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Slow $\dot{V}O_2$ kinetics in acute hypoxia are not related to a hyperventilation-induced hypocapnia

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Running Head: Effects of hypocapnia on $\dot{V}O_{2p}$ kinetics in hypoxia

Abstract (*160 words*)

We examined whether slower pulmonary O_2 uptake ($\dot{V}O_{2p}$) kinetics in hypoxia is a consequence of: a) hypoxia alone (lowered arterial O_2 pressure), b) hyperventilation-induced hypocapnia (lowered arterial CO_2 pressure), or c) a combination of both. Eleven participants performed 3-5 repetitions of step-changes in cycle ergometer power output from 20W to 80% lactate threshold in the following conditions: i) normoxia (CON; room air); ii) hypoxia (HX, inspired O_2 =12%; lowered end-tidal O_2 pressure [$P_{ET}O_2$] and end-tidal CO_2 pressure [$P_{ET}CO_2$]); iii) hyperventilation (HV; increased $P_{ET}O_2$ and lowered $P_{ET}CO_2$); and iv) normocapnic hypoxia (NC-HX; lowered $P_{ET}O_2$ and $P_{ET}CO_2$ matched to CON). Ventilation was increased (relative to CON) and matched between HX, HV, and NC-HX conditions. During each condition $\dot{V}O_{2p}$ was measured and phase II $\dot{V}O_{2p}$ kinetics were modeled with a mono-exponential function. The $\dot{V}O_{2p}$ time constant was different ($p<0.05$) amongst all conditions: CON, 26±11s; HV, 36±14s; HX, 46±14s; and NC-HX, 52±13s. Hypocapnia may prevent further slowing of $\dot{V}O_{2p}$ kinetics in hypoxic exercise.

Key words: O_2 uptake kinetics, near-infrared spectroscopy, hypocapnia, CO_2, exercise, end-tidal

1. Introduction

Breathing a gas mixture containing a fractional oxygen (O_2) concentration lower than that of air (i.e., hypoxia) reduces the partial pressure of inspired O_2 (P_iO_2) and O_2 concentration of the arterial blood (C_aO_2). Without an adequate compensatory response, hypoxia may limit O_2 supply to the peripheral tissues. When a step-increase in moderate-intensity exercise power output is performed in conditions of acute hypoxia (12-15% inspired fraction of O_2), the rate of adjustment of pulmonary O_2 uptake ($\dot{V}O_{2p}$) (i.e., as described by the phase II time constant [$\tau\dot{V}O_{2p}$]) is slowed relative to the same step-transition performed in normoxic conditions (Bowen et al., 2013; Engelen et al., 1996; Hughson and Kowalchuk, 1995; MacDonald et al., 2000; Perrey et al., 2005; Spencer et al., 2012a) suggesting that convective and/or diffusive O_2 delivery to the active muscles may limit muscle O_2 utilization ($\dot{V}O_{2m}$), necessitating a greater contribution of substrate level phosphorylation to the exercise energetics. Hypoxia, CO_2 retention, slow $\dot{V}O_{2p}$ kinetics, and low exercise tolerance are characteristics common to several highly prevalent heart and respiratory pathologies such as heart failure (Bowen et al., 2012) and restrictive or obstructive lung disease (Nery et al., 1982). The hypoxia and CO_2 retention that frequently accompanies these conditions limits the adjustment of $\dot{V}O_{2p}$ on transition to exercise, but the mechanisms by which these contribute to slow $\dot{V}O_{2p}$ kinetics remains incomplete.

Relative to normoxia, hypoxic exercise induces a greater rate of ventilation ($\dot{V}_E$) (Adams and Welch, 1980; Engelen et al., 1996; MacDonald et al., 2000) due to reflex-activation of low P_aO_2-sensitive peripheral chemoreceptors (Buckler and Vaughan-jones, 1994; Cunningham et al., 1986; Duffin, 1990). A consequence of an increased ventilatory drive (relative to metabolic demand) is a reduction in the partial pressure of arterial CO_2 (P_aCO_2) (Chin et al., 2007), which causes a shift in the carbonic anhydrase-catalyzed equilibrium reaction between CO_2 and

bicarbonate (HCO_3^-) with an increase in flux in the direction of CO_2 formation (law of mass action). The subsequent reduction in arterial [HCO_3^-] and hydrogen ions ([H^+]) results in a state of respiratory alkalosis. For example, Parolin et al., (2000) reported a near two-fold greater $\dot{V}_E$ concomitant with a 15 mmHg (~35%) reduction in end-tidal CO_2 pressure ($P_{ET}CO_2$, a reasonable surrogate for P_aCO_2 in a healthy lung (Jones et al., 1979)) during steady-state submaximal exercise in hypoxia ($F_IO_2 = 0.12$). Additionally, considerable reductions in arterial [H^+] (Adams and Welch, 1980; Parolin et al., 2000) and muscle [H^+] (Green et al., 1992) have been observed during submaximal exercise in hypoxic relative to normoxic conditions. Thus, hyperventilation-induced hypocapnic alkalosis is a physiological consequence of exercise in hypoxia.

When a hyperventilation manoeuvre (decreasing $P_{ET}CO_2$ and increasing $P_{ET}O_2$) is performed during exercise whilst breathing room air, $\dot{V}O_{2p}$ kinetics are slowed (Chin et al., 2013, 2010a, 2010b, 2007; Hayashi et al., 1999; Ward et al., 1983) by a magnitude similar to that reported during hypoxic exercise (i.e., twofold increase in $\tau\dot{V}O_{2p}$). Furthermore, the proposed mechanisms by which hyperventilation or hypoxia slow the on-transient $\dot{V}O_{2p}$ response are also very similar. For example, effects have been attributed to reductions in convective and diffusive O_2 delivery in both conditions of acute hypoxia (DeLorey et al., 2004; Koskolou et al., 1997; MacDonald et al., 2000) and hyperventilation-induced hypocapnic alkalosis (Chin et al., 2013, 2010a, 2010b; Hayashi et al., 1999). Furthermore, similar increases in the rates of glycogenolysis and glycolysis, and decreases in the rates of pyruvate oxidation, also have been reported (relative to a control) during submaximal exercise performed in either conditions (LeBlanc et al., 2002; Linnarsson et al., 1974; Parolin et al., 2000), implicating a delayed activation of rate-limiting enzymes (e.g., pyruvate dehydrogenase) as a reason for the observed slower $\dot{V}O_{2p}$ kinetics.

Therefore, the slowing of $\dot{V}O_{2p}$ kinetics in hyperventilation or hypoxia (relative to normal conditions) may stem from similar hypocapnia-specific mechanisms.

It is unclear whether the slower $\dot{V}O_{2p}$ kinetics observed during hypoxia is a consequence of: a) lower arterial O_2 availability, b) lower arterial CO_2, or c) a combination of both. Recently, Chin et al., (2013) demonstrated that hyperventilation in normoxia performed with CO_2 added to the inspirate prevented the hypocapnia and induced-alkalosis and restored the slower $\dot{V}O_{2p}$ kinetics back towards control values. Therefore, here we examined the role of hyperventilation and hypocapnia in the hypoxia-induced slowing of $\dot{V}O_{2p}$ kinetics. It was hypothesized that the slower $\dot{V}O_{2p}$ kinetics in hypoxia compared to a normoxic control was related to effects of hypocapnia in addition to reduced O_2 delivery, and that addition of inspired CO_2 during hypoxic exercise (i.e., normocapnic hypoxia) would return (i.e., speed) $\dot{V}O_{2p}$ kinetics back towards control (normocapnic normoxia) values.

