Influence of hyperoxia on muscle metabolic responses and the power-duration relationship during severe-intensity exercise in humans: a ^{31}P magnetic resonance spectroscopy study

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Abstract

Severe-intensity constant-work-rate exercise results in the attainment of $\dot{V}_{O_2}$ max but the muscle metabolic milieu at the limit of tolerance (T_{lim}) remains to be elucidated. We hypothesized that T_{lim} during severe intensity exercise would be associated with the attainment of consistently low values of intramuscular phosphocreatine ([PCr]) and pH, as determined using ^{31}P-MRS, irrespective of the work rate and the inspired O_2 fraction. We also hypothesized that hyperoxia would increase the asymptote of the hyperbolic power-duration relationship (the critical power, CP) without altering the curvature constant (W′). Seven subjects (mean ± SD, age 30 ± 9 years) completed four constant-work-rate, knee-extension exercise bouts to the limit of tolerance (T_{lim}, range: 3-10 min) both in normoxia (N) and hyperoxia (H; 70% O_2) inside the bore of 1.5 T superconducting magnet. The [PCr] (~5-10% of resting baseline) and pH (~6.65) at the limit of tolerance during each of the four trials was not significantly different either in normoxia or hyperoxia. At the same fixed work rate, the overall rate at which [PCr] fell with time was attenuated in hyperoxia (mean response time, N: 59 ± 20 *vs*. H: 116 ± 46 s; $P<0.05$). The CP was higher (N: 16.1 ± 2.6 *vs*. H: 18.0 ± 2.3 W; $P<0.05$) and the W′ was lower (N: 1.92 ± 0.70 *vs*. H: 1.48 ± 0.31 kJ; $P<0.05$) in hyperoxia compared to normoxia. These data indicate that T_{lim} during severe intensity exercise is associated with the attainment of consistently low values of muscle [PCr] and pH. The CP and W′ parameters of the power-duration relationship were both sensitive to the inspiration of hyperoxic gas.

Key Words: Critical power, endurance, bioenergetics, exercise tolerance, muscle fatigue, NIRS.

Introduction

The severe exercise-intensity domain encompasses a finite range of constant work rates which result in the attainment of maximal oxygen uptake ($\dot{V}_{O_2}$ max) with the limit of tolerance (T_{lim}) reached shortly thereafter (Poole et al. 1988; Hill et al. 2002; Wilkerson et al. 2004). Exercise tolerance within the severe domain is predictable according to the hyperbolic relationship between power output and time to exhaustion (Monod & Scherrer, 1965; Poole et al. 1988; Hill et al. 2002). This relationship has been reported both in different animal species (Full, 1986; Lauderdale & Hinchcliff, 1999; Billat et al. 2005) and in a number of exercise modalities in humans (Moritani et al. 1981; Le Chevalier et al. 2000; Smith & Jones, 2001; Hill et al. 2003). The power-asymptote of the power-duration relationship, termed the 'critical power' (CP), indicates the lower boundary of the severe domain (Poole et al. 1988; Jones et al. 2008) which is closely associated with the so-called 'maximal (lactate) steady state' (Smith & Jones, 2001). The CP represents a physiological threshold above which pulmonary $\dot{V}_{O_2}$, blood acid-base balance, and intramuscular metabolite concentrations such as phosphocreatine ([PCr]), inorganic phosphate ($[P_i]$) and $[H^+]$, cannot be stabilized (Poole et al. 1988; Wilkerson et al. 2004; Jones et al. 2008). The exercise tolerance >CP is defined by the curvature constant of the power-duration relationship (W′), which indicates a fixed amount of work that can be performed above the CP, irrespective of the rate of its expenditure (Fukuba et al. 2003; Vanhatalo & Jones, 2009).

It has been proposed that exercise tolerance within the severe intensity domain may be linked to the (rate of) decline of some intramuscular 'fatigue-related' factor(s), such as pH and [PCr], towards some consistently low, limiting value (Poole et al. 1988). Given the close association between the dynamics of pulmonary $\dot{V}_{O_2}$ and intramuscular [PCr] (Rossiter et al. 2001, 2002) and the fact that $\dot{V}_{O_2}$ consistently attains its maximum at T_{lim} in the severe domain, it is reasonable to consider that muscle [PCr] should attain a consistent 'nadir' for any work rate within the severe domain. We have recently demonstrated that severe intensity exercise (10% >CP) performed to the limit of tolerance was associated with a progressive depletion of intramuscular PCr and continued decrease in pH (Jones et al. 2008). However, the hypothesis that these metabolites reach the same low, limiting values at different constant-work-rates within the severe intensity domain remains to be tested.

The inspiration of a hyperoxic gas enhances peripheral O_2 diffusion by increasing the O_2 pressure gradient between the microvasculature and the mitochondria. Improved exercise tolerance in hyperoxia has been reported during both incremental (Hogan et al. 1999) and high-intensity constant-work-rate exercise (Linnarsson et al. 1974; Wilkerson et al. 2006). Hyperoxia also results in a considerable reduction in the amplitude of the $\dot{V}_{O_2}$ slow component (MacDonald et al. 1997; Wilkerson et al. 2006) and the related intramuscular [PCr] slow component (Haseler et al. 2004). Given that the slow component amplitude is most pronounced in the lower bound of the severe domain (Poole et al. 1988; Wilkerson et al. 2004), it is possible that hyperoxia enhances exercise tolerance by 'trimming out' the [PCr] and $\dot{V}_{O_2}$ slow components and thus increasing the CP. In contrast, because the W′ is believed to reflect a work capacity which is chiefly derived through substrate-level phosphorylation (Moritani et al. 1981; Miura et al. 1999), hyperoxia would not be expected to alter the W′.

The purpose of the present investigation was to use ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) to explore the mechanistic bases of exercise tolerance within the severe domain by assessing the kinetics of intramuscular phosphate-linked metabolites and pH during severe-intensity exercise bouts performed both in normoxia and in hyperoxia. We tested the hypotheses that 1) the [PCr] and pH would reach the same values at T_{lim} irrespective of the work rate within the severe domain or the inspired O_2 fraction; 2) the T_{lim} at a fixed severe-intensity work rate would be extended in hyperoxia compared to normoxia due to a reduced rate of change of [PCr] and pH towards their terminal values; and 3) hyperoxia would alter the power-duration relationship by increasing the CP but without altering the W′. The CP is believed to be a parameter of oxidative metabolic function whereas the W′ is believed to reflect, at least in part, the anaerobic work capacity such that it should be O_2 dependent and therefore insensitive to interventions which alter muscle O_2 delivery.

Methods

Subjects

Seven habitually active male volunteers (mean ± standard deviation: age 30 ± 9 years, height 1.78 ± 0.04 m, body mass 80.6 ± 9.9 kg) were informed of the benefits and possible

risks associated with the study and provided written consent. The study was approved by the local research ethics committee and was conducted in accordance with the Declaration of Helsinki. The subjects were familiarized to the exercise protocol prior to data collection and were instructed to avoid strenuous exercise for 24 h prior to testing. Subjects were also advised to arrive at the laboratory adequately hydrated, and having abstained from consuming alcohol for 24 h, and food or caffeine for 3 h, before each scheduled test.

Experimental procedures

Preliminary testing was undertaken in order to familiarize subjects with the single-leg knee-extension exercise using the same ergometer as for the experimental trials. Each subject performed at least one constant-work-rate familiarization trial where volitional exhaustion occurred between 2 and 12 min. All subsequent trials were performed inside a whole-body MR scanner. Subjects completed two sets of four exhaustive trials which were used to establish the power-duration relationship in normoxia (whilst breathing ambient air) and in hyperoxia (whilst breathing gas containing 70% O_2 in balance N_2). The hyperoxic gas was supplied to the subject through a low-resistance mouthpiece and tubing assembly from a Douglas bag, which was supplied from a gas cylinder fixed outside the scanner room. Subjects inhaled the hyperoxic gas for 5 min before the commencement of the experimental protocol. The subjects also wore the mouthpiece and tubing assembly during the normoxic trials. All trials were presented in a blind randomized order, at a similar time of day (± 3 h) and were separated by a minimum of 24 h.

