance accepted at \(P < 0.05\). All values are means ± SD.

RESULTS

“Steady-State” Responses Across Time During Hypo and Con Trials

Group-averaged profiles across time during Hypo and Con are shown for respiratory measures (Fig. 1), blood flow-related measures and heart rate (Fig. 2), and NIRS data (Fig. 3). Group means (±SD) are included for specific time points representing the end points for each of the step transitions of the protocol.

Respiratory measures. During the resting accommodation and through to the end of the exercise protocol, subjects were able to decrease \((P < 0.05)\) and maintain \(PETCO_2\) at \(\approx 20\) mmHg in Hypo by doubling \((P < 0.05)\) their ventilation (Ve) compared with Con (Fig. 1). At end-exercise, tidal volume was higher \((P < 0.05)\) in Hypo \((1.9 \pm 0.8\) l/breath) than in Con \((1.3 \pm 0.2\) l/breath), whereas breathing frequency was higher \((P < 0.05)\) in Hypo \((32 \pm 15\) breaths/min) compared with Con \((21 \pm 3\) breaths/min). No difference was observed in \(\dot{V}O_2p\) between Hypo and Con conditions throughout the protocol, whereas overall \(\dot{V}CO_2\) was higher \((P < 0.05)\) in Hypo compared with Con.

Blood flow-related and HR measures. After the onset of hyperventilation, measured MBV was higher \((P < 0.05)\) during Hypo than Con (Fig. 2). The FA diameter remained unchanged throughout the entire protocol during Con \((-10.5\)
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The average moderate WR intensity in the present study was 47 (±12) W, representing 78 (±7)% of \( \dot{V}\text{O}_2 \). Figure 4 presents the normalized group mean response profiles and model fits at the exercise transition for \( \dot{V}\text{O2p} \), MBV, and LBF and individual values for the respective time constants (\( \tau \)) with group means (±SD). The parameter estimates for the kinetics of \( \dot{V}\text{O2p} \), MBV, LBF, and HR are presented in Table 1, whereas parameter estimates for \( \Delta \text{HHb} \) are presented in Table 2.

\( \dot{V}\text{O2p} \). The amplitude for \( \dot{V}\text{O2p} \) was similar between conditions (Table 1), whereas the phase 2 time constant of \( \dot{V}\text{O2p} \) (\( \tau\dot{V}\text{O2p} \)) was greater (\( P < 0.05 \)) during Hypo (49 ± 26 s) than during Con (28 ± 8 s) (Fig. 4), with differences between conditions being greater than the 95% confidence intervals for each condition (Table 1), reflecting a slower adjustment of \( \dot{V}\text{O2p} \) during Hypo.

**MBV, LBF, and HR.** The MBV amplitude and time constant were not different between Hypo and Con conditions (Table 1). The LBF amplitude (Hypo, 1.21 ± 0.35 l/min; Con, 1.22 ± 0.41 l/min) and ratio (\( \Delta \text{LBF} \times 2/\Delta \dot{V}\text{O2p} \)) (~4.5 liters of blood per liter of \( \text{O2} \) uptake for Hypo and Con) were similar between conditions; however, as a result of the gradual increase in FA diameter during Hypo, the adjustment of LBF was slower (\( P < 0.05 \)) in Hypo (\( \tau\text{LBF} \), 46 ± 16 s) than in Con (\( \tau\text{LBF} \), 23 ± 6 s) (Fig. 4).

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mm). However, during Hypo, the FA diameter (which was not different from that during Con at the preaccommodation period) began to decrease during the accommodation period such that the FA diameter at each time point measured was smaller (\( P < 0.05 \)) than preaccommodation values and those during Con (postaccommodation: Hypo, 9.8 ± 1.2 mm; Con, 10.5 ± 1.1 mm). During the transition to Mod, the FA diameter began to increase in an exponential-like manner (\( \text{MRT} = 99 ± 17 \) s) toward that observed in Con; the FA diameter at the end of exercise (10.3 ± 1.2 mm) was smaller (\( P < 0.05 \)) than in Con (10.5 ± 1.1 mm) but greater than that measured during the postaccommodation, passive, and baseline periods (~9.8 mm) in Hypo. A main effect of condition for LBF was observed, with an overall higher (\( P < 0.05 \)) LBF in Hypo compared with Con. HR values were higher (\( P < 0.05 \)) in Hypo compared with Con at the end of the accommodation period and during passive exercise.