2. Materials and Methods

2.1 Participants

Eleven healthy young adult men (age, 24 ± 4 yrs (mean ± SD); body mass, 81 ± 8 kg; height, 182 ± 5 cm; peak $\dot{V}O_2$, 44.2 ± 6.1 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered and gave written informed consent to participate in the study. All procedures were approved by The University of Western Ontario Ethics Committee for Research on Human Subjects. All subjects were non-smokers who were free of any musculoskeletal, respiratory, cardiovascular, and metabolic conditions and who were not taking any medications that might influence cardiorespiratory or metabolic responses to exercise.

2.2 Pre-experimental Protocol

On the first visit, each subject reported to the laboratory to perform a ramp incremental exercise test to volitional exhaustion (20 W baseline for 4 min followed by a 25 $W \cdot min^{-1}$ ramp) on an electronically-braked cycle ergometer (model: Velotron, RacerMate Inc., Seattle, WA, USA) for determination of peak $\dot{V}O_{2p}$ ($\dot{V}O_{2peak}$), peak power output (PO_{peak}) and to estimate the lactate threshold ($\hat{\theta}_L$). Participants were asked to maintain a cadence of ~60 rpm during the test. $\dot{V}O_{2peak}$ was defined as the greatest 20-s $\dot{V}O_{2p}$ computed from a rolling average and peak power output was defined as the power output achieved at termination of the ramp incremental test. $\hat{\theta}_L$ was determined by visual inspection using standard ventilatory and gas exchange indices as previously described (Beaver et al., 1986).

Before beginning the experiments, subjects were familiarized with the hyperventilation manoeuvre. Subjects were instructed to breathe in time with an audible que by metronome, while tidal volumes were continuously displayed on a monitor. Subjects were required to meet target volumes during each inspiration and expiration in time with the metronome. Tidal volumes and breathing frequencies were manipulated so as to lower and maintain $P_{ET}CO_2$ at ~30 mmHg – a value previously reported during exercise using $F_iO_2 = 0.12$, as used here (Lador et al., 2013; Parolin et al., 2000).

2.3 Experimental Conditions

The study used four experimental conditions: control (CON), hyperventilation (HV), hypoxia (HX), and normocapnic hypoxia (NC-HX). Each condition involved different requirements for ventilation and inspired gas mixtures (Table 1), with multiple repetitions performed in each condition (see *Exercise Protocol*). All conditions began with a resting period of 22 min, during which subjects breathed quietly for the first 2 min (pre-accommodation period), then either continued to breath normally (i.e., CON) or were instructed to hyperventilate (i.e., HV, HX, and

NC-HX) for the next 20 min (accommodation period) at a constant breathing frequency and tidal volume sufficient to reduce and maintain $P_{ET}CO_2$ at ~30 mmHg. This target ventilation was used in all subsequent HV, HX, and NC-HX trials to ensure equivalence in breathing frequency and tidal volume and thus, total ventilation for these conditions.

For all conditions, subjects began at the pre-accommodation period by breathing room air. To blind subjects to the testing condition, in CON they breathed gas from a Douglas bag (filled with air: ~0.03% CO_2, ~21% O_2, balance N_2). A three-way T-shaped stopcock and hose (Hans Rudolph, Kansas City, MO, USA) joined the Douglas bag to the breathing apparatus, which included a two-way Y-shaped non-rebreathing valve (Two-way NRB Y-valve 2730; Hans Rudolph, Kansas City, MO, USA), a pneumotach, volume turbine and mouthpiece (see *Data Collection*). At the start of the accommodation period, an assistant manually changed (i.e., HX and NC-HX trials only) or performed a "dummy" change (i.e., CON and HV trials only) of the direction of the respiratory flow path such that subjects switched from breathing room air to: a) breathing a hypoxic gas mixture from the Douglas bag (i.e., HX and NC-HX trials), or b) continuing to breathe room air (i.e., CON and HV trials). Additionally, during NC-HX trials, an assistant continuously monitored and maintained $P_{ET}CO_2$ around CON values by introducing an inspirate containing ~ 5% CO_2, 12% O_2, and balance N_2 from a pressurized tank via a small hose introduced distal to the inflow port on the two-way non-rebreathing valve.

Breath-by-breath respiratory variables were displayed (PowerLab Chart v.7.3.1; ADInstruments Inc., Colorado, CO, USA), allowing continuous feedback to the subject for maintenance of required tidal volumes. The $P_{ET}CO_2$ and $P_{ET}O_2$ were not visible to the subjects during any trial to avoid alerting the subject to the intervention being used. However, subjects

were not blinded to the CON condition where they breathed spontaneously, with no instruction given regarding rate and depth of breathing.

2.4 Exercise Protocol

Subjects were seated on the cycle ergometer throughout the accommodation period. After 20 min subjects began cycling at 20 W for 6 min after which the power output was increased instantaneously to a power output equivalent to 80% $\hat{\theta}_L$ for an additional 6 min. During cycling all subjects were instructed to maintain a cadence of 60 rpm. The protocol was repeated 3-5 times per condition (i.e., CON, HV, HX, NC-HX) to improve the signal-to-noise ratio and thus the confidence in the measured responses and kinetic parameter estimates. In order to match $P_{ET}CO_2$ between CON and NC-HX, it was necessary to precede the first NC-HX trial by at least one CON trial; thereafter all conditions were randomized.

2.5 Data Collection.

During each trial participants wore a noseclip and breathed through a mouthpiece for breath-by-breath gas-exchange measurements. Inspired and expired volumes and flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies, VMM 110) and pneumotach (Hans Rudolph, Model 4813) positioned in series from the mouthpiece (total apparatus dead space was 150 mL); respired air was continuously sampled at the mouth and analysed by mass spectrometry (Innovision, AMIS 2000, Lindvedvej, Denmark) for fractional concentrations of O_2 and CO_2. The volume turbine was calibrated before each test using a syringe of known volume (3 L) over a range of flow rates and the pneumotach was adjusted for zero flow. Gas concentrations were calibrated with precision-analyzed gas mixtures. The time delay between an instantaneous square-wave change in fractional gas concentration at the sampling inlet and its detection by the mass spectrometer was measured electronically by

computer. Respiratory volumes, flow and gas concentrations were recorded in real-time at a sampling frequency of 100 Hz and transferred to a computer, which aligned gas concentrations with volume signals as measured by the turbine. Flow from the pneumotach was used to resolve inspiratory-expiratory phase transitions and the turbine was used for volume measurement. The computer executed a peak-detection program to determine end-tidal PO_2, end-tidal PCO_2 and inspired and expired volumes and durations to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated using the algorithms of Swanson (1980).

