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Title: Effect of heavy-intensity 'priming' exercise on oxygen uptake and muscle deoxygenation kinetics during moderate-intensity step-transitions initiated from an elevated work rate

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Highlights

1. Priming exercise speeds O_2 uptake kinetics in those whose kinetics are slow
2. Slower muscle deoxygenation kinetics accompany this faster rate of adjustment
3. O_2 uptake kinetics are slower when initiated from elevated intensities
4. Priming mitigates this work-to-work effect in those with slow but not fast kinetics
5. Mechanistically, the work-to-work effect differs depending on initial kinetics

Abstract

We examined the effect of heavy-intensity 'priming' exercise on the rate of adjustment of pulmonary O_2 uptake ($\tau\dot{V}O_{2p}$) initiated from elevated intensities. Fourteen men (separated into two groups: $\tau\dot{V}O_{2p}\leq25$s [Fast] or $\tau\dot{V}O_{2p}>25$s [Slow]) completed step-transitions from 20W-to-45%lactate threshold (LT; lower-step, LS) and 45%-to-90%LT (upper-step, US) performed (i) without; and (ii) with US preceded by heavy-intensity exercise (HUS). Breath-by-breath $\dot{V}O_{2p}$ and near-infrared spectroscopy-derived muscle deoxygenation ([HHb+Mb]) were measured. Compared to LS, $\tau\dot{V}O_{2p}$ was greater ($p<0.05$) in US in both Fast (LS, 19±4s; US, 30±4s) and Slow (LS, 25±5s; US, 40±11s) with $\tau\dot{V}O_{2p}$ in US being lower ($p<0.05$) in Fast. In HUS, $\tau\dot{V}O_{2p}$ in Slow was reduced (28±8s, $p<0.05$) and was not different ($p>0.05$) from LS or Fast group US. In Slow, τ[HHb+Mb] increased ($p<0.05$) in US relative to HUS; this finding coupled with a reduced $\tau\dot{V}O_{2p}$ indicates a priming-induced improvement in matching of muscle O_2 delivery-to-O_2 utilization during transitions from elevated intensities in those with Slow but not Fast $\dot{V}O_{2p}$ kinetics.

Keywords: O_2 uptake kinetics, near-infrared spectroscopy, muscle oxygenation, priming exercise

Effect of heavy-intensity 'priming' exercise on oxygen uptake and muscle deoxygenation kinetics during moderate-intensity step-transitions initiated from an elevated work rate

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Abstract

We examined the effect of heavy-intensity 'priming' exercise on the rate of adjustment of pulmonary O_2 uptake ($\tau\dot{V}O_{2p}$) initiated from elevated intensities. Fourteen men (separated into two groups: $\tau\dot{V}O_{2p}\leq25$s [Fast] or $\tau\dot{V}O_{2p}>25$s [Slow]) completed step-transitions from 20W-to-45%lactate threshold (LT; lower-step, LS) and 45%-to-90%LT (upper-step, US) performed (i) without; and (ii) with US preceded by heavy-intensity exercise (HUS). Breath-by-breath $\dot{V}O_{2p}$ and near-infrared spectroscopy-derived muscle deoxygenation ([HHb+Mb]) were measured. Compared to LS, $\tau\dot{V}O_{2p}$ was greater ($p<0.05$) in US in both Fast (LS, 19±4s; US, 30±4s) and Slow (LS, 25±5s; US, 40±11s) with $\tau\dot{V}O_{2p}$ in US being lower ($p<0.05$) in Fast. In HUS, $\tau\dot{V}O_{2p}$ in Slow was reduced (28±8s, $p<0.05$) and was not different ($p>0.05$) from LS or Fast group US. In Slow, τ[HHb+Mb] increased ($p<0.05$) in US relative to HUS; this finding coupled with a reduced $\tau\dot{V}O_{2p}$ indicates a priming-induced improvement in matching of muscle O_2 delivery-to-O_2 utilization during transitions from elevated intensities in those with Slow but not Fast $\dot{V}O_{2p}$ kinetics.

Keywords: O_2 uptake kinetics, near-infrared spectroscopy, muscle oxygenation, priming exercise

1. Introduction

When exercise transitions are initiated from a higher compared to lower baseline metabolic rate within the moderate-intensity exercise domain (i.e., intensities that do not engender appreciable lactate accumulation), the "fundamental" (phase II) component of the pulmonary O_2 uptake ($\dot{V}O_{2p}$) response (reflecting the dynamic adjustment of muscle $\dot{V}O_2$) adjusts more slowly (greater phase II $\dot{V}O_{2p}$ time constant, $\tau\dot{V}O_{2p}$) and with a greater $\dot{V}O_{2p}$ gain (larger $\Delta\dot{V}O_{2p}/\Delta WR$) than when the same transition is initiated from a lower baseline (Bowen et al., 2011; Brittain et al., 2001; Hughson and Morrissey, 1982; Keir et al., 2016a, 2016b, 2014; MacPhee et al., 2005; Williams et al., 2013). These responses have been attributed to starting the exercise from a less favourable intramuscular 'energetic state' consequent to the elevated level of muscle metabolism (Bowen et al., 2011; Grassi et al., 2011; Meyer and Foley, 1996; Wüst et al., 2014), to slowed adjustments in convective muscle O_2 delivery to support oxidative metabolism (Hughson and Morrissey, 1982; MacPhee et al., 2005), and to recruitment of motor units which are positioned higher in the muscle recruitment hierarchy and that are comprised of muscle fibre pools having lower metabolic efficiency and slower dynamic adjustment characteristics (Brittain et al., 2001; Wilkerson and Jones, 2006).

In individuals presenting with slower $\dot{V}O_{2p}$ kinetics (i.e., $\tau\dot{V}O_{2p} \geq 20$ s) within the moderate-intensity domain, prior heavy exercise was shown to have a 'priming effect' on the $\dot{V}O_{2p}$ response with kinetics becoming faster (i.e., a lower phase II $\tau\dot{V}O_{2p}$) in the 'primed' compared to the 'unprimed' condition (DeLorey et al., 2007; Gurd et al., 2006, 2005; Murias et al., 2011; Spencer et al., 2013, 2012). Also, the 'speeding' was greater in individuals presenting with slower $\dot{V}O_{2p}$ kinetics in the 'unprimed' condition (Gurd et al., 2009, 2006, 2005; Murias et al., 2014, 2011). This effect has been linked to priming-induced: i) acute improvements in the

coordination of microvascular blood flow and O_2 delivery (Murias et al., 2011; Spencer et al., 2012); ii) acute reductions in the activation time for oxidative phosphorylation (Behnke et al., 2002; Korzeniewski and Rossiter, 2015) through activation of rate limiting enzymes and greater delivery of oxidative substrate to mitochondria (Gurd et al., 2006; Howlett et al., 1999; Timmons et al., 1998); and iii) a combination of both mechanisms (Gurd et al., 2005).

Few studies have examined the effects of heavy-intensity priming exercise on phase II $\dot{V}O_{2p}$ kinetics during transitions from elevated baselines. In those studies, prior exercise was demonstrated to be ineffective at reducing phase II $\tau\dot{V}O_{2p}$ during 'work-to-work' transitions (DiMenna et al., 2008; DiMenna et al., 2009; DiMenna et al., 2010b), suggesting that limitations in local muscle O_2 availability are unlikely to contribute to the response. However, in these studies transitions were from moderate-intensity baselines into either the heavy- or severe-intensity exercise domains where $\dot{V}O_{2p}$ responses (and likely regulation of $\dot{V}O_{2p}$) are markedly different from responses confined within the moderate-intensity domain (Poole et al., 2008). Our group has demonstrated that in individuals with relatively slow $\dot{V}O_{2p}$ kinetics, a prior bout of heavy-intensity exercise results in faster $\dot{V}O_{2p}$ kinetics during the subsequent transition to moderate-intensity exercise (Gurd et al., 2005; Murias et al., 2011; Scheuermann et al., 2002). Therefore, it remains uncertain as to what effect a heavy-intensity priming intervention may have on $\dot{V}O_{2p}$ kinetics when transitioning from elevated baselines within the moderate-intensity domain – particularly in individuals having slow $\dot{V}O_{2p}$ kinetics.

Recently, Williams et al., (2013) reported that four weeks of high-intensity interval training caused a reduction in $\tau\dot{V}O_{2p}$ (relative to pre-training measures) in both the lower- (LS: from 24 s to 14 s) and upper-step (US: from 45 s to 25 s) of a moderate-intensity 'double-step' protocol. The relative speeding of $\dot{V}O_{2p}$ kinetics was accompanied by an unchanged rate of

adjustment in muscle deoxygenation ([HHb+Mb]; derived using near-infrared spectroscopy) in both LS and US (compared to pre-training measures) and that the $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratio 'overshoot' (reflecting a transient decrease in microvascular blood flow-to-muscle O_2 utilization and increased O_2 extraction) was eliminated in US. Despite the apparent rectification of any muscle O_2 delivery limitations in US, $\dot{V}O_{2p}$ kinetics remained slower than LS suggesting that the fundamental cause of slower $\dot{V}O_{2p}$ kinetics may not be related to limitations in regional O_2 delivery.

In light of these observations, we examined the effects of heavy-intensity priming exercise on the $\dot{V}O_{2p}$ and [HHb+Mb] responses to transitions from elevated baseline intensities constrained within the moderate-intensity domain whilst considering individuals with both faster and slower $\dot{V}O_{2p}$ kinetics. Such an experimental design could serve to elucidate the mechanisms contributing to: i) the slowing of $\dot{V}O_{2p}$ kinetics with transitions from elevated baseline intensities; and ii) the speeding of moderate-intensity on-transient kinetics with heavy intensity priming exercise. We hypothesized that: 1) $\tau\dot{V}O_{2p}$ would be greater in US vs LS in both the faster and slower groups; 2) heavy-intensity priming exercise would lead to a reduction in $\tau\dot{V}O_{2p}$ of US (relative to unprimed control) in the slower, but not fast group; 3) the speeding of $\dot{V}O_{2p}$ kinetics in US in the slower group would be accompanied by a slowing in the kinetics of [HHb+Mb].

