



Deposited via The University of Leeds.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/91938/>

Version: Accepted Version

Article:

Keir, DA, Robertson, TC, Benson, AP et al. (2016) The influence of metabolic and circulatory heterogeneity on the expression of pulmonary oxygen uptake kinetics in humans. *Experimental Physiology*, 101 (1). pp. 176-192. ISSN: 0958-0670

<https://doi.org/10.1113/EP085338>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

1 **The influence of metabolic and circulatory heterogeneity on the expression of pulmonary**
2 **$\dot{V}O_2$ kinetics in humans**

3
4 Daniel A. Keir^{1,2}, Taylor C. Robertson^{1,2}, Alan P. Benson⁴, Harry B. Rossiter⁵, & John M.
5 Kowalchuk^{1,2,3}

6
7 ¹Canadian Centre for Activity and Aging, ²School of Kinesiology, ³Department of Physiology
8 and Pharmacology, The University of Western Ontario, London, ON, Canada, ⁴School of
9 Biomedical Sciences, University of Leeds, Leeds, United Kingdom, and ⁵Rehabilitation Clinical
10 Trials Center, Division of Respiratory & Critical Care Physiology & Medicine, Los Angeles
11 Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA.

12
13 *Running Title:* $\dot{V}O_{2p}$ kinetics are slowed dependent on the pre-transition work rate

14
15 Key words: O_2 uptake kinetics, near-infrared spectroscopy, metabolic rate, muscle

16
17 Corresponding author: Dr. J. M. Kowalchuk
18 School of Kinesiology
19 The University of Western Ontario
20 London, Ontario, Canada
21 N6A 3K7
22 e-mail: jkowalch@uwo.ca
23 phone: (519) 661-1605

24 **New findings**

25

26 *What is the central question of this study?*

27 That pulmonary $\dot{V}O_2$ kinetics on transition to moderate exercise is invariant and exponential is
28 consistent with a first-order reaction controlling $\dot{V}O_2$. However, slowed $\dot{V}O_2$ kinetics when
29 initiating exercise from raised baseline intensities challenges this notion.

30

31 *What is the main finding and its importance?*

32 Here we demonstrate how a first-order system can respond with non-first-order response
33 dynamics. Data suggest that progressive recruitment of muscle fibre populations having
34 progressively lower mitochondrial density and slower microvascular blood flow kinetics can
35 unify the seemingly contradictory evidence for the control of pulmonary $\dot{V}O_2$ on transition to
36 exercise.

37

38 **Abstract**

39 We examined the relationship amongst baseline work rate (WR), phase II pulmonary $\dot{V}O_2$ time
40 constant ($\tau\dot{V}O_{2p}$) and functional gain (G_p : $\Delta\dot{V}O_{2p}/\Delta WR$) during moderate-intensity exercise.
41 Transitions were initiated from a constant or variable baseline WR. A validated circulatory
42 model was used to examine the role of heterogeneity in muscle metabolism ($\dot{V}O_{2m}$) and blood
43 flow (\dot{Q}_m) in determining $\dot{V}O_{2p}$ kinetics. We hypothesized that $\tau\dot{V}O_{2p}$ and G_p would be invariant
44 in the constant baseline condition, but would increase linearly with increased baseline WR.
45 Fourteen men completed 3-5 repetitions of $\Delta 40W$ step-transitions initiated from 20, 40, 60, 80,
46 100, and 120 W on a cycle ergometer. The $\Delta 40W$ step-transitions from 60, 80, 100, and 120 W
47 were preceded by 6 minutes of 20 W cycling from which the progressive ΔWR transitions
48 (constant baseline condition) were examined. $\dot{V}O_{2p}$ was measured breath-by-breath using mass
49 spectrometry and volume turbine. For a given ΔWR , both $\tau\dot{V}O_{2p}$ (22 to 35s) and G_p (8.7 to 10.5
50 $\text{mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$) increased ($p<0.05$) linearly as a function of baseline WR (20 to 120 W). $\tau\dot{V}O_{2p}$
51 was invariant ($p<0.05$) in transitions initiated from 20 W, but G_p increased with ΔWR ($p<0.05$).
52 Modeling the summed influence of multiple muscle compartments revealed that $\tau\dot{V}O_{2p}$ could
53 appear fast (24s), and similar to *in vivo* measurements ($22\pm 6s$), despite being derived from
54 $\tau\dot{V}O_{2m}$ values ranging 15-40s and $\tau\dot{Q}_m$ ranging 20-45s suggesting that within the moderate-
55 intensity domain phase II $\dot{V}O_{2p}$ kinetics are slowed dependent on the pre-transition WR, and are
56 strongly influenced by muscle metabolic and circulatory heterogeneity.

57

58

59 **Introduction**

60 After a step-change in moderate-intensity exercise work rate (WR) (i.e., below the lactate
61 threshold), pulmonary O₂ uptake ($\dot{V}O_{2p}$) increases in an exponential-like manner towards a new
62 steady-state O₂ requirement following a brief cardiodynamic period (phase I). The exponential
63 increase in $\dot{V}O_{2p}$ (phase II) is closely associated with the dynamic adjustment of skeletal muscle
64 $\dot{V}O_2$ ($\dot{V}O_{2m}$) (Barstow *et al.* 1990; Grassi *et al.* 1996; Krstrup *et al.* 2009) and is characterized
65 by both its rate (quantified by the time constant for $\dot{V}O_{2p}$ [$\tau\dot{V}O_{2p}$]) and amplitude (quantified by
66 the functional $\dot{V}O_{2p}$ gain [$G_P: \Delta\dot{V}O_{2p}/\Delta WR$]) (Whipp *et al.* 1982).