Local muscle oxygenation of the quadriceps vastus lateralis muscle was measured using a frequency domain multi-distance near-infrared spectroscopy (NIRS) system (Oxiplex TS, Model 92505, ISS, Champaign, USA) as described elsewhere (Spencer et al., 2012b). Briefly, the system was comprised of a single channel consisting of eight laser diodes operating at two wavelengths (λ = 690 and 828 nm, four at each wavelength) which were pulsed in a rapid succession down a photomultiplier tube. A rigid plastic NIRS probe (connected to laser diodes and photomultiplier tube by optical fibers) consisted of two parallel rows of light emitter fibers and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. The probe was placed on the belly of the muscle, at the distal end of the vastus lateralis muscle. NIRS measurements were collected continuously for the entire duration of each trial. The NIRS was calibrated at the beginning of each testing session following an instrument warm-up period of at least 20 min. Throughout each testing session, continuous measurements of the absolute scattering (μ_s) and absorption (μ_a) coefficients were determined from the measured intensity (AC) and phase shift of the light entering and traversing the tissue (at both wavelengths) such that the absolute concentrations of HHb and O_2Hb (in μM) could be

derived. Data were stored at an output frequency of 25 Hz, but were reduced to 1-s bins for all subsequent analyses.

Heart rate (HR) was collected using a Polar Wearlink Chest Strap, H1 Heart Rate Sensor and SP0180 Polar Transmitter (Polar Electro Inc., Lachine, QC, Canada). Arterial O_2 saturation ($\%O_2Sat$) was measured using finger pulse oximetry (model 8600, Nonin Medical, Plymouth, MN, USA). Both HR and $\%O_2sat$ were continuously monitored using PowerLab Chart v.7.3.1 (ADInstruments Inc., Colorado, CO, USA).

2.6 Data Analysis

Data were analysed in a similar manner to those of Keir et al., (2016b). The on-transient responses for $\dot{V}O_{2p}$ and [HHb] were modeled with the following mono-exponential function:

$$Y(t) = Y_{BSL} + A_p \cdot (1 - e^{-(t\text{-}TD)/\tau}) \quad (1)$$

where, $Y(t)$ is the value of the dependent variable at any time during the transition, Y_{BSL} is the pre-transition baseline value, A_p is the steady-state increase in Y above the baseline value, τ is the time constant of the response or the time for Y to increase to 63% of the absolute change in Y, and TD is the time delay. For $\dot{V}O_{2p}$, phase I was excluded from the fitting window by progressively moving the window (from ~ 30 s) back towards time zero while examining the flatness of the residual profile and values of CI_{95} and χ^2 (Rossiter et al., 2001). The end of the fitting window was set to ~5 times the estimated time constant in order to restrict the modeling to data lying within the transient phase. Once the optimal fitting window was established a non-linear least squares regression analysis was performed using Origin 8.5 (OriginLab, Northampton, MA). In order to determine CI_{95} for $\tau\dot{V}O_{2p}$, model convergence was established with the A_p and TD parameters first allowed to vary; subsequently, the model was iterated again with these parameters constrained to best fit values. The mean response time (MRT) of $\dot{V}O_{2p}$

described the overall time course of $\dot{V}O_{2p}$ during the exercise transition and was estimated using the function described in equation 1, with the inclusion of all $\dot{V}O_{2p}$ data but with the TD constrained to 0 s.

The TD for the [HHb] ([HHb]-TD) response was determined using second-by-second data and corresponded to the time after the onset of exercise at which the [HHb] signal increased above 1 SD of the pre-transition baseline value. Determination of the [HHb]-TD was made on each individual's ensemble-averaged response and the data were modeled using equation 1. Different fitting strategies ranging from 90-180 s into a transition resulted in minimal differences in estimates of τ[HHb]. The MRT for [HHb] (MRT-[HHb]) described the overall time for [HHb] to increase from the start of exercise to 63% of the "steady-state" value and was calculated as the sum of τ[HHb] and [HHb]-TD.

2.7 Statistical Analysis

Data are presented as mean ± SD. A two-way analysis of variance (ANOVA) for repeated measures was used to compare gas exchange and muscle oxygenation variables with the main effects of condition and time. Comparison of all kinetic parameter estimates were made using a one-way ANOVA for repeated measures. Where significant effects were found, a Student Newman-Keul's *post hoc* analysis was performed for multiple comparisons. All statistical analyses were performed using SigmaPlot Version 11.0, (Systat Software Inc., San Jose, CA). Statistical significance was accepted at $p < 0.05$.

3. Results

The group mean $\dot{V}O_{2peak}$ from the ramp-incremental exercise protocol was 3.6 ± 0.5 $L \cdot min^{-1}$ (44.2 ± 6.1 $mL \cdot kg^{-1} \cdot min^{-1}$). This occurred at a PO_{peak} of 336 ± 52 W and corresponded to a peak

220 $\dot{V}_E$ of 169 ± 33 L·min^{-1}, peak HR of 196 ± 31 beats·min^{-1}, and a peak respiratory exchange ratio
221 of 1.33 ± 0.05.

222 *3.1 Comparison amongst conditions across time*

223 The mean responses across time for select gas exchange variables are displayed in Figure
224 1. Table 1 displays the average responses for ventilatory data, $P_{ET}O_2$, $P_{ET}CO_2$, and O_2 saturation
225 in the last 2 min of baseline and moderate exercise conditions. There were no differences in any
226 of the gas exchange variables amongst any of the conditions in the pre-accommodation period
227 ($p>0.05$). Thereafter, relative to CON, breathing frequency and expired tidal volume were
228 manipulated in HV, HX, and NC-HX such that $\dot{V}_E$ did not differ ($p>0.05$) amongst the three
229 experimental conditions (HV, HX, NC-HX) at any time point during the accommodation and
230 exercise periods of the protocol (Fig. 1A-C). After the pre-accommodation period, $P_{ET}O_2$ was
231 greater in HV (~113 ± 3 mmHg) compared to CON (~97 ± 4 mmHg, $p<0.05$) and both were
232 greater than in the HX and NC-HX conditions (~52 ± 3 and ~55 ± 3 mmHg, respectively, Fig.
233 1D). After pre-accommodation, $P_{ET}CO_2$ was reduced in both HV and HX to ~29 ± 2 mmHg, but
234 was maintained at ~40 ± 1 mmHg in NC-HX such that it was not different from CON in the
235 accommodation and baseline periods (Fig. 1E). During moderate exercise, $P_{ET}CO_2$ was lower
236 ($p<0.05$) in NC-HX relative to CON (Fig. 1E and Table 1).

237 The NIRS-derived variables were not different for any of the conditions during the pre-
238 accommodation period. The NIRS-derived [Hb_{tot}] was not different amongst conditions at any
239 time point during the protocol however there was a main effect of time such that [Hb_{tot}] was
240 greater ($p<0.05$) in exercise compared to the pre-accommodation, accommodation, and baseline
241 periods (Fig. 2A). There was a significant condition x time interaction for [O_2Hb] such that CON
242 was greater than all other conditions in moderate exercise ($p<0.05$) but not during any other time

periods (Fig. 2B). Similarly, there was a significant condition x time interaction for [HHb] with the response being greater in NC-HX and HX relative to CON and HV ($p<0.05$) during exercise (Fig. 2C) but not at any other times. The muscle tissue O_2 saturation index (TOI, %) was not different amongst conditions ($p>0.05$) until exercise, thereafter TOI was lower in NC-HX and HX compared to CON and HV ($p<0.05$), with TOI being greater in CON than HV ($p<0.05$, Fig 2D).