2. Methods

2.1 Participants. Fourteen healthy young men (age: 25 ± 2 yr; $\dot{V}O_{2peak}$: 49.5 ± 5.3 $mL \cdot kg^{-1} \cdot min^{-1}$; mean ± SD) volunteered and provided written informed consent to participate in this study. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research involving Human Subjects. Participants were recreationally

active non-smokers, who had no known cardiovascular, respiratory, metabolic or musculoskeletal disease and who were not taking any medications that might affect cardiorespiratory and hemodynamic responses to exercise. Participants were instructed not to consume food or caffeine two hours prior to visits to the laboratory for data collection and to avoid exercise 24 hours prior to testing.

2.2 Preliminary Testing. Each participant performed a ramp incremental exercise test (20-25 W/min) to the limit of tolerance on a cycle ergometer (model: H-300-R Lode; Lode B.V., Groningen, The Netherlands) for determination of peak $\dot{V}O_{2p}$ ($\dot{V}O_{2peak}$) and estimated lactate threshold ($\hat{\theta}_L$); the ramp portion of the protocol was initiated after 4 min of baseline cycling at 20 W. Participants were asked to maintain a cycling cadence between 60 - 70 rpm. The $\hat{\theta}_L$ was estimated by visual inspection using a combination of standard gas exchange and ventilatory measures as previously described (Beaver et al., 1986). Each participant was assigned work rates (WR) corresponding to the $\dot{V}O_{2p}$ associated with: i) ~90% $\hat{\theta}_L$ (WR90); ii) 50% of the difference between 20 W and WR90 (WR50); and iii) Δ50% (i.e., WR corresponding to ~50% of the difference between $\hat{\theta}_L$ and $\dot{V}O_{2peak}$).

2.3 Experimental Protocol. Three separate experimental protocols were performed by each participant. Each exercise protocol began with 6 min of baseline cycling at 20 W after which distinct series of step-changes in WR were performed as follows (Fig.1): Protocol A) two step-changes in WR to 90% $\hat{\theta}_L$ (i.e., WR90) (MOD_1, MOD_2) each lasting 6 min, separated by a 6 min bout of heavy-intensity exercise at a WR corresponding to Δ50%, as previously described (Scheuermann et al., 2002); Protocol B) two equal step-changes in WR performed in series from 20W→WR50 (LS) and from WR50→WR90 (US), each lasting 6 min; Protocol C) two equal step-changes in WR performed in series from 20W→WR50 (LS) and from WR50→WR90

(HUS), each lasting 6 min, but separated by a 6 min bout of heavy-intensity exercise at Δ50%, and a subsequent 6 min bout at the previous workload (LS: 20W→WR50, WR50→Δ50%, Δ50%→WR50; HUS: WR50→90% $\hat{\theta}_L$). During all trials, participants maintained a cadence of ~70 rpm. Each participant completed 3-6 repeats of each protocol in a randomized order. The larger amplitude of the $\dot{V}O_{2p}$ response due to a larger increase in WR resulted in each participant completing 3 repeats of Protocol A. In order to ensure a high signal-to-noise ratio for a protocol utilizing smaller $\dot{V}O_{2p}$ amplitude, 6 repeats were performed for Protocols B and C. In all cases, only one exercise trial was performed per visit.

2.4 Data Collection

During each trial participants wore a noseclip and breathed through a mouthpiece for breath-by-breath gas-exchange measurement. Inspired and expired volumes and flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies, VMM 110) and pneumotach (Hans Rudolph, Model 4813) positioned in series from the mouthpiece (total apparatus dead space was 150 mL); respired air was sampled continuously at the mouth and analysed by mass spectrometry (Innovision, AMIS 2000, Lindvedvej, Denmark) for fractional concentrations of O_2, CO_2 and N_2. The volume turbine was calibrated before each test using a syringe of known volume (3 L) over a range of flow rates and the pneumotach was adjusted for zero flow. Gas concentrations were calibrated with precision-analyzed gas mixtures. The time delay between an instantaneous, square-wave change in fractional gas concentration at the sampling inlet and its detection by the mass spectrometer was measured electronically by computer. Respiratory volumes, flows, and gas concentrations were recorded in real-time used to build a profile of each breath. Alveolar gas exchange was calculated on a breath-by-breath basis using the algorithms of Swanson (1980).

2.5 Near-infrared spectroscopy. Local muscle deoxygenation ([HHb+Mb]) of the vastus lateralis muscle was monitored continuously with a frequency-domain multi-distance near-infrared spectroscopy (NIRS) system (Oxiplex TS, Model 95205, ISS, Champaign, IL, USA) as described elsewhere (Spencer et al., 2012). The probe was placed on the belly of the muscle, midway between the lateral epicondyle and greater trochanter of the femur; it was secured in place with an elastic strap and bandage tightened to prevent movement and covered with an optically-dense, black vinyl sheet, thus minimizing the intrusion of extraneous light and loss of NIR light. The NIRS measurements were collected continuously for the entire duration of each trial. Briefly, the system comprised a single channel of eight laser diodes operating at two wavelengths (λ = 690 and 828 nm, four at each wavelength) pulsed in a rapid succession (110 MHz) and a photomultiplier tube. The lightweight plastic NIRS probe (connected to laser diodes and photomultiplier tube by optical fibers) consisted of two parallel rows of light emitter fibers and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths.

The NIRS was calibrated in accordance with manufacturer guidelines at the beginning of each testing session following an instrument warm-up period of at least 20 min. Calculation of [HHb+Mb] reflected continuous measurements of a reduced scattering coefficient (μ_s') made throughout each testing session (i.e., constant scattering value not assumed). To improve the signal-to-noise ratio a moving average was applied to the NIRS signal with a measurement averaging period of 1000 ms and a scatter averaging period of 50,000 ms. Data were stored online at an output frequency of 25 Hz, but were reduced to 1 s bins for all subsequent analyses.

2.6 Data Analysis

Breath-by-breath $\dot{V}O_{2p}$ data were edited by removing data that lay outside 3 SD of the local mean (Lamarra et al., 1987). The remaining data were interpolated to 1 s intervals, and time-aligned such that time "zero" represented the initiation of the step-increase in WR. The remaining data were linearly interpolated on a second-by-second basis, using the protocol where values removed by editing were replaced by data joined by straight-line segments (refer to Keir et al., 2014). Like-trials were ensemble-averaged and further averaged into 5 s time bins. The on-transient responses for $\dot{V}O_{2p}$ and [HHb+Mb] were modelled using the following exponential equation:

$$Y_{(t)} = Y_{BSL} + A\,(1 - e^{-(t-TD)/\tau}) \qquad \text{Equation 1}$$

where $Y_{(t)}$ represents the value of the dependent variable at any given time (t); Y_{BSL} is the steady-state baseline value of Y before an increase in WR (given as the average Y value in the 60 s period immediately prior to a transition); A is the amplitude of the increase in Y above Y_{BSL}; τ is the time constant representing the time to attain 63% of the steady-state amplitude; and TD represents the mathematically generated time delay at which the exponential model is predicted to intersect Y_{BSL}. The functional gain (G) of the phase II $\dot{V}O_{2p}$ response was calculated as $\Delta\dot{V}O_{2pss}/\Delta WR$ ($ml \cdot min^{-1} \cdot W^{-1}$), where $\dot{V}O_{2pss}$ is steady-state increase in $\dot{V}O_{2p}$ above baseline and ΔWR is the change in WR (in W). Data were modelled from the phase I-phase II transition to the end of the 6 min exercise transition using non-linear least-squares regression (Origin 8.5; OriginLab, Northampton, MA). The 95% confidence interval (CI_{95}) for the estimated time constant was determined after preliminary fit of the data with Y_{BSL}, A and TD constrained to the best-fit values and the τ allowed to vary. Phase I was excluded from the fitting window by progressively moving the window (from ~35 s) back towards time zero while examining the flatness of the residual profile and values of CI_{95} [where CI_{95} is equal to the SE (derived from the

sum of squared residuals from the model parameter estimates) multiplied by the t-distribution value for the 2.5% two-tailed dimensions]. The window that yielded the flattest residuals (visual inspection) and most reduced CI_{95} was considered as the mono-exponential region (Rossiter et al., 2001); note that there are other methods that the influence of phase I may be avoided (Murias et al., 2011b). The mean response time (MRT-$\dot{V}O_{2p}$) of $\dot{V}O_{2p}$ was characterized from a fit of the $\dot{V}O_{2p}$ response from t=0 to the end of the exercise. The NIRS-derived [HHb+Mb] profiles were time-aligned and ensemble-averaged into 5 s bins to yield a single response time for each subject. The time-course of adjustment for the [HHb+Mb] profile has been previously described as consisting of a time delay at the onset of exercise, with a subsequent "exponential-like" increase in the signal with time of exercise (DeLorey et al., 2003). The time delay for the [HHb+Mb] response (TD-[HHb+Mb]) was determined visually using second-by-second data and corresponded to the time, after the onset of exercise, at which the [HHb+Mb] signal increased above 1 SD of the pre-transition baseline value. Determination of the TD-[HHb+Mb] was made on individual response profiles and averaged over the number of trial repeats for that individual. The ensemble-averaged [HHb+Mb] responses were modeled from TD-[HHb+Mb] with a monoexponential function of the form in Eq. 1 to determine the time course of muscle [HHb+Mb] (τ[HHb+Mb]). Baseline [HHb+Mb] ($[HHb+Mb]_{BSL}$) values were fixed as the mean value in the 60 s period leading up to a transition; similar to $\dot{V}O_{2p}$ described previously. Whereas the τ[HHb+Mb] describes the time course for the increase in [HHb+Mb], the effective time constant, or MRT, of [HHb+Mb] (MRT-[HHb+Mb] = TD-[HHb+Mb] + τ[HHb+Mb]) described the overall time course of the [HHb+Mb] from the onset of each step transition. The [HHb+Mb] at the end of each step ($[HHb+Mb]_{end\text{-}step}$) was computed from the average of the last 60 s of each step transition.

205 2.7 Statistics

206 Values are presented as mean ± SD. Parameter estimates for $\dot{V}O_{2p}$ and NIRS-derived
207 [HHb+Mb] data using a two-way (Group x Condition) repeated measures analyses of variance
208 (ANOVA) to determine statistical significance for the dependent variables. When interactions
209 were identified, Tukey's post-hoc analysis was used. Pearson product moment correlation
210 coefficients were used to determine the degree of association amongst key variables.