67 During exercise initiated from a common, low, baseline WR, both phase II $\tau\dot{V}O_{2p}$ and G_P
68 are invariant, and independent of the subsequent moderate-intensity WR performed (Özyener *et*
69 *al.* 2001; Spencer *et al.* 2013). This kinetic behavior implies that phase II $\dot{V}O_{2p}$ kinetics are
70 consequent to a first order rate reaction; specifically, linked, to mitochondrial ADP feedback
71 (Rossiter, 2011; Wüst *et al.* 2011). However, when exercise is initiated from a raised baseline
72 WR and metabolic rate, $\dot{V}O_{2p}$ kinetics are slowed: both $\tau\dot{V}O_{2p}$ and G_P are increased (Brittain *et*
73 *al.* 2001; MacPhee *et al.* 2005; Spencer *et al.* 2011; Bowen *et al.* 2011; Williams *et al.* 2013;
74 Keir *et al.* 2014). This behavior was suggested to be evidence that $\dot{V}O_{2p}$ kinetics do not conform
75 to a first order rate reaction (Robergs, 2014). However, others interpreted this kinetic slowing to
76 be reflective of the physiological properties of the activated musculature (Brittain *et al.* 2001),
77 where individual fibers operate through a first-order ADP-feedback reaction, but where
78 mitochondrial volume-density differs among the activated muscle fibers (Rossiter, 2011). In
79 other words, preferential activation of highly oxidative muscle fibers at low baseline WR will
80 have inherently faster $\dot{V}O_{2p}$ kinetics than less-oxidative fibers activated at higher work rates; yet
81 within each fiber $\dot{V}O_{2p}$ kinetics are first order (Brittain *et al.* 2001).

82 Recently, Keir *et al.* (2014) reported that moderate-intensity $\dot{V}O_{2p}$ kinetics were strongly
83 related to baseline metabolic rate, but not to the baseline WR or the magnitude of the increase in
84 WR during the exercise bout (ΔWR). This suggested that the intramuscular metabolic status (e.g.
85 [ADP]/[ATP], and/or free energy of ATP), rather than kinetic characteristics of the recruited
86 motor units, influence $\dot{V}O_{2p}$ kinetics; a conclusion supported by Bowen *et al.* (2011) using a
87 model of incomplete muscle recovery, and by Wüst *et al.* (2014) with exercise transitions in
88 electrically-stimulated dog hind limb muscle. However, DiMenna *et al.* (2010), using high-
89 intensity exercise, found that baseline metabolic rate did not influence subsequent $\dot{V}O_{2p}$ kinetics,
90 supporting the recruitment hypothesis.

91 To shed light on this controversy we aimed to determine the relationship between
92 baseline metabolic rate and $\dot{V}O_{2p}$ kinetics during exercise initiated from several different rates of
93 baseline metabolism, each using an identical work rate increment (ΔWR) within the moderate-
94 intensity domain; previous investigations having used only two different baseline work rates.
95 This would allow us to determine whether $\dot{V}O_{2p}$ kinetics are slowed in relation to baseline $\dot{V}O_{2p}$.
96 Additionally, to account for potential confounding effects of regional O_2 delivery on muscle
97 $\dot{V}O_{2p}$ (Hughson & Morrissey, 1982; MacPhee *et al.* 2005; Koga *et al.* 2007; Wüst *et al.* 2014),
98 we simultaneously measured regional muscle deoxygenation (hemoglobin (Hb) + myoglobin
99 (Mb); hereafter referred to as “[HHb]”) by near-infrared spectroscopy, which reflects the
100 relationship between microvascular O_2 delivery to O_2 utilization (Murias *et al.* 2014). In addition,
101 it is well understood that $\dot{V}O_{2m}$ kinetics can be modulated by circulatory dynamics on transition
102 to their pulmonary expression as phase II $\dot{V}O_{2p}$ (Hoffmann *et al.* 2013; Benson *et al.* 2013). To
103 account for this we used a validated circulatory model (Benson *et al.* 2013) to determine whether

104 circulatory distortions could explain a progressive slowing of $\dot{V}O_{2p}$ kinetics with progressively
105 greater baseline work rate.

106 Thus, the purpose of this study was to determine in the same participants: 1) $\dot{V}O_{2p}$ and
107 [HHb] kinetics over a range of moderate-intensity WR increments initiated from a constant
108 baseline metabolic rate, i.e., the same baseline metabolism with variable ΔWR (constant baseline
109 condition); 2) the relationship between baseline metabolic rate and $\dot{V}O_{2p}$ and [HHb] kinetics for
110 a constant WR increment within the moderate-intensity domain, i.e., variable baseline
111 metabolism with the same ΔWR (variable baseline condition); and 3) the extent to which
112 circulatory dynamics may contribute to the slowed $\dot{V}O_{2p}$ kinetics from progressively increasing
113 baseline metabolic rate. We hypothesized that, unlike in the constant baseline condition, in the
114 variable baseline condition $\tau\dot{V}O_{2p}$ and G_p would increase linearly with baseline $\dot{V}O_{2p}$
115 independent of [HHb] dynamics, and that these differences could not be explained by circulatory
116 modulation of muscle $\dot{V}O_2$ kinetics. Accepting this hypothesis would suggest an association
117 between instantaneous muscle metabolism and $\tau\dot{V}O_{2p}$ and G_p in moderate intensity exercise,
118 which is inconsistent with simple first order kinetic control theory.

119

120 **Methods**

121 *Ethical approval.* The study was conducted according to the Declaration of Helsinki and all
122 procedures were approved by The University of Western Ontario Ethics Committee for Research
123 on Human Subjects. All participants volunteered and gave informed written consent to
124 participate in the study.

125

126 *Participants.* Fourteen healthy young adult men (mean \pm SD values: age, 24 ± 3 yrs; body mass,
127 83 ± 5 kg; height, 180 ± 8 cm; $\dot{V}O_{2\text{peak}}$, 48.1 ± 5.9 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) volunteered for the study. All
128 participants were non-smokers who were free of any known musculoskeletal, respiratory,
129 cardiovascular, and metabolic conditions, and who were not taking any medications that might
130 influence cardiorespiratory or metabolic responses to exercise. Participants were selected on the
131 basis that their WR at the estimate lactate threshold ($\hat{\theta}_L$) was at least 130 W, allowing all step-
132 transitions used in the protocol to begin within the moderate-intensity domain.

133

134 *Ramp Incremental Exercise.* Each participant reported to the laboratory to perform a symptom-
135 limited ramp incremental (RI) exercise test (20 W baseline for 4 min followed by a 25 W \cdot min $^{-1}$
136 ramp) on a magnetically-braked cycle ergometer (model: Velotron, RacerMate Inc., Seattle, WA,
137 USA) for determination of peak $\dot{V}O_{2p}$ ($\dot{V}O_{2\text{peak}}$) and peak WR (WR_{peak}). Participants were asked
138 to maintain a cadence of ~ 60 rpm during the test. $\dot{V}O_{2\text{peak}}$ was defined as the greatest 20 s $\dot{V}O_{2p}$
139 computed from a rolling average and WR_{peak} was defined as the WR achieved at termination of
140 the RI test. Additionally, the $\hat{\theta}_L$ was determined by visual inspection using standard ventilatory
141 and gas exchange indices as previously described (Beaver *et al.* 1986).

