EFFECT OF HYPEROXIA ON CRITICAL POWER AND $\dot{V}O_2$ KINETICS DURING UPRIGHT CYCLING

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Running head: Hyperoxia and the power-duration relationship

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ABSTRACT

Introduction/Purpose: Critical power (CP) is a fundamental parameter defining high-intensity exercise tolerance, however its physiological determinants are incompletely understood. The present study determined the impact of hyperoxia on CP, the time constant of phase II pulmonary oxygen uptake kinetics ($\tau_{\dot{V}O2}$), and muscle oxygenation (assessed by near-infrared spectroscopy) in 9 healthy men performing upright cycle ergometry. **Methods:** CP was determined in normoxia and hyperoxia (fraction of inspired O_2 = 0.5) via 4 severe-intensity constant load exercise tests to exhaustion on a cycle ergometer, repeated once in each condition. During each test, $\tau_{\dot{V}O2}$ and the time constant of muscle deoxyhaemoglobin kinetics ($\tau_{[HHb]}$), alongside absolute concentrations of muscle oxyhaemoglobin ([HbO_2]), were determined. **Results:** CP was greater (hyperoxia: 216 ± 30 vs. normoxia: 197 ± 29W; P < 0.001) whereas W' was reduced (hyperoxia: 15.4 ± 5.2 kJ, normoxia: 17.5 ± 4.3 W; P = 0.037) in hyperoxia compared to normoxia. $\tau_{\dot{V}O2}$ (hyperoxia: 35 ± 12 vs normoxia: 33 ± 10 s; P = 0.33) and $\tau_{[HHb]}$ (hyperoxia: 11 ± 5 vs. normoxia: 14 ± 5 s; P = 0.65) were unchanged between conditions, whereas [HbO_2] during exercise was greater in hyperoxia compared to normoxia (hyperoxia: 73 ± 20 vs. normoxia: 66 ± 15 μM; P = 0.001). **Conclusion:** This study provides novel insights into the physiological determinants of CP and by extension, exercise tolerance. Microvascular oxygenation and CP were improved during exercise in hyperoxia compared with normoxia. Importantly, the improved microvascular oxygenation afforded by hyperoxia did not alter $\tau_{\dot{V}O2}$, suggesting that microvascular O_2 availability is an independent determinant of the upper limit for steady-state exercise, i.e. CP.

Keywords: critical power, exercise tolerance, oxidative metabolism, oxygen uptake kinetics, power-duration relationship, hyperoxia.

INTRODUCTION

The relationship between power output and the tolerable duration of high-intensity exercise takes the form of a rectangular hyperbola and is defined by two parameters: critical power (CP), representing the asymptote of the curve, and *W'*, the rectangular constant of the hyperbola representing the finite work capacity available above CP (1). CP represents the boundary delineating the heavy and severe exercise intensity domains (1,2). During heavy-intensity exercise, a delayed steady-state can be attained for pulmonary oxygen uptake ($\dot{V}O_2$) and the intramuscular metabolic responses to exercise (1–3). In contrast, the challenge to system homeostasis during severe-intensity exercise is such that a steady-state cannot be attained for $\dot{V}O_2$, with the slow component driving $\dot{V}O_2$ towards its maximally attainable value ($\dot{V}O_2$max) with the limit of tolerance being reached shortly thereafter. The pulmonary $\dot{V}O_2$ response is reflective of the intramuscular metabolic responses during exercise above CP, with muscle lactate ($[L^-]$) and inorganic phosphate ($[P_i]$) reaching maximal values and intramuscular phosphocreatine ([PCr]) and pH reaching a nadir immediately prior to the limit of tolerance (1,2). CP and W' therefore conflate to determine the tolerable duration of severe intensity exercise, which is predictably limited as a function of the power output above CP and the size of the *W'*.

At the onset of exercise, pulmonary $\dot{V}O_2$ kinetics are well-characterised by an exponential function, following a brief delay termed phase I (4). This "fundamental" increase in $\dot{V}O_2$ can be characterised by a time constant ($\tau_{\dot{V}O2}$) that, in healthy humans, has previously been shown to reflect the kinetics of muscle $\dot{V}O_2$ ($\dot{V}O_{2m}$) (5). We have recently provided evidence to suggest that $\tau_{\dot{V}O2}$ is an independent determinant of CP (6–9). Specifically, when $\tau_{\dot{V}O2}$ was acutely reduced, CP increased (7,9), whereas when $\tau_{\dot{V}O2}$ was acutely increased, CP correspondingly decreased (6,8). A potential explanation for this seemingly causal

relationship between $\tau_{\dot{V}O2}$ and CP is that CP represents the highest work rate for which accumulation of the O_2 deficit can be stabilised (10). Hence, as $\tau_{\dot{V}O2}$ determines the magnitude of the O_2 deficit, a smaller $\tau_{\dot{V}O2}$ would enable the same O_2 deficit accumulation to be stabilised for a higher work rate, thus increasing CP.

The inspiration of a hyperoxic gas mixture increases the driving pressure for peripheral diffusion of O_2 from capillary to mitochondria (11). As such, improved high-intensity exercise performance (12,13) has been reported during hyperoxia. However, hyperoxia does not appear to speed the pulmonary $\dot{V}O_2$ kinetics during upright cycling (13–15), which is seemingly inconsistent with the putative linkage between $\tau_{\dot{V}O2}$ and CP previously described (6–9). Nevertheless, Vanhatalo et al. (16) previously demonstrated that CP was increased when determined in hyperoxia compared to normoxia, suggesting a central role for O_2 availability *per se* in determining CP. However, Vanhatalo et al. (16) did not determine $\dot{V}O_2$ kinetics, and the prone position employed in this study would have impaired perfusion pressure (17), raising the possibility that the increased CP these authors observed in hyperoxia may have been due to faster $\dot{V}O_2$ kinetics in this condition, rather than improved O_2 availability *per se*. Furthermore, in a recent study we demonstrated that hyperoxia speeded pulmonary $\dot{V}O_2$ kinetics and increased CP during supine exercise, but that the change in $\tau_{\dot{V}O2}$ did not correlate linearly with the change in CP (9). Taken together, the relative, independent contributions of $\tau_{\dot{V}O2}$ and O_2 availability in determining CP remain uncertain.

A convenient means by which to investigate the dependency of CP on O_2 availability, independent of the effects of $\tau_{\dot{V}O2,}$ is via the use of hyperoxia in young healthy individuals performing upright cycle exercise, where a speeding of $\dot{V}O_2$ kinetics would not be expected (13, 15). Hence, if O_2 availability is an independent determinant of CP, then hyperoxia would be expected to increase CP without a concomitant reduction in $\tau_{\dot{V}O2.}$ Conversely, if the role

of O_2 availability in determining CP is merely via its contribution to $\tau_{\dot{V}O2}$ then no change in CP between conditions of hyperoxia and normoxia would be expected.