211 All statistical analyses were performed using SigmaPlot 11 (Systat, California, USA).
212 Statistical significance was accepted at $p<0.05$.

213 **3. Results**

214 By design, participants were separated into two groups based on $\dot{V}O_{2p}$ kinetics from
215 MOD1: $\tau\dot{V}O_{2p}$ < 25 s (Fast group; n = 6; $\tau\dot{V}O_{2p}$ range: 19 s - 24 s) and $\tau\dot{V}O_{2p}$ > 25 s (Slow
216 group; n =8; $\tau\dot{V}O_{2p}$ range: 26 s - 48 s). The two groups were not different for $\dot{V}O_{2peak}$ (Fast, 4.04
217 ± 0.21 $L{\cdot}min^{-1}$; Slow, 3.93 ± 0.37 $L{\cdot}min^{-1}$), WR_{peak} (Fast, 345 ± 17 W; Slow, 331 ± 12 W), and
218 $\dot{V}O_{2p}$ and WR associated with $\hat{\theta}_L$ (Fast, 2.17 ± 0.32 $L{\cdot}min^{-1}$ and 131 ± 34 W, respectively; Slow,
219 2.19 ± 0.27 $L{\cdot}min^{-1}$ and 130 ± 27 W, respectively). As such, the WRs corresponding to WR50
220 (i.e., WR used for LS), WR90 (i.e., WR used for MOD, US, and HUS) and Δ50% were not
221 different between the Fast (WR50, 75 ± 17 W; WR90, 131 ± 34 W; Δ50%, 232 ± 34 W) and
222 Slow groups (WR50, 75 ± 13 W; WR90, 130 ± 27 W; Δ50%, 231 ± 30 W). The steady-state
223 $\dot{V}O_{2pss}$ associated with all moderate-intensity WRs did not exceed the $\dot{V}O_{2p}$ corresponding to $\hat{\theta}_L$
224 in any of the participants.

225 *3.1 $\dot{V}O_{2p}$* kinetics

226 3.1.1 MOD1-MOD2 Transition. The $\dot{V}O_{2p}$ kinetic parameter estimates are displayed in
227 Table 1. By design, $\tau\dot{V}O_{2p}$ in MOD1 was lower ($p<0.05$) in Fast (21 ± 2 s) compared to Slow

(32 ± 7 s). After heavy-intensity 'priming' exercise there was no change in $\tau\dot{V}O_{2p}$ in Fast but in Slow $\tau\dot{V}O_{2p}$ was reduced (to 24 ± 2 s; $p<0.05$) and not different from Fast MOD1 and MOD2. The reduction $\tau\dot{V}O_{2p}$ between MOD1 and MOD2 was positively correlated ($r = 0.76$, $p<0.05$) with the initial $\tau\dot{V}O_{2p}$ in MOD1 (Figure 2A).

3.1.2 LS-US Transition. The parameter estimates for the on-transient $\dot{V}O_{2p}$ responses and the group mean ensemble-averaged $\dot{V}O_{2p}$ profiles to LS-US for the Fast and Slow groups are presented in Table 2 and Figure 3 (A, B), respectively. The transition to US was initiated from an elevated $\dot{V}O_{2pbsl}$ and despite identical ΔWR for LS and US, both $\tau\dot{V}O_{2p}$ and G were greater in US compared to LS in both groups ($p<0.05$).

3.1.3 LS-HUS Transition. The parameter estimates for the on-transient $\dot{V}O_{2p}$ responses and the group mean ensemble-averaged $\dot{V}O_{2p}$ profiles to LS-HUS for the Fast and Slow groups are presented in Table 2 and Figure 3 (C, D), respectively. The LS to HUS transition consisted of two equal step-changes in WR performed in series from 20W→WR50 (LS) and from WR50→WR90 (HUS), each lasting 6 min, but separated by a 6 min bout of heavy-intensity exercise at Δ50% and a subsequent 6 min bout at the previous workload. A Student's t-test confirmed that there were no differences in both $\dot{V}O_{2p}$ and NIRS-derived parameter estimates for LS from both protocols B and C ($p<0.05$), therefore these data were averaged into a single value (LS) for all subsequent comparisons

In the Fast group, after 'priming' exercise, $\tau\dot{V}O_{2p}$ remained greater ($p<0.05$) in HUS (30 ± 5 s) than in LS (19 ± 4 s) but was not different from US (30 ± 4 s). However, in the Slow group, 'priming' exercise resulted in a speeding of $\dot{V}O_{2p}$ kinetics such that the $\tau\dot{V}O_{2p}$ in HUS (28 ± 8 s) was not different to LS (21 ± 5 s) and less than the $\tau\dot{V}O_{2p}$ in US (40 ± 11 s) ($p<0.05$). The reduction in $\tau\dot{V}O_{2p}$ between HUS and US was linearly correlated with $\tau\dot{V}O_{2p}$ in the 'unprimed'

US condition ($r = 0.73$; $p<0.05$; Figure 2B). In both the Fast and Slow groups, after the heavy-intensity 'priming' exercise, the G in HUS was not different to LS but lower than in US ($p<0.05$).

3.2 [HHb+Mb] Kinetics

3.2.1 MOD1-MOD2 Transition. The parameter estimates for muscle [HHb+Mb] kinetics are presented in Table 1. There were no differences in any [HHb+Mb] kinetic parameters between the Fast and Slow groups during MOD1 (Table 1). In both groups, the $[HHb+Mb]_{bsl}$ was lower ($p<0.05$) but the $[HHb+Mb]_{amp}$ was greater ($p<0.05$) in MOD2 than in MOD1. Also, in both groups, TD-[HHb+Mb] was shorter ($p<0.05$) and the τ[HHb+Mb] was greater ($p<0.05$) in MOD2 than in MOD1, and as a consequence the overall τ'[HHb+Mb] was not different in the Fast group in MOD1 and MOD2 but was greater ($p<0.05$) in MOD2 in the Slow group (Table 1).

3.2.2 LS-US Transition. The group mean [HHb+Mb] kinetic parameter estimates and group mean profiles for the LS-US transition are displayed in Table 3 and Figure 4A. For both groups, the $[HHb+Mb]_{bsl}$ was elevated ($p<0.05$) and the $[HHb+Mb]_{amp}$ was not different in US compared to LS. Also, in both groups, a shorter TD-[HHb+Mb] ($p<0.05$) and greater τ[HHb+Mb] ($p<0.05$) in US than in LS resulted in a not different overall τ'[HHb+Mb] in US and LS. However, while the τ[HHb+Mb] and τ'[HHb+Mb] were not different for both groups in LS, during US the τ[HHb+Mb], but not τ'[HHb+Mb], was shorter ($p<0.05$) in the Slow than in the Fast group (Table 3).

3.2.3 LS-HUS Transition. In both groups, TD-[HHb+Mb] was shorter ($p<0.05$) and τ[HHb+Mb] was greater ($p<0.05$) in HUS than in LS (Table 3). τ[HHb+Mb] in HUS and US were not different in the Fast group, but in the Slow group τ[HHb+Mb] was greater ($p<0.05$) in HUS than in US (Table 3). Also, in the Fast group, the overall τ'[HHb+Mb] was not different in

HUS, LS and US, but in the Slow group, τ'[HHb+Mb] was greater ($p<0.05$) in HUS compared to both LS and US, and was greater ($p<0.05$) than the Fast group in HUS but not US.

4. Discussion

In this study, the effect of heavy-intensity 'priming' exercise on $\dot{V}O_{2p}$ and muscle deoxygenation kinetics was examined in response to moderate-intensity step-transitions initiated from a raised baseline WR in individuals with slow, compared to fast, $\dot{V}O_{2p}$ kinetics. Young adults (mean age, 25 yrs) were grouped according to whether they expressed slower ($\tau\dot{V}O_{2p} > 25$ s) or faster ($\tau\dot{V}O_{2p} < 25$ s) $\dot{V}O_{2p}$ kinetics based on a preliminary step-transition to a WR corresponding to ~90% $\hat{\theta}_L$ (WR90). Consistent with previous studies (Bowen et al., 2011; Brittain et al., 2001; Keir et al., 2016b, 2014; MacPhee et al., 2005; Williams et al., 2013) all participants in both the Fast and Slow groups demonstrated a greater phase II $\tau\dot{V}O_{2p}$ and greater G when step-transitions of similar ΔWR were initiated from a high (US) compared to a lower (LS) baseline intensity, each within the moderate-intensity domain. The novel finding was that heavy-intensity 'priming' exercise was effective in reducing $\tau\dot{V}O_{2p}$ (i.e., speeding $\dot{V}O_{2p}$ kinetics) in the Slow group, but not the Fast group, during exercise on-transitions from low (MOD1 → MOD2: Slow, 32 s → 24 s; Fast, 21 s → 21 s) and elevated baseline WRs (US → HUS: Slow, 40 s → 28 s; Fast, 30 s → 30 s). The speeding of VO_{2p} kinetics after 'priming' exercise in the Slow group was accompanied by a slowing of [HHb+Mb] kinetics (longer τ and τ') suggesting that the dynamics of muscle O_2 utilization were enhanced consequent to improved muscle perfusion which reduces the reliance on O_2 extraction during the early transition to exercise

These findings suggest that for the Slow group, but not the Fast group, any limitation imposed on the adjustment of muscle O_2 utilization during US was overcome consequent to a

bout of heavy-intensity ‘priming’ exercise. Furthermore, after ‘priming’ exercise, the $\dot{V}O_{2p}$ kinetics in US were not different from LS for the Slow group.

That $\dot{V}O_{2p}$ kinetics were faster in LS compared to US is consistent with other studies that examined “multi-step” exercise within the moderate-intensity domain (Bowen et al., 2011; Brittain et al., 2001; Keir et al., 2016b, 2014; MacPhee et al., 2005; Williams et al., 2013). Furthermore, the finding that heavy-intensity ‘priming’ exercise was associated with a greater reduction in $\tau\dot{V}O_{2p}$ during subsequent moderate-intensity exercise in those individuals having “slower” compared to “faster” $\dot{V}O_{2p}$ kinetics also is consistent with previous findings (Chin et al., 2010; Gurd et al., 2009, 2006, 2005; Murias et al., 2011). Therefore, this discussion will focus on the novel finding: a bout of heavy-intensity priming exercise is effective at speeding $\dot{V}O_{2p}$ kinetics of moderate-intensity work-to-work transitions in those with slow but not fast $\dot{V}O_{2p}$ kinetics.