There was a significant condition x time interaction for HR, with HR increasing from pre-accommodation to accommodation period ($p<0.05$) and then again from baseline to exercise ($p<0.05$) in all conditions. No differences were observed between conditions during pre-accommodation or accommodation periods ($p>0.05$). During the baseline period, HR was elevated in HX and NC-HX relative to the CON and HV conditions ($p<0.05$) and these differences persisted during exercise. As expected by design, following the pre-accommodation period, O_2 saturation (Fig. 1F) was reduced ($p<0.05$) in HX and NC-HX relative to CON and HV. The reduction in O_2 saturation in the hypoxic conditions remained constant until exercise, where it was further reduced ($p<0.05$).

3.2 $\dot{V}O_{2p}$ and muscle deoxygenation kinetic responses to moderate-intensity exercise

Mean parameter estimates for the kinetic responses of $\dot{V}O_{2p}$ are presented in Table 2. Figure 3 displays the group mean on-transient responses of $\dot{V}O_{2p}$ for each condition with phase 2 exponential model fits derived from the group mean parameter estimates superimposed over the data. The phase II $\dot{V}O_{2p}$ time constant was different amongst all conditions (CON, 26 ± 11 s; HV, 36 ± 14 s; HX, 46 ± 14 s; and NC-HX, 52 ± 13 s; $p<0.05$; Table 2). The mean $\hat{\theta}_L$ was 1.90 ± 0.38 L·min^{-1} (53 ± 11%$\dot{V}O_{2peak}$) and the mean exercise power output (102 ± 24 W) corresponded to ~80%$\hat{\theta}_L$ which elicited a steady-state $\dot{V}O_{2p}$ ($\dot{V}O_{2pSS}$) ranging from ~1.50 ± 0.2 L·min^{-1} (CON

and HV) to ~1.56 ± 0.2 L·min^{-1} (HX and NC-HX) or ~79% and ~82% of the $\dot{V}O_{2p}$ at $\hat{\theta}_L$. The $\dot{V}O_{2pSS}$ was greater in HX and NC-HX relative to CON ($p<0.05$).

Mean parameter estimates for the kinetic responses of [HHb] can be seen in Table 3. Baseline [HHb] was different between CON and HX only. The [HHb] amplitude was only greater ($p<0.05$) in HX compared to CON. The τ[HHb] was greater ($p<0.05$) in HX relative to CON and there were no differences ($p>0.05$) amongst conditions for the overall response (MRT-[HHb]) or the [HHb]-TD parameters.

4. Discussion

Relative to a normoxic control condition, the adjustment of pulmonary (and presumably muscle) $\dot{V}O_2$ is slowed in response to a step-change in moderate-intensity exercise under conditions of: i) hypoxic breathing (Bowen et al., 2013; Engelen et al., 1996; Hughson and Kowalchuk, 1995; MacDonald et al., 2000; Perrey et al., 2005; Spencer et al., 2012a), and ii) volitional hyperventilation and accompanying hypocapnia and respiratory alkalosis (Chin et al., 2013, 2010a, 2010b, 2007; Hayashi et al., 1999; Ward et al., 1983). A common physiological feature consequent to both experimental interventions is hyperventilation and hyperventilation-induced hypocapnic alkalosis. We examined whether the slowing of $\dot{V}O_{2p}$ kinetics in hypoxia was associated simply with reduced O_2 delivery, or whether the response was related, in part, to hyperventilation and its associated hypocapnia. To do so, we examined the effects of hypoxic breathing or volitional hyperventilation (with induced hypocapnia) alone and the effects of hypoxic breathing with added CO_2 to maintain normocapnia. Recent work in normoxia (Chin et al., 2013) demonstrated that eucapnic-hyperventilation partially restored the slower $\dot{V}O_{2p}$ kinetics back towards control values. Therefore, the present study was designed to examine the

289 effects of: a) lower arterial O_2 availability, b) lower arterial CO_2, and c) a combination of both on
290 $\dot{V}O_{2p}$ and muscle oxy-/deoxygenation response dynamics during the transition to moderate-
291 intensity exercise.

292 The main findings of this study were that $\dot{V}O_{2p}$ kinetics were slowed with hypoxia (HX)
293 and with hyperventilation (HV), but the addition of CO_2 to the inspirate to maintain normocapnia
294 during hypoxia did not speed $\dot{V}O_{2p}$ kinetics back towards control values; in fact, it slowed $\dot{V}O_{2p}$
295 kinetics even further. In both HX and NC-HX, $\dot{V}_E$ was matched such that the work of breathing
296 between these experimental interventions was assumed not different at any time point during the
297 protocol. Furthermore, both conditions involved inspiring gas composed of the same fractional
298 concentration of O_2 (12%) that led to a similar $P_{ET}O_2$ (and presumably P_aO_2) in both conditions
299 ($P_{ET}O_2$ ~55 mmHg; O_{2sat} ~80%), but which was less than normal. The only difference between
300 conditions was that, in HX, $P_{ET}CO_2$ was allowed to fall to ~30 mmHg (a value consistent with
301 the literature for the same level of hypoxia and exercise intensity (Lador et al. 2013; Parolin et al.
302 2000)) but in NC-HX, the inspired gas was supplemented with CO_2 such that $P_{ET}CO_2$ remained
303 near control values (i.e., 40 mmHg). In contrast to our hypothesis, $\dot{V}O_{2p}$ kinetics were slower in
304 NC-HX ($\tau\dot{V}O_{2p}$ ~52 s) compared to HX ($\tau\dot{V}O_{2p}$ ~46 s); although this difference should be
305 interpreted with caution because it is very close to the minimally important difference in $\tau\dot{V}O_{2p}$
306 for interventional studies (i.e., 5 s) (Benson et al., 2017a). This also is in contrast to the speeding
307 of $\dot{V}O_{2p}$ kinetics reported by Chin and coworkers (2013) with normocapnic hyperventilation.

308 The $\tau\dot{V}O_{2p}$ in HX increased relative to control by ~75% ($\tau\dot{V}O_{2p}$ = 46 s vs. 26 s, for HX
309 vs. CON, respectively) which is consistent with previous work using a similar level of
310 normobaric hypoxia (Bowen et al., 2013; Engelen et al., 1996; Hughson and Kowalchuk, 1995).
311 Furthermore, $\dot{V}O_{2p}$ kinetics were slower in HX ($\tau\dot{V}O_{2p}$ = 46 s) compared to HV ($\tau\dot{V}O_{2p}$ = 36 s).