The aim of this study was therefore to assess the effect of hyperoxia on pulmonary $\dot{V}O_2$ kinetics and CP during upright cycle exercise. Our hypotheses were threefold: 1) CP would be greater in hyperoxia compared to normoxia; 2) $\tau_{\dot{V}O2}$ would not differ between hyperoxia and normoxia; and 3) microvascular oxygenation (as assessed by near-infrared spectroscopy; NIRS) would be improved in hyperoxia compared to normoxia.

METHODS

Nine healthy male subjects (mean ± SD, age = 23 ± 3 years; height = 179 ± 8 cm; mass = 77 ± 8 kg) who were recreationally active provided written informed consent for participation. The experiment was approved by the Institutional Research Ethics Committee. Participants were asked to avoid alcohol and strenuous exercise 24 h prior to each visit, not to consume caffeine 3 h prior to each visit, and to arrive 3 h postprandial. Tests were separated by at least 24 h, with each test performed at the same time of day (± 2 h).

Procedures. All tests took place in a temperature-controlled laboratory that was maintained between 18-21 ^{o}C. The experiment involved nine visits over a 3-5 week period, including one preliminary trial and eight experimental trials. All tests were performed on the same electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). The ergometer seat and handlebar configuration were recorded at the first visit and replicated during each subsequent visit. Throughout all exercise tests, participants were instructed to cycle at a self-selected cadence between 70-90 rev/min (which was recorded and replicated in subsequent visits), with task failure being defined as the point at which the cadence dropped below 50 rev/min. Time to task failure was recorded to the nearest second in all tests.

120 *Preliminary trial.* Height and body mass were recorded, after which participants undertook an
121 incremental ramp test to task failure to determine $\dot{V}O_2$ max and the gas exchange threshold
122 (GET), such that the power outputs for subsequent visits could be calculated. The ramp test
123 consisted of 3 min baseline pedalling at 30 W, followed by a ramped increase in power of 30
124 $W.min^{-1}$ until task failure occurred. Ventilatory and gas exchange variables were measured
125 continuously breath-by-breath throughout each test. $\dot{V}O_2$ max was defined as the highest 30 s
126 value. The GET was estimated via visual determination of the time point at which the
127 following occurred: 1) excessive CO_2 output ($\dot{V}CO_2$) relative to $\dot{V}O_2$, 2) increased minute
128 ventilation ($\dot{V}E$) relative to $\dot{V}O_2$ ($\dot{V}E/\dot{V}O_2$) without an increase in $\dot{V}E/\dot{V}CO_2$, and 3) an
129 increase in end tidal O_2 tension without decreasing end tidal CO_2 tension. The mean response
130 time (MRT) was determined as the time between the beginning of the ramp test and
131 intersection between baseline $\dot{V}O_2$ (average $\dot{V}O_2$ measured during last 30 s of baseline; $\dot{V}O_{2b}$)
132 and backwards extrapolation of the $\dot{V}O_2$-time relationship (18). This technique was also used
133 to calculate power outputs for subsequent visits.

134 *Experimental trials.* The subsequent eight visits required exhaustive exercise at one of four
135 fixed severe-intensity power outputs, each repeated twice: once in normoxia (breathing
136 atmospheric air) and once in hyperoxia (FiO_2 0.5, in balance N_2, British Oxygen Company).
137 These power outputs were selected to span a range of 50%Δ (i.e. 50% of the difference
138 between the GET and $\dot{V}O_2$ max) – 110% $\dot{V}O_2$ max, such that the range of exercise tolerance
139 times was 2-15 minutes for each subject (19). When a particular test deviated from this range,
140 the power output was modified and the test was repeated on a separate day. These power
141 outputs are subsequently referred to as WR1, WR 2, WR 3, and WR 4, with WR 1 being the
142 lowest and WR 4 being the highest power outputs, respectively. The power outputs were
143 presented in random order, and participants alternated between hyperoxic and normoxic
144 conditions. In both conditions, tests began with 3 minutes of baseline pedalling at 20 W,

followed by a step increase in power output to 70% GET for 6 minutes for the characterisation of the $\dot{V}O_2$ kinetics during moderate exercise. Following these 6 minutes of moderate cycling, the power output was decreased to 20 W for 6 minutes, after which a step increase in power was applied to the desired severe-intensity power output, and participants exercised until task failure occurred.

Pulmonary gas exchange and ventilation were measured breath-by-breath throughout all tests using a metabolic cart (Blue Cherry, Geratherm Respiratory, GmbH, Germany), with participants wearing a silicone face mask (Hans Rudolph, Kansas, United States) attached to a differential pressure flow sensor (Geratherm Respiratory, GmbH, Germany, resistance <0.12 kPa, dead space < 32 mL) The metabolic cart was connected to the participant via a capillary line connected to the flow sensor. Expired gases were measured using an electrochemical cell O_2 analyser (rise time: t10-90 < 90 ms) and a principle infrared spectroscopy CO_2 analyser (rise time: t10-90 < 90 ms), which were calibrated before and after each test using gases of known concentration. The gas sampling rate was 125 MHz. Flow sensors were calibrated using a 3-liter syringe (Hans Rudolph, Kansas City, MO). The software of the metabolic unit was specifically adapted to measure FiO_2 during both inspiration and expiration (instead of assuming constant FiO_2), whereas the delay time between airflow and gas concentration signals was first determined during calibration and the synchronization between these signals was continuously optimized during every inspiration throughout each test. The flow sensor was attached to a two-way non-rebreathing valve (Hans Rudolph T-Shape Two-Way Non-Rebreathing Valve Series 2600; Hans Rudolph, Kansas, United States) via rubber tubing. The inlet port of this valve was connected to a 200 L Douglas bag. In the hyperoxic condition, the Douglas bag was continuously filled with the hyperoxic gas mixture, and in the normoxic condition the Douglas bag was bypassed so that participants breathed room air; participants were not informed of the condition they were

exercising in. In both conditions participants rested quietly on the ergometer for 10 minutes prior to the commencement of exercise, breathing either the hyperoxic inspirate or normoxic room air, to allow equilibration of body O_2. 20 μL of blood was drawn from the thumb of the right hand at rest, during the final minute of baseline pedalling before the onset of severe exercise, and immediately following task failure into sodium heparinized plastic capillary tubes (EKF Diagnostics, Cardiff, Wales, UK) before being placed into an Eppendorf containing a glucose/ L^- haemolysing solution and being vigorously shaken until the sample had mixed adequately.. Whole blood L^- was determined using a Biosen lactate analyser (Biosen C-Line, EKF, Germany).