**NIRS measures.** There was a main effect of condition for the NIRS-derived \( \Delta \text{HHb} \), with an overall lower (\( P < 0.05 \)) value in Hypo than in Con (Fig. 3). The \( \Delta \text{O2Hb} \) in Hypo was lower (\( P < 0.05 \)) at baseline and end-exercise compared with Con, whereas the \( \Delta \text{HHb} \) tended to be similar between conditions.

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**Fig. 1.** Averaged profiles across time of respiratory measures during normal breathing (control, Con; ○) and hyperventilation-induced hypocapnic alkalosis (Hypo; ●). Dotted lines indicate the transitions between the resting preaccommodation period (Pre-Acc), resting accommodation period (Acc), passive exercise (Pass), baseline knee-extension (KE) exercise (Bsl), and moderate-intensity KE exercise (Mod). Specific points represent group means ± SD. *\( P < 0.05 \), different from Con. †\( P < 0.05 \), different from Pre-Acc. ‡\( P < 0.05 \), different from Acc. †\( P < 0.05 \), different from Pass. SP < 0.05, different from Bsl. \( \text{VCO2} \), \( \text{CO2} \) output; PET\( \text{CO2} \), end-tidal partial pressure of \( \text{CO2} \). Frequency is breathing frequency given in breaths/min.

**Fig. 2.** Averaged profiles across time of blood flow-related measures and heart rate (HR; beats/min) during Con (○) and Hypo (●). Dotted lines indicate the transitions between Pre-Acc, Acc, Pass, Bsl, and Mod. Specific points represent group means ± SD. *\( P < 0.05 \), different from Con. †\( P < 0.05 \), different from Pre-Acc. ‡\( P < 0.05 \), different from Acc. †\( P < 0.05 \), different from Pass. SP < 0.05, different from Bsl. MBV, mean blood velocity; LBF, leg blood flow.
blood flow (and microvascular blood flow) in Hypo, there was a greater reliance on O₂ extraction in this condition, which would be expected to lower microvascular O₂ content and P O₂ and thus slow O₂ diffusion to the mitochondrial cytochrome c oxidase. Therefore, with an attenuated O₂ diffusion, a greater provision of one or more of the other oxidative substrates (i.e., reducing equivalents, ADP, P i) would be required to achieve the required rate of oxidative ATP production and contribute to the slow adjustment of muscle O₂ utilization, and VO₂p, during Hypo conditions.

**Hyperventilation-Induced Hypocapnic Alkalosis and LBF**

*At rest.* Previous studies that measured blood flow at rest during Hypo yielded inconsistent results, with studies reporting an increase (12, 16, 17, 46, 57, 58), a decrease (11, 31, 38, 40, 42), or an unchanged blood flow response (41). In the present study, measured LBF at the end of the resting accommodation period was similar to preaccommodation LBF, as a result of a higher (~27%) MV and a lower (~7%) FA diameter that accompanied the fall in PaCO₂ (and the decrease in arterial CO₂ and [H⁻¹]) (13).

The constriction of blood vessels is dependent on vascular smooth muscle (VSM) contraction in response to an increase in intracellular calcium concentration ([Ca²⁺] i) (4). This is in turn related to an increase in intracellular and extracellular pH (4, 73). Plasma pHi influences the extracellular pH to which VSM are exposed (73), and although plasma pHi was not measured in the present study, estimations can be made from PaCO₂. Based on our previous study (13), in which blood was sampled during a hyperventilation protocol identical to that used in this study, postaccommodation and end-exercise arterial pH was 7.63 and 7.54, respectively (compared with 7.51 and 7.41, respectively, in Con). Thus, in the present study, a pH effect on VSM is likely. Support for a Hypo-induced FA vasoconstriction comes from studies that demonstrated increases in [Ca²⁺] i (2, 7, 63, 64, 70) and tension (2, 3) following alkalization of VSMs (either by removal of CO₂ from the superfusate or exposure to NH₄Cl or NaOH).