The work rates for the trials were selected in order to yield a range of T_{lim} varying from ~3 min for the shortest trial to ~10 min for the longest trial (Hill, 1993). The work rates for the trials performed in normoxia and hyperoxia were therefore adjusted test-by-test with the aim of acquiring a similar range of T_{lim} for both conditions. Accurate quantification of the power-duration relationship requires that the predicting exercise bouts are strictly within the severe exercise intensity domain (Hill, 1993). Because we hypothesized that the same work rate would be sustainable for longer in hyperoxia, it was necessary to adjust the work rates in order that the range of T_{lim} was similar in both conditions. However, to allow for comparisons to be made in T_{lim} and muscle metabolic responses between conditions, it was ensured that each individual performed one trial at the same absolute work rate in normoxia and in hyperoxia. To this end, a work rate that resulted in a T_{lim} of 4-5 min in normoxia for

a given subject was reproduced in hyperoxia (or *vice versa*, depending on randomized order of trials). During the trials, subjects received strong verbal encouragement to continue exercising for as long as possible. The T_{lim} was recorded to the nearest second, defined as the time at which the subject failed to keep pace with the set rate of contractions (see below). Subjects were not informed of the work rates or their performance until the entire project had been completed. Individual CP and W′ estimates were derived from the prediction trial data by least squares fitting of the following regression models:

1) Non-linear power versus time model: $T = W'/(P-CP)$ *Eq. 1*

2) Linear work versus time model: $W = CP \cdot T + W'$ *Eq. 2*

The parameter estimates from Eq. 1 and Eq. 2 were compared to ensure goodness of fit and the model with the lowest SEE was chosen for further analysis (Hill & Smith, 1994).

Equipment and MRS measurements

The single-leg knee-extension exercise was performed in the prone position, with the subject secured to the ergometer bed with Velcro straps at the thigh, buttocks and lower back to minimize extraneous movement during the exercise protocol. The in-house constructed ergometer consisted of a nylon frame secured on top of the bed close to the subject's feet, and a base unit placed at the distal end of the bed. The subject's right foot was connected to a rope running along the top of the frame to the base unit on which a mounted pulley system permitted brass weight plates to be lifted and lowered. Exercise was performed at the rate of 40 contractions per minute, with the subject lifting and lowering the weight over a distance of ~0.22 m in accordance with a visual cue projected on the scanner room wall. A shaft encoder (type BDK-06, Baumer Electronics, Swindon, UK) was fitted within the pulley system to record the distance traveled by the load, alongside a nonmagnetic load cell (type F250, Novatech Measurements, St. Leonards-On-Sea, UK) to record applied forces, which were then used to calculate the work rate.

The MRS was performed at the Peninsula Magnetic Resonance Research Unit (Exeter, UK) using a 1.5 T superconducting MR scanner (Intera, Philips, The Netherlands). A 6 cm ^{31}P transmit/receive surface coil was placed within the ergometer bed and the subject was positioned such that the coil was centered over the quadriceps muscle of the right leg.

Initially, fast field echo images were acquired to determine correct positioning of the muscle relative to the coil. Placement of cod liver oil capsules, which yield high-intensity signal points within the image, adjacent to the coil, allowed its orientation relative to the muscle volume under examination to be assessed. A number of preacquisition steps were carried out to optimize the signal from the muscle under investigation. Tuning and matching of the coil were performed to maximize energy transfer between the coil and the muscle. An automatic shimming protocol was then undertaken within a volume that defined the quadriceps muscle to optimize homogeneity of the local magnetic field, thereby leading to maximum signal collection. To ensure that the muscle was consistently at the same distance from the coil at the time of data sampling, the subjects was visually cued via a display consisting of two vertical bars, one that moved at a constant frequency of 0.67 Hz and one that monitored foot movement via a sensor within the ergometer pulley system. Following the start of exercise, subjects matched the movements of these two bars. The contraction phase of the knee extensors and the ^{31}P-MRS examination of the quadriceps were synchronized by a trigger pulse sent from the phosphorous MR amplifier at the time of data acquisition to the visual display.

Before and during exercise, data were acquired every 1.5 s with a spectral width of 1,500 Hz and 1,000 data points. Phase cycling with four phase cycles was employed, leading to a spectrum being acquired every 6 s. The subsequent spectra were quantified by peak fitting, with the assumption of prior knowledge, using the jMRUI (version 2) software package and the AMARES fitting algorithm. Spectra were fitted with the assumption that P_i, PCr, ATP and phosphodiester peaks were present. In all cases, relative amplitudes were corrected for partial saturation due to the repetition time relative to T1. The T1 saturation was corrected via a spectrum that was acquired with a long relaxation time prior to the beginning of data acquisition.

Intracellular pH was calculated using the chemical shift of the P_i spectra relative to the PCr peak (Taylor et al. 1983). The [PCr] and [P_i] were expressed as percent change relative to resting baseline, which was assumed to represent 100%. Resting and end-exercise values of [PCr], [P_i], and pH were calculated over the last 30 s of the rest or exercise period. ADP concentration was calculated as described by Kemp et al. (2001).

Near infra-red spectroscopy measurements

The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using near infra-red spectroscopy (NIRS; model NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan). Optodes (inter-optode spacing of 5 cm) were placed on the belly of the muscle 20 cm above the fibular head, and secured in position with adhesive tape and an elasticated wrap to minimize any extraneous light which could influence the signal and to ensure that the optodes did not move during exercise. Pen marks were made around the probes to enable reproduction of the placement in subsequent tests. The probe gain was set with the subject at rest in the supine position with leg fully extended prior to the exercise bout, and NIRS data were collected continuously throughout the bout. Four different wavelength laser diodes provided the light source (776, 826, 845 and 905 nm) and the light returning from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate concentration changes from the resting baseline for oxygenated hemoglobin/myoglobin ($[HbO_2]$), deoxygenated hemoglobin/myoglobin ([HHb]), total tissue hemoglobin/myoglobin ([tHb], i.e. $[HbO_2]$ + [HHb]), and oxygenation index ($[HbO_2]$-[HHb]). The [HHb] signal derived from NIRS measurements reflects the balance between O_2 delivery and O_2 utilization in the field of interrogation and has been used to provide a non-invasive estimate of fractional O_2 extraction in the microcirculation during exercise (De Blasi et al. 1993; Grassi et al. 2003). The [HHb] signal can be regarded as being essentially blood-volume insensitive during exercise and was therefore assumed to provide an estimate of changes in intramuscular oxygenation status and fractional O_2 extraction in the field of interrogation (De Blasi et al. 1993; Grassi et al. 2003).

Modeling Procedures

For analysis of the [PCr] kinetics during exercise at the same fixed work rate, the [PCr] data were first expressed as the % change relative to the initial resting baseline which was assumed to represent 100%. The [PCr] responses were then modeled using non-linear least-squares regression techniques. Briefly, an exponential function of the form:

$$\Delta[PCr]_{(t)} = [PCr]_0 - \Delta[PCr]_{ss}(1-e^{-t/\tau}) \qquad \textit{Eq. 2}$$

where $[PCr]_0$ is the value of [PCr] at time zero (onset of exercise), $\Delta[PCr]_{ss}$ is the projected asymptotic value, and τ is the time constant of the response, was fit to the data. The first fit contained only the first 60 s of exercise data but the fitting window was then increased iteratively until there was a clear departure of the measured data from the model fit, as judged from visual inspection of a plot of the residuals. In this way, the best-fit exponential for the fundamental component of the response was established. The magnitude of any possible [PCr] slow component was then calculated as the difference between the asymptotic amplitude of the fundamental [PCr] response at the point of exhaustion as indicated by the modeled fit and the mean [PCr] measured over the last 12 s of exercise at that work-rate. In addition, a mono-exponential model (see Eq. 2) was fitted from the onset of exercise through the entire data to provide information on the overall response kinetics (mean response time, MRT).