Absolute concentrations of muscle and microvascular deoxyhaemoglobin + deoxymyoglobin ([HHb + Mb]), oxyhaemoglobin +oxymyoglobin ([HbO_2 + MbO_2]), and total haemoglobin + total myoglobin ([THb + Mb]) were determined using a frequency-domain multidistance NIRS system (OxiplexTS, ISS, Chapaign, IL, USA). This technique has been described in detail previously (7,20). The device measures and incorporates dynamic reduced scattering coefficients to provide absolute concentrations of [HHb + Mb] and [HbO_2 + MbO_2]. The NIRS probe was calibrated prior to each test according to the manufacturer's instructions. Two flexible NIRS probes were placed on the participant; one longitudinally along the belly of the right vastus lateralis (VL), the other longitudinally along the belly of the rectus femoris (RF) muscle. The probes were held firmly in place via Velcro strapping, and the area underneath the probe was cleaned, shaved and marked with pen such that probe position could be accurately replicated for each trial. To account for the influence of adipose tissue thickness (ATT) on the NIRS signal, we utilised the correction factor of Bowen et al. (21), albeit with separate correction factors for the RF and VL.

Data analysis. Raw breath-by-breath $\dot{V}O_2$ were edited to remove data points lying more than 4 standard deviations (SD) outside the local 5-breath mean (22). Edited $\dot{V}O_2$ were then

subsequently linearly interpolated to provide second-by-second values. During moderate intensity exercise, second-by-second $\dot{V}O_2$ and [HHb + Mb] data for the four identical transitions were averaged together to produce a single dataset for each condition. The severe-intensity exercise bouts were not repeated and therefore were modelled separately. The $\dot{V}O_2$ and [HHb + Mb] responses to transitions were modelled utilising the following mono-exponential function:

$$Y_{(t)} = Y_{(b)} + A_Y * (1 - e^{-(t - TD/\tau)}) \quad (1)$$

Where $Y_{(t)}$ is the value of the independent variable at time t, $Y_{(b)}$ is the baseline value measured over the final 30 seconds of baseline, A_Y is the amplitude of increase in Y above baseline, TD is the time delay relative to the onset of exercise and τ is the time constant of the response.

$\dot{V}O_2$ data preceding the time point at which a drop in respiratory exchange ratio and end-tidal O_2 pressure was observed were excluded from the modelling process. $\dot{V}O_2$ responses to moderate exercise were fit to the end of exercise whereas for severe-intensity exercise, the onset of the slow component was determined by iteratively lengthening the fitting window in 1 second intervals from 60 seconds to end-exercise. The onset of the slow component was taken as the point at which there was a departure from a plateau in the plot of $\tau_{\dot{V}O2}$ and χ^2 versus time, as described previously (7,17) with TD, τ and A_y determined from this fitting window. The magnitude of the $\dot{V}O_2$ slow component was calculated as the difference between end exercise $\dot{V}O_2$ (i.e. mean $\dot{V}O_2$ over final 30 s of exercise) and $A_y + Y_{(b)}$.

The onset of the fundamental rise in [HHb + Mb] was taken as the time point at which the [HHb + Mb] signal increased above 1 SD of the pretransition baseline value. On occasions where [HHb + Mb] decreased after the exercise onset, the onset of the fundamental increase

in [HHb + Mb] was taken as the first point following the nadir showing a sustained increase in [HHb + Mb]. This time-point defined TD for [HHb + Mb] kinetics, with data preceding this being excluded from the modelling process, during which TD was allowed to vary. For moderate exercise, [HHb + Mb] responses were fit with equation 1 using the iterative procedures described for the determination of the $\dot{V}O_2$ kinetics but with the fitting window commencing at 20 s. This modelling strategy thus allows for the determination of the optimum "phase II" fitting window even in the presence of a [HHb + Mb] overshoot. By plotting the resultant $\tau_{[HHb+Mb]}$ values against time and identifying the point at which a sustained decrease (overshoot) or increase in $\tau_{[HHb+Mb]}$ was observed alongside a sharp increase in the χ^2 value. For severe-intensity exercise, the model window was constrained to the TD before the onset of the $\dot{V}O_2$ slow component. The amplitude of the [HHb + Mb] during severe exercise was calculated by subtracting $Y_{(b)} + A_Y$ from the mean value of Y during the final 30 s of exercise. The spatial heterogeneity of $TD_{[HHb+Mb]}$ and $\tau_{[HHb+Mb]}$ was calculated for each participant using the intersite coefficient of variation (CV% = 100 * SD/ mean of the two sites). Confidence intervals for all τ parameters were obtained in Origin 6.0 (OriginLab Corporation, MA, USA). For [HbO_2 + MbO_2] and [THb + Mb] during moderate exercise, 30 second averages were determined at baseline, and every 30 seconds thereafter until the end of the transition. For severe exercise, mean [HbO_2 + MbO_2] and [THb + Mb] was determined at baseline, at 30 and 120 seconds into the transition (15 second bins centred on each time point), and at end-exercise (final 30 seconds) to allow comparisons between conditions.

CP and *W'* were determined by inputting power output, time to task failure and work done into three models: the hyperbolic power-time (P-T) model (Eq. 2), the linear work-time (W-T) model (Eq. 3), and the linear power versus the inverse-of-time (1/T) models:

(2) $$P = W' / T + CP$$

244 (3) $W = CP * T + W'$

245 (4) $P = W' * (1/T) + CP$

246 The standard errors of the estimates (SEE) associated with CP and W' were expressed as a
247 coefficient of variation (CV) relative to the parameter estimate. Best individual fit parameter
248 estimates were obtained for each participant by selecting the model that produced the lowest
249 summed CV for both parameters across conditions.

250 *Statistical analyses.* All kinetic parameters (i.e. $\dot{V}O_2$, [HHb + Mb]) and spatial heterogeneity
251 of [HHb + Mb] during severe exercise, blood [L^-], [HbO_2 + MbO_2] and [THb + Mb] were
252 analysed using two-way -way repeated measures ANOVAs (condition * work rate, condition
253 * muscle, condition * time, work rate * time, work rate * muscle, muscle * time), as
254 appropriate. Where significant differences were found, planned repeated and simple contrasts
255 were used to determine where the differences were located. $\dot{V}O_2$ and spatial heterogeneity of
256 [HHb + Mb] for moderate exercise as well as differences in CP and *W'* between conditions
257 were compared using student's paired t-tests. Pearson's correlation coefficient was used to
258 determine relationships between variables of interest. All data are presented as mean ± SD
259 unless otherwise stated. For clarity, and to highlight values for parameters measured across
260 all four severe-intensity work rates, the overall mean across work rates ± SD is presented in
261 text, with work rate-specific mean ± SD presented in tables. Statistical significance was
262 accepted at $P < 0.05$.