312 At first glance, this finding supports an additive contribution of lower arterial CO_2 and lower
313 arterial O_2 to slower $\dot{V}O_{2p}$ kinetics: that is, relative to CON ($\tau\dot{V}O_{2p}$ = 26 s), hypoxia increased
314 $\tau\dot{V}O_{2p}$ by 10 s and hypocapnia by an additional 10 s (26 + 10 + 10 = 46 s). However, were this to
315 be a truly additive interaction, elimination of one or both of these factors should reduce or
316 completely restore $\dot{V}O_{2p}$ kinetics, respectively, towards control values. Although eliminating low
317 arterial O_2 (i.e., HV) restored $\dot{V}O_{2p}$ kinetics towards control values ($\tau\dot{V}O_{2p}$ decreased from 46 to
318 36 s), the same effect was not observed with the removal of lower arterial CO_2 (i.e., NC-HX;
319 $\tau\dot{V}O_{2p}$ increased from 46 to 52 s). Given that $\dot{V}_E$ was matched in all of these conditions, our
320 findings indicate that the addition of low arterial CO_2 in conditions of low arterial O_2 may
321 paradoxically prevent a further slowing $\dot{V}O_{2p}$ kinetics in acute hypoxia.

322 The postulated mechanisms responsible for the slowed adjustment of pulmonary (and
323 muscle) $\dot{V}O_2$ in acute hypoxia are a reduction in both convective and diffusive O_2 delivery to
324 muscle (Bowen et al., 2013; DeLorey et al., 2004; Lador et al., 2013; Perrey et al., 2005; Roach
325 et al., 1999), and slow activation of PDH (Parolin et al., 2000; and possibly other rate-limiting
326 enzymes). We reasoned that any of these limiting factors could be rectified by preventing
327 hypocapnia during hypoxic exercise. In theory, preventing hypocapnia with CO_2-breathing
328 should facilitate greater peripheral O_2 delivery by preventing a hypocapnic-induced leftward-
329 shift in the oxyhemoglobin dissociation curve (the Bohr effect). In this instance, for the same
330 $\dot{V}O_{2p}$ and O_2 extraction requirement, microvascular PO_2 should remain higher and facilitate
331 diffusive transport into muscle. The slower $\dot{V}O_{2p}$ kinetics in NC-HX relative to HX were
332 accompanied by no differences in the rate (τ; MRT) or magnitude of change (amplitude) in
333 [HHb] during the on-transient. This suggests that microvascular O_2 delivery increased at a slower
334 rate in NC-HX. Therefore, it is possible that any enhancement in microvascular PO_2 and thus

diffusive O_2 delivery consequent to preventing hypocapnia with CO_2-supplementation in the hypoxic condition was offset by a reduction in convective O_2 delivery. For example, in hypoxia, a decrease HbO_2 affinity is expected with the higher CO_2 (and H^+), that accompanies CO_2 supplementation, which could contribute to a lower HbO_2 and thus convective O_2 delivery. At the same time, the presence of more CO_2 (and H^+) in hypoxia could increase competition for binding sites on Hb via the Haldane effect, resulting in a greater formation of carbamino compounds (Roughton, 1935). However, in our study the NIRS-derived HbO_2 was not different in HX and NC-HX. Alternatively, maintaining eucapnia during hypoxia with CO_2-supplementation may augment the systemic vasodilatory effect of low PO_2 directly by CO_2-mediated vasodilation (Clifford and Hellsten 2004). Lower systemic vascular resistance of non-active tissue in NC-HX (relative to HX) could impair the distribution of cardiac output, and thus convective O_2 delivery to active muscle during the transition to moderate exercise.

Alternatively, maintenance of eucapnia during hypoxic exercise should improve activation of rate limiting enzymes (e.g., PDH) via a prevention of a cellular respiratory alkalosis (Parolin et al., 2000). Thus, although PDH activity is depressed during hypoxia, preventing intracellular alkalosis by CO_2 breathing may alleviate the alkalosis-induced inhibition of PDH (LeBlanc et al., 2002) resulting in faster $\dot{V}O_{2p}$ kinetics in NC-HX relative to HX. For instance, Parolin et al. (2000) observed that submaximal exercise performed with hypoxic breathing was associated with an attenuated activation of PDH (a rate-limiting step in the delivery of carbohydrate derived substrate to the mitochondria). Similarly, mismatch between cytosolic pyruvate production and mitochondrial pyruvate oxidation (evidence of attenuated PDH activation) was also observed consequent to volitional hyperventilation-induced hypocapnic alkalosis during an identical exercise protocol (LeBlanc et al. 2002). However, in the present

study maintenance of normocapnia during hypoxic breathing did not speed $\dot{V}O_{2p}$ kinetics in NC-HX relative to HX suggesting that a hypocapnia-induced inhibition of PDH was not the cause of slower $\dot{V}O_{2p}$ kinetics in the hypoxic conditions. Therefore, the mechanism by which $\dot{V}O_{2p}$ kinetics are slowed in hypoxia appears not to be a result of reduced P_aCO_2. On the contrary, low P_aCO_2 in hypoxia appears to be mildly protective of slowing $\dot{V}O_{2p}$ kinetics, which may be a consequence of microvascular O_2 delivery being less well matched to demand during eucapnic hypoxia compared with hypocapnic hypoxia.

The alveolar air equation predicts that for the same F_iO_2 (0.12) and respiratory exchange ratio, a 10 mmHg increase in P_aCO_2 would result in a 12 mmHg decrease in alveolar PO_2. Therefore, it is possible that hypoxia may have been more severe in NC-HX relative to HX. Although this could explain slower $\dot{V}O_{2p}$ kinetics in NC-HX relative to HX, both O_2 saturation, and $P_{ET}O_2$ were not different between conditions suggesting that arterial PO_2 was not different and the NIRS-derived muscle microvascular data also were not different between NC-HX and HX refuting differences in the level of hypoxia at the muscle.

4.1 Limitations

Although phase II $\dot{V}O_{2p}$ kinetics are reflective of the muscle $\dot{V}O_2$ kinetics, they are expressed at the lung in conjunction with phase I. Phase I is largely determined by pulmonary blood flow kinetics and the O_2 concentration of mixed venous blood arriving at the lung (Benson et al., 2013). Even though we matched $\dot{V}_E$ between experimental conditions, such that the work of breathing should be similar, it is possible that differences in the partial pressure of inspired (and presumably arterial) O_2 and CO_2 between conditions could have influenced the early dynamics of blood flow and therefore phase I $\dot{V}O_{2p}$ kinetics (i.e., amplitude and time constant). Indeed there are clear differences between phase I $\dot{V}O_{2p}$ amplitude among CON and

experimental conditions (phase I is greater in HX and NC-HX than COND and HV; Figure 3). Dynamic circulatory differences (if present) influence phase I and phase II $\dot{V}O_{2p}$ kinetics directly, and could contribute to condition-specific dissociations between muscle and pulmonary $\dot{V}O_2$ (Benson et al., 2017b, 2013). In addition, control of ventilation and inspired O_2 and CO_2 were used to manipulate the PO_2 and PCO_2 at the arterial and muscle level. However, the different combinations of low and high $P_{ET}O_2$ and $P_{ET}CO_2$ that were used could have led to unintended condition-specific physiological adjustments (e.g., via acid-base changes in various tissue compartments or activation of the sympathetic nervous system, differences in respiratory muscle work) that could conflate to influence the $\dot{V}O_{2p}$ kinetics of each condition.

5. Conclusion

Exercise in hypoxia incurs conditions of both lower arterial PO_2 and lower arterial PCO_2. Because the addition of inspired CO_2 during hypoxic exercise did not speed $\dot{V}O_{2p}$ kinetics, our findings suggesting that the hypoxia-induced hyperventilation with associated hypocapnia does not contribute to the slowing of $\dot{V}O_{2p}$ kinetics in the hypoxic condition. In fact, hypocapnia actually may prevent a further slowing of $\dot{V}O_{2p}$ kinetics when arterial O_2 availability is lowered.