There were studies, however, that found vasodilation with alkalization of VSMs (36, 66), whereas others observed a biphasic response, where a transient vasoconstriction (lasting from 1 (50) to 10 min (20)) preceded sustained vasoconstriction. A biphasic response was observed by Kontos et al. (42) in humans during voluntary hyperventilation, where an induced vasoconstriction in the forearm was followed, after 4 min, by vasoconstriction and a reduction in forearm blood flow. In the present study, the reduction in FA diameter was not significant until ~4 min after the start of hyperventilation. Therefore, the apparent time-dependent vasoconstrictor response with hyperventilation may explain the varied findings of previous studies at rest, where the duration of hyperventilation varied between 1 and 20 min (12, 16, 17, 40–42, 46, 57, 58) and 1–2 h (11, 31, 38).

**During mod exercise.** To our knowledge, no studies have reported blood flow kinetics in humans during transitions to exercise during Hypo. In the present study, steady-state FA (i.e., bulk) LBF tended to be higher in Hypo than in Con during baseline and exercise, due to the elevated MBV. However, during the exercise transition, the adjustment of LBF was slower in Hypo than in Con, which was related more to the

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**Fig. 3.** Averaged near-infrared spectrometry (NIRS) profile across time during Con (shaded curves) and Hypo (solid curves). Dotted lines indicate the transitions between Pre-Acc, Acc, Pass, Bsl, and Mod. Specific points (Con; ○; Hypo; •) represent group means ± SD. *P < 0.05, different from Con. Δ[HHb], Δ[O₂-Hb], Δ[HHb], concentration changes of total, oxygenated, and deoxygenated hemoglobin-myoglobin; a.u., arbitrary unit.

Slower VO₂p kinetics during the transition to Mod exercise were shown previously to occur with hyperventilation-induced hypocapnic alkalosis, although the mechanism(s) related to this slower response was not established (13, 14, 33). In agreement with our previous findings, the present study reported slower VO₂p kinetics in Hypo than in Con conditions, whereas Δ[HHb] kinetics were not different between conditions but the Δ[HHb] amplitude (Δ[HHb]/ΔVO₂p) was greater in Hypo. The new findings reported in the present study were that 1) absolute MBV was higher in Hypo than in Con, but MBV kinetics were similar between conditions; 2) FA diameter remained at preaccommodation levels throughout the protocol in Con (consistent with previous studies), but in Hypo, FA diameter decreased during the accommodation period and increased in an “exponential-like” manner back toward, but did not reach, control levels during the transition to Mod (the response time for vasodilation being ~100 s); and 3) leg femoral conduit artery (bulk) blood flow kinetics during the exercise transition were slower in Hypo than in Con. These data suggest that as a consequence of the slower adjustment of femoral artery (bulk)
time course of the increase in FA diameter, since MBV, although tending to be slower, was not different between conditions (τMBV: Hypo, 30 s; Con, 23 s).

During Con, the FA diameter during MOD remained unchanged from rest, as observed in previous studies (39, 47, 49, 55, 56). Indeed, under control conditions, the initial increase in blood flow during submaximal exercise is achieved by microcirculatory vasodilation via a muscle contraction-induced mechanical compression or distortion of resistance vessels and/or contraction-induced release of vasodilator substances (69), which negates the need to increase conduit artery diameters (56). During Hypo, however, the restoration of FA diameter from its constricted state occurred with an exponential-like time course (effective MRT, 99 ± 17 s) at the onset of Mod (Fig. 2). Although the hyperventilation protocol was maintained throughout Mod (thereby maintaining PetCO2 at ~20 mmHg), microvessels in close proximity to recruited muscle fibers will be exposed to CO2 released by muscle fibers. Thus, as mitochondrial CO2 production increases during the transition to exercise, it is expected that CO2 diffusion out of active muscle fibers will contribute to local arteriolar vasodilation with a time course somewhat slower than the activation of mitochondrial oxidative phosphorylation. The vascular network (consisting of arterioles, capillaries, and venules) is arranged such that microvessels are in close proximity to each other (22, 53), thus allowing diffusion of CO2 to occur between neighboring microvessels. Furthermore, the vasodilatory signal originating