142

143 *Constant Work Rate Exercise Protocols.* Participants performed 3-5 repetitions of 40W step-
144 increments (i.e., $\Delta 40$) from a baseline WR of 20, 40, 60, 80, 100, and 120 W (i.e., variable
145 baseline condition). Step-transitions were 6 min of baseline WR followed by an instantaneous
146 step-increase in WR to 40 W above the baseline for 6 min. With the exception of the step-
147 transitions from 20 W and 40 W, all other transitions were preceded by 6 min of baseline cycling
148 at 20 W, which provided an opportunity to examine step-transitions from a common 20 W

149 baseline and different WR increments (i.e., $\Delta 40$, $\Delta 60$, $\Delta 80$, $\Delta 100$; constant baseline condition).
150 Therefore, one-third of the exercise protocols were 12 min in duration (those initiated from 20
151 and 40 W) and the other two-thirds were 18 min (see Figure 1). A maximum of two exercise
152 protocols were completed per visit with a minimum of 20 min seated rest between exercise
153 bouts. For each participant, the sequence of exercise protocols was randomized in a
154 counterbalanced order and it was assumed that exercise duration did not affect physiological
155 outcome measures.

156 *Data Collection.* During each trial participants wore a noseclip and breathed through a
157 mouthpiece for breath-by-breath gas-exchange measurements. Inspired and expired volumes and
158 flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha
159 Technologies, VMM 110) and pneumotach (Hans Rudolph, Model 4813) positioned in series
160 from the mouthpiece (total apparatus dead space was 150 mL); respired air was continuously
161 sampled at the mouth and analysed by mass spectrometry (Innovision, AMIS 2000, Lindvedvej,
162 Denmark) for fractional concentrations of O₂ and CO₂. The volume turbine was calibrated before
163 each test using a syringe of known volume (3 L) over a range of flow rates and the pneumotach
164 was adjusted for zero flow. Gas concentrations were calibrated with precision-analyzed gas
165 mixtures. The time delay between an instantaneous, square-wave change in fractional gas
166 concentration at the sampling inlet and its detection by the mass spectrometer was measured
167 electronically by computer. Respiratory volumes, flow, and gas concentrations were recorded in
168 real-time used to build a profile of each breath. Alveolar gas exchange was calculated on a
169 breath-by-breath basis using the algorithms of Swanson (1980).

170 Local muscle deoxygenation ([HHb]) of the quadriceps *vastus lateralis* muscle was
171 measured using a frequency domain multi-distance near-infrared spectroscopy (NIRS) system

172 (Oxiplex TS, Model 92505, ISS, Champaign, USA) as described elsewhere (Spencer *et al.*
173 2012). Briefly, the system was comprised of a single channel consisting of eight laser diodes
174 operating at two wavelengths ($\lambda = 690$ and 828 nm, four at each wavelength) that were pulsed in
175 a rapid succession down a photomultiplier tube. A rigid plastic NIRS probe (connected to laser
176 diodes and photomultiplier tube by optical fibers) consisted of two parallel rows of light emitter
177 fibers and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5,
178 3.0, and 3.5 cm for both wavelengths. The probe was placed on the belly of the muscle, at the
179 distal end of the *vastus lateralis* muscle. NIRS measurements were collected continuously for the
180 entire duration of each trial. The NIRS was calibrated at the beginning of each testing session
181 following an instrument warm-up period of at least 20 min. Throughout each testing session,
182 continuous measurements of the absolute scattering (μ_s) and absorption (μ_a) coefficients for each
183 wavelength were determined by measuring the intensity of modulated light entering and
184 traversing the tissue (determined from the average component of the modulated light waves
185 [DC], amplitude of the modulation [AC], and the phase shift [Φ] between those two signals) at
186 the four source - detector distances (R). The coefficients of AC and Φ were plotted as a function
187 of distance R at both wavelengths to determine μ_s and μ_a , which were used to resolve the
188 absolute concentrations of HHb (in μM) using the Beer-Lambert equation. Data were stored
189 online at an output frequency of 25 Hz, but were reduced to 1 s bins for all subsequent analyses.

190

191 *Data Analysis.* Breath-by-breath $\dot{V}O_{2p}$ data were edited on an individual basis by removing
192 aberrant data that lay 3 SD from the local mean (Lamarra *et al.* 1987). Within each participant,
193 like-repetitions were linearly interpolated on a second-by-second basis, ensemble-averaged and

194 time-aligned such that time “zero” represented the onset of the transition. The on-transient of
195 each profile was modeled with the following mono-exponential function:

196

$$197 \quad y(t) = y_{\text{BSL}} + A \cdot (1 - e^{-(t-\text{TD})/\tau}) \quad (1)$$

198

199 where, $y(t)$ is the value of the dependent variable at any time during the transition, y_{BSL} is the
200 pre-transition baseline value (determined from the ~60 s before a transition), A is the steady-state
201 increase in y above the baseline value, TD is the time delay, and τ is the time constant of the
202 response (or the time for y to increase to 63% of the new steady-state after TD). The functional
203 gain (G_p : $\Delta\dot{V}O_{2p}/\Delta\text{WR}$) of the response was determined by dividing A (in $\text{mL}\cdot\text{min}^{-1}$) by the
204 change in WR (in W). Data were fit using the Levenberg-Marquardt algorithm to find the
205 minimum sum of squared residuals between the mono-exponential function and the experimental
206 data (using Origin 8.5; OriginLab, Northampton, MA). The end of the fitting window was set to
207 ~ 5 times the estimated time constant in order to restrict the modeling to data lying within the
208 transient phase. For those individuals who transitioned into the heavy-intensity domain, the end
209 of the phase II fitting window was determined by examining the change in τ , CI_{95} , χ^2 , and plotted
210 residuals in response to progressive increases in the end of the fitting window. The point
211 immediately preceding a systematic increase in τ , CI_{95} , and χ^2 was considered as the end of phase
212 II. The mean response time ($\text{MRT}-\dot{V}O_{2p}$) of $\dot{V}O_{2p}$ was characterized from a fit of the $\dot{V}O_{2p}$
213 response from $t=0$ to the end of the exercise

214 The TD for the $[\text{HHb}]$ ($[\text{HHb}]-\text{TD}$) response was determined using second-by-second
215 data and corresponded to the time, after the onset of exercise, at which the $[\text{HHb}]$ signal
216 increased above 1 SD of the pre-transition baseline value. Determination of the $[\text{HHb}]-\text{TD}$ was

217 made on each individual's ensemble-averaged response and the data were modeled using
218 equation 1. Different fitting strategies ranging from 90-180 s into a transition resulted in minimal
219 differences in estimates of τ [HHb]. The MRT for [HHb] (MRT-[HHb]) described the overall
220 time for [HHb] to increase from the start of exercise to 63% of the "steady-state" value and was
221 calculated as the sum of τ [HHb] and [HHb]-TD.