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512

Figure Captions

Figure 1. Time course of group mean ventilation ($\dot{V}_E$), breathing frequency (f_{br}), expired tidal volume (V_T), end-tidal pressure of O_2 ($P_{ET}O_2$), end-tidal pressure of CO_2 ($P_{ET}CO_2$), and O_2 saturation (O_2sat) for CON (*white circles*), HV (*light grey squares*), HX (*dark grey triangles*) and NC-HX (*black circles*) conditions. Vertical dashed lines separate the *pre-accommodation* (seated rest), *accommodation* (seated rest), *baseline* (20 W cycling), and *moderate exercise* (cycling at 85% estimated lactate threshold) periods.

Figure 2. Ensemble-averaged group mean response of NIRS-derived variables: total hemoglobin ([Hb_{tot}]), oxygenated hemoglobin ([O_2Hb]), deoxygenated hemoglobin ([HHb]), and tissue oxygen index (TOI) for CON (*white circles*), HV (*light grey circles*), HX (*dark grey circles*) and NC-HX (*black circles*) conditions. Vertical dashed lines separate the *pre-accommodation* (seated rest), *accommodation* (seated rest), *baseline* (20 W cycling), and *moderate exercise* (cycling at 85% estimated lactate threshold) periods.

Figure 3. Ensemble-averaged group mean responses for $\dot{V}O_{2p}$ in all four conditions. Vertical dashed lines indicate the onset of moderate exercise (time = 0 s) transition. The group mean phase II kinetic responses for each condition are superimposed over the data (*dark lines*, fitted with a mono-exponential function using group mean parameter estimates) and the group mean phase II fit of the control condition (*dashed black line*) is displayed on the hyperventilation, hypoxia, and normocapnic hypoxia panels. Group mean (± SD) $\tau\dot{V}O_{2p}$ values are inset under each transition and residuals are shown about y = 0 (*grey line*).

533 **Table 1.** Comparison of parameters for each test condition.

Condition	Control	Hyperventilation	Hypoxia	Normocapnic Hypoxia
Ventilatory requirement	Normal	hyperventilation	hyperventilation	hyperventilation
Percentage of inspired O_2 (%)	20.9	20.9	12.0	12.0
Percentage of inspired CO_2 (%)	0.03	0.03	0.03	5.0
		Baseline		
Frequency (breaths·min^{-1})	$14 \pm 5^{b,c,d}$	24 ± 5^{a}	24 ± 1^{a}	24 ± 5^{a}
Tidal Volume (L)	1.7 ± 0.4	1.7 ± 0.4	1.6 ± 0.4	1.7 ± 0.4
Ventilation (L·min^{-1})	$22 \pm 4^{b,c,d}$	39 ± 5^{a}	37 ± 6^{a}	40 ± 5^{a}
End-tidal PO_2 (mmHg)	$98 \pm 3^{b,c,d}$	$115 \pm 2^{a,c,d}$	$52 \pm 4^{a,b}$	$55 \pm 3^{a,b}$
End-tidal PCO_2 (mmHg)	$40 \pm 3^{b,c}$	$28 \pm 2^{a,d}$	$28 \pm 2^{a,d}$	$40 \pm 1^{b,c}$
O_2 saturation (%)	$97 \pm 1^{c,d}$	$98 \pm 0^{c,d}$	$85 \pm 5^{a,b}$	$85 \pm 2^{a,b}$
		End-exercise		
Frequency (breaths·min^{-1})	$18 \pm 5^{b,c,d}$	26 ± 2^{a}	25 ± 1^{a}	26 ± 2^{a}
Tidal Volume (L)	2.3 ± 0.6	2.5 ± 0.3	2.4 ± 0.3	2.6 ± 0.3
Ventilation (L·min^{-1})	$40 \pm 4^{b,c,d}$	66 ± 7^{a}	64 ± 9^{a}	69 ± 10^{a}
End-tidal PO_2 (mmHg)	$95 \pm 6^{b,c,d}$	$113 \pm 3^{a,c,d}$	$51 \pm 2^{a,b}$	$53 \pm 3^{a,b}$
End-tidal PCO_2 (mmHg)	$45 \pm 4^{b,c,d}$	$29 \pm 2^{a,d}$	$30 \pm 2^{a,d}$	$41 \pm 1^{a,b,c}$
O_2 saturation (%)	$97 \pm 1^{c,d}$	$99 \pm 1^{c,d}$	$79 \pm 5^{a,b}$	$80 \pm 4^{a,b}$

534

Table 2. Kinetics parameters for pulmonary O_2 uptake responses at rest and during moderate-intensity exercise in each condition: Control, Hyperventilation, Hypoxia, and Normocapnic hypoxia.

Condition	Control	Hyperventilation	Hypoxia	Normocapnic Hypoxia
$\dot{V}O_{2p\ acc}$ ($L \cdot min^{-1}$)	0.37 ± 0.04	0.39 ± 0.04	0.40 ± 0.03	0.38 ± 0.05
$\dot{V}O_{2p\ bsl}$ ($L \cdot min^{-1}$)	0.76 ± 0.07	0.77 ± 0.07	0.81 ± 0.05	0.77 ± 0.04
$\dot{V}O_{2pss}$ ($L \cdot min^{-1}$)	1.50 ± 0.21	1.52 ± 0.21	1.56 ± 0.19[a]	1.56 ± 0.23[a]
A_p ($L \cdot min^{-1}$)	0.73 ± 0.23[d]	0.75 ± 0.22	0.75 ± 0.22	0.79 ± 0.24[a]
TD (s)	9 ± 7	2 ± 9	-7 ± 10	-13 ± 10
$\tau\dot{V}O_{2p}$ (s)	26 ± 11[b,c,d]	36 ± 14[a,c,d]	46 ± 14[a,b,d]	52 ± 13[a,b,c]
CI_{95} (s)	3 ± 1	3 ± 1	3 ± 1	3 ± 1
$\Delta\dot{V}O_{2p}$ /ΔPO ($mL \cdot min^{-1} \cdot W^{-1}$)	8.9 ± 0.4	9.1 ± 0.4	9.4 ± 1.0	9.6 ± 0.6[a]