from arterioles (62, 71, 72), capillaries (9, 18), or venules (19) is capable of being transmitted through endothelial and smooth muscle cells via gap junctions (6, 15, 24) and ascending up the vascular network to produce dilation of the feed artery (71). This could explain the increase in FA diameter that was seen at the start of Mod in the present study. As discussed above, CO2 has the ability to evoke changes in VSM cells through alterations in intra- and extracellular pH and subsequent changes in [Ca^{2+}]_i. In particular, VSM cells respond to lowered pH by reducing [Ca^{2+}]_i and promoting relaxation (2, 3, 50, 64).

Another possible contributor to the increase in FA diameter is lactate⁻, which is capable of causing vasodilation independently of acid-base changes (1, 34, 51, 52). Lactate⁻ accumulation in arterial plasma was greater in Hypo (~5 mM) than in Con (~3 mM) during Mod cycling exercise (13, 45), a consequence of a slower activation of pyruvate dehydrogenase (PDH; requiring a greater substrate level phosphorylation) with Hypo (45) and enhanced transport out of the muscle via the sarcolemma monocarboxylate transporter (37). A lactate⁻-induced vasodilation has been shown to cause a decrease in systemic vascular resistance in pigs (1) and relaxation of isolated precontracted mesenteric arteries in rats (51) and coronary arteries in dogs and pigs (34, 52). Furthermore, vasodilation was observed with lactate⁻ concentrations as low as 1 mM (34), with vascular relaxation occurring in a concentration-dependent manner (51, 52). Thus, because the present study shares an exercise protocol similar to that of the study by Chin et al. (13), it is reasonable to assume that an increase in arterial plasma lactate⁻ concentration over the course of the Mod bout in Hypo conditions might contribute to the gradual increase in FA diameter observed in the present study.

Hyperventilation-Induced Hypocapnic Alkalosis and NIRS

The slower \( \dot{V}_{O_2} \) kinetics during exercise in Hypo observed in the present study are consistent with the findings from other published reports (13, 14, 33) and reflect slower muscle O\(_2\) utilization kinetics. In addition, Forbes et al. (26) reported that muscle PCr breakdown kinetics [a proxy measure of muscle O\(_2\) utilization (59)] was slowed and the fall in muscle PCR content was greater in Hypo during the transition to moderate-intensity plantar-flexion exercise, supporting the relationship between \( \dot{V}_{O_2} \) and muscle O\(_2\) utilization kinetics (5, 28, 43).

Hayashi et al. (33) proposed that slower \( \dot{V}_{O_2} \) kinetics observed during Mod cycling with prior hyperventilation of 2 min was the result of a leftward shift of the oxyhemoglobin dissociation curve, which attenuated the offloading of O\(_2\) from hemoglobin. Therefore, a lower microvascular PO\(_2\) would be expected for any level of O\(_2\) extraction in Hypo compared with Con. In addition, LeBlanc et al. (45), using a hyperventilation protocol similar to that used in the present study, reported that the active form of the mitochondrial PDH complex was lower during the first minute of cycling exercise at 55% \( \dot{V}_{O_2}\text{max} \) compared with a normal control condition. Although \( \dot{V}_{O_2} \) was not measured in that study, the slower activation of PDH, which regulates the conversion of pyruvate to acetyl-CoA and provision of carbohydrate-derived substrates for the tricarboxylic acid cycle and reducing equivalents (i.e., NADH, FADH\(_2\)) to the electron transport chain (65), suggests a metabolic explanation for slower \( \dot{V}_{O_2}\) kinetics observed during respiratory alkalosis. Hence, slower activation of PDH may contribute to slowed \( \dot{V}_{O_2} \) adjustment during hyperventilation and Mod; however, PDH was not measured in the present study.