263 **RESULTS**

264 $\dot{V}O_2$ max determined from the ramp test was 3.99 ± 0.70 $L.min^{-1}$ (51 ± 5 $mL.kg^{-1}.min^{-1}$), and
265 this was achieved at a peak work-rate of 322 ± 36 W. The GET was 1.93 ± 0.14 $L.min^{-1}$ (108
266 ± 15 W), and thus the moderate exercise bouts at 70% GET were conducted at 76 ± 11 W.
267 Blood [L^-] did not differ between rest and baseline (normoxia rest: 1.40 ± 0.20, hyperoxia

rest: 1.14 ± 0.21, normoxia baseline: 1.44 ± 0.18, hyperoxia baseline: 1.53 ± 0.50 mmol.L^{-1}), however blood [L^-] was increased at end-exercise (normoxia: 10.81 ± 1.85, hyperoxia: 11.07 ± 2.26 mmol.L^{-1}; main effect of time, $P < 0.001$). There was no main effect of condition on blood L^- ($P = 0.91$).

Individual fit optimisation resulted in the hyperbolic P-T model being used for 7 participants, the W-T model for 1 participant, and the 1/T model for 1 participant. CP was greater in hyperoxia than in normoxia (hyperoxia: 216 ± 30, normoxia: 197 ± 29 W; $P < 0.001$; Figure 1*A*), whereas *W'* was reduced in hyperoxia compared to normoxia (hyperoxia: 15.4 ± 5.2 kJ, normoxia: 17.5 ± 4.3 W; $P = 0.037$; Figure 1*B*).

The group mean $\dot{V}O_2$ responses to moderate exercise in each condition are displayed in Figure 2*A*, whereas $\dot{V}O_2$ responses to severe exercise at a representative work rate from a representative participant in each condition are displayed in Figure 2*B*. $\tau_{\dot{V}O2}$ did not differ between conditions during moderate (hyperoxia: 25 ± 6, normoxia: 24 ± 9 s; $P = 0.49$) or severe exercise (hyperoxia: 35 ± 12, normoxia: 33 ± 10 s; $P = 0.33$). There were also no differences between conditions for any of the other parameters of $\dot{V}O_2$ kinetics during moderate exercise (Figure 2*A*). For severe exercise, there was no difference in $\dot{V}O_2$ peak between constant work rate trials within each condition or between any of the constant work rate trials in normoxia and the $\dot{V}O_2$ peak obtained in the ramp incremental test (Table 1, both $P > 0.05$). $A_{\dot{V}_{O2}}$ and $\dot{V}O_2$ peak were greater in hyperoxia compared to normoxia (Table 1, Figure 2*B*; both $P < 0.001$), however there were no other differences in the parameters of the $\dot{V}O_2$ kinetics during severe exercise (Table 1). $\tau_{\dot{V}O2}$ during moderate exercise was inversely correlated with CP in normoxia ($R^2 = 0.85$; $P < 0.001$), and in hyperoxia ($R^2 = 0.56$; $P = 0.021$). End-tidal PO_2 was increased in hyperoxia compared to normoxia at baseline

(hyperoxia: 308 ± 7, normoxia: 106 ± 4 mmHg; $P < 0.001$) and end-exercise (hyperoxia: 315 ± 9, normoxia: 121 ± 4 mmHg; $P < 0.001$).

[HbO_2 + MbO_2] was increased during both moderate (hyperoxia: 74 ± 21 μM, normoxia: 68 ± 18; main effect of condition, $P < 0.001$, Figure 4*A*) and severe (hyperoxia: 73 ± 20 μM, normoxia: 66 ± 15; main effect of condition, $P = 0.039$, Figure 4*B*) exercise. [THb + Mb] was unchanged during both moderate (hyperoxia: 99 ± 18 μM, normoxia: 99 ± 21; no main effect of condition, $P = 0.97$) and severe (hyperoxia: 106 ± 13 μM, normoxia: 104 ± 17; no main effect of condition, $P = 0.88$) exercise. Baseline and end-exercise [HHb + Mb] were reduced during moderate exercise (Figure 3*A*, both $P < 0.001$), however there were no differences between conditions with regard to any of the other [HHb + Mb] kinetic parameters during either moderate (Figure 3*A*) or severe exercise (Figure 3*B*, Table 2). Furthermore, the spatial heterogeneity of $\tau_{[HHb + Mb]}$ and $TD_{[HHb + Mb]}$ did not differ between conditions during moderate nor severe exercise (all $P > 0.05$). CP did not correlate with [HbO_2 + MbO_2] in either the RF or VL in either condition or at any time point (R^2 range, RF: 0.09 – 0.43, VL: 0.10 – 0.31, $P > 0.05$ for all comparisons), whereas the change in CP between conditions did not correlate with changes in [HbO_2 + MbO_2] between conditions in either the RF or VL at any time point (R^2 range, RF: 0.01 – 0.15, VL: 0.06 – 0.37), $P > 0.05$ for all comparisons).

DISCUSSION

Recent work from our laboratory has provided strong evidence that pulmonary $\dot{V}O_2$ kinetics are an independent determinant of CP: we have demonstrated that a speeding of the $\dot{V}O_2$ kinetics increases CP (7,9), and also that a slowing of the $\dot{V}O_2$ kinetics decreases CP (6,8). However, since two of these previous studies involved interventions that also enhance O_2 availability (i.e. priming exercise and hyperoxia), whether the speeding of $\dot{V}O_2$ kinetics we

noted previously (7,9) was the sole cause of the increases in CP observed in these studies, or whether muscle O_2 availability also independently determines CP, was unclear. The present study therefore sought to determine whether CP is primarily determined by $\tau_{\dot{V}O2}$, or whether microvascular O_2 availability also independently determined CP. Given that in healthy, young individuals performing upright cycle exercise, muscle O_2 availability does not appear to be the rate-limiting factor for the speed of the $\dot{V}O_2$ kinetics (15, 26), we employed hyperoxia as a means to test the independent effect of increased microvascular O_2 availability on CP during upright cycling. Hyperoxia improved microvascular oxygenation during exercise (assessed via NIRS), increased the fundamental phase $\dot{V}O_2$ amplitude, and increased CP when compared to normoxia. $\tau_{\dot{V}O2}$ was significantly related to CP in both normoxia and hyperoxia, consistent with the notion that $\tau_{\dot{V}O2}$ is an independent determinant of CP. However, $\tau_{\dot{V}O2}$ was unchanged between conditions. These findings therefore suggest that in addition to $\tau_{\dot{V}O2}$, microvascular oxygenation is also an independent determinant of CP.