In the present study, a bout of heavy-intensity 'priming' exercise resulted in a speeding of $\dot{V}O_{2p}$ kinetics in HUS in those with slow (>25 s) $\dot{V}O_{2p}$ kinetics. In this group, the ‘priming’ bout resulted in both faster $\dot{V}O_{2p}$ kinetics in HUS ($\tau\dot{V}O_{2p}$ ~28s) relative to US ($\tau\dot{V}O_{2p}$ ~40s) and an increase in both τ[HHb+Mb] and τ’[HHb+Mb] in HUS relative to US (τ[HHb+Mb]: 14 vs 24 s and τ’[HHb+Mb] : 21 vs 26 s, for US vs HUS, respectively). The faster adjustment of muscle O_2 utilization (inferred from the smaller phase II $\tau\dot{V}O_{2p}$) was associated with an earlier onset of muscle deoxygenation (smaller TD-[HHb+Mb]) but a slower time course of fractional O_2 extraction (larger τ[HHb+Mb] and τ’[HHb+Mb]) suggesting that muscle microvascular perfusion likely was enhanced in US after ‘priming’ exercise (a slower rate of deoxygenation in the presence of faster rate of muscle O_2 utilization is consistent with a greater O_2 delivery). MacPhee et al., (2005) demonstrated that transitions of moderate-intensity knee-extension

exercise initiated from high vs. low baseline metabolic rates were associated with slower kinetics of both $\dot{V}O_{2p}$ and femoral (conduit) artery blood flow, as well as a lower steady-state blood flow-to-$\dot{V}O_{2p}$ ratio. Heavy-intensity 'priming' exercise could contribute to speeding of $\dot{V}O_{2p}$ kinetics during a subsequent US exercise bout via i) greater bulk muscle (conduit artery) blood flow and local muscle microvascular blood flow and O_2 delivery, ii) rightward-shift of the oxyhemoglobin dissociation curve (induced via changes in acidosis, PCO_2 and temperature), iii) greater O_2 flux from the capillary into muscle consequent to a greater muscle microvascular blood flow-to-$\dot{V}O_2$ ratio facilitating a greater capillary PO_2 and O_2 driving pressure, iv) improved muscle O_2 diffusing capacity related to an increase in functional capillary surface area (i.e., related to a greater capillary red blood cell volume in contact with the muscle membrane), v) greater intracellular PO_2 and O_2 flux across the mitochondrial membrane, or vi) more rapid activation of rate-limiting oxidative enzymes and/or enhanced delivery of oxidative substrate to the mitochondrial tricarboxylic acid (TCA) cycle and electron transport system (ETS) (Gerbino et al., 1996; Burnley et al., 2000; DiMenna et al., 2010c; Spencer et al., 2012 Gurd et al., 2006; Gurd et al., 2009). Our data suggest that microvascular blood flow distribution may have been improved in HUS and, at least in part, contributed to a speeding of $\dot{V}O_{2p}$ kinetics during work-to-work transitions, but in the Slow group only. However, faster adjustments and flux through metabolic pathways in the Slow, but not the Fast, group cannot be discounted. Alterations of microvascular blood flow distribution between the Slow and Fast groups may accompany our observation that young, healthy individuals can present with a broad range of initial $\tau\dot{V}O_{2p}$ values, in agreement with previous findings (Murias et al., 2011; Nederveen et al., 2014). Although an inverse association between $\tau\dot{V}O_{2p}$ and $\dot{V}O_{2peak}$ has been reported previously (Chillibeck et al., 1996; Gurd et al., 2005; Murias et al., 2011), in the present study no

relationship was observed between fitness ($\dot{V}O_{2peak}$) and $\tau\dot{V}O_{2p}$ (-0.43, $p>0.05$) - in fact, some individuals presenting with the highest $\dot{V}O_{2peak}$ (e.g., 56 mL kg^{-1} min^{-1}) were in the Slow group (i.e., τ $\dot{V}O_{2p}$ = 39 s). Findings by Wust et al. (2013) suggest that the $\dot{V}O_{2p}$ rate constant ($k = 1/\tau$) in single muscle cells is linearly correlated with the cellular $\dot{V}O_{2max}$, a finding that is reflected at the whole body level across multiple species ranging widely in $\dot{V}O_{2max}$ (reviewed by Poole and Jones, 2012). In the present study, we did not find a significant relationship between k and $\dot{V}O_{max}$ ($R = 0.362$, $p > 0.05$), perhaps as a consequence of the relatively narrow ranges for both $\dot{V}O_{max}$ and $\tau\dot{V}O_{2p}$ (and thus k). Although not a focus of the present study, the lack of relationship between measures of fitness and $\dot{V}O_{2p}$ kinetics (as assessed by the tau or k) may not be surprising, leading one to speculate that the speed of adjustment of $\dot{V}O_{2p}$ might be an important independent predictor of health, fitness and tolerance for exercise and daily activities (Rossiter, 2011).

We make the observation that while the Slow group exhibited faster $\dot{V}O_{2p}$ kinetics following 'priming' exercise, $\dot{V}O_{2p}$ kinetics were not affected in the Fast group – this despite the $\tau\dot{V}O_{2p}$ being ~60% greater in US than in LS. Furthermore, in Fast, [HHb+Mb] kinetics were not different between US and HUS. Taken together, it appears that any priming-induced increase in O_2 delivery may contribute (but not completely eliminate) the slower $\dot{V}O_{2p}$ kinetics in the HUS in the Slow group, but have no effect on HUS $\dot{V}O_{2p}$ kinetics in Fast. However, there appears to a limit to these improvements in O_2 delivery in Slow (~28s $\tau\dot{V}O_{2p}$) following priming, such that they are similar to observed in Fast (~30s $\tau\dot{V}O_{2p}$). Therefore, regardless of whether or not individuals were sped following a priming bout of exercise, these data suggest that there may be mediators other than O_2 delivery that limit the adjustment to mitochondrial oxidative phosphorylation and contribute to slowed $\dot{V}O_{2p}$ when exercise transitions are initiated from an

elevated metabolic rate. A raised metabolic rate in muscle is associated with a disruption to the metabolic "stability" within the active muscle (i.e., increased muscle $[H^+]$, $[ADP_{free}]$, $[P_i]$, $[AMP_{free}]$, $[IMP_{free}]$, and reduction in [PCr], [ATP], and less negative ΔG_{ATP}), and presumably would occur in individuals regardless of whether their initial $\tau\dot{V}O_{2p}$ could be considered fast or slow. Collectively, a greater perturbation of the metabolic environment prior to the onset of exercise transitions, in part, may be responsible for greater $\tau\dot{V}O_{2p}$ elicited during transitions from elevated levels of metabolism.

Alternatively, transitions from elevated baseline metabolic rates recruits additional motor units, and thus the slower $\dot{V}O_{2p}$ kinetics and greater G may reflect the metabolic and contractile characteristics of the newly recruited muscle fibres (Brittain et al., 2001; Wilkerson and Jones 2006; Keir et al., 2016a, Keir et al., 2016b). In this scenario, according to Henneman's size principle of motor unit recruitment, muscle fibres associated with low threshold motor units, possessing lower $\tau\dot{V}O_2$ and G, would be recruited preferentially with exercise transitions initiated for a lower baseline WRs (e.g., LS) with muscle fibres having greater $\tau\dot{V}O_{2p}$ and G characteristics being recruited from transitions initiated from higher baseline WRs (e.g., as in US and HUS) (Rossiter, 2011). Also, higher order muscle units may have metabolic profiles that are less oxidative and which are perfused by vascular units having lower and slower contraction-induced hyperemic responses (Behnke et al., 2003; Ferreira et al., 2006; McDonough et al., 2005), and are be associated with slower $\dot{V}O_{2p}$ kinetics in both human (Barstow et al., 1996; Pringle et al., 2003) and animal (Crow and Kushmerick, 1982; Wüst et al., 2013) models. Therefore, 'priming' exercise should be most effective in situations where $\dot{V}O_{2p}$ kinetics are slowed because of inadequate muscle O_2 availability.

In conclusion, we showed that a bout of heavy-intensity 'priming' exercise was associated with a speeding of $\dot{V}O_{2p}$ kinetics (smaller $\tau\dot{V}O_{2p}$) in individuals presenting with slower (Slow, $\tau\dot{V}O_{2p}$ > 25s), but not faster (Fast, $\tau\dot{V}O_{2p}$ < 25s), $\dot{V}O_{2p}$ kinetics during step-transitions into the moderate-intensity domain initiated from an elevated baseline metabolic rate. This reduction in $\tau\dot{V}O_{2p}$ subsequent to the 'priming' exercise also was associated with a slower rate of muscle deoxygenation (i.e., increased τ- and τ'-[HHb+Mb]) in the Slow, but not the Fast, group suggesting that improved microvascular O_2 delivery and distribution within the active muscle fibers during the exercise on-transient may have contributed to the faster adjustment of $\dot{V}O_{2p}$. However, in the Fast group microvascular O_2 delivery appears not to limit muscle $\dot{V}O_2$ kinetics.

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References

Barstow, T.J., Jones, A.M., Nguyen, P.H., Casaburi, R., 1996. Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. J. Appl. Physiol. 81, 1642–1650.

Beaver, W.L., Wasserman, K., Whipp, B.J., 1986. A new method for detecting anaerobic threshold by gas exchange. J. Appl. Physiol. 60, 2020–2027.