The [HHb] response was modeled to provide information on muscle oxygenation during trials performed in normoxia and hyperoxia. The mean ± SD [HHb] was calculated for the 60-second period immediately prior to exercise onset. The baseline [HHb] so derived allowed for identification of the initiation of the [HHb] response to exercise, which was defined as the first datum greater than one standard deviation above the baseline mean. Only data collected from this point forward was included in the exponential model fit. A nonlinear least-square algorithm was used to fit the data, as described in the following bi-exponential equation:

$$[HHb](t) = [HHb]_{baseline} + A_p(1-e^{-(t-TDp)/\tau_p}) + A_s(1-e^{-(t-TDs)/\tau_s}) \qquad \textit{Eq. 3}$$

where [HHb] (t) represents the absolute [HHb] at a given time t; $[HHb]_{baseline}$ represents the mean [HHb] in the baseline period (see above); A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively, describing the initial increase in [HHb] above baseline; and A_s, TD_s, and τ_s represent the amplitude of, time delay before the onset of, and time constant describing the development of, the [HHb] slow component, respectively. An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values.

Statistical analyses

End-exercise [PCr], pH, [P_i] and [ADP] were compared for the four bouts of severe-intensity exercise in normoxia and hyperoxia using two-way repeated measures ANOVAs. Paired samples t-tests were used to assess differences between normoxia and hyperoxia in T_{lim}, end-exercise muscle metabolite concentrations and the kinetic features of the [PCr] and [HHb] responses at the same fixed work rate. Differences in the parameters of the power-duration relationship between normoxia and hyperoxia were also assessed using paired samples t-tests. The relationship between changes in the CP and W′ was assessed using the Pearson product moment correlation coefficient. Data are presented as mean ± SD. Significance was accepted at $P<0.05$.

Results

Effects of hyperoxia on muscle oxygenation

The NIRS responses provided evidence that muscle oxygenation was altered by hyperoxia (Figure 1). The [HbO_2] was higher in hyperoxia at 120 s of exercise ($P<0.05$) and the oxygenation index was significantly higher in hyperoxia after 60 s, 120 s and at the end of exercise (all $P<0.05$). Importantly, the parameters derived from modeling the [HHb] response were consistent with the interpretation that hyperoxia enhanced muscle O_2 supply. Hyperoxia resulted in a significant increase in the [HHb] time delay (N: 13 ± 2 *vs*. H: 17 ± 3 s; $P<0.05$) and a significant reduction in the [HHb] amplitude (N: 290 ± 130 *vs*. H: 201 ± 108 AU; $P<0.01$). The difference in the [HHb] time constant (N: 9 ± 3 *vs*. H: 13 ± 3 W) and the amplitude of the [HHb] slow component (N: 226 ± 73 *vs*. H: 175 ± 47 AU) did not attain statistical significance.

Effects of hyperoxia on muscle metabolic responses to exercise

The end-exercise [PCr], [P_i], [ADP] and pH were not significantly different at the different severe-intensity work rates and were not altered by the inspired O_2 fraction (Table 1). The end-exercise [PCr] (~5-11% of initial baseline) was not significantly different at different work rates either within or between conditions (Table 1). The [PCr] responses of one subject to the bouts of severe-intensity exercise in normoxia are shown in Figure 2. The

end-exercise pH (~6.65), [P_i] (~ 500% above the initial baseline) and [ADP] values were also not significantly different at different work rates either within or between conditions (Table 1).

At the same fixed work rate (23 ± 3 W), the T_{lim} was significantly greater in hyperoxia than in normoxia (N: 280 ± 50 *vs*. H: 332 ± 71 s; $P<0.05$; Table 2), with no differences between conditions in the end-exercise [PCr] (N: 10 ± 9 *vs*. H: 10 ± 9 %; $P=0.84$; Figure 3A), pH (N: 6.74 ± 0.15 *vs*. H: 6.70 ± 0.21; $P=0.53$; Figure 3B) or [P_i] (N: 412 ± 131 *vs*. H: 501 ± 109 %; $P=0.16$). At this fixed work rate, the sparing of [PCr] for the same iso-time along with the extended T_{lim} in hyperoxia resulted in differences in the MRT (N: 59 ± 20 *vs*. H: 116 ± 46 s; $P<0.01$) and T_{50} (N: 30 ± 11 *vs*. H: 71 ± 32 s; $P<0.05$) between the conditions. The time constant for the fundamental phase decrement in [PCr] was significantly longer in hyperoxia (N: 23 ± 9 *vs*. H: 37 ± 14 s; $P<0.05$), but there were no significant differences in the amplitudes of the fundamental (N: 64 ± 13 *vs*. H: 58 ± 17 %) or slow (N: 25 ± 11 *vs*. H: 31 ± 15 %) phases of the response.

Effect of hyperoxia on the power-duration relationship

There were no significant differences in the power-duration parameter estimates derived from non-linear and linear models (Eq. 1 and 2) in normoxia or in hyperoxia. However, the estimates derived using the non-linear power-time model (Eq. 1) were associated with lower standard errors of estimate and were therefore used for further analysis.

The CP was higher in hyperoxia (N: 16.1 ± 2.6 *vs*. H: 18.0 ± 2.3 W; $P<0.05$), and the W′ was lower in hyperoxia (N: 1.92 ± 0.70 *vs*. H: 1.48 ± 0.31 kJ; $P<0.05$). The individual CP and W′ values in normoxia and hyperoxia are shown in Table 3. The difference in CP between normoxia and hyperoxia (ΔCP) was negatively correlated to the difference in W′ between normoxia and hyperoxia (ΔW′; $r = -0.88$, $P<0.05$). The alterations in the power-duration relationship in hyperoxia resulted in enhanced exercise tolerance, but only for work rates less than 24.4 ± 3.8 W where the T_{lim} was greater than 232 ± 125 s. Figure 4 illustrates the effect of hyperoxia on the power-duration relationship in a representative individual.

Discussion

The principal novel finding of this investigation was that [PCr] and pH reached low values at T_{lim} which were not significantly different irrespective of the work rate within the severe domain or the inspired O_2 fraction. At a fixed severe-intensity work rate, there was a reduced rate of change of [PCr] towards its terminal value in hyperoxia compared to normoxia and T_{lim} was extended. These data are consistent with our hypotheses and indicate that the inability to continue severe intensity exercise might be linked to the attainment of critically low values of muscle [PCr] and/or pH (or that these serve as 'proxy variables' for other changes in the muscle metabolic milieu that presage exercise intolerance). The inspiration of hyperoxic gas resulted in significant changes to the power-duration relationship: the CP was increased and the W′ was reduced suggesting, in contrast to our hypothesis, that *both* parameters are sensitive to muscle O_2 availability.

We utilized NIRS to provide information on changes in muscle oxygenation during exercise in normoxia and hyperoxia. The data indicated that the muscle oxygenation index was higher when subjects breathed the hyperoxic inspirate. Moreover, the muscle [HHb] response was blunted in hyperoxia (i.e. the kinetics were slower and the response amplitude was reduced). With the assumption that muscle O_2 utilization was similar in hyperoxia compared to normoxia (Savasi et al. 2002; Stellingwerff et al. 2005; Wilkerson et al. 2006), these data indicate that local muscle O_2 delivery was higher in hyperoxia such that muscle fractional O_2 extraction (as estimated by the NIRS [HHb] signal; Grassi et al. 2003) was reduced accordingly.

Fatigue during severe intensity exercise

^{31}P-MRS provides a means of investigating changes in intramuscular metabolites non-invasively and with high temporal resolution and can therefore provide insight into the metabolic determinants of muscle fatigue (e.g., Hogan et al. 1999; Jones et al. 2007; Jones et al. 2008). We hypothesized that the intramuscular [PCr] and pH would reach the same low, possibly limiting, values at T_{lim} irrespective of the work rate and the fractional concentration of O_2 in the inspirate during exercise within the severe exercise intensity domain. This hypothesis was confirmed. While it is not possible to identify the exact cellular mechanism(s) responsible for the subjects' eventual failure to maintain the

requisite muscle power output, and it is recognized that muscle fatigue is a multi-faceted process (Fitts, 1994; Bangsbo et al. 1996; Westerblad & Allen, 2003; Amann & Calbet, 2008), the present data indicate that the point at which subjects volitionally terminated exercise was associated with the attainment of low values of [PCr] and pH (and high values of $[P_i]$). This was true for all subjects irrespective of both the work rate within the severe domain and the fraction of O_2 in the inspirate. That the fatigue process in this range of work rates is associated with changes in [PCr], pH and $[P_i]$ is underscored by the fact that the values at exhaustion in either normoxia or hyperoxia were not significantly different despite the rate of change in these variables being greater at the higher work rates, and attenuated for the same fixed work rate in hyperoxia.