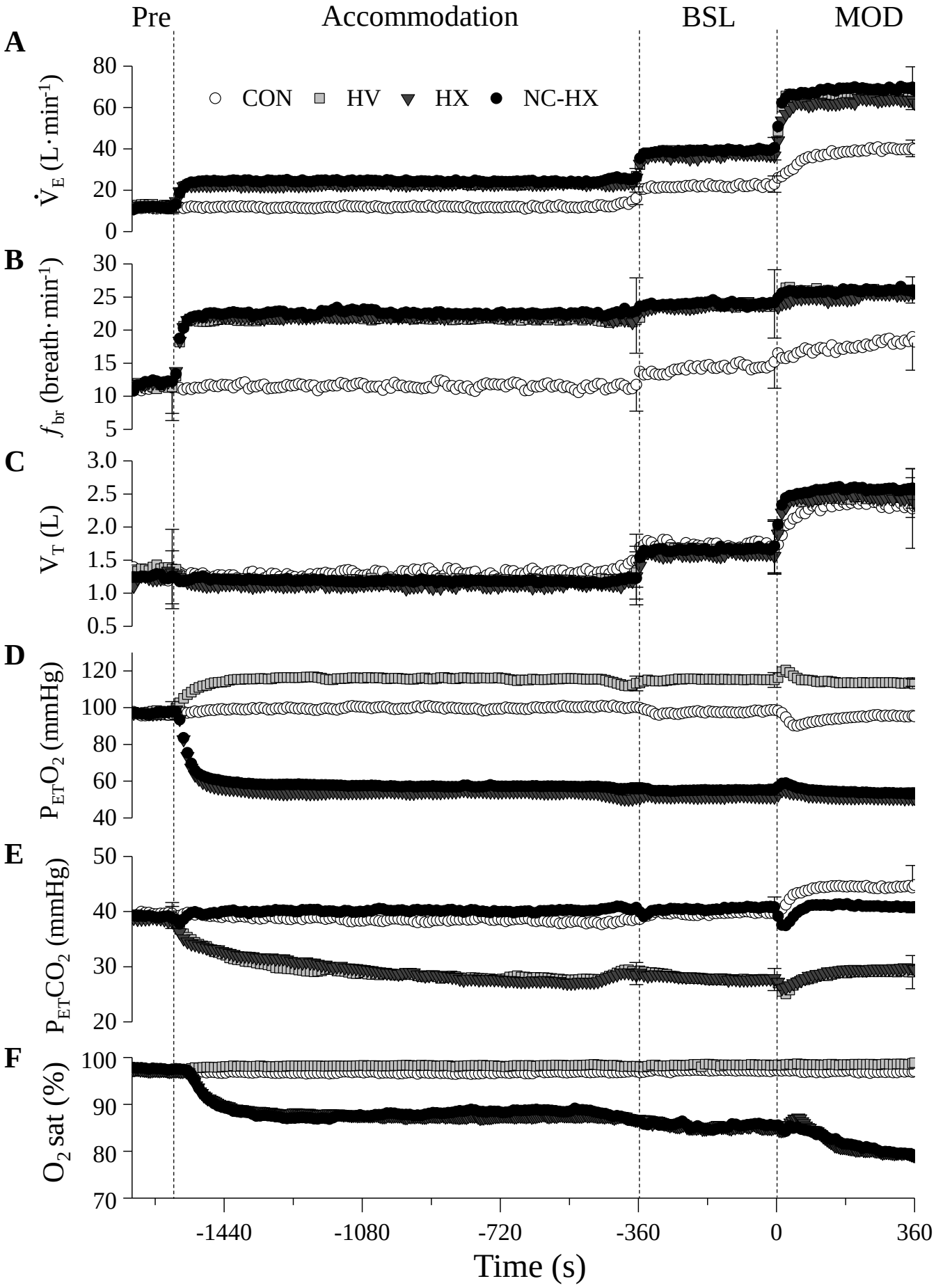
Values are means ± SD. $\dot{V}O_{2p}$, pulmonary O_2 uptake; $\dot{V}O_{2p\ acc}$, resting $\dot{V}O_{2p}$; $\dot{V}O_{2p\ bsl}$, baseline $\dot{V}O_{2p}$; $\dot{V}O_{2p\ ss}$, steady-state $\dot{V}O_{2p}$; A_p, amplitude of $\dot{V}O_{2p}$ response; TD, time delay; $\tau\ \dot{V}O_{2p}$, time constant for $\dot{V}O_{2p}$ response; CI_{95}, 95% confidence interval for $\tau\dot{V}O_{2p}$; $\Delta\dot{V}O_{2p}$ /ΔPO, functional gain. [a] significant ($p < 0.05$) difference from control. [b] significant ($p < 0.05$) difference from hyperventilation. [c] significant ($p < 0.05$) difference from hypoxia. [d] significant ($p < 0.05$) difference from normocapnic hypoxia.

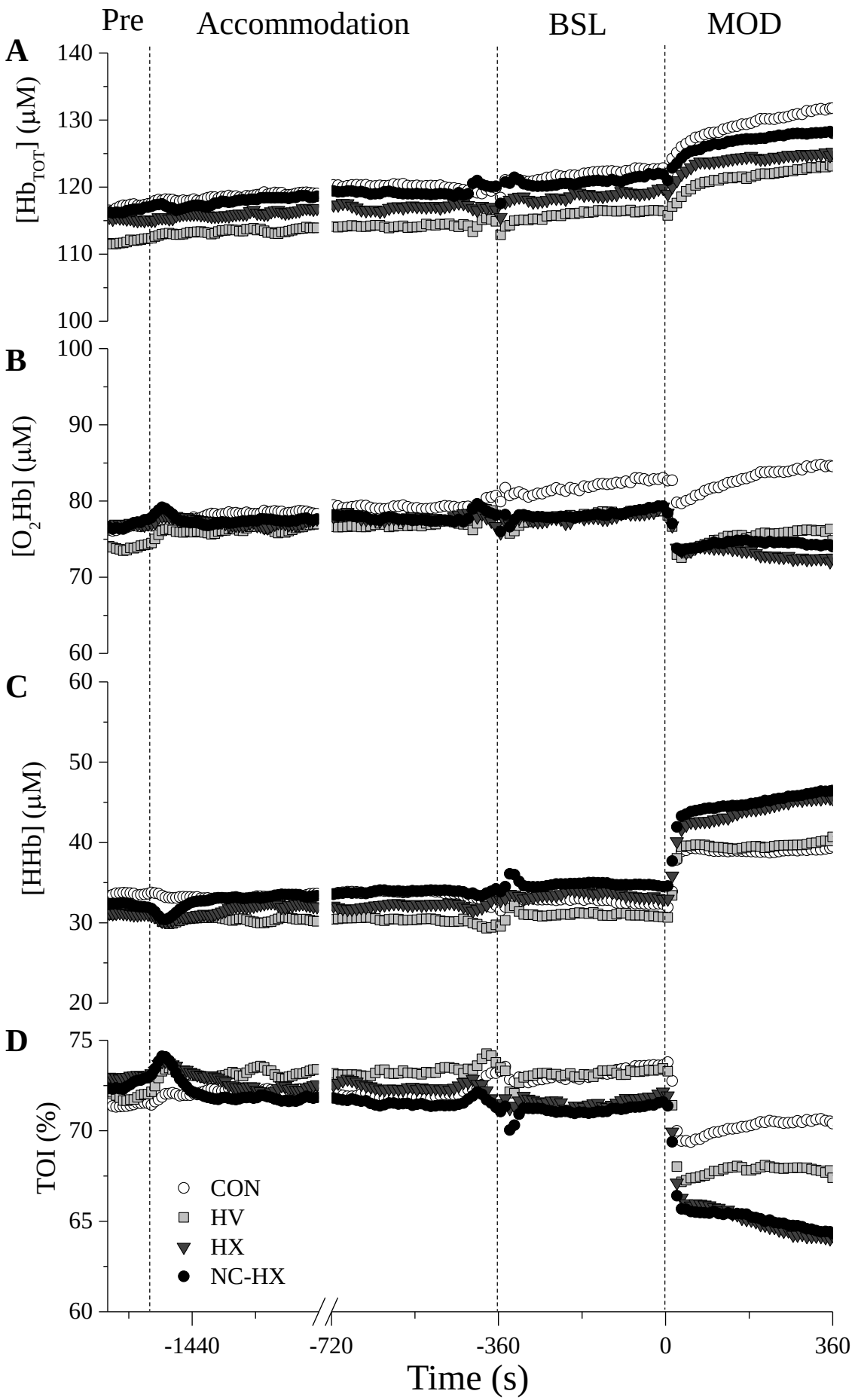
543 **Table 3**. Kinetic parameters for vastus lateralis muscle deoxygenation ([HHb]) from Control, Hyperventilation, Hypoxia, and
544 Normocapnic hypoxia.
545