Behnke, B.J., Kindig, C.A., Musch, T.I., Sexton, W.L., Poole, D.C., 2002. Effects of prior contractions on muscle microvascular oxygen pressure at onset of subsequent contractions. J. Physiol. 539, 927–934. doi:10.1113/jphysiol.2001.013165

Behnke, B.J., McDonough, P., Padilla, D.J., Musch, T.I., Poole, D.C., 2003. Oxygen exchange profile in rat muscles of contrasting fibre types. J. Physiol. 549, 597–605. doi:10.1113/jphysiol.2002.035915

Bowen, T.S., Murgatroyd, S.R., Cannon, D.T., Cuff, T.J., Lainey, A.F., Marjerrison, A.D., Spencer, M.D., Benson, A.P., Paterson, D.H., Kowalchuk, J.M., Rossiter, H.B., 2011. A raised metabolic rate slows pulmonary O_2 uptake kinetics on transition to moderate-intensity exercise in humans independently of work rate. Exp. Physiol. 96, 1049–1061. doi:10.1113/expphysiol.2011.058321

Brittain, C.J., Rossiter, H.B., Kowalchuk, J.M., Whipp, B.J., 2001. Effect of prior metabolic rate on the kinetics of oxygen uptake during moderate-intensity exercise. Eur. J. Appl. Physiol. 86, 125–134.

Burnley, M., Jones, a M., Carter, H., Doust, J.H., 2000. Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. J. Appl. Physiol. 89, 1387–1396.

Chilibeck, P.D., Paterson, D.H., Petrella, R.J., Cunningham, D.A., 1996. The influence of age and cardiorespiratory fitness on kinetics of oxygen uptake. Canadian journal of applied physiology = Revue canadienne de physiologie appliquee 21, 185-196.

Chin, L.M., Heigenhauser, G.J., Paterson, D.H., Kowalchuk, J.M., 2010. Effect of hyperventilation and prior heavy exercise on O2 uptake and muscle deoxygenation kinetics during transitions to moderate exercise. Eur. J. Appl. Physiol. 108, 913–925. doi:10.1007/s00421-009-1293-1

Crow, M.T., Kushmerick, M.J., 1982. Chemical energetics of slow- and fast-twitch muscles of the mouse. J. Gen. Physiol. 79, 147–166.

DeLorey, D.S., Kowalchuk, J.M., Heenan, A.P., Dumanoir, G.R., Paterson, D.H., 2007. Prior exercise speeds pulmonary O2 uptake kinetics by increases in both local muscle O2 availability and O2 utilization. J. Appl. Physiol. (Bethesda, Md. 1985) 103, 771–778. doi:10.1152/japplphysiol.01061.2006

DeLorey, D.S., Kowalchuk, J.M., Paterson, D.H., 2003. Relationship between pulmonary O2 uptake kinetics and muscle deoxygenation during moderate-intensity exercise. J. Appl. Physiol. (Bethesda, Md. 1985) 95, 113–120. doi:10.1152/japplphysiol.00956.2002

DiMenna, F.J., Fulford, J., Bailey, S.J., Vanhatalo, A., Wilkerson, D.P., Jones, A.M., 2010. Influence of priming exercise on muscle [PCr] and pulmonary O2 uptake dynamics during

"work-to-work" knee-extension exercise. Respir. Physiol. Neurobiol. 172, 15–23. doi:10.1016/j.resp.2010.04.017

DiMenna, F.J., Wilkerson, D.P., Burnley, M., Bailey, S.J., Jones, A.M., 2010. Priming exercise speeds pulmonary O2 uptake kinetics during supine "work-to-work" high-intensity cycle exercise. J. Appl. Physiol. 108, 283–292. doi:10.1152/japplphysiol.01047.2009

DiMenna, F.J., Wilkerson, D.P., Burnley, M., Jones, A.M., 2008. Influence of priming exercise on pulmonary O2 uptake kinetics during transitions to high-intensity exercise from an elevated baseline. J. Appl. Physiol. 105, 538 –546. doi:10.1152/japplphysiol.90357.2008.

DiMenna, F.J.J., Wilkerson, D.P.P., Burnley, M., Jones, A.M.M., Bailey, S.J., Jones, A.M.M., 2009. Influence of priming exercise on pulmonary O2 uptake kinetics during transitions to high-intensity exercise at extreme pedal rates. J. Appl. Physiol. 106, 432–442. doi:10.1152/japplphysiol.91195.2008

Ferreira, L.F., McDonough, P., Behnke, B.J., Musch, T.I., Poole, D.C., 2006. Blood flow and O2 extraction as a function of O2 uptake in muscles composed of different fiber types. Respir. Physiol. Neurobiol. 153, 237–249. doi:10.1016/j.resp.2005.11.004

Gerbino, A., Ward, S.A., Whipp, B.J., 1996. Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. J. Appl. Physiol. (Bethesda, Md. 1985) 80, 99–107.

Grassi, B., Porcelli, S., Salvadego, D., Zoladz, J.A., 2011. Slow VO_2 kinetics during moderate-intensity exercise as markers of lower metabolic stability and lower exercise tolerance. Eur. J. Appl. Physiol. 111, 345–355. doi:10.1007/s00421-010-1609-1; 10.1007/s00421-010-1609-1

Gurd, B.J., Peters, S.J., Heigenhauser, G.J.F., LeBlanc, P.J., Doherty, T.J., Paterson, D.H., Kowalchuk, J.M., 2009. Prior heavy exercise elevates pyruvate dehydrogenase activity and muscle oxygenation and speeds O2 uptake kinetics during moderate exercise in older adults. Am. J. Physiol. Regul. Integr. Comp. Physiol. 297, R877–84. doi:10.1152/ajpregu.90848.2008; 10.1152/ajpregu.90848.2008

Gurd, B.J., Peters, S.J., Heigenhauser, G.J.F., LeBlanc, P.J., Doherty, T.J., Paterson, D.H., Kowalchuk, J.M., 2006. Prior heavy exercise elevates pyruvate dehydrogenase activity and speeds O2 uptake kinetics during subsequent moderate-intensity exercise in healthy young adults. J. Physiol. 577, 985–996. doi:10.1113/jphysiol.2006.112706

Gurd, B.J., Scheuermann, B.W., Paterson, D.H., Kowalchuk, J.M., 2005. Prior heavy-intensity exercise speeds VO2 kinetics during moderate-intensity exercise in young adults. J. Appl. Physiol. (Bethesda, Md. 1985) 98, 1371–1378. doi:10.1152/japplphysiol.01028.2004

Howlett, R.A., Heigenhauser, G.J., Hultman, E., Hollidge-Horvat, M.G., Spriet, L.L., 1999. Effects of dichloroacetate infusion on human skeletal muscle metabolism at the onset of exercise. Am. J. Physiol. 277, E18–25.

Hughson, R.L., Morrissey, M., 1982. Delayed kinetics of respiratory gas exchange in the transition from prior exercise. J. Appl. Physiol. 52, 921–929.

Keir, D.A., Benson, A.P., Love, L.K., Robertson, T.C., Rossiter, H.B., Kowalchuk, J.M., 2016a. Influence of muscle metabolic heterogeneity in determining the VO_{2p} kinetic response to ramp-incremental exercise. J. Appl. Physiol. 120, 503–513.

491 doi:10.1152/japplphysiol.00804.2015

492 Keir, D.A., Nederveen, J.P., Paterson, D.H., Kowalchuk, J.M., 2014. Pulmonary O_2 uptake
493 kinetics during moderate-intensity exercise transitions initiated from low versus elevated
494 metabolic rates: insights from manipulations in cadence. Eur. J. Appl. Physiol. 114, 2655–
495 2665. doi:10.1007/s00421-014-2984-9

496 Keir, D.A., Robertson, T.C., Benson, A.P., Rossiter, H.B., Kowalchuk, J.M., 2016b. The
497 influence of metabolic and circulatory heterogeneity on the expression of pulmonary VO2
498 kinetics in humans. Exp. Physiol. 101, 176–192. doi:10.1113/EP085338

499 Korzeniewski, B., Rossiter, H.B., 2015. Each-step activation of oxidative phosphorylation is
500 necessary to explain muscle metabolic kinetic responses to exercise and recovery in
501 humans. J. Physiol. 593, 5255–68. doi:10.1113/JP271299

502 Lamarra, N., Whipp, B.J., Ward, S.A., Wasserman, K., 1987. Effect of interbreath fluctuations
503 on characterizing exercise gas exchange kinetics. J. Appl. Physiol. 62, 2003–2012.

504 MacPhee, S.L., Shoemaker, J.K., Paterson, D.H., Kowalchuk, J.M., 2005. Kinetics of O_2 uptake,
505 leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower
506 region of the moderate-intensity exercise domain. J. Appl. Physiol. 99, 1822–1834.
507 doi:10.1152/japplphysiol.01183.2004

508 McDonough, P., Behnke, B.J., Padilla, D.J., Musch, T.I., Poole, D.C., 2005. Control of
509 microvascular oxygen pressures in rat muscles comprised of different fibre types. J. Physiol.
510 563, 903–913. doi:10.1113/jphysiol.2004.079533

511 Meyer, R.A., Foley, J.M., 1996. Cellular Processes integrating the metabolic response to
512 exercise, in: Rowell, L.B., Shepherd, J.T. (Eds.), Handbook of Physiology. Exercise:
513 Regulation and Integration of Multiple Systems. Am. Physiol. Soc., Bethesda, MD, pp.
514 841–869.

515 Murias, J.M., Spencer, M.D., Delorey, D.S., Gurd, B.J., Kowalchuk, J.M., Paterson, D.H., 2011.
516 Speeding of VO_2 kinetics during moderate-intensity exercise subsequent to heavy-intensity
517 exercise is associated with improved local O_2 distribution. J. Appl. Physiol. 111, 1410–
518 1415. doi:10.1152/japplphysiol.00607.2011.

519 Murias, J.M., Spencer, M.D., Kowalchuk, J.M., Paterson, D.H., 2011b. Influence of phase I
520 duration on phase II VO2 kinetics parameter estimates in older and young adults. American
521 journal of physiology. Regulatory, integrative and comparative physiology 301, R218-224.

522 Murias, J.M., Spencer, M.D., Paterson, D.H., 2014. The critical role of O_2 provision in the
523 dynamic adjustment of oxidative phosphorylation. Exerc. Sport Sci. Rev. 42, 4–11.
524 doi:10.1249/JES.0000000000000005; 10.1249/JES.0000000000000005

525 Nederveen, J.P., Major, B., Paterson, D.H., Murias, J.M., 2014. Faster VO(2) kinetics after
526 eccentric contractions is explained by better matching of O(2) delivery to O(2) utilization.
527 European journal of applied physiology 114, 2169-2181.