In this present study we found that CP was ~19 W greater in hyperoxia when compared to normoxia. This finding is consistent with previous reports of improved aerobic exercise performance in hyperoxia (12,13). Furthermore, Vanhatalo et al. (16) previously demonstrated an increase in CP of ~10% in hyperoxia during small muscle mass exercise in the prone position, similar to the magnitude reported herein. CP has also been shown to be reduced in hypoxia (24), and thus it appears CP is highly dependent on FiO_2 and thus the state of O_2 availability. However, prior to the present study it was unknown whether the influence of FiO_2 on CP was due to enhanced O_2 availability *per se*, or mediated by the potential effects of FiO_2 on pulmonary $\dot{V}O_2$ kinetics.

The present findings therefore extend those of Vanhatalo et al. (16) by demonstrating that CP is improved by hyperoxia during large muscle mass, upright cycle exercise and provides

339 further evidence to the growing body of literature demonstrating that CP is an important
340 parameter of aerobic function (6,10,16,20,25). In the present study, end-tidal PO_2 (and,
341 therefore, alveolar PO_2) was enhanced in hyperoxia at baseline and end-exercise when
342 compared to normoxia. Resultant increases in microvascular oxygenation are demonstrated
343 by a reduced baseline and steady-state [HHb + Mb] during moderate exercise in hyperoxia,
344 and an increased [HbO_2 + MbO_2] in hyperoxia during exercise at all intensities, when
345 compared to normoxia. These observations are consistent with studies demonstrating that
346 hyperoxia increases arterial O_2 concentration, capillary O_2 pressure (PO_2) (26,27), and
347 intracellular PO_2 (11), suggesting that the capillary driving pressure for O_2 diffusion was
348 increased in this condition. Additionally, we saw no between-condition differences in the CV
349 for $\tau_{[HHb + Mb]}$ and $TD_{[HHb + Mb]}$, suggesting that the spatial distribution of O_2 delivery was
350 unaffected by hyperoxia. This combination of enhanced microvascular oxygenation (as
351 inferred from the reduced [HHb + Mb] and increased [HbO_2 + MbO_2]) with an unchanged
352 spatial distribution of O_2 would thus likely have improved the overall potential for peripheral
353 O_2 diffusion in hyperoxia. The finding of no between-condition differences with respect to
354 $\tau_{\dot{V}O2}$ during either moderate or severe upright cycle exercise is consonant with previous
355 reports (13,14,28), and bolsters the notion that O_2 availability is generally not the crucial rate-
356 limiting step for oxidative metabolism in physically active, young individuals undertaking
357 upright exercise (29). Hence the present data suggests that the differences in CP observed
358 between conditions are instead likely attributable to the increased microvascular oxygenation
359 observed in the hyperoxic condition. Taken together, the results of the present experiment
360 therefore suggest that O_2 availability within the exercising musculature is, in addition to $\tau_{\dot{V}O2}$
361 (6–9), an independent determinant of CP. Alternatively, the increased CP observed in the
362 hyperoxic condition in the present study may instead have been attributable to the subsequent
363 effects of increased microvascular oxygenation on the fundamental phase $\dot{V}O_2$ amplitude

observed in this condition. The fundamental phase $\dot{V}O_2$ amplitude was enhanced in hyperoxia relative to normoxia, consistent with previous observations that the fundamental $\dot{V}O_2$ amplitude is sensitive to manipulations in O_2 delivery (13). However, interventions which alter the fundamental phase $\dot{V}O_2$ amplitude, do not consistently affect CP (7). Therefore it appears problematic to ascribe the presently observed effects of hyperoxia on CP to operate via their impact on the fundamental $\dot{V}O_2$ amplitude. Hence, we suggest that the improvement in CP in hyperoxia noted herein was primarily due to the increased microvascular oxygenation observed in this condition, rather than the secondary effects of improved microvascular oxygenation on $\dot{V}O_2$ kinetics.

Our previously demonstrated dependence of CP on the speed of the $\dot{V}O_2$ kinetics (6–8) may be, at least in part, explained by the [ADP] - $\dot{V}O_2$ relationship. During exercise, increases in [ADP] stimulate $\dot{V}O_2$ via a relationship that has been shown to be sigmoidal *in vivo* (30). At high metabolic rates therefore the "plateau" region of the curve is approached, and thus the $\dot{V}O_2$ response to a given increment in [ADP] becomes progressively smaller with increasing metabolic rate. Thus, a potential explanation for our previously noted dependence of CP on $\tau_{\dot{V}O2}$ may be that CP represents the work-rate at which a critical [ADP] is attained beyond which the $\dot{V}O_2$ response to further elevations in [ADP] is ultimately insufficient to meet the demands for ATP turnover, causing a cascade of metabolic events that prohibit the attainment of steady state (10). $\tau_{\dot{V}O2}$ therefore determines CP since a smaller $\tau_{\dot{V}O2}$ (i.e. faster $\dot{V}O_2$ kinetics) will lessen the rise in intracellular [ADP] during a given exercise transition (31), thus increasing the work-rate at which a "critical [ADP]" is attained. Despite this hypothesis, the precise mechanisms underpinning the determining effect of $\tau_{\dot{V}O2}$ on CP remains to be elucidated. Indeed in the present study, $\tau_{\dot{V}O2}$ was unchanged between conditions despite an increase in CP with hyperoxia. Nevertheless, this remains consistent with the suggestion of

388 an underlying critical [ADP] because [ADP] is highly dependent upon intracellular PO_2 (32–
389 35), such that a greater intracellular PO_2 increases the $\dot{V}O_2$ achieved for a given change in
390 intracellular [ADP] (12). In the present study we therefore suggest that the increased CP in
391 hyperoxia was due to a reduced perturbation to [ADP] during the rest-to-exercise transition of
392 the criterion bouts, consequent to an elevated microvascular and intracellular PO_2 (indirectly
393 inferred from the elevated [HbO_2 + MbO_2]). This would be predicted to increase CP by virtue
394 of an increase in the work-rate at which either a critical [ADP] is attained during the rest-to-
395 exercise transition. Hence the present data suggest that O_2 availability, in addition to $\tau_{\dot{V}O2}$ (6–
396 9), is an independent determinant of CP. Furthermore, the role of O_2 availability *per se* in
397 determining CP is also implied by the data of Mitchell et al. (25), which demonstrated that
398 CP was strongly positively correlated with the number of capillary contacts per type I muscle
399 fibre. A high number of capillary contacts per type I muscle fibre would enhance the potential
400 for peripheral O_2 diffusion, and thus also raise the intracellular PO_2, in muscle fibres
401 recruited at the onset of exercise.