The failure to sustain severe intensity exercise was associated with significant depletion of muscle PCr (mean [PCr] at exhaustion of 8 %). However, although the [PCr] value at T_{lim} was similar *within* subjects, there was an appreciable range of [PCr] values at T_{lim} *between* subjects (range: 0 to 26 %). It is known that the depletion of muscle high-energy phosphates during exercise displays regional heterogeneity (Sahlin et al. 1997). It is therefore possible that [PCr] fell to sufficiently low values in at least some of the recruited muscle fibers during severe exercise that the required muscle force could not be maintained even when the "mean" [PCr] in the region of muscle interrogated was appreciably above "zero". Severe intensity exercise intolerance might also be attributed to the extent, or the rate, of accumulation of metabolites that have been associated with the fatigue process, such as P_i and H^+. A reduction in muscle pH has been linked to fatigue through H^+ competition with Ca^{2+} for binding to troponin, interference with Ca^{2+} release from the sarcoplasmic reticulum, and inhibition of phosphofructokinase (Trivedi & Danforth, 1966; Fuchs et al. 1970; Fitts, 1994). Recently, the possible role of P_i accumulation (specifically, in its diprotonated form) has received increasing attention (Cooke et al. 1988; Nosek et al. 1987; Wilson et al. 1988; Westerblad & Allen, 2003). In the present study, muscle $[P_i]$ increased and pH decreased with time until the termination of exercise and it is possible that metabolite accumulation was responsible for, or at least contributed to, exercise intolerance. It should be noted also that the rate of development of peripheral muscle fatigue (which is itself influenced by arterial O_2 content; Knight et al. 1993; Hogan et al. 1999; Richardson et al. 1999) might impact on central motor drive and motor unit recruitment (i.e., central fatigue; Amann & Calbet, 2008).

At the same fixed work rate, the rate of change in [PCr] and pH was blunted in hyperoxia (Figure 3), which possibly enabled exercise to be sustained for longer. Enhanced muscle O_2 availability (such as would be expected to occur with hyperoxia; Figure 1) would enable the same metabolic rate to be achieved with greater redox and phosphorylation potentials and a reduced perturbation of muscle adenine nucleotides (Hogan et al. 1992). A reduced rate of decline of muscle [PCr] towards some critically-low value, along with a reduced rate of increase of $p\dot{V}_{O_2}$ towards the elevated $p\dot{V}_{O_2}$ max (Poole et al. 1988; Wilkerson et al. 2004), would be expected to increase the T_{lim} at a constant severe-intensity work rate (Burnley & Jones, 2007), as was indeed observed in the present study. In addition to direct effects of enhanced muscle O_2 availability on the phosphorylation potential, a reduced rate of glycogenolysis in hyperoxia (Stellingwerff et al. 2006), leading to a better preservation of pH homeostasis (Figure 3B), would also require muscle PCr to fall less to provide the same ADP stimulus for oxidative phosphorylation (Conley et al. 2001). Our data are consistent with the study of Hogan et al. (1999) in which it was reported that the end-exercise values of [PCr], [P_i] and pH were not significantly different when incremental exercise was performed to volitional exhaustion in hypoxia (10% O_2), normoxia, and hyperoxia (100% O_2), despite the T_{lim} being shortest in hypoxia and longest in hyperoxia. These authors also concluded that exhaustion occurred when a particular intracellular environment was achieved and that the extent of the disturbance to homeostasis was a function of cellular oxygenation (Hogan et al. 1999).

The blunting of the overall rate at which [PCr] was depleted to its terminal value in hyperoxia, along with the greater T_{lim}, resulted in a significant slowing of the overall [PCr] kinetics (assessed using the MRT or T_{50}). Interestingly, the time constant describing the initial fall in [PCr] was also significantly greater in hyperoxia than in normoxia (~37 *vs*. 23 s). This finding differs from that of Haseler et al. (2004) who reported that the [PCr] time constant was not different when subjects breathed 100% O_2 compared to normoxia during knee-extension exercise. This may be explained by the fact that the intensity of exercise was higher in our study (severe) than in theirs (ostensibly heavy); changes in muscle PO_2 evoked by hyperoxia might be expected to impact more on the muscle phosphorylation potential at work rates that are closer to maximal (Richardson et al. 1999). The available data indicate that the rate at which muscle $\dot{V}_{O_2}$ (Evans et al. 2001; Savasi et al. 2002; Stellingwerff et al. 2005) or pulmonary $\dot{V}_{O_2}$ (MacDonald et al. 1997; Wilkerson et al. 2006) increases over the first 1-3 min following the onset of exercise is not altered by hyperoxia.

Since muscle [PCr] kinetics is often considered as a proxy for muscle $\dot{V}_{O_2}$ kinetics (Rossiter et al. 2002), the slower [PCr] kinetics we observed in hyperoxia in the present study suggests that the kinetics of muscle [PCr] and $\dot{V}_{O_2}$ might have been dissociated by the intervention. This is likely a consequence of the direct effects of hyperoxia on the muscle phosphorylation potential which enables the same metabolic rate to be sustained with a reduced perturbation of muscle adenine nucleotides (Wilson et al. 1977; Hogan et al. 1992).

Influence of hyperoxia on the parameters of the power-duration relationship

In the present study, subjects completed four exercise bouts to the limit of tolerance in the severe exercise intensity domain both in normoxia and in hyperoxia, thus enabling us to assess the effect of inspired O_2 fraction on the asymptote (the CP) and the curvature constant (W′) of the power-duration relationship. Consistent with our hypothesis, the CP was significantly increased in the hyperoxic condition. Surprisingly, only one previous study has manipulated the inspired O_2 fraction to investigate the dependency of CP on muscle O_2 availability. Moritani et al. (1981) reported that the CP was reduced but the W′ was unchanged in two subjects when exercise was performed in hypoxia (9% and 12% O_2) compared to normoxia. Collectively, those results along with the present findings indicate that the CP is sensitive to muscle O_2 availability and support the notion that the CP is a parameter of oxidative metabolic function.

In contrast to our hypothesis, we also observed a considerable reduction in the W′. Indeed, our data indicate that exercise tolerance might only be enhanced in hyperoxia for exercise durations greater than approximately four minutes and might even be slightly impaired for shorter durations of exhaustive exercise. The explanation for this is not immediately apparent. One possibility is that the energy yield from substrate phosphorylation was reduced in hyperoxia. Stellingwerff et al. (2006) have reported that glycogenolysis was significantly reduced by hyperoxia during cycle exercise at 70% $\dot{V}_{O_2}$ max. If the maximal glycogenolytic rate is impaired in hyperoxia, this might impair performance during very high intensity exercise thereby affecting the shortest duration trials. Another explanation for the possible reduction in exercise tolerance at very high work rates in hyperoxia is that contractile function is impaired by increased oxidative stress. It is known, for example, that excessive accumulation of reactive oxygen species (which is likely to be exacerbated in hyperoxia) can inhibit muscle force production during fatiguing exercise, possibly

through effects of reactive oxygen species and also nitric oxide on muscle redox status (Reid & Durham, 2002).

The significant inverse correlation between the changes in CP and W′ induced by hyperoxia in the present study (r = -0.88) closely resembles previous data on the effect of training on these same parameters (Jenkins & Quigley, 1992; Vanhatalo et al. 2008). Jenkins and Quigley (1992) demonstrated a 30% increase in CP in conjunction with a (non-significant) 26% reduction in W′ following endurance training, and Vanhatalo et al. (2008) reported an inverse relationship between ΔCP and ΔW′ (r = -0.75) after interval training. Collectively, these results may be explained by the relative changes induced by a given intervention on the CP (the lower boundary of the severe domain) and the $\dot{V}_{O_2}$ max (the upper boundary of the severe domain). If the increase in CP is greater than the increase in $\dot{V}_{O_2\,max}$ (i.e., the range of work rates encompassing the severe domain is reduced) the W′ must decrease (Burnley & Jones, 2007). The tendency for W′ to be reduced following interventions such as training and hyperoxia which are effective in altering both CP and $\dot{V}_{O_2\,max}$ (Linnarsson et al. 1974; Jenkins & Quigley, 1992; Richardson et al. 1999; Vanhatalo et al. 2008), might thus reflect the relative changes in the lower and upper limits of the severe domain. Both interventions might be expected to have a greater effect on 'sub-maximal' indices of aerobic fitness (such as the CP) than on $\dot{V}_{O_2}$ max (Linnarsson et al. 1974; Jones & Carter, 2004; Wilkerson et al. 2006; Vanhatalo et al. 2008). These data suggest that the W' may not represent a fixed 'anaerobic' substrate store (Monod & Scherrer, 1965; Miura et al. 1999) but rather a mechanical work capacity which can be performed whilst the $\dot{V}_{O_2}$ and [PCr] kinetics project towards their respective maximum and nadir values.