528 Poole, D.C., Barstow, T.J., McDonough, P., Jones, A.M., 2008. Control of oxygen uptake during
529 exercise. Med. Sci. Sports Exerc. 40, 462–474. doi:10.1249/MSS.0b013e31815ef29b;
530 10.1249/MSS.0b013e31815ef29b

531 Pringle, J.S.M., Doust, J.H., Carter, H., Tolfrey, K., Jones, A.M., 2003. Effect of pedal rate on
532 primary and slow-component oxygen uptake responses during heavy-cycle exercise. J.

Appl. Physiol. (Bethesda, Md. 1985) 94, 1501–1507. doi:10.1152/japplphysiol.00456.2002

Rossiter, H.B., Ward, S.A., Kowalchuk, J.M., Howe, F.A., Griffiths, J.R., Whipp, B.J., 2001. Effects of prior exercise on oxygen uptake and phosphocreatine kinetics during high-intensity knee-extension exercise in humans. The Journal of physiology 537, 291-303.

Rossiter, H.B., 2011. Exercise: Kinetic Considerations for Gas Exchange. Compr. Physiol. 1, 203–244.

Scheuermann, B.W., Bell, C., Paterson, D.H., Barstow, T.J., Kowalchuk, J.M., 2002. Oxygen uptake kinetics for moderate exercise are speeded in older humans by prior heavy exercise. J. Appl. Physiol. 92, 609–616. doi:10.1152.japplphysiol.00186.2001

Spencer, M.D., Keir, D.A., Nederveen, J.P., Murias, J.M., Kowalchuk, J.M., Paterson, D.H., 2013. Prolonged moderate-intensity exercise oxygen uptake response following heavy-intensity priming exercise with short- and longer-term recovery. Appl. Physiol. Nutr. Metab. 38, 566–573. doi:10.1139/apnm-2012-0244; 10.1139/apnm-2012-0244

Spencer, M.D., Murias, J.M., Grey, T.M., Paterson, D.H., 2012. Regulation of VO2 kinetics by O2 delivery: insights from acute hypoxia and heavy-intensity priming exercise in young men. J. Appl. Physiol. (Bethesda, Md. 1985) 112, 1023–1032. doi:10.1152/japplphysiol.01215.2011

Swanson, G.D., 1980. Breath-to-breath considerations for gas exchange kinetics, in: Cerretelli, P., Whipp, B.J. (Eds.), Exercise Bioenergetics and Gas Exchange. Elsevier, Amsterdam, pp. 211–222.

Timmons, J.A., Gustafsson, T., Sundberg, C.J., Jansson, E., Greenhaff, P.L., 1998. Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. Am. J. Physiol. 274, E377–80.

Wilkerson, D.P., Jones, A.M., 2006. Influence of initial metabolic rate on pulmonary O_2 uptake on-kinetics during severe intensity exercise. Respir. Physiol. Neurobiol. 152, 204–219. doi:10.1016/j.resp.2005.10.001

Williams, A.M., Paterson, D.H., Kowalchuk, J.M., 2013. High-intensity interval training speeds the adjustment of pulmonary O_2 uptake, but not muscle deoxygenation, during moderate-intensity exercise transitions initiated from low and elevated baseline metabolic rates. J. Appl. Physiol. 114, 1550–1562. doi:10.1152/japplphysiol.00575.2012

Wüst, R.C., McDonald, J.R., Sun, Y., Ferguson, B.S., Rogatzki, M.J., Spires, J., Kowalchuk, J.M., Gladden, L.B., Rossiter, H.B., 2014. Slowed muscle oxygen uptake kinetics with raised metabolism are not dependent on blood flow or recruitment dynamics. J. Physiol. 592, 1857–1871. doi:10.1113/jphysiol.2013.267476

Wüst, R.C.I., van der Laarse, W.J., Rossiter, H.B., 2013. On-off asymmetries in oxygen consumption kinetics of single Xenopus laevis skeletal muscle fibres suggest higher-order control. J. Physiol. 591, 731–744. doi:10.1113/jphysiol.2012.241992

570 **Figure Captions**

571

572 **Figure 1.** Schematic of three experimental exercise protocols. Left panel: MOD1 – Hvy –
573 MOD2 protocol; MOD, moderate-intensity exercise (~90% $\hat{\theta}_L$), Hvy, heavy-intensity "priming"
574 exercise. Middle panel: LS – US protocol; LS (~45% $\hat{\theta}_L$), US (~90% $\hat{\theta}_L$). Right panel: LS –
575 Hvy – HUS protocol; LS (~45% $\hat{\theta}_L$), Hvy, HUS (~90% $\hat{\theta}_L$). By design, work rates at both LS
576 and US/HUS were identical for participants.

577

578 **Figure 2.** Panel (A); relationship between the changes (Δ) in $\tau\dot{V}O_{2p}$ from MOD1 to MOD2 and
579 initial MOD1 $\tau\dot{V}O_{2p}$ ($p<0.05$). Panel (B); relationship between the changes (Δ) in $\tau\dot{V}O_{2p}$ from US
580 to HUS and initial US $\tau\dot{V}O_{2p}$ ($p<0.05$). Open circles denote individual data, filled square denotes
581 group mean ± SD). Dashed diagonal line on each graph represents the line of best fit.

582

583 **Figure 3.** Ensemble average group mean responses (~5s average, open circles) for $\dot{V}O_{2p}$ in
584 response to experimental conditions. Vertical dashed lines indicate the onset of the work
585 transition. The group mean phase II $\dot{V}O_{2p}$ kinetic response for each condition are superimposed
586 over the data (black lines, fitted with a mono-exponential function). $\tau\dot{V}O_{2p}$ values (±SD) are inset
587 under each transition and residuals are shown about y = 0. Panel (A) denotes the Slow group (n =
588 8) response to LS-US transitions; Panel (C) denotes the Slow group response to LS-Hvy-HUS
589 transitions. Panel (B) denotes the Fast group (n = 6) response to LS-US transitions; Panel (D)
590 denotes the Fast group response to LS-Hvy-HUS transitions

591

592 **Figure 4.** Ensemble average group mean response for the adjustments of deoxyhemoglobin
593 concentration ([HHb+Mb], μm) in response to experimental conditions. Vertical dashed lines
594 indicate the onset of the work transition. Panel (A) denotes the ensemble average group mean for

Slow group (black line) and for the Fast group (grey line) in response to LS-US transitions. Panel (B) denotes the ensemble average group mean for the Slow group (black line) and for the Fast group (grey line) in response to LS-HUS transitions.

Table

Table 1. Kinetic parameter estimates for $\dot{V}O_{2p}$ and [HHb+Mb] in Fast and Slow groups during MOD1 and MOD2

Parameter	FAST (n=6)		SLOW (n=8)	
	MOD1	MOD2	MOD1	MOD2
$\dot{V}O_{2p}$				
$\dot{V}O_{2pbsl}$ ($L \cdot min^{-1}$)	0.86 ± 0.07	0.98 ± 0.09†	0.87 ± 0.16	1.05 ± 0.16†
$\dot{V}O_{2pss}$, ($L \cdot min^{-1}$)	1.89 ± 0.42	1.96 ± 0.41†	1.88 ± 0.21	1.97 ± 0.25†
A_p, ($L \cdot min^{-1}$)	1.00 ± 0.39	0.93 ± 0.39†	1.00 ± 0.39	0.93 ± 0.39†
$\tau\dot{V}O_{2p}$ (s)	21 ± 2	21 ± 2	32 ± 7*	24 ± 2†
C_{95} (s)	5 ± 2	7 ± 2	5 ± 2	6 ± 2
G ($mL \cdot min^{-1} \cdot W^{-1}$)	9.3 ± 1.8	8.8 ± 1.8†	9.2 ± 0.34	8.3 ± 0.5†
[HHb+Mb]				
$[HHb+Mb]_{bsl}$ (μM)	23.7 ± 6.8	21.0 ± 5.3†	19.7 ± 4.9	17.5 ± 3.85†
$[HHb+Mb]_{ee}$, (μM)	29.1 ± 8.7	29.0 ± 9.0	25.9 ± 5.1	27.4 ± 5.0
$[HHb+Mb]_{amp}$ (μM)	7.5 ± 3.6	10.7 ± 6.0†	5.4 ± 2.8	8.9 ± 4.0†
TD-[HHb+Mb] (s)	8 ± 2	5 ± 4†	8 ± 3	3 ± 2†
τ[HHb+Mb] (s)	11 ± 3	15 ± 4†	9 ± 3	18 ± 4†
τ'[HHb+Mb] (s)	19 ± 3	20 ± 4	17 ± 4	21 ± 2†
C_{95} (s)	2 ± 1	2 ± 1	3 ± 1	2 ± 1

Values are mean ± SD. $\dot{V}O_{2p}$, pulmonary O_2 uptake; $\dot{V}O_{2pbsl}$, baseline $\dot{V}O_{2p}$; $\dot{V}O_{2pss}$, steady-state $\dot{V}O_{2p}$; A_p, amplitude of $\dot{V}O_{2p}$ response; TD, time delay; $\tau\dot{V}O_{2p}$, time constant for $\dot{V}O_{2p}$ response; C_{95}, 95% confidence interval for $\tau\dot{V}O_{2p}$; G, functional gain ($\Delta\dot{V}O_{2p}/\Delta WR$). [HHb+Mb], deoxyhemoglobin+myoglobin concentration; $[HHb+Mb]_{bsl}$, baseline [HHb+Mb]; $[HHb+Mb]_{ee}$, end-exercise [HHb+Mb]; $[HHb+Mb]_{amp}$, amplitude of [HHb+Mb]; TD-[HHb+Mb], time delay of [HHb+Mb]; τ[HHb+Mb], time constant for [HHb+Mb] response; τ'[HHb+Mb], effective time constant (τ + TD) for [HHb+Mb]; C_{95}, 95% confidence interval for τ[HHb+Mb].