402 Despite the finding that hyperoxia increased microvascular oxygenation (inferred via NIRS)
403 and enhanced critical power, we found no relationship between the changes in CP and [HbO_2
404 + MbO_2] (i.e. ΔCP vs. Δ[HbO_2 + MbO_2]) between conditions. This finding may suggest that
405 other factor(s) may have been responsible for the increased CP in hyperoxia, rather than an
406 increase in microvascular, and thus intracellular, PO_2. An alternative explanation is that our
407 NIRS measurements did not have sufficient spatial resolution to quantitatively capture the
408 increase in microvascular O_2 availability across the entire exercising muscle mass. Skeletal
409 muscle is a structurally and functionally heterogeneous tissue, and muscle deoxygenation and
410 activation have been shown to be regionally heterogeneous during exercise (29). Thus, whilst
411 we have improved our spatial resolution by measuring two muscle sites, NIRS interrogates a
412 relatively superficial portion of muscle and a small fraction of the exercising muscle mass.

Consequently, ΔCP and $\Delta[HbO_2 + MbO_2]$ do not scale with each other because the NIRS parameters are a poor reflection of the precise value of oxygen availability to the recruited muscle cells. Notwithstanding this, the notion that whole-body and microvascular oxygenation was enhanced in hyperoxia in the present study is supported by the increased end-tidal PO_2, increased fundamental phase $\dot{V}O_2$ amplitude, $[HbO_2 + MbO_2]$ and reduced [HHb + Mb] in hyperoxia compared to normoxia.

We observed a ~2 kJ reduction in *W'* in hyperoxia, consistent with previous reports (16). A possible explanation for this finding is that hyperoxia increased CP to a greater extent than the $\dot{V}O_2$ max. This would necessitate a decrease in *W'*, because the applicable range of work-rates in the severe domain would be reduced (16). However, the increase in $\dot{V}O_2$ peak in hyperoxia in the present study (~ 0.25 $L.min^{-1}$) was similar to the increase in $\dot{V}O_2$ that would correspond with the average increase in CP of 19 W (assuming a gain of 10 – 13 $ml.min^{-1}.W^{-1}$, 38) also observed in this condition. An alternative explanation, therefore, is that the amount of metabolic energy available from anaerobic metabolism is dependent upon the degree of O_2 availability (37). For example, anaerobic energy release was greater during all-out sprint exercise for durations of < 120 seconds in hypoxia, such that performance was maintained relative to normoxia (37). A greater O_2 availability in hyperoxia in the present study may therefore have reduced the potential for anaerobic energy release. This would have impaired performance at the higher work-rates where the tolerable duration was short, thus accounting for the decrease in *W'*.

In conclusion, the present study provides unique insight into the physiological determinants of the upper limit for steady-state exercise, i.e. CP. The inspiration of hyperoxic gas resulted in improved microvascular oxygenation (determined by NIRS) when compared to normoxia, and as a result, CP was increased. Crucially, $\tau_{\dot{V}O2}$ was unchanged between conditions. These

results underscore the importance of CP as a parameter capable of reflecting aerobic function, and suggest that, in addition to $\tau_{\dot{V}O2}$, microvascular O_2 availability is an independent determinant of CP.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest. The results of the present study do not constitute endorsement by ACSM. The results of the present study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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FIGURE LEGENDS

Figure 1. Group mean ± SD critical power (*A*) and *W'* (*B*) in normoxia and hyperoxia. Open bars represent group means, whereas dashed black lines represent individual changes in critical power and *W'* between conditions. * indicates significant difference between conditions. The power-duration relationship of a representative participant in both conditions is also displayed (*C;* clear circles: hyperoxia, black circles: normoxia).

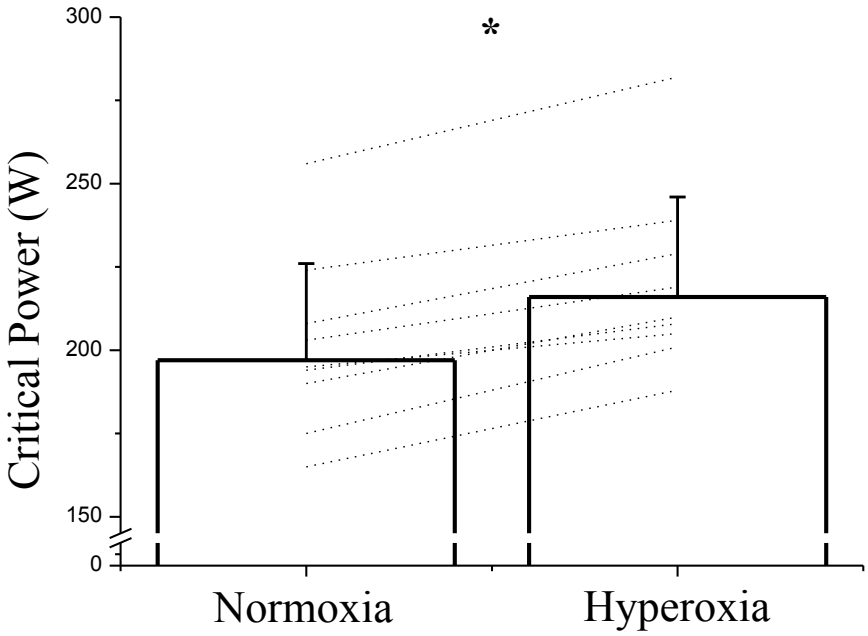
Figure 2. *A*: Group mean pulmonary oxygen uptake ($\dot{V}O_2$) responses to moderate exercise in normoxia (black circles) and hyperoxia (clear circles). Group mean exponential fits are overlaid onto the $\dot{V}O_2$ responses as solid curved lines. Error bars represent SD. *B*: Pulmonary oxygen uptake ($\dot{V}O_2$) responses and best-fit modelled responses of a representative participant at a single work rate in the normoxic (black circles) and hyperoxic (clear circles) conditions. Solid curved lines represent modelled fits, horizontal dashed lines represent the

574 condition-specific $\dot{V}O_2$ peak, and vertical dashed lines represent the limit of tolerance. Lines
575 of residuals are displayed at the bottom for normoxia (black) and hyperoxia (grey).

576 Figure 3. *A*: Group mean ± SD [deoxyhaemoglobin + myoglobin] ([HHb + Mb]) responses to
577 moderate exercise for the rectus femoris (black triangles: normoxia; clear triangles:
578 hyperoxia) and vastus lateralis (black circles: normoxia; clear circles: hyperoxia) in both
579 conditions. Group mean exponential fits are overlaid onto the $\dot{V}O_2$ responses as solid curved
580 lines. Error bars represent SD. Vertical dashed black line represents exercise onset. *B*: Muscle
581 [deoxyhaemoglobin + myoglobin] ([HHb + Mb]) responses to severe exercise in a
582 representative participant at a representative work rate for the rectus femoris and vastus
583 lateralis in both conditions. Residual lines are displayed at the bottom for normoxia (RF:
584 black dashed line; VL: solid black line) and hyperoxia (RF: grey dashed line; VL: solid grey
585 line). * indicates significant main effect of condition and # indicates significant main effect of
586 muscle on both baseline and end-exercise [HHb + Mb] ($P < 0.05$).