In conclusion, this study has shown that the limit of tolerance within the severe exercise intensity domain was associated with muscle [PCr] and pH reaching a nadir, and [P_i] reaching a peak, the values of which were neither different between work rates nor affected by the fraction of O_2 in the inspirate. The rate of change in [PCr] and pH at a fixed severe work rate was reduced in hyperoxia compared to normoxia, and these alterations were associated with an elevated CP and a reduced W′ in hyperoxia. These data provide further support for the notion that T_{lim} in the severe-intensity exercise domain is linked, directly or indirectly, to the attainment of some critical level of intra-muscular [PCr], [P_i] and/or pH.

References

Amann M & Calbet JA (2008). Convective oxygen transport and fatigue. *J Appl Physiol* **104**, 861-870.

Bangsbo J, Madsen K, Kiens B & Richter EA (1996). Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J Physiol* **495**, 587–596.

Billat VL, Mouisel E, Roblot N & Melki J (2005). Inter- and intra-strain variation in mouse critical running speed. *J Appl Physiol* **98**, 1258–1263.

Burnley M & Jones AM (2007). Oxygen uptake kinetics as a determinant of sports performance. *Eur Journal Sports Sci* **7**, 63-79.

Conley KE, Kemper WF & Crowther GJ (2001). Limits to sustainable muscle performance: interaction between glycolysis and oxidative phosphorylation. *J Exp Biol* **204**, 3189–3194.

Cooke R, Franks K, Luciani GB & Pate E (1988). The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J Physiol* **395**, 77–97.

De Blasi RA, Cope M, Elwell C, Safoue F & Ferrari M (1993). Noninvasive measurement of human forearm oxygen consumption by near infrared spectroscopy. *Eur J Appl Physiol Occup Physiol* **67**, 20–25.

Evans MK, Savasi I, Heigenhauser GJ & Spriet LL (2001). Effects of acetate infusion and hyperoxia on muscle substrate phosphorylation after onset of moderate exercise. *Am J Physiol Endocrinol Metab* **281**, E1144-1150.

Fitts RH (1994). Cellular mechanisms of muscle fatigue. *Physiol Rev* **74**, 49–94.

Fuchs F, Reddy Y & Briggs FN (1970). The interaction of cations with the calcium-binding site of troponin. *Biochim Biophys Acta* **221**, 407–409.

Fukuba Y, Miura A, Endo M, Kan A, Yanagawa K & Whipp BJ (2003). The curvature constant parameter of the power-duration curve for varied-power exercise. *Med Sci Sports Exerc* **35**, 1413–1418.

Full RJ (1986). Locomotion without lungs: energetics and performance of a lungless salamander. *Am J Physiol Regul Integr Comp Physiol* **251**, R775–R780.

Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C & Cerretelli P (2003). Muscle oxygenation and pulmonary gas exchange kinetics during cycle exercise on-transitions in humans. *J Appl Physiol* **95**, 149-158.

Haseler LJ, Kindig CA, Richardson RS & Hogan MC (2004). The role of oxygen in determining phosphocreatine onset kinetics in exercising humans. *J Physiol* **558**, 985–992.

Hill DW (1993). The critical power concept. A review. *Sports Med* **16**, 237-254.

Hill DW, Alain C & Kennedy MD (2003). Modeling the relationship between velocity and time to fatigue in rowing. *Med Sci Sports Exerc* **35**, 2098–2105.

Hill DW, Poole DC & Smith JC (2002). The relationship between power and the time to achieve $\dot{V}_{O_2}$ max. *Med Sci Sports Exerc* **34**, 709-714.

Hill DW & Smith JC (1994). A method to ensure the accuracy of estimates of anaerobic capacity derived using the critical power concept. *J Sports Med Phys Fitness* **34**, 23-37.

Hogan MC, Richardson RS & Haseler LJ (1999). Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a ^{31}P-MRS study. *J Appl Physiol* **86**, 1367-1373.

Hogan MC, Arthur PG, Bebout DE, Hochachka PW & Wagner PD (1992). Role of O_2 in regulating tissue respiration in dog muscle working in situ. *J Appl Physiol* **73**, 728-36.

Jenkins DG & Quigley BM (1992). Endurance training enhances critical power. *Med Sci Sports Exerc* **24**, 1283–1289.

Jones AM & Carter H (2004). The effect of endurance training on parameters of aerobic fitness. *Sports Med* **29**, 373-386.

Jones AM, Wilkerson DP, Berger NJ & Fulford J (2007). Influence of endurance training on muscle [PCr] kinetics during high-intensity exercise. *Am J Physiol Regul Integr Comp Physiol* **293**, R392–R401.

Jones AM, Wilkerson DP, DiMenna F, Fulford J & Poole DC (2008). Muscle metabolic responses to exercise above and below the "critical power" assessed using 31P-MRS. *Am J Physiol Regul Integr Comp Physiol* **294**, R585-593.

Jones AM, Wilkerson DP & Fulford J (2008). Influence of prior exercise on muscle [phosphorylcreatine] and deoxygenation kinetics during high-intensity exercise in men. *Exp Physiol* **93**, 468-478.

Kemp GJ, Roussel M, Bendahan D, Le Fur Y & Cozzone PJ (2001). Interrelations of ATP synthesis and proton handling in ischaemically exercising human forearm muscle studied by 31P magnetic resonance spectroscopy. *J Physiol* **535**, 901-928.

Knight DR, Schaffartzik W, Poole DC, Hogan MC, Bebout DE & Wagner PD (1993). Effects of hyperoxia on maximal leg O_2 supply and utilization in men. *J Appl Physiol* **75**, 2586-2594.

Lauderdale MA & Hinchcliff KW (1999). Hyperbolic relationship between time-to-fatigue and workload. *Equine Vet J Suppl* **30**, 586–590.

Le Chevalier JM, Vandewalle H, Thépaut-Mathieu C, Stein JF & Caplan L (2000). Local critical power is an index of local endurance. *Eur J Appl Physiol* **81**, 120-127.

Linnarsson D, Karlsson J, Fagraeus L & Saltin B (1974). Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *J Appl Physiol* **36**, 399-402.

MacDonald M, Pedersen PK & Hughson RL (1997). Acceleration of $\dot{V}_{O_2}$ kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. *J Appl Physiol* **83**, 1318-1325.

Miura A, Kino F, Kajitani S, Sato H & Fukuba Y (1999). The effect of oral creatine supplementation on the curvature constant parameter of the power-duration curve for cycle ergometry in humans. *Jpn J Physiol* **49**, 169–174.

Monod H & Scherrer J (1965). The work capacity of a synergic muscle group. *Ergonomics* **8**, 329–338.

Moritani T, Nagata A, deVries HA & Muro M (1981). Critical power as a measure of physical work capacity and anaerobic threshold. *Ergonomics* **24**, 339–350.

Nosek TM, Fender KY & Godt RE (1987). It is diprotonated inorganic phosphate that depresses force in skinned skeletal muscle fibers. *Science* **236**, 191–193.

Poole DC, Ward SA, Gardner GW & Whipp BJ (1988). Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* **31**, 1265–1279.

Reid MB & Durham WJ (2002). Generation of reactive oxygen and nitrogen species in contracting skeletal muscle: potential impact on aging. *Ann N Y Acad Sci* **959**, 108-116.

Richardson RS, Grassi B, Gavin TP, Haseler LJ, Tagore K, Roca J & Wagner PD (1999). Evidence of O_2 supply-dependent $\dot{V}_{O_2}$ max in the exercise-trained human quadriceps. *J Appl Physiol* **86**, 1048-1053.