* difference from FAST ($p < 0.05$)

† difference from MOD1 ($p < 0.05$)

Table 2. $\dot{V}O_{2p}$ kinetic parameters for lower step (LS) and upper steps (US, HUS) moderate-intensity exercise transitions

	FAST (n=6)			SLOW (n=8)		
Parameter	LS	US	HUS	LS	US	HUS
$\dot{V}O_{2p\ bsl}$ ($L \cdot min^{-1}$)	0.82 ± 0.09	1.30 ± 0.25[a]	1.47 ± 0.24[a]	0.81 ± 0.10	1.32 ± 0.17[a]	1.47 ± 0.14[a]†
$\dot{V}O_{2p\ ss}$, ($L \cdot min^{-1}$)	1.31 ± 0.23	1.86 ± 0.43[a]	1.96 ± 0.38[a]	1.30 ± 0.17	1.91 ± 0.25[a]	1.93 ± 0.23[a]
A_p, ($L \cdot min^{-1}$)	0.49 ± 0.18	0.56 ± 0.19[a]	0.49 ± 0.17	0.48 ± 0.13	0.59 ± 0.09[a]	0.45 ± 0.10
TD (s)	15 ± 2	6 ± 4[a]	8 ± 8	13 ± 3	6 ± 6[a]	10 ± 7
$\tau\dot{V}O_{2p}$ (s)	19 ± 4	30 ± 4[a]	30 ± 5[a]	25 ± 5	40 ± 11[a]*	28 ± 8†
C_{95} (s)	7 ± 2	7 ± 2	7 ± 2	7 ± 1	7 ± 2	7 ± 2
G ($mL \cdot min^{-1} \cdot W^{-1}$)	8.8 ± 1.4	10.1 ± 1.1[a]	8.8 ± 0.9†	8.8 ± 1.5	10.9 ± 1.3[a]	8.3 ± 0.6†
O_2 deficit (mL)	273 ± 80	338 ± 135[a]	300 ± 93†	300 ± 53	441 ± 116 [a]	288 ± 101†

Values are means ± SD. LS, lower step; US, upper step; HUS, upper step following heavy-intensity; $\dot{V}O_{2p}$, pulmonary O_2 uptake; $\dot{V}O_{2pbsl}$, baseline $\dot{V}O_{2p}$; $\dot{V}O_{2pss}$, steady-state $\dot{V}O_{2p}$; A_p, amplitude of $\dot{V}O_{2p}$ response; TD, time delay; $\tau\dot{V}O_{2p}$, time constant for $\dot{V}O_{2p}$ response; C_{95}, 95% confidence interval for $\tau\dot{V}O_{2p}$; G, functional gain ($\Delta\dot{V}O_{2p}/\Delta WR$).

[a] difference from LS ($p<0.05$)

* difference from the Fast group ($p<0.05$)

† difference from US ($p<0.05$)

Table 3. Muscle de-oxygenation ([HHb+Mb]) kinetic parameters for lower step (LS) and upper step (US, HUS) moderate-intensity exercise transitions

	FAST (n=6)			SLOW (n=8)		
Parameter	LS	US	HUS	LS	US	HUS
$[HHb+Mb]_{bsl}$ (μM)	22.0 ± 6.1	26.8 ± 8.5[a]	23.8 ± 6.7 [a]	19.7 ± 3.5	22.5 ± 4.8[a]	22.8 ± 5.2 [a]
$[HHb+Mb]_{ee}$ (μM)	26.1 ± 8.9	31.1 ± 11.0[a]	29.0 ±10.4[a]	22.8 ± 4.7	25.9 ± 6.2[a]	27.8 ± 8.1 [a]
$[HHb+Mb_{amp}$ (μM)	3.9 ± 2.5	4.2 ± 2.0	5.5 ± 3.3 [a]†	3.1 ± 1.4	2.9 ± 1.4	5.0 ± 2.9 [a]†
TD-[HHb+Mb] (s)	13 ± 5	5 ± 3[a]	5 ± 3[a]	12 ± 3	7 ± 5[a]	4 ± 2[a]
τ[HHb+Mb] (s)	8 ± 2	21 ± 5[a]	19 ± 9 [a]	10 ± 4	14 ± 5[a]*	24 ± 7 [a]†
τ'[HHb+Mb] (s)	21 ± 3	26 ± 5	24 ± 9	22 ± 4	21 ± 6	28 ± 8[a]†
C_{95} (s)	4 ± 1	4 ± 1	3 ± 2	4 ± 1	4 ± 2	3 ± 2

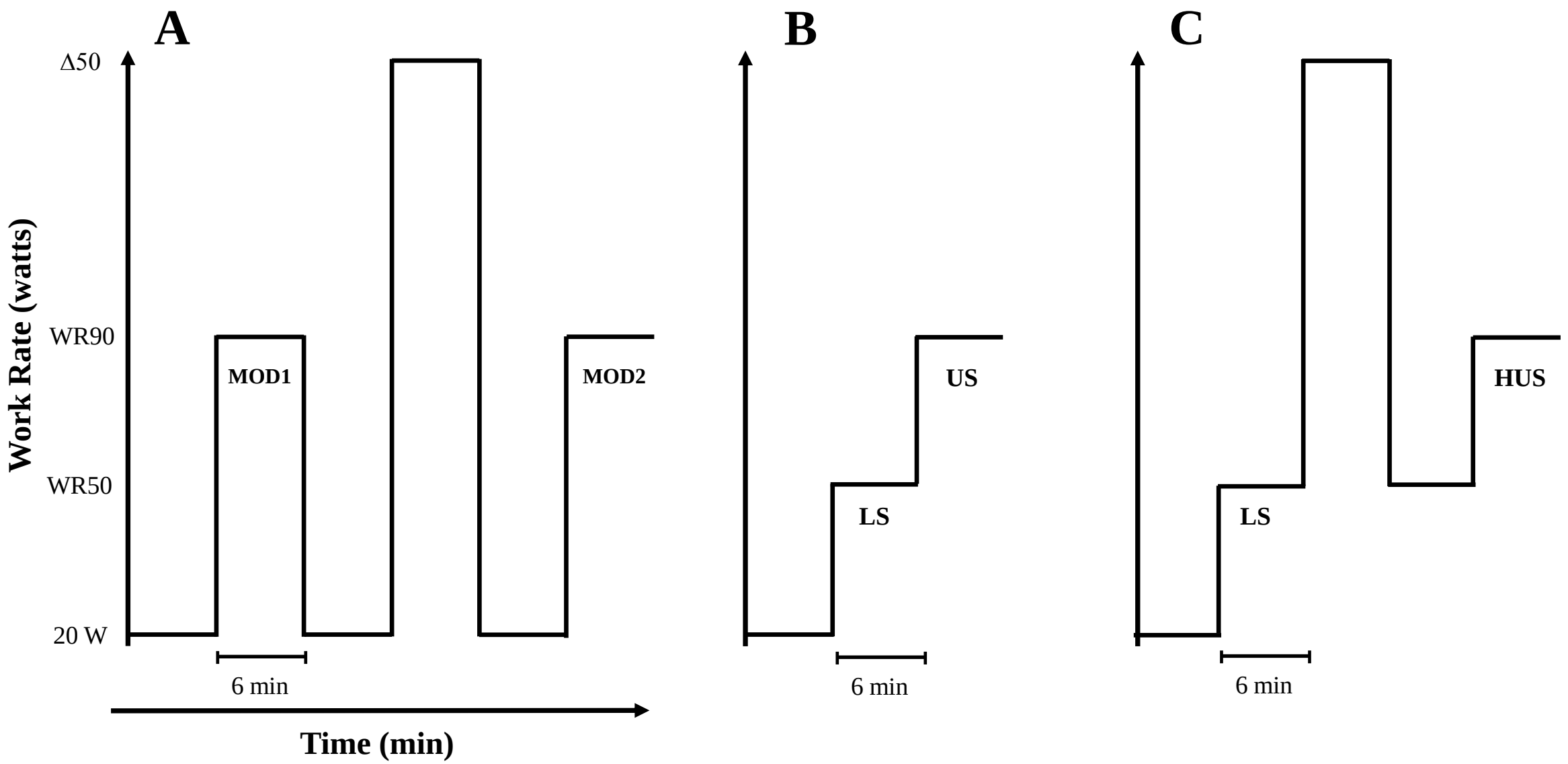
Values are means ± SD. LS, lower step; US upper step; HUS, upper step following heavy-intensity; [HHb+Mb], deoxyhemoglobin+myoglobin concentration; $[HHb+Mb]_{bsl}$, baseline [HHb+Mb]; $[HHb+Mb]_{ee}$, end-exercise [HHb+Mb]; $[HHb+Mb]_{amp}$, amplitude of [HHb+Mb]; TD-[HHb+Mb], time delay for [HHb+Mb]; τ[HHb+Mb], time constant for [HHb+Mb] response; τ'[HHb+Mb], effective time constant (τ + TD) for [HHb+Mb]; C_{95}, 95% confidence interval for τ[HHb+Mb].