587 Figure 4. *A*: Group mean ± SD [oxyhaemoglobin +myoglobin] ([HbO_2 + MbO_2]) responses
588 to moderate exercise (black triangles: normoxia; clear triangles: hyperoxia) and vastus
589 lateralis (black circles: normoxia; clear circles: hyperoxia) in both conditions. Vertical dashed
590 line represents exercise onset. Error bars respresent SD. *B*: Muscle [oxyhaemoglobin +
591 oxymyoglobin] ([HbO_2 + MbO_2]) responses to severe exercise in a representative participant
592 at a representative work rate for the rectus femoris and vastus lateralis in both conditions. *
593 indicates significant main effect of condition, # indicates significant main effect of muscle (P
594 < 0.05).

595



596

597 **Figure 1**

598

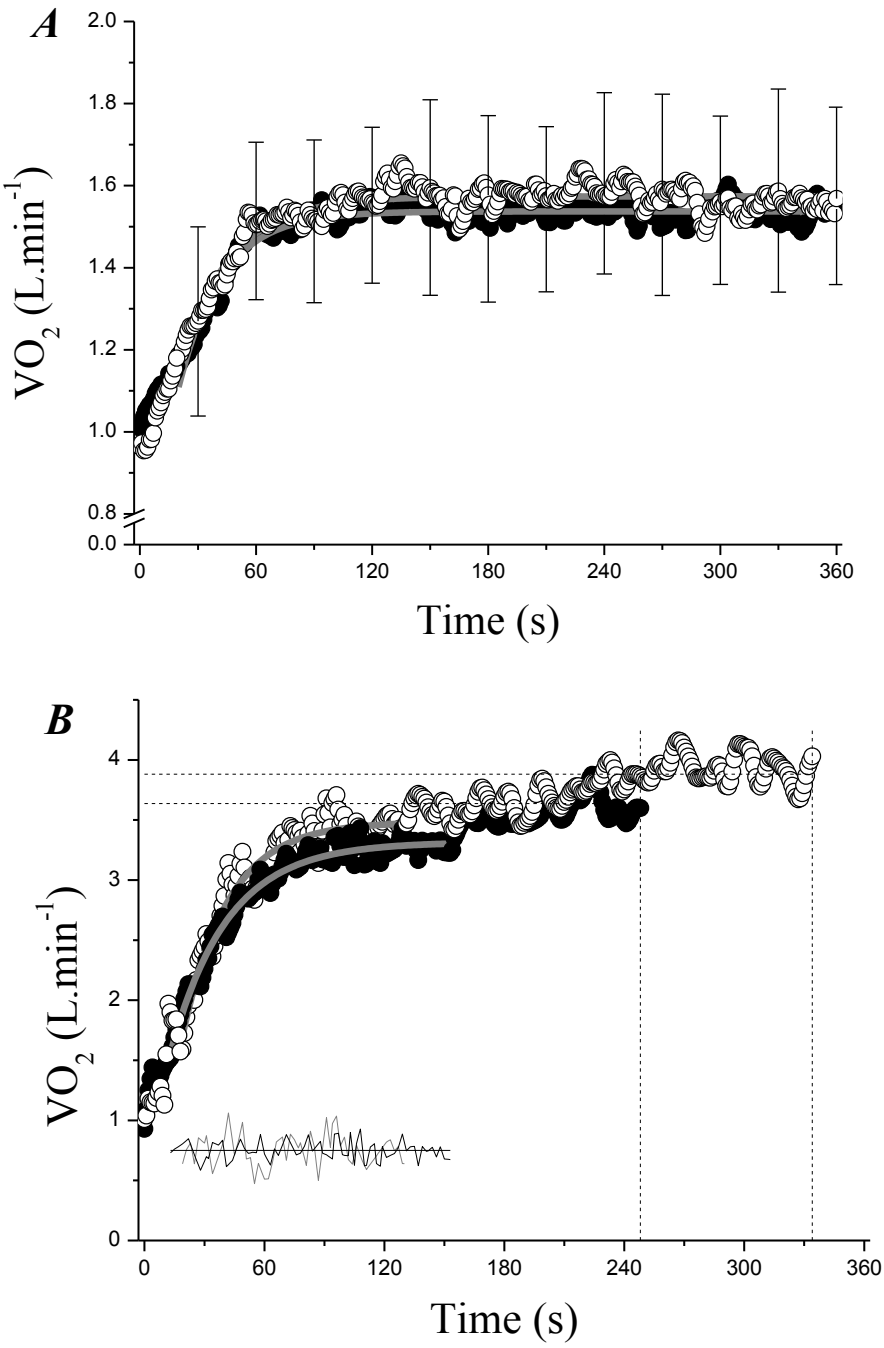

A
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.0
VO2 (L.min-1)
0
60
120
180
240
300
360
Time (s)
B
4
3
2
1
0
VO2 (L.min-1)
0
60
120
180
240
300
360
Time (s)