222

223 *In silico simulations.* The validated multi-compartmental model (MCM) of Benson *et al.* (2013)
224 was used to investigate the dissociation between muscle and pulmonary $\dot{V}O_2$ kinetics. Briefly,
225 mathematical expressions for O_2 delivery (\dot{Q}), muscle O_2 uptake, and a lumped compartment
226 representing the rest of the body were joined in parallel by a single arterial compartment and
227 multiple compartments on the venous side (representing blood draining the muscle, the rest of
228 the body, and a mixed compartment approximating the blood volume proximal to the common
229 femoral veins). The fractions of total metabolic rate (from muscle: 0.57; from body 0.43) and
230 cardiac output (\dot{Q} ; to muscle: 0.57; to body 0.43) for unloaded pedaling and venous blood
231 volume associated with muscle (0.7 L), body (0.1 L), and mixed compartments (2.3 L) were
232 fixed while the WR and kinetic variables for muscle and body \dot{Q} (\dot{Q}_m and \dot{Q}_b) and $\dot{V}O_2$ ($\dot{V}O_{2m}$
233 and $\dot{V}O_{2b}$) were manipulated experimentally to mimic the pre-transition conditions of the *in vivo*
234 experiments and predict phase I and phase II $\dot{V}O_{2p}$ kinetics. The output of the model was
235 instantaneous $\dot{V}O_{2p}(t)$:

236

$$237 \quad \dot{V}O_{2p}(t) = \dot{Q}(t) \cdot (C_aO_2 - C_vO_2)(t) \quad (2)$$

238

239 where C_aO_2 is arterial content (fixed to 20 mL O_2 per 100 mL of blood), C_vO_2 is mixed venous
240 O_2 content. During the simulated exercise step-transitions, \dot{Q}_m and $\dot{V}O_{2m}$ increased exponentially
241 according to equation 1 (with $TD = 0$), toward their WR-dependent steady-state values with a
242 steady-state relationship of 6 $L \cdot \text{min}^{-1}$ per 1 $L \cdot \text{min}^{-1}$ of $\dot{V}O_2$. The intercept of the \dot{Q} -to- $\dot{V}O_2$
243 relationship was set to 3.6 $L \cdot \text{min}^{-1}$ (Benson *et al.* 2013). Values of $\dot{V}O_2$ were dependent on WR
244 inputs and functional gains (i.e., $\Delta\dot{V}O_2/\Delta W$) of 9.5, 11.0, and -1.5 $\text{mL} \cdot \text{min}^{-1} \cdot \text{W}^{-1}$ for $\dot{V}O_{2p}$, $\dot{V}O_{2m}$,
245 and $\dot{V}O_{2b}$, respectively as previously described (Benson *et al.* 2013). Values for \dot{Q} in the various
246 compartments were based on the aforementioned \dot{Q} -to- $\dot{V}O_2$ relationship.

247 Exercise was simulated for moderate-intensity transitions by specifying a step-change in
248 WR. Initially, the steady-state $\dot{V}O_{2p}$ baseline for 20 W was set to 0.75 $L \cdot \text{min}^{-1}$ and $\tau\dot{V}O_{2m}$ and
249 $\tau\dot{Q}_m$ were modulated in order to generate a $\tau\dot{V}O_{2p}$ value that corresponded to the group mean
250 $\tau\dot{V}O_{2p}$ from the 20 W to 60 W step. Thereafter, simulations were done for $\Delta 40W$ step-changes
251 from 40, 60, 80, 100, and 120 W and for $\Delta 60W$, $\Delta 80W$, and $\Delta 100W$ step-changes from 20 W
252 with fixed parameter inputs for $\tau\dot{V}O_{2m}$ and $\tau\dot{Q}_m$ in order to determine the degree by which
253 circulatory dynamics modulate the dynamics of $\dot{V}O_{2p}$ at a constant $\tau\dot{V}O_{2m}$ and varying moderate-
254 intensity baseline WR and WR increments. In addition, simulations were produced to examine
255 the influence of slowing \dot{Q}_m dynamics on the $\dot{V}O_{2p}$ profile; for each of the $\Delta 40W$ step-changes,
256 $\tau\dot{V}O_{2m}$ was fixed to each of 20, 30, and 40 s, and a series of simulations of $\dot{V}O_{2p}$ was produced
257 where $\tau\dot{Q}_m$ was varied (MacPhee *et al.* 2005). In all experimental conditions, the phase II region
258 of the $\dot{V}O_{2p}$ output from the simulations was fitted to equation 1, with the end of phase I being
259 identified precisely from the profile of simulated C_vO_2 .

260 To investigate how the homogenized $\dot{V}O_{2p}$ response to a step-change in WR from 20 to
261 120 W may be influenced by multiple muscle compartments with heterogeneous metabolic and

262 circulatory dynamics, the group mean dynamic responses of $\dot{V}O_{2p}$ and [HHb] from each of the
263 equal WR increment steps (variable baseline condition) were used to infer the $\dot{V}O_{2m}$ and \dot{Q}_m
264 kinetics of six theoretical muscle compartments ($\tau\dot{V}O_{2m} = 15, 20, 25, 30, 35, 40$ s and $\tau\dot{Q}_m = 20,$
265 $25, 30, 35, 40, 45$ s for compartments 1 – 6, respectively) assuming that both $\tau\dot{V}O_{2m}$ and $\tau\dot{Q}_m$
266 linearly increase as a function of WR. Assuming simultaneous activation and equal contributions
267 of each of the six compartments, the homogenized $\dot{V}O_{2p}$ response to a step-change in WR from
268 20 to 120 W was simulated for each compartment and combined into a single homogenized
269 response. The averaged $\dot{V}O_{2p}$ output was modeled as previously mentioned and kinetics were
270 compared to the actual group mean $\dot{V}O_{2p}$ response to the step-change in WR from 20 to 120 W.