Hyperventilation-Induced Hypocapnic Alkalosis and NIRS

Microvascular changes within a muscle can be inferred from NIRS measures. The kinetics of muscle deoxygenation (\([\Delta [HHb]]\)) reflect fractional muscle O\(_2\) extraction (21) and, when considered in combination with \( \dot{V}_{O_2} \) kinetics, provide information on the adjustment of muscle microvascular blood flow (21, 25, 32). In the present study, the time course of \([\Delta [HHb]]\) was similar between conditions despite slower \( \dot{V}_{O_2} \) kinetics (reflecting slower adjustment of muscle O\(_2\) utilization), which is consistent with our previous findings (13, 14, 26). In addition, the \([\Delta [HHb]]\) amplitude and \([\Delta [HHb]]\)-to-\( \dot{V}_{O_2} \) ratio were greater during Hypo, reflecting a greater fractional O\(_2\) extraction with hyperventilation. These findings, along with the observation that conduit FA LBF kinetics were slowed in Hypo, are consistent with slowed adjustment of muscle blood flow at the microvascular level during Hypo, thereby requiring a greater fractional O\(_2\) extraction to meet the muscle O\(_2\) requirements. This in turn would potentiate the fall in microvascular PO\(_2\), thereby reducing O\(_2\) diffusive flux into the muscle.

Table 1. Kinetic parameter estimates of \( \dot{V}_{O_2p} \), MBV, LBF, and HR during Con and Hypo conditions

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Baseline</th>
<th>Amplitude</th>
<th>( \tau ), s</th>
<th>( C_{95} ), s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>0.50 ± 0.03</td>
<td>0.55 ± 0.17</td>
<td>28 ± 8</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Hypo</td>
<td>0.48 ± 0.05</td>
<td>0.55 ± 0.18</td>
<td>49 ± 26*</td>
<td>4 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBV, cm/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>13 ± 2</td>
<td>23 ± 7</td>
<td>23 ± 6</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Hypo</td>
<td>17 ± 3*</td>
<td>22 ± 5</td>
<td>30 ± 13</td>
<td>4 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBF, l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>0.68 ± 0.14</td>
<td>1.22 ± 0.41</td>
<td>23 ± 6</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Hypo</td>
<td>0.74 ± 0.14*</td>
<td>1.21 ± 0.35</td>
<td>46 ± 16*</td>
<td>5 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>70 ± 4</td>
<td>22 ± 6*</td>
<td>21 ± 15</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Hypo</td>
<td>73 ± 6</td>
<td>19 ± 5*</td>
<td>35 ± 19*</td>
<td>5 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SD for parameter estimates of pulmonary O\(_2\) uptake (\( \dot{V}_{O_2p} \)), mean blood velocity (MBV), leg blood flow (LBF), and heart rate (HR) during normal breathing (control; Con) and hyperventilation-induced hypocapnic alkalosis (Hypo). Baseline values are the average of the last 120 s of 3-W knee-extension exercise. \( \tau \), Time constant; \( C_{95} \), 95% confidence interval. *\( P < 0.05 \), different from Con.

Table 2. Kinetic parameter estimates of \([\Delta [HHb]]\) (model fit to 90 s) during Con and Hypo

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Baseline, a.u.</th>
<th>Amplitude, a.u.</th>
<th>MRT, s</th>
<th>TD, s</th>
<th>( \tau ), s</th>
<th>( C_{95} ), s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>1.49 ± 0.079</td>
<td>6.69 ± 6.31</td>
<td>24 ± 3</td>
<td>15 ± 2</td>
<td>10 ± 4</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Hypo</td>
<td>2.24 ± 2.51</td>
<td>8.05 ± 7.47*</td>
<td></td>
<td></td>
<td>10 ± 4</td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SD for parameter estimates of muscle deoxymyoglobin concentration changes (\([\Delta [HHb]]\)) during Con and Hypo. Baseline values are the average of the last 120 s of 3-W KE exercise. MRT, mean response time (TD + \( \tau \)); TD, time delay. *\( P < 0.05 \), different from Con.
Also, a lower $\Delta[Hb_{Tot}]$ and $\Delta[O_2Hb]$, as seen in this study during Hypo, would suggest a lower muscle blood (hemoglobin) volume and $O_2$ availability in muscle (compared with Con) before and during Mod. Because red blood cell (RBC) spacing in capillaries contributes to the functional capillary surface area for $O_2$ diffusion and thus is an important determinant of $O_2$ diffusive conductance ($D_{O2}$) (35), a lower $\Delta[Hb_{Tot}]$ might reflect a lower $D_{O2}$, which, combined with a lower microvascular $P_{O2}$, would impair $O_2$ flux between the RBC and muscle mitochondrial cytochrome $c$ oxidase.