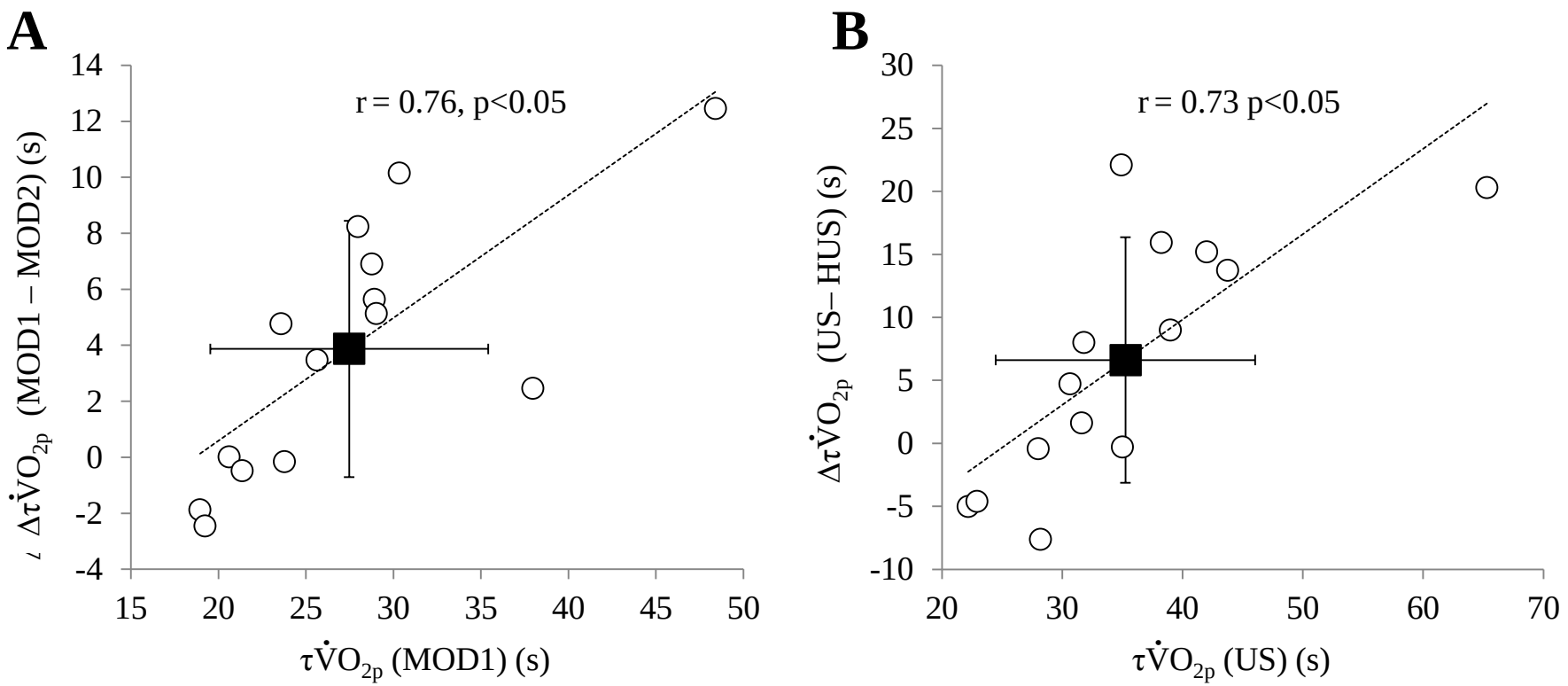
[a] difference from LS ($p<0.05$)

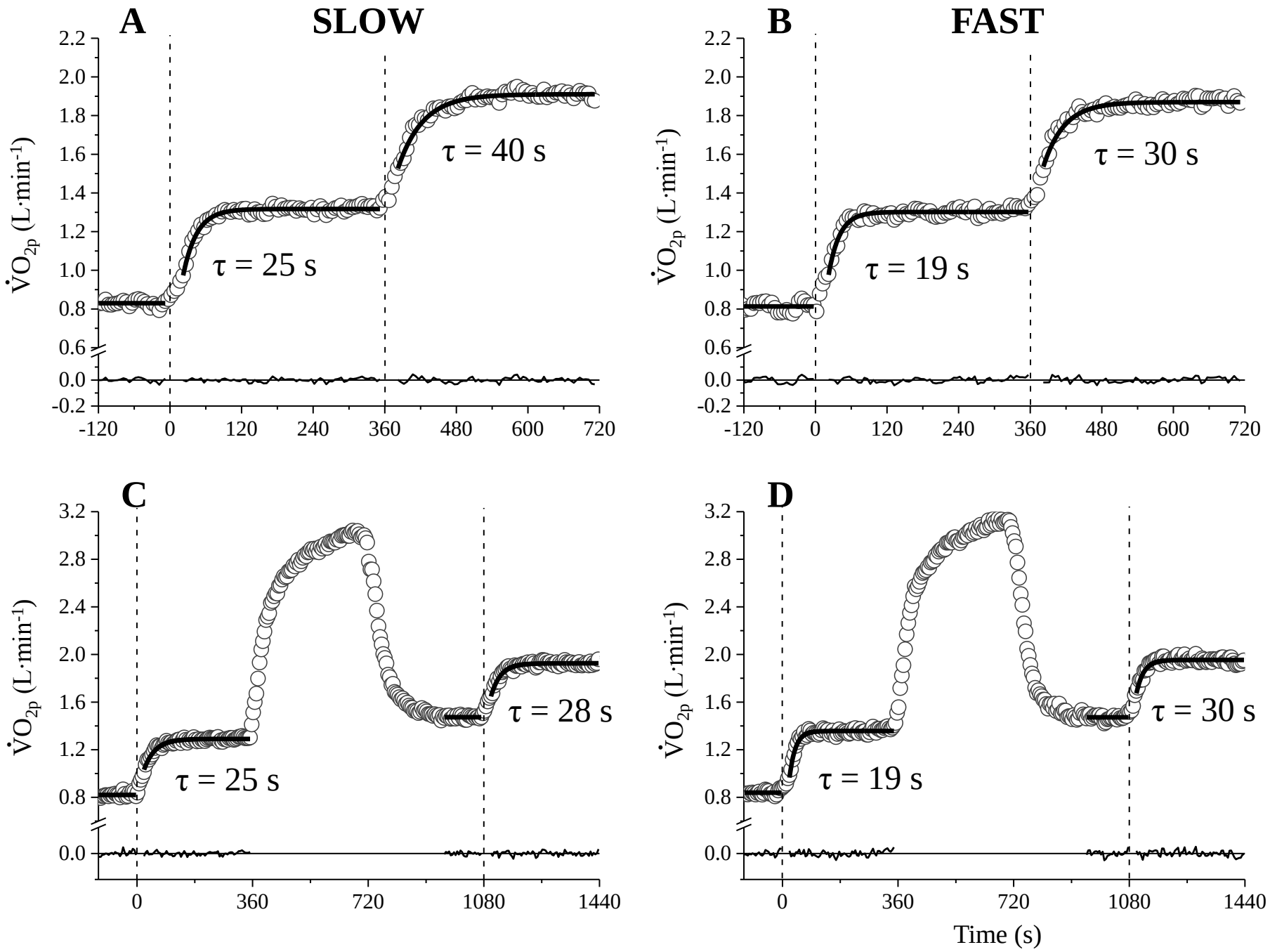
* difference from the Fast group ($p<0.05$)

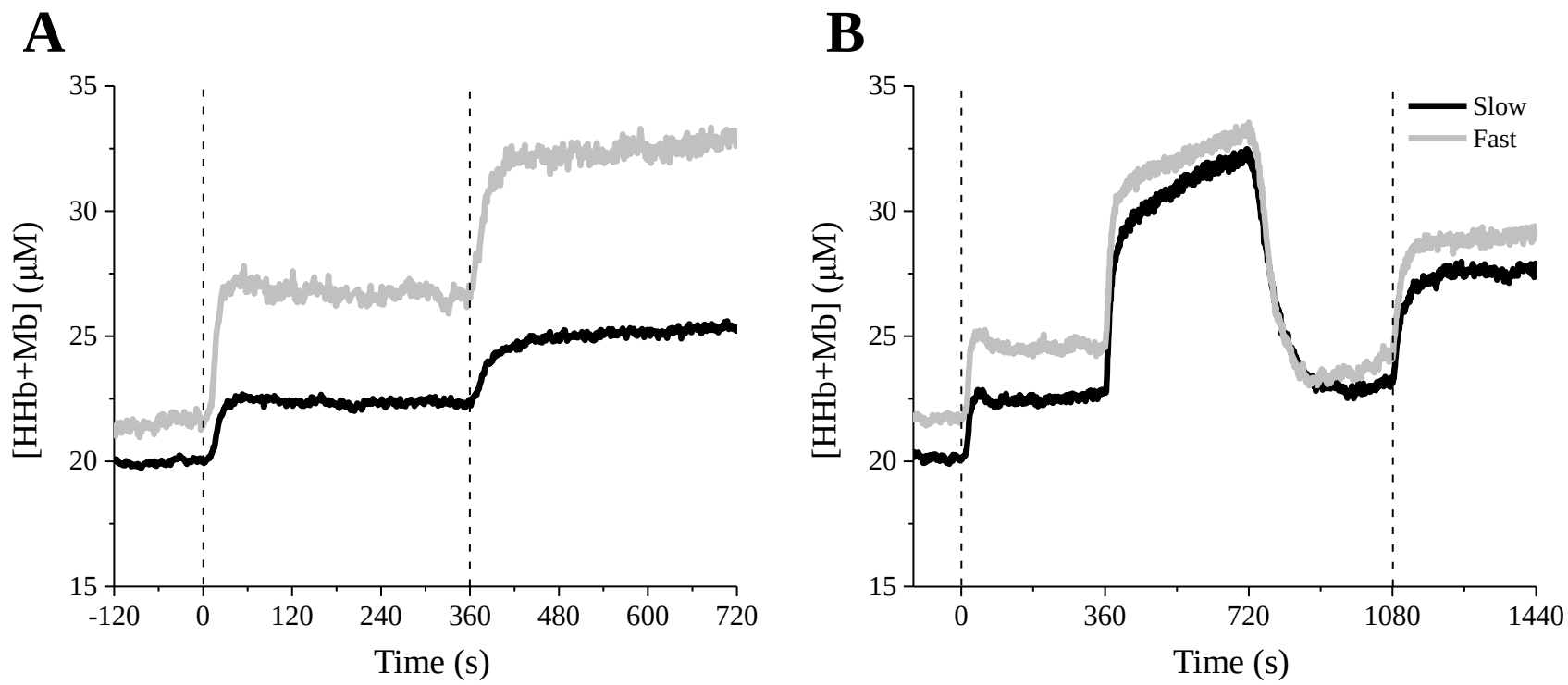
† difference from US ($p<0.05$)

Figure


A
B
C
Δ50
WR90
WR50
20 W
Work Rate (watts)
MOD1
MOD2
US
LS
LS
HUS
6 min
6 min
6 min
Time (min)


A
r = 0.76, p<0.05
Δτ V̇O2p (MOD1 – MOD2) (s)
τ V̇O2p (MOD1) (s)
B
r = 0.73 p<0.05
ΔτV̇O2p (US– HUS) (s)
τV̇O2p (US) (s)


A
SLOW
B
FAST
C
D
$\dot{V}O_{2p}$ (L·min^{-1})
τ = 40 s
τ = 25 s
τ = 30 s
τ = 19 s
τ = 28 s
τ = 30 s
τ = 19 s
Time (s)


A
B
[HHb+Mb] (μM)
Time (s)
Slow
Fast
35
30
25
20
15
-120
0
120
240
360
480
600
720
1080
1440