271
272 *Statistical Analysis.* Data are presented as means \pm SD. A one-way analysis of variance
273 (ANOVA) for repeated measures was used to compare kinetic parameters and variables between
274 conditions. Where significant main effects were found, a Tukey's *post hoc* analysis was
275 performed for multiple comparisons testing. All statistical analyses were performed using
276 SigmaPlot Version 11.0, (Systat Software Inc., San Jose, CA). Statistical significance was
277 accepted at an alpha level of 5%.

278

279 **Results**

280 The group mean $\hat{\theta}_L$ was 2.22 ± 0.32 L \cdot min $^{-1}$ (range: 1.85 – 3.00 L \cdot min $^{-1}$), which
281 corresponded to a mean WR of 161 ± 34 W (range: 130 – 240 W). Therefore, the WR associated
282 with $\hat{\theta}_L$ for all participants was greater than the maximum baseline WR from which step-
283 transitions were initiated in the experimental protocol (i.e., 120 W). The 40 W transitions from
284 baselines of 20, 40, 60, 80, 100, and 120 W corresponded to $35 \pm 6, 43 \pm 7, 51 \pm 8, 60 \pm 9, 68 \pm$

285 10, 78 ± 12 % of $\dot{V}O_{2p}$ at $\hat{\theta}_L$, and the steady-state $\dot{V}O_{2p}$ achieved corresponded to 50 ± 8 , 60 ± 9 ,
286 68 ± 10 , 78 ± 11 , 87 ± 12 , 97 ± 14 % of $\hat{\theta}_L$. Based on the steady-state $\dot{V}O_{2p}$ achieved, four (of
287 14) participants transitioned into the heavy-intensity domain at 160 W and one at 140 W.
288 However, removal of these data *post hoc* did not alter the overall study outcomes described
289 below.

290 Table 1 shows the phase II $\dot{V}O_{2p}$ parameters for each of the variable baseline conditions.
291 The ensemble-averaged group profiles of $\dot{V}O_{2p}$ for the variable baseline condition are displayed
292 in Figure 2. In each panel, a mono-exponential fit derived from the group mean parameter
293 estimates (from equation 1) is superimposed over the data. As designed, $\dot{V}O_{2pBSL}$ increased
294 ($p < 0.05$) progressively from the 20 W to 120 W baseline transitions (Table 1). Despite constant
295 ΔWR , the parameter estimates for A, G_p and $\tau \dot{V}O_{2p}$ increased progressively with increasing
296 baseline WR ($p < 0.05$; see Table 1 and Figure 3 for pairwise differences).

297 The group mean parameter estimates from the phase II $\dot{V}O_{2p}$ fits for the constant baseline
298 condition exercise transitions are displayed in Table 2. Figure 4 shows the mean $\dot{V}O_{2p}$ responses
299 for $\Delta 40$, $\Delta 60$, $\Delta 80$, $\Delta 100$ W transitions from a 20 W baseline. As designed, $\dot{V}O_{2pBSL}$ did not
300 change ($p > 0.05$) amongst these step-transitions and A increased ($p < 0.05$) with increasing WR
301 increment. The G_p increased ($p < 0.05$, main effect) during transitions to increasing moderate-
302 intensity WRs, but there was no difference in $\tau \dot{V}O_{2p}$ among the different ΔWR conditions
303 ($p > 0.05$). The relationships between A, $\tau \dot{V}O_{2p}$ or G_p , and the WR increment are shown in Figure
304 5.

305 The kinetic parameters of [HHb] under the variable and constant baseline conditions are
306 shown in Tables 3 and 4 with group mean responses displayed in Figures 6 and 7. Similar to
307 $\dot{V}O_{2p}$, $\tau[HHb]$ increased with increasing $\dot{V}O_{2pBSL}$ ($p < 0.05$, main effect). Baseline [HHb]

308 increased ($p < 0.05$, main effect) and [HHb]-TD decreased ($p < 0.05$, main effect) with increasing
309 baseline WR (see Table 3 for multiple comparisons). The Δ [HHb] and MRT-[HHb] were not
310 different ($p > 0.05$) when the WR increment was constant (variable baseline condition; Table 3)
311 but, as expected, Δ [HHb] increased ($p < 0.05$) with increasing Δ WR (constant baseline condition;
312 Table 4). There was no influence of WR increment ($p > 0.05$) on [HHb]_{BSL}, [HHb]-TD, MRT-
313 [HHb], or τ [HHb] in the constant baseline condition (Table 4).

314 The relationship between phase II $\dot{V}O_{2p}$ kinetics and [HHb] kinetics was examined by
315 computing the difference in “time to reach steady-state” for both signals (i.e., $(4 * \tau \dot{V}O_{2p})$ for
316 $\dot{V}O_{2p}$ and $([HHb]-TD + 4 * \tau [HHb])$ for [HHb], in s) (Figure 8). A “steady-state” for [HHb] was
317 reached ~ 45 s earlier than for $\dot{V}O_{2p}$ for all $\Delta 40$ W WR transitions (Figure 8A) and ~ 37 s earlier
318 for WR transitions from a common 20 W baseline (Figure 8B); responses were similar between
319 and within variable and constant baseline conditions ($p > 0.05$, Figures 8A and 8B).

320 The *in silico* simulations using the *in vivo* estimated parameters for $\dot{V}O_{2p}$ kinetics are
321 presented in Figure 9. First, the outcomes from simulations performed to determine values of
322 $\tau \dot{V}O_{2m}$ and $\tau \dot{Q}_m$ that would elicit a $\tau \dot{V}O_{2p}$ of 22 s for the $\Delta 40$ W step-transition from 20 W were
323 26.1 s and 28.5 s for $\tau \dot{V}O_{2m}$ and $\tau \dot{Q}_m$, respectively. These values were then used to determine the
324 extent to which $\tau \dot{V}O_{2p}$ would be modified by increasing WR increment (constant baseline
325 condition; Figure 9A) and baseline WR (variable baseline condition; Figure 9B) assuming $\dot{V}O_{2m}$
326 and \dot{Q}_m kinetics were unaltered. As shown in Figure 9A, the influence on increasing the WR
327 increment in the constant baseline condition is that $\tau \dot{V}O_{2p}$ is reduced from 22.0 s (at $\Delta 40$ W) to
328 20.3 s (at $\Delta 100$ W). The opposite occurs in the variable baseline condition with common $\Delta 40$ W
329 WR increments, where $\tau \dot{V}O_{2p}$ increases from 22.0 s (at 20 W baseline) to 24.4 s (at 120 W
330 baseline) (Figure 9B). In addition, we also tested the effect of allowing \dot{Q}_m kinetics to become

331 slower as baseline WR was increased ($\tau\dot{Q}_m$ was increased by 5 s per 20 W increase in baseline).
332 Figure 9C shows that the difference between $\tau\dot{V}O_{2m}$ and $\tau\dot{V}O_{2p}$ tends to become smaller as the
333 baseline WR is increased (variable baseline condition).