Richardson RS, Leigh JS, Wagner PD & Noyszewski EA (1999). Cellular PO_2 as a determinant of maximal mitochondrial O_2 consumption in trained human skeletal muscle. *J Appl Physiol* **87**, 325-31.

Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR & Whipp BJ (2002). Dynamic asymmetry of phosphocreatine concentration and O_2 uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. *J Physiol* **541**, 991-1002.

Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR & Whipp BJ (2001). Effects of prior exercise on oxygen uptake and phosphocreatine kinetics during high-intensity knee-extension exercise in humans. *J Physiol* **537**, 291-303.

Sahlin K, Soderlund K, Tonkonogi M & Hirakoba K (1997). Phosphocreatine content in single fibers of human muscle after sustained submaximal exercise. *Am J Physiol Cell Physiol* **273**, C172–C178.

Savasi I, Evans MK, Heigenhauser GJ & Spriet LL (2002). Skeletal muscle metabolism is unaffected by DCA infusion and hyperoxia after onset of intense aerobic exercise. *Am J Physiol Endocrinol Metab* **283**, E108-115.

Smith CG & Jones AM (2001). The relationship between critical velocity, maximal lactate steady-state velocity and lactate turnpoint velocity in runners. *Eur J Appl Physiol* **85**, 19–26.

Stellingwerff T, Glazier L, Watt MJ, LeBlanc PJ, Heigenhauser GJ & Spriet LL (2005). Effects of hyperoxia on skeletal muscle carbohydrate metabolism during transient and steady-state exercise. *J Appl Physiol* **98**, 250-256.

Stellingwerff T, LeBlanc PJ, Hollidge MG, Heigenhauser GJ & Spriet LL (2006). Hyperoxia decreases muscle glycogenolysis, lactate production, and lactate efflux during steady-state exercise. *Am J Physiol Endocrinol Metab* **290**, E1180-1190.

Taylor DJ, Bore PJ, Styles P, Gadian DG & Radda GK (1983). Bioenergetics of intact human muscle. A ^{31}P nuclear magnetic resonance study. *Mol Biol Med* **1**, 77–94.

Trivedi B & Danforth WH (1966). Effect of pH on the kinetics of frog muscle phosphofructokinase. *J Biol Chem* **241**, 4110–4112.

Vanhatalo A, Doust JH & Burnley M (2008). A 3-min all-out cycling test is sensitive to a change in critical power. *Med Sci Sports Exerc* **40**, 1693-1699.

Vanhatalo A & Jones AM (2009). Influence of prior sprint exercise on the parameters of the 'all-out critical power test' in men. *Exp Physiol* **94**, 255-263.

Westerblad H & Allen DG (2003). Cellular mechanisms of skeletal muscle fatigue. *Adv Exp Med Biol* **538**, 563–570.

Wilkerson DP, Berger JA & Jones AM (2006). Influence of hyperoxia on pulmonary O_2 uptake kinetics following the onset of exercise in humans. *Resp Physiol Neurobiol* **153**, 92-106.

Wilkerson DP, Koppo K, Barstow TJ & Jones AM (2004). Effect of work rate on the functional "gain" of phase II pulmonary O_2 uptake response to exercise. *Respir Physiol Neurobiol* **142**, 211–223.

Wilson DF, Erecinska M, Drwn C & Silver IA (1977). Effect of oxygen tension on cellular energetics. *Am J Physiol* **233**, 1347-1353.

Wilson JR, McCully KK, Mancini DM, Boden B & Chance B (1988). Relationship of muscular fatigue to pH and diprotonated P_i in humans: a ^{31}P-NMR study. *J Appl Physiol* **64**, 2333–2339.

Figure Legends

Figure 1: Group mean muscle oxygenation measures derived from NIRS at the same fixed work rate in normoxia (●) and hyperoxia (○). Panel A: oxygenated Hb/Mb, panel B: total Hb/Mb ([HbO_2/Mb]+[HHb/Mb]), panel C: deoxygenated Hb/Mb and D: oxygenation index ([HbO_2/Mb]-[HHb/Mb]). The final data point in each figure indicates the end-exercise values (±SD).

Figure 2: Muscle [PCr] responses to severe-intensity constant-work-rate exercise in normoxia in a representative subject. Notice that [PCr] falls more steeply towards its nadir at higher work rates but that the [PCr] reaches a similar value at the T_{lim}.

Figure 3: Group mean (n=7) muscle [PCr] (panel A) and pH (panel B) responses to severe-intensity exercise at a fixed work rate (23 ± 3 W) in normoxia (●) and hyperoxia (○). The T_{lim} was significantly greater in hyperoxia (N: 280 ± 50 *vs*. H: 332 ± 71 s; $P<0.05$) but the end-exercise [PCr] and pH values were not significantly different. The final data point in each figure indicates the end-exercise values (±SD).

Figure 4: The hyperbolic power-duration (panel A; *Eq.* 1) and the linear work-time (panel B; *Eq.* 2) relationships during severe-intensity knee-extension exercise in normoxia (●) and hyperoxia (○) in subject #1. In panel A the CP is indicated by the power-asymptote and the W′ is the curvature constant. In panel B the CP is the slope and the W′ is the y-intercept of the linear regression.

Table 1: ***Mean ± SD end-exercise [PCr], pH, [Pi] and [ADP] during severe-intensity exercise in normoxia and hyperoxia***

	Normoxia				**Hyperoxia**			
Power (W)	26 ± 3	24 ± 3	22 ± 3	20 ± 3	26 ± 3	24 ± 3	22 ± 3	20 ± 3
[PCr] (%)	7 ± 8	8 ± 10	11 ± 12	11 ± 10	5 ± 6	6 ± 7	11 ± 7	7 ± 9
pH	6.63 ± 0.16	6.66 ± 0.19	6.65 ± 0.2	6.70 ± 0.14	6.60 ± 0.13	6.65 ± 0.17	6.73 ± 0.16	6.64 ± 0.13
[Pi] (%)	512 ± 70	478 ± 141	431 ± 120	476 ± 111	480 ± 82	513 ± 82	536 ± 137	463 ± 105
[ADP] (µM)	509 ± 398	440 ± 230	323 ± 327	307 ± 318	589 ± 355	478 ± 357	340 ± 339	494 ± 598

Table 2: ***Mean ± SD [PCr] kinetics at a fixed severe-intensity work rate (23 ± 3 W) in normoxia and hyperoxia***