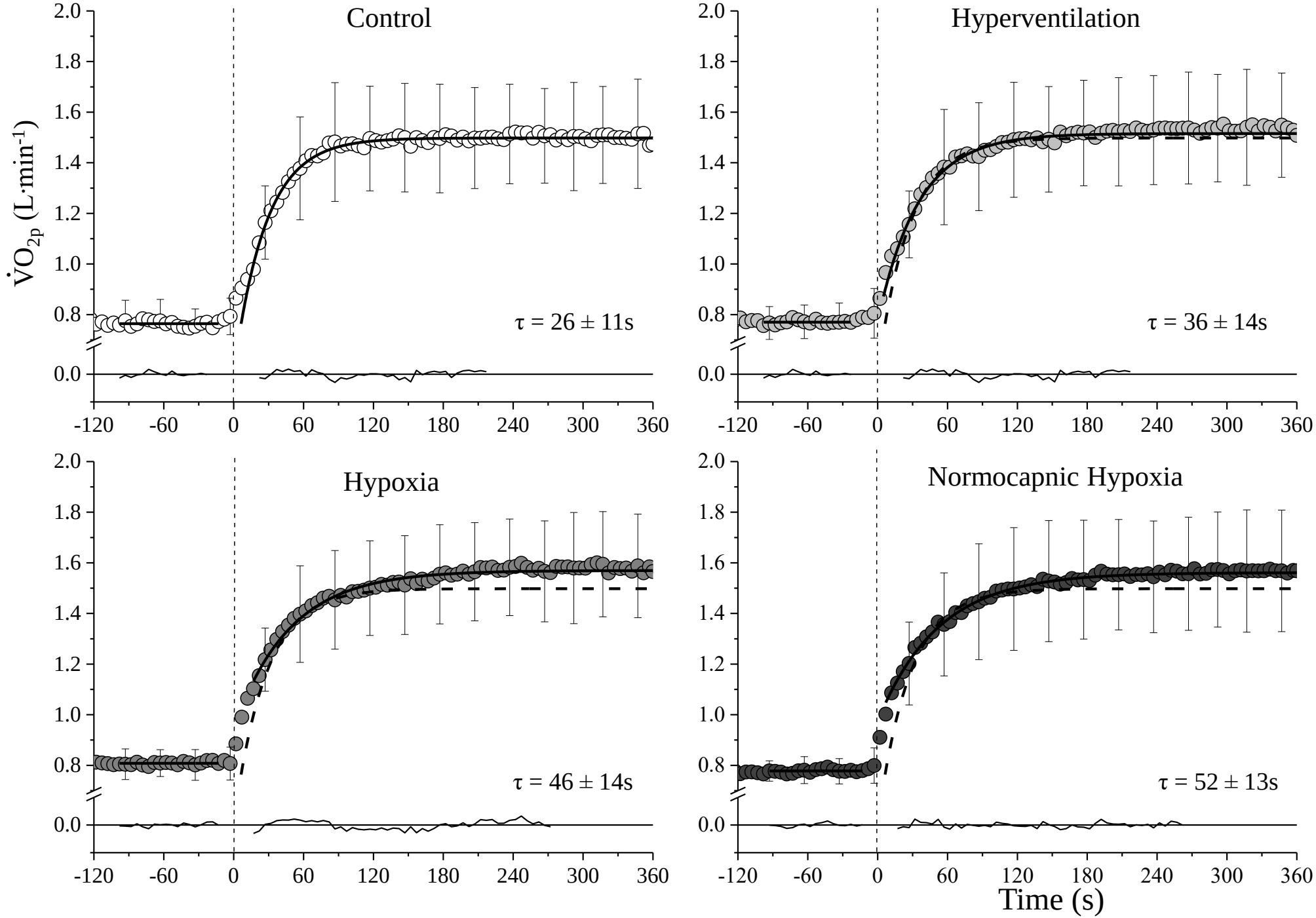
Condition	Control	Hyperventilation	Hypoxia	Normocapnic Hypoxia
$[HHb]_{bsl}$ (μM)	33 ± 9	31 ± 8[d]	33 ± 9	35 ± 9[b]
$[HHb]_{amp}$ (μM)	7 ± 5[c]	9 ± 6	10 ± 7[a]	9 ± 7
[HHb]-TD (s)	12 ± 3	12 ± 4	10 ± 2	11 ± 3
τ[HHb] (s)	8 ± 3[c]	9 ± 3	13 ± 4[a]	10 ± 3
MRT-[HHb] (s)	20 ± 3	21 ± 5	23 ± 5	21 ± 4
CI_{95} (s)	2 ± 1	1 ± 1	2 ± 0	2 ± 1

546

547 Values are means ± SD. [HHb], deoxyhemoglobin concentration; $[HHb]_{bsl}$, baseline [HHb]; $[HHb]_{amp}$, amplitude of [HHb], [HHb]-
548 TD, time delay; τ[HHb], time constant for [HHb] response; MRT-[HHb], mean response time (τ[HHb] + [HHb]-TD); C_{95}, 95%
549 confidence interval for τ[HHb]. [a] significant ($p < 0.05$) difference from control. [b] significant ($p < 0.05$) difference from
550 hyperventilation. [c] significant ($p < 0.05$) difference from hypoxia. [d] significant ($p < 0.05$) difference from normocapnic hypoxia.


Pre
Accommodation
BSL
MOD
A
B
C
D
E
F
CON
HV
HX
NC-HX
$\dot{V}_E$ (L·min^{-1})
f_{br} (breath·min^{-1})
V_T (L)
$P_{ET}O_2$ (mmHg)
$P_{ET}CO_2$ (mmHg)
O_2 sat (%)
Time (s)


Pre
Accommodation
BSL
MOD
A
[Hb$_{TOT}$] (μM)
140
130
120
110
100
B
[O$_2$Hb] (μM)
100
90
80
70
60
C
[HHb] (μM)
60
50
40
30
20
D
TOI (%)
75
70
65
60
CON
HV
HX
NC-HX
-1440
-720
-360
0
360
Time (s)


Control
Hyperventilation
Hypoxia
Normocapnic Hypoxia
$\dot{V}O_{2p}$ (L·min^{-1})
τ = 26 ± 11s
τ = 36 ± 14s
τ = 46 ± 14s
τ = 52 ± 13s
Time (s)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.0
-120
-60
0
60
120
180
240
300
360