334 Figure 10 displays the results of the simulated six muscle compartment model to a step
335 change from 20 to 120 W. Based on the dynamic responses of $\dot{V}O_{2p}$ and [HHb] from the group
336 means of the equal WR increment steps, the $\dot{V}O_{2m}$ and \dot{Q}_m kinetics for six theoretical muscle
337 compartments were determined: $\tau\dot{V}O_{2m} = 15, 20, 25, 30, 35, 40$ s and $\tau\dot{Q}_m = 20, 25, 30, 35, 40,$
338 45 s (for compartments 1 – 6, respectively). The resultant $\tau\dot{V}O_{2p}$ of the averaged $\dot{V}O_{2p}$ output
339 was 23.5 s, which was less than the mean $\tau\dot{V}O_{2m}$ of all compartments (27.5 s) and very similar to
340 the measured $\dot{V}O_{2p}$ response of 21.8 s (Figure 10).

341 **Discussion**

342 The purpose of this study was to examine the relative stability of phase II $\dot{V}O_{2p}$ and
343 [HHb] kinetics under a wide range of conditions with varying baseline work rate and work rate
344 increment during moderate-intensity exercise. The main findings of the study were that: i) in the
345 variable baseline condition, exercise transitions from a progressively greater baseline WR were
346 associated with progressive increases in both $\tau\dot{V}O_{2p}$ and G_p – the relationships between baseline
347 WR and $\tau\dot{V}O_{2p}$ or G_p were approximately linear; ii) in the constant baseline condition, but with
348 progressively greater Δ WR increments, $\tau\dot{V}O_{2p}$ did not vary, but G_p increased, with increasing
349 WR increment; iii) [HHb] kinetics were consistently faster than $\dot{V}O_{2p}$ kinetics by a magnitude
350 that was essentially constant under all experimental conditions (i.e., a slowing of $\dot{V}O_{2p}$ kinetics
351 was concomitant with a slowing of [HHb] kinetics) suggesting that both $\dot{V}O_{2m}$ and \dot{Q}_m kinetics
352 slowed in concert as baseline work rate increased; and iv) *in silico* simulations of the circulatory
353 influences dissociating $\tau\dot{V}O_{2p}$ from $\tau\dot{V}O_{2m}$ showed that the slowed $\dot{V}O_{2p}$ kinetics observed in the

354 variable baseline condition were unlikely to be consequent to altered circulatory dynamics. Thus,
355 these data suggest that $\dot{V}O_{2p}$ kinetics may be dependent on the instantaneous metabolic rate from
356 which they are initiated. However, the *in silico* simulations suggest that metabolic and
357 circulatory dynamics can turn a heterogeneous (or summed) $\dot{V}O_{2m}$ response containing
358 kinetically slow compartments into a uniformly fast $\dot{V}O_{2p}$ response. Therefore, we propose that
359 intracellular O_2 utilization dynamics are first order and are intrinsically slower (in a progressive
360 manner) in muscle fibres located at higher positions within the fibre recruitment hierarchy.

361 *Mechanisms of $\dot{V}O_2$ kinetic control*

362 Intriguingly, our finding that $\dot{V}O_{2p}$ kinetics were constant during different WR
363 increments initiated from a common baseline (20 W) is consistent with the traditional suggestion
364 that a first order rate reaction controls $\dot{V}O_{2p}$ kinetics (Whipp & Mahler, 1980; Barstow & Mole,
365 1991; Özyener *et al.* 2001; Scheuermann & Barstow, 2003; Spencer *et al.* 2013). However, the
366 finding that $\dot{V}O_{2p}$ kinetics increased linearly with increasing baseline work rate is also consistent
367 with the frequent and long-standing observation that moderate-intensity $\dot{V}O_{2p}$ kinetics are not
368 first order (Hughson & Morrissey, 1982; Brittain *et al.* 2001; Robergs, 2014). Importantly, these
369 findings were made within the same group of participants, ruling-out individual participant
370 differences as an explanation for differences when comparing amongst published studies.

371 When two identical step-WR increments (between 20 W and 90% $\hat{\theta}_L$) bisecting the
372 moderate-intensity domain are performed, the kinetics of $\dot{V}O_{2p}$ are consistently slower in the
373 upper compared to the lower step-transition (Brittain *et al.* 2001; MacPhee *et al.* 2005; Bowen *et*
374 *al.* 2011; Williams *et al.* 2013; Keir *et al.* 2014). By examining a range of pre-transition baseline
375 work and metabolic rates spanning the entire moderate-intensity domain, the present study
376 extends previous findings to show that both $\tau\dot{V}O_{2p}$ and G_p increase as a function of pre-transition

377 baseline WR and metabolic rate in an approximately linear manner. This phenomenon has been
378 previously explained by two hypotheses. The first is a fibre activation pattern favouring
379 “kinetically-faster”, more efficient fibres, to perform lower intensities and a progressive
380 incorporation of “kinetically-slower” fibres to perform greater external WRs (Brittain *et al.*
381 2001). An alternative view proposes that $\dot{V}O_{2p}$ kinetics are influenced by the pre-transition
382 metabolic rate such that greater reductions in intracellular energy state cause less free energy
383 delivery from the mitochondria and increase the demand for mitochondrial ATP production
384 (Bowen *et al.* 2011; Wüst *et al.* 2014). It currently is not possible to know whether the muscle
385 motor units recruited to transition to low work rates are the same or different from those
386 recruited to transition to greater work rates. Thus, we cannot distinguish whether the present
387 results are due to recruitment of motor units innervating muscle fibres with inherently different
388 mitochondrial volume-density, or whether our findings were consequent to a progressive
389 contribution of increasing metabolic rate within a uniform fibre population.