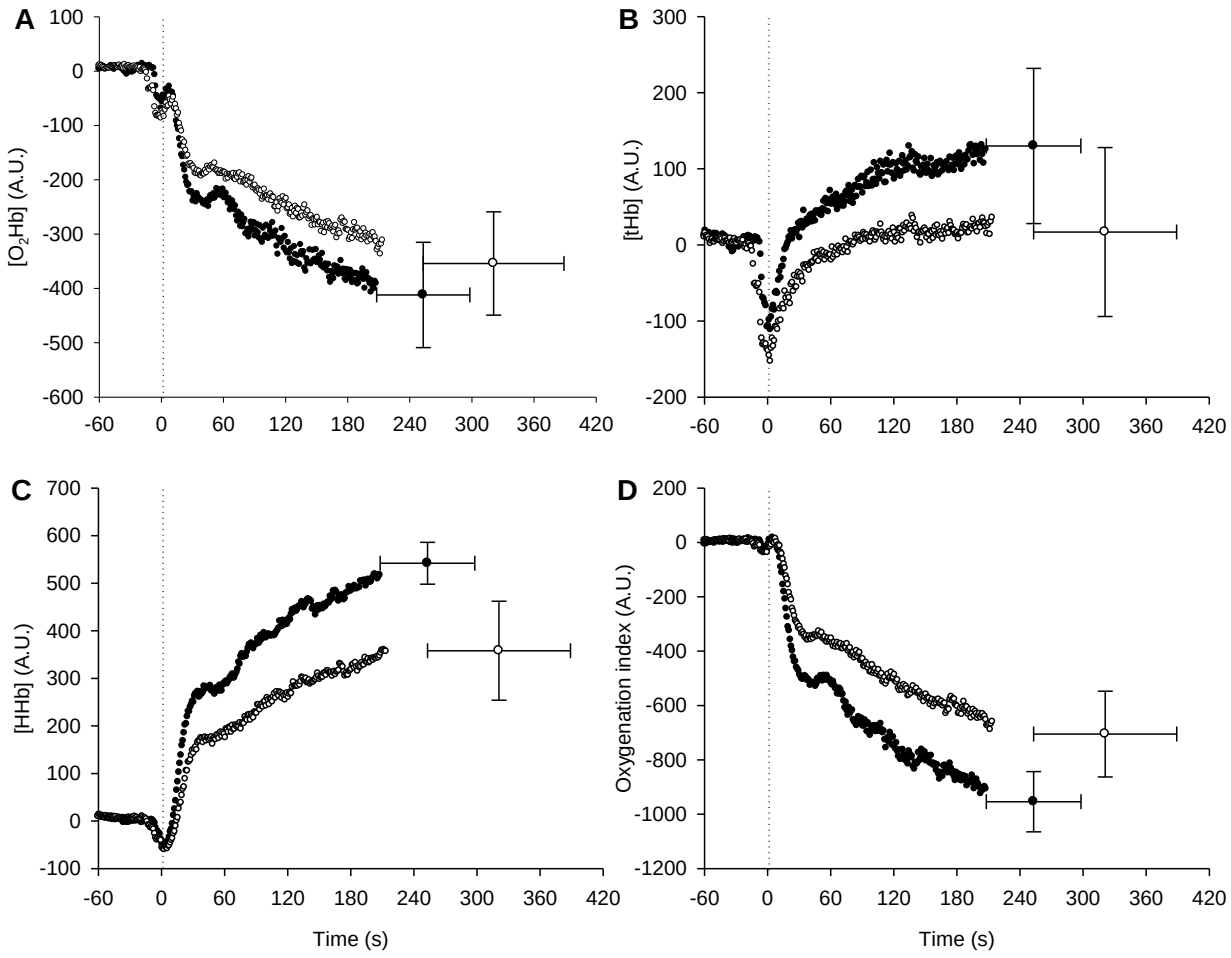
	Normoxia	**Hyperoxia**
Baseline [PCr] (%)	100 ± 0	100 ± 0
Fundamental Amplitude (%)	64 ± 13	58 ± 17
Time constant (s)	23 ± 9	37 ± 14 *
Amplitude / Time Constant (%/s)	2.8 ± 1.3	1.6 ± 1.0 *
Slow Phase Amplitude (%)	25 ± 11	31 ± 15
[PCr] at end-exercise (%)	11 ± 9	10 ± 9
Mean Response Time (s)	59 ± 20	116 ± 46 *

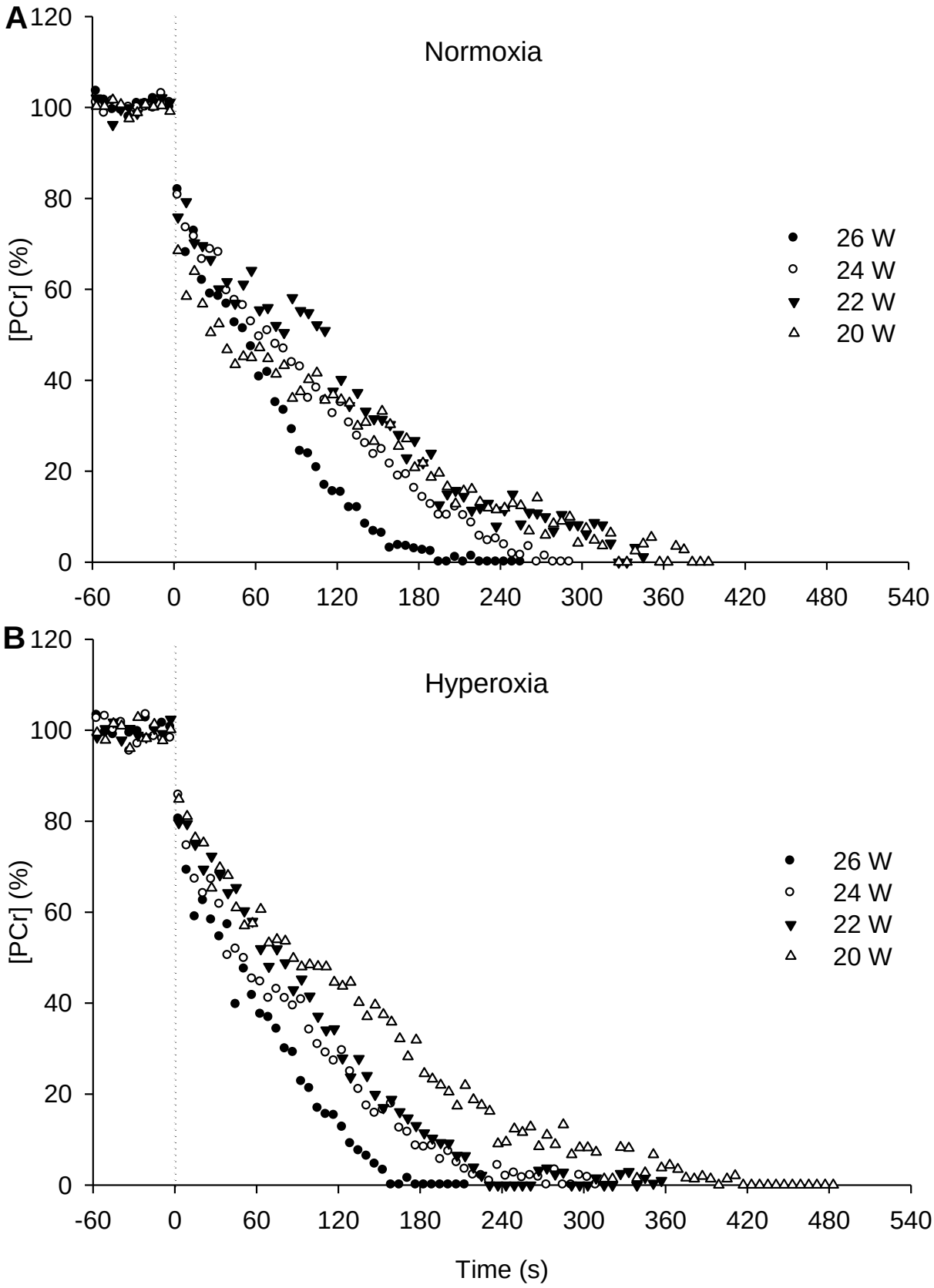
* = $P<0.05$. Time constant refers to the [PCr] kinetics in the fundamental phase of the response whereas mean response time refers to the [PCr] kinetics over the entire exercise bout.