Microvascular blood volume in humans (13) and microcirculation in animals (31, 40) at rest were previously shown to be lower with Hypo. However, this is in contrast to bulk (conduit artery) blood flow measured at the FA in the present study, where steady-state blood flow was not attenuated during Hypo (as discussed above). Interestingly, however, a reduction of the diameter of microvessels within the ear chamber of rabbits recently was demonstrated with hypocapnia ($P_{ETCO_2}$, $\sim$15–20 mmHg) (40), consistent with the FA constriction observed at rest during Hypo in the present study. Therefore, although conduit arteries and peripheral microvessels appear to respond in a similar manner to Hypo, the latter appear to be affected by Hypo to a greater extent.

Hyperventilation-Induced Hypocapnic Alkalosis and HR

HR was not different between the conditions, although the adjustment of HR was slower in Hypo, suggesting a slower adjustment of cardiac output could have contributed to the slower LBF kinetics, and thus the slower $V_{O2p}$ kinetics, seen in Hypo in the present study. This finding is in contrast to that of Chin et al. (13, 14) and Hayashi et al. (33), where no differences in HR kinetics were reported between conditions. In the present study and those of others (13, 14, 33), the exercise intervention was in the Mod domain. However, the present study used KE exercise rather than leg cycling exercise (13, 14, 33), where end-exercise HR reached $\sim$90 beats/min compared with $\sim$115 beats/min for leg cycling. Importantly, autonomic control of HR increases may differ somewhat between these two exercise modalities. Parasympathetic (i.e., vagal) withdrawal contributes to the increase in HR up to $\sim$100 beats/min, whereas further increases in HR to above $\sim$100 beats/min rely on sympathetic activation (60). Vagal tone has been shown to be attenuated with hyperventilation (27), suggesting that vagal reactivity has been altered and that there is a diminished ability to increase HR by vagal withdrawal during Hypo. Indeed, older inactive adults who display reduced vagal tone (61) have shown slower increases in HR in response to exhaustive isometric hand-grip exercise (67) relative to younger controls. Since in the present study the increase in HR during moderate KE exercise relies mainly on parasympathetic withdrawal, an attenuated vagal reactivity associated with hyperventilation may contribute to slower HR kinetics during Hypo compared with Con. Although the slower HR kinetics (and cardiac output) may have contributed to slower LBF kinetics in the present study, we believe that the hyperventilation-induced reduction in FA diameter and subsequent slow rate of FA (and microvascular) dilation at exercise onset may play a more important role, since other studies using leg cycling exercise reported slowed $V_{O2p}$ kinetics with hyperventilation in the absence of slowed HR kinetics (13, 14, 33).

Conclusion

In the present study, a prolonged hyperventilation (Hypo) maneuver (>20 min) was associated with a slowing of $V_{O2p}$ and conduit artery (FA) LBF kinetics but not with the kinetics of muscle fractional $O_2$ extraction (as assessed by NIRS-derived $\Delta[Hb]$). Also, FA diameter was reduced at rest during accommodation to Hypo, and an exponential-like increase in FA diameter toward Con values during the transition to Mod contributed, in part, to the slower LBF kinetics in Hypo, since the adjustment of MBV was not different between conditions. In addition, slower microvascular blood flow kinetics (inferred from the faster and greater $\Delta[Hb]-to-V_{O2p}$) suggests a slowed microcirculatory adjustment during the transition to moderate-intensity exercise. These findings support the concept that the adjustment of muscle $O_2$ utilization (as reflected by $V_{O2p}$ kinetics) may be affected by peripheral vascular adjustments that control muscle conduit artery (bulk) and microvascular blood flow and thus local convective and diffusive $O_2$ delivery to contracting skeletal muscle.

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DISCLOSURES

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

REFERENCES