390 It has been suggested that the free energy associated with ATP hydrolysis (ΔG_{ATP} , i.e.,
391 $\sim 58 \text{ kJ}\cdot\text{mol}^{-1}$) is greater than the activation energy required for acto-myosin crossbridge cycling
392 ($\sim 40 \text{ kJ}\cdot\text{mol}^{-1}$ (Sheetz *et al.* 1984) but close to that for SERCA ATPase function ($\sim 52 \text{ kJ}\cdot\text{mol}^{-1}$)
393 under normal physiological conditions (Grassi *et al.* 2015). Since the concentration of
394 metabolites that affect ΔG_{ATP} (i.e., $[\text{ADP}_{free}]$ and $[\text{Pi}]$) are elevated at greater baseline metabolic
395 rates, less negative ΔG_{ATP} could prevent some acto-myosin crossbridge or SERCA ATPase
396 regions from receiving sufficient energy to activate. The resultant effect would be a
397 progressively larger and possibly less “square wave” change in ATP required for a given change
398 in WR, thereby altering the exponential-nature of the response leading to an increase in both
399 $\tau\dot{V}O_{2p}$ and G_p . However, this hypothesis is difficult to reconcile in moderate-intensity cycling

400 exercise, where muscle fatigue appears to be absent (Cannon *et al.* 2011). An alternative
401 mechanism could be that $\dot{V}O_{2m}$ of individual fibers does operate through a unimolecular reaction
402 of ADP-feedback to its mitochondrial network; when these same fibers are recruited at a greater
403 level of metabolic activity, the intracellular $[ADP_{free}]$ exceeds the K_m and thus the sensitivity of
404 $\dot{V}O_{2m}$ to increases in $[ADP_{free}]$ may decrease, manifesting as a slower rate of adjustment in $\dot{V}O_{2p}$
405 with a greater baseline metabolic rate. Nevertheless, this also seems unlikely in moderate
406 exercise where intramyocellular disturbance in $[ADP_{free}]$ in the active fibres is likely small and
407 thus an alternative explanation is required.

408 *In silico simulations*

409 Using *in silico* simulations based on a validated multi-compartmental model of
410 circulatory dynamics, Benson *et al.* (2013) demonstrated that kinetic dissociations between phase
411 II $\dot{V}O_{2p}$ and $\dot{V}O_{2m}$ can occur dependent on both the dynamics of muscle and whole body \dot{Q} and
412 flow-weighted mixing of muscle and whole body venous effluents. In the present study, this
413 model was applied to assess whether altered circulatory dynamics with transitions from
414 increasing baseline WRs might distort, by “slowing”, the dynamic adjustment of the $\dot{V}O_{2p}$ profile
415 despite $\dot{V}O_{2m}$ kinetics being “constant” when transitioning from progressively higher starting
416 baseline metabolic rates. When assuming constant kinetics for $\dot{V}O_{2m}$ ($\tau\dot{V}O_{2m}$, 26.1 s) and \dot{Q}_m
417 (28.5 s) (see Methods), simulations showed that constant $\Delta 40W$ step-transitions initiated from
418 progressively higher baseline WRs (from 20 to 120W) were associated with a slight ~ 2 s
419 increase in $\tau\dot{V}O_{2p}$ (22.0 to 24.2 s); however, this effect was not large enough to explain the
420 observed $\tau\dot{V}O_{2p}$ which increased from 22 s to 35 s under the same conditions. This suggests that
421 significant “distortion” of the $\dot{V}O_{2m}$ profile at the lung because of circulatory dynamics and
422 mixing of muscle and other body venous compartments, while potentially contributory, likely

423 were not responsible for the major progressive slowing of $\dot{V}O_{2p}$ kinetics observed in this study
424 (Bowen *et al.* 2011).

425 That [HHb] kinetics were consistently faster than $\dot{V}O_{2p}$ kinetics suggests that, overall,
426 during the on-transient phase, a greater rate of O_2 extraction was required relative to the rate of
427 muscle O_2 utilization. Since, the magnitude by which kinetics of both signals differed did not
428 change as a function of baseline WR (Figure 8) suggests that the underlying microvascular blood
429 flow (\dot{Q}_m) dynamics were slightly slower than $\dot{V}O_{2m}$ and that \dot{Q}_m became progressively slower
430 with transitions from greater baseline WRs. Given that MacPhee *et al.* (2005) also reported a
431 twofold increase in the time constant for bulk leg blood flow when equal WR increment step-
432 transitions were initiated from WRs midway between 20W and 90% $\hat{\theta}_L$ compared to a baseline
433 of 20W, we examined the effect of progressively increasing $\tau\dot{Q}_m$ (by a factor of ~ 5 s per 20W
434 increase in baseline; MacPhee *et al.* (2005)) on the modulation of $\tau\dot{V}O_{2p}$ for a range of $\tau\dot{V}O_{2m}$
435 (from 20 – 40 s; Figure 9C). For any given $\tau\dot{V}O_{2m}$, $\tau\dot{V}O_{2p}$ was reduced as baseline WR and $\tau\dot{Q}_m$
436 increased (a relationship opposite to that observed experimentally in the present study). This
437 further supports the notion that the slowing of $\dot{V}O_{2p}$ kinetics coincident with higher baseline
438 metabolic rates, seen in this and other studies, reflects an actual slowing of $\dot{V}O_{2m}$ kinetics, and is
439 not a consequence of circulatory-induced “distortions” of an unchanging $\dot{V}O_{2m}$ profile.

440 Since It has been suggested that the dynamics of O_2 delivery are regionally
441 heterogeneous within human quadriceps muscle (Koga *et al.* 2007), we further used this model to
442 provide an additional explanation to reconcile why $\dot{V}O_{2p}$ kinetics were slowed (from ~ 22 s to \sim
443 35 s) in transitions initiated from progressively higher baseline WRs but are fast (~ 20 s) and
444 unchanged with transitions initiated from a 20 W baseline but increasing ΔWR increment. In
445 accordance with Koga *et al.* (2007), we proposed that “kinetically-faster” fibre pools