Figure 2

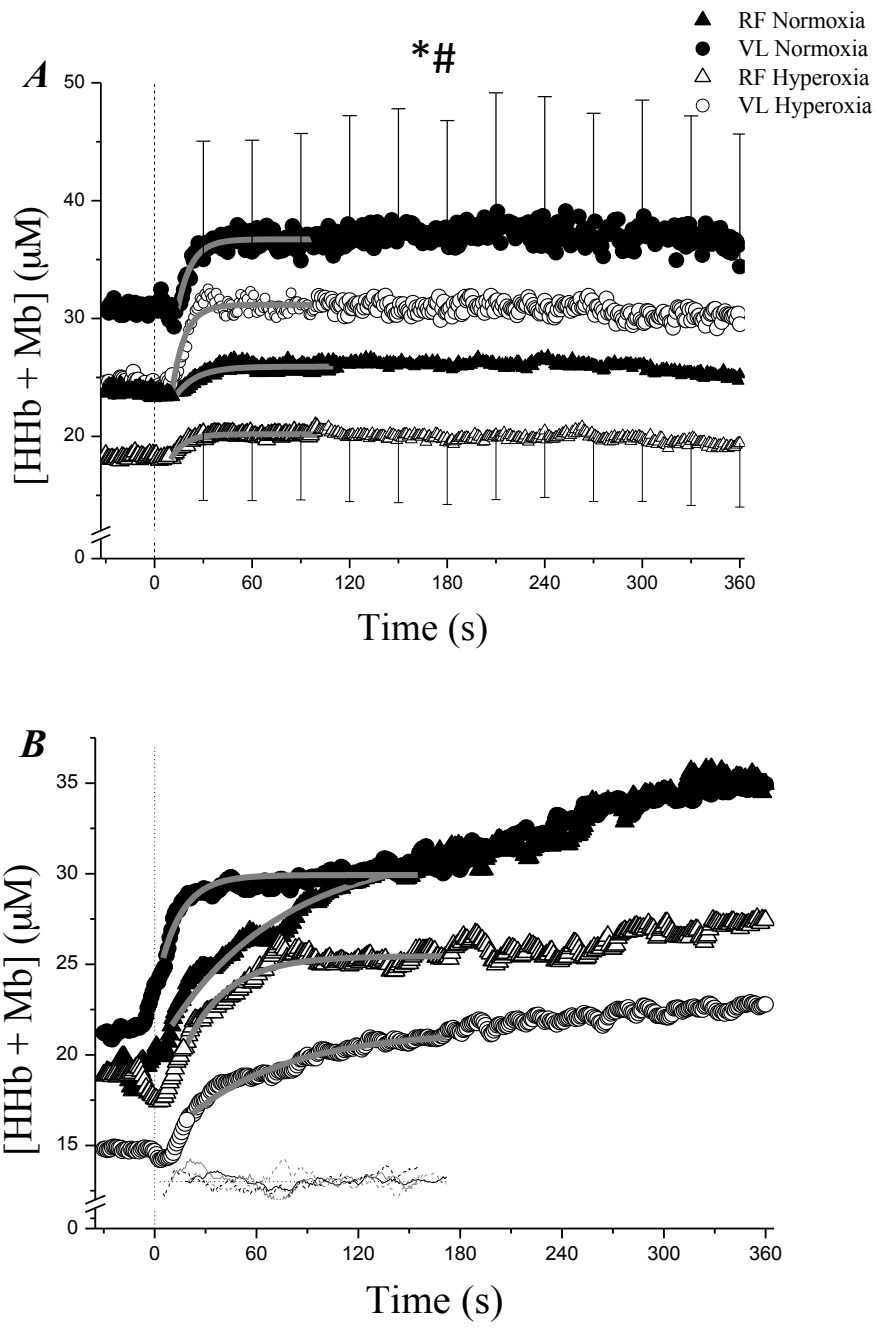
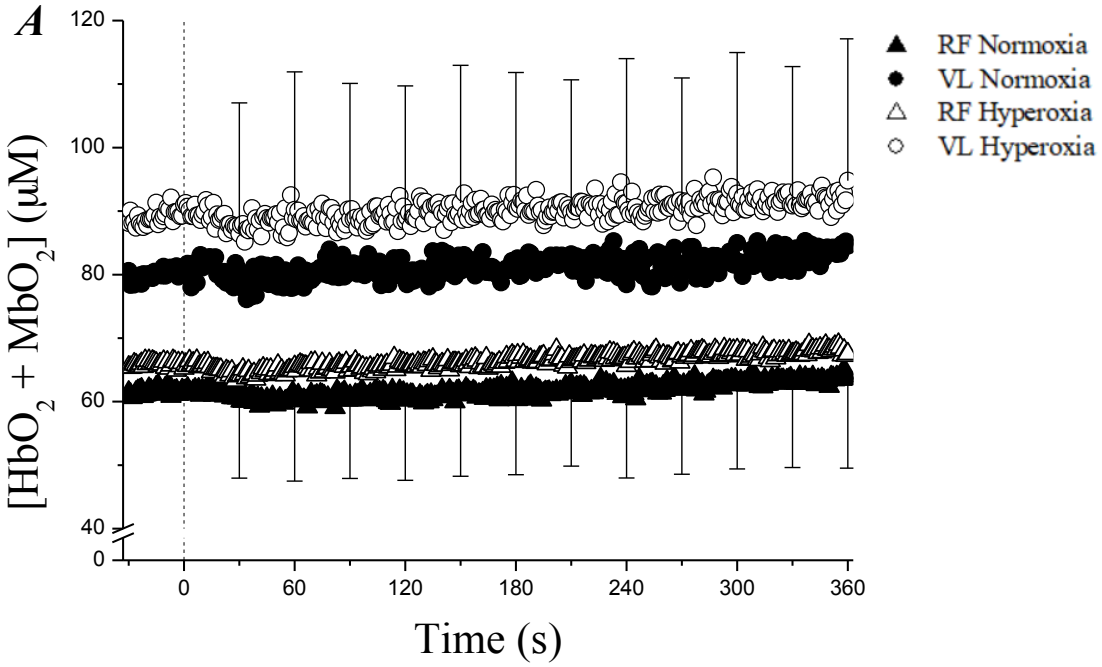

A
*#
RF Normoxia
VL Normoxia
RF Hyperoxia
VL Hyperoxia
[HHb + Mb] (μM)
Time (s)
B
[HHb + Mb] (μM)
Time (s)

Figure 3



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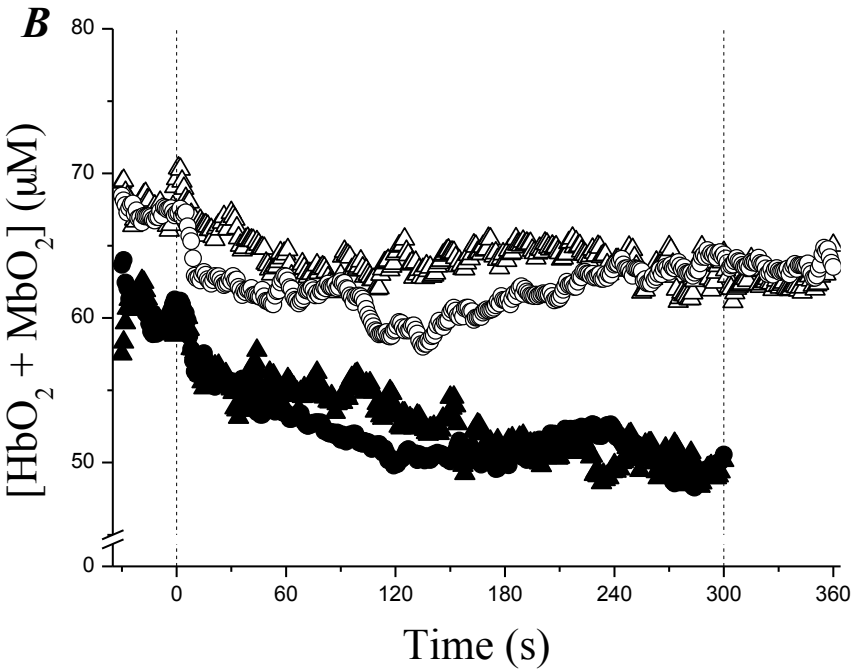

637

638 **Figure 4**