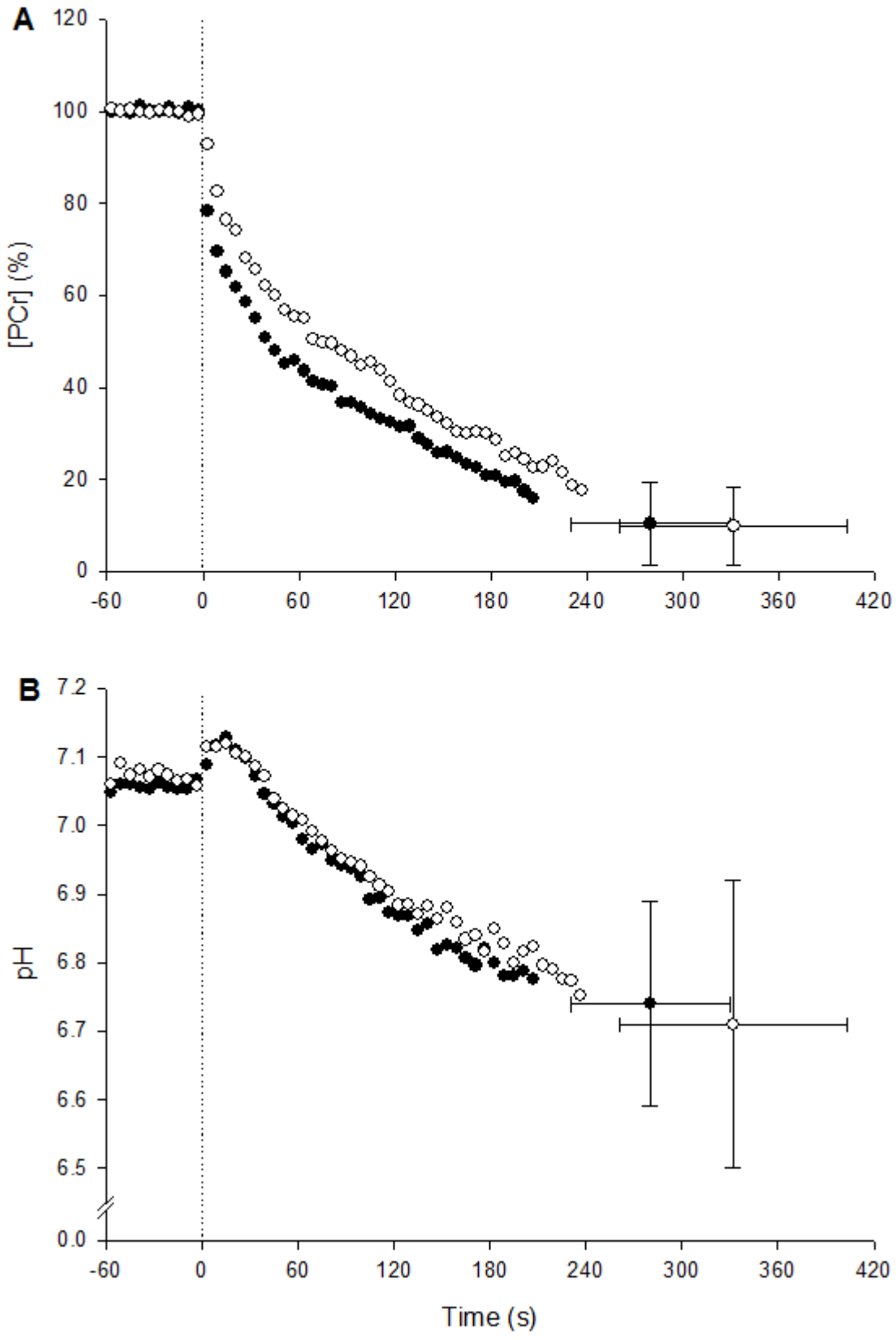
Table 3: ***The CP and W' estimates for each individual in normoxia and hyperoxia***

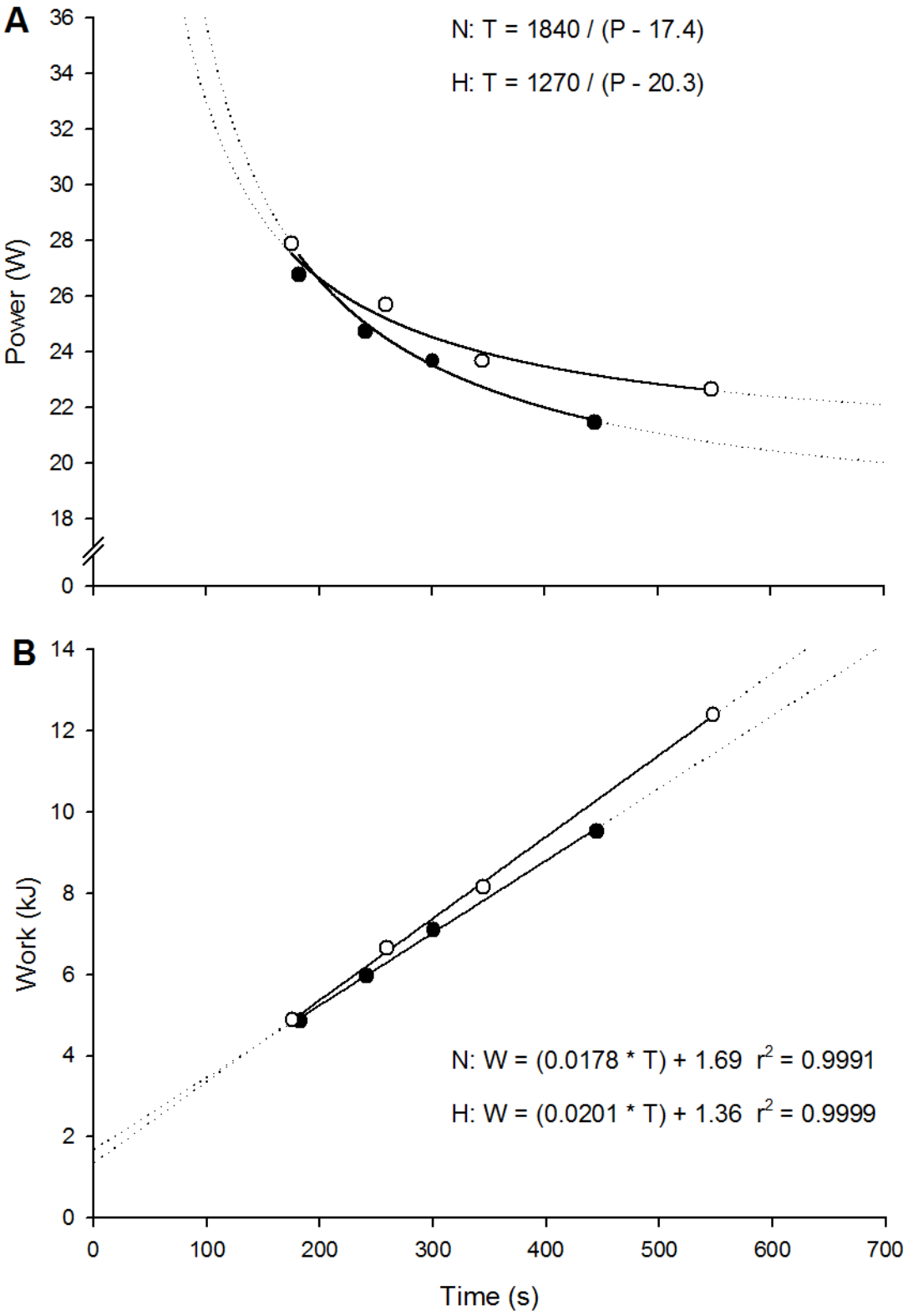
	Critical Power (W)			**W′ (kJ)**		
Subject	***N***	***H***	***Δ (%)***	***N***	***H***	***Δ (%)***
1	17.4	20.3	16.7	1.84	1.27	-31.0
2	18.8	18.9	0.5	1.58	1.56	-0.9
3	17.4	18.8	8.0	2.59	1.91	-26.1
4	18.7	20	7.0	1.78	1.57	-12.0
5	13.4	17.9	33.6	3.11	1.79	-42.6
6	14.9	16.5	10.7	1.45	1.14	-21.3
7	12.3	13.6	10.6	1.10	1.14	3.4
Mean	**16.1**	**18.0 ***	**12.4**	**1.92**	**1.48 ***	**-18.7**
SD	**2.6**	**2.3**	**10.5**	**0.70**	**0.31**	**16.5**

* $P<0.05$. N = normoxia; H = hyperoxia; Δ is the % difference between conditions.


A
B
C
D
[O2Hb] (A.U.)
[tHb] (A.U.)
[HHb] (A.U.)
Oxygenation index (A.U.)
Time (s)
Time (s)


A
Normoxia
[PCr] (%)
120
100
80
60
40
20
0
-60
0
60
120
180
240
300
360
420
480
540
26 W
24 W
22 W
20 W
B
Hyperoxia
[PCr] (%)
Time (s)


A
120
100
80
60
40
20
0
[PCr] (%)
-60
0
60
120
180
240
300
360
420
B
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
0.0
pH
-60
0
60
120
180
240
300
360
420
Time (s)


A
N: T = 1840 / (P - 17.4)
H: T = 1270 / (P - 20.3)
Power (W)
36
34
32
30
28
26
24
22
20
18
0
B
Work (kJ)
14
12
10
8
6
4
2
0
N: W = (0.0178 * T) + 1.69 r2 = 0.9991
H: W = (0.0201 * T) + 1.36 r2 = 0.9999
0
100
200
300
400
500
600
700
Time (s)