446 (presumably those with a greater mitochondrial volume and better blood supply) dominate the
447 collective, or homogenized, $\dot{V}O_{2p}$ response by exerting a greater influence on C_vO_2 of the blood
448 draining the muscle during the early portion of the transition. To explore this suggestion we used
449 a model broadly based on the six muscle “compartments” derived from the responses to the six
450 $\Delta 40W$ steps that were examined in this study. We assumed that each compartment was of equal
451 size but varied in $\dot{V}O_{2m}$ and \dot{Q}_m kinetics. We examined the average $\dot{V}O_{2p}$ response to a step-
452 change in WR from 20 to 120 W derived from the simultaneous activation of these
453 compartments (see Methods). $\dot{V}O_{2m}$ and \dot{Q}_m kinetics for each compartment were determined *ad-*
454 *hoc* and were selected to reflect the kinetics (as inferred from $\dot{V}O_{2p}$ and [HHb]) from the group
455 means of the equal WR increment steps ($\tau\dot{V}O_{2m} = 15, 20, 25, 30, 35, 40$ s and $\tau\dot{Q}_m = 20, 25, 30,$
456 $35, 40, 45$ s for compartments 1 – 6, respectively). There was a slight discrepancy between the
457 $\dot{V}O_{2p}$ model output and the measured $\dot{V}O_{2p}$ response (Figure 10) in the early phase (phase I) of
458 the transition (possibly due to differences in the blood flow profile during abrupt transitions from
459 $\Delta 100W$ compared to $\Delta 40W$). However, phase II $\tau\dot{V}O_{2p}$ was 23.5 s which was less than the mean
460 $\tau\dot{V}O_{2m}$ of all compartments (27.5 s) and very similar to the measured $\dot{V}O_{2p}$ response of 22 s. This
461 suggests that the conflation of \dot{Q}_m dynamics amongst compartments may modulate the
462 “summed” $\dot{V}O_{2m}$ kinetics towards a faster $\tau\dot{V}O_{2p}$ via disproportionate and time-dependent
463 contributions (of those compartments) to the dynamics of C_vO_2 of blood draining the muscle.
464 Therefore, it is possible that the $\tau\dot{V}O_{2p}$ from the larger step-changes (i.e., greater WR increments)
465 may have been influenced by the blood flow and metabolic heterogeneity and differences in
466 circulatory and metabolic dynamics existing within the pool of muscle fibres recruited to support
467 the change in WR. Although \dot{Q}_m dynamics were not directly measured, microvascular O_2
468 pressure ($P_{mv}O_2$) has been shown to drop at a faster rate and to a greater extent at the onset of

469 contraction in rat muscle comprised predominately of fast- compared to slow-muscle fibres
470 (Behnke *et al.* 2003; McDonough *et al.* 2005). Therefore, it is conceivable that human skeletal
471 muscle may also express diversity in \dot{Q}_m dynamics such that muscle fibres with slow $\dot{V}O_{2m}$
472 kinetics may also have slower blood flow dynamics.

473 *Reconciling models of $\dot{V}O_{2p}$ kinetic control*

474 Thus, while distinguishing among the various hypotheses for $\dot{V}O_{2p}$ kinetic control will
475 require complex and detailed descriptions of $\dot{V}O_{2m}$ and intramyocellular signalling dynamics
476 under a range of conditions (such as those used in this study), our combined *in vivo* and *in silico*
477 data propose a unifying hypothesis. That $\dot{V}O_{2p}$ kinetics were invariant in the moderate domain
478 with constant baseline WR is consistent with first order control. While the progressive increase
479 in $\tau\dot{V}O_{2p}$ with increasing baseline work rates appears to subvert the requirements of a first order
480 system, we propose that these findings can be reconciled by the understanding of the
481 heterogeneity in both dynamics of muscle motor unit recruitment (of fibres with different a
482 volume of mitochondrial network) and of blood flow (where the microvascular blood flow
483 dynamics may vary to a similar magnitude to $\dot{V}O_{2p}$ kinetics). Thus, the transit and mixing of
484 blood draining kinetically variable motor units are later combined to form a $\dot{V}O_{2p}$ kinetic
485 response that is dominated by the influence of the motor units where both $\dot{V}O_{2m}$ and \dot{Q}_m kinetics
486 are fast. These features can explain both the main findings of $\dot{V}O_{2p}$ kinetic behaviour in this
487 study and remain consistent with the assumptions of apparent first order control.

488 *Conclusion*

489 The main finding of the present study was that $\dot{V}O_{2p}$ kinetics are not altered when step-
490 changes are initiated from a 20 W baseline WR, but get progressively slower with increasing
491 baseline work rate. The observed slowing of $\dot{V}O_{2p}$ kinetics with increasing baseline intensity

492 could not be attributed to limitations in O₂ delivery (since the magnitude of the difference
493 between kinetic adjustments of [HHb] and $\dot{V}O_{2p}$ was unchanged regardless of baseline WR or
494 Δ WR increment). However, a model combining heterogeneous metabolism and blood flow
495 kinetics was able to reconcile these apparently conflicting findings. Collectively, these data
496 suggest that within the moderate-intensity domain phase II $\dot{V}O_{2p}$ kinetics are influenced by pre-
497 transition WR, becoming slower as baseline WR increases, and are strongly influenced by
498 heterogeneity in the dynamic metabolic and circulatory properties of the active muscles. Taken
499 together, these data suggest that the work rate-dependent non-linear $\dot{V}O_{2p}$ responses may be
500 attributable to “heterogeneity” within the range of muscle fibres recruited to address the exercise
501 challenge. Importantly, each of these muscle fibres may independently behave as a linear, first
502 order system.

503 **Acknowledgments:** We would like to express our gratitude to the participants in this study. We
504 also extend our gratitude to Professor P.A. Robbins, University of Oxford, for providing the
505 “End-tidal Forcing” software for breath-by-breath pulmonary oxygen uptake measurement.

506

507 **Grants:** This study was supported by the National Science and Engineering Research Council of
508 Canada (NSERC) research and equipment grants (RGPGP-2015-00084). Daniel A. Keir was
509 supported by a Post-Graduate Doctoral Scholarship from NSERC.

510

511 **Disclosures:** No conflicts of interest, financial or otherwise, are declared by the authors.

512

513 **Author Contributions:** D.A.K and J.M.K. conceived and designed the study; D.A.K and T.C.R
514 collected the data; A.P.B collected the computational data; all authors interpreted the results of
515 the experiment/simulations; D.A.K. prepared the figures and the first draft of the manuscript; all
516 authors edited and approved the final version of the manuscript.

517

518 **References**

- 519 Barstow TJ, Lamarra N & Whipp BJ (1990). Modulation of muscle and pulmonary O₂ uptakes
520 by circulatory dynamics during exercise. *J Appl Physiol* **68**, 979–989.
- 521 Barstow TJ & Mole PA (1991). Linear and nonlinear characteristics of oxygen uptake kinetics
522 during heavy exercise. *J Appl Physiol* **71**, 2099–2106.
- 523 Beaver WL, Wasserman K & Whipp BJ (1986). A new method for detecting anaerobic threshold
524 by gas exchange. *J Appl Physiol* **60**, 2020–2027.
- 525 Behnke BJ, McDonough P, Padilla DJ, Musch TI & Poole DC (2003). Oxygen exchange profile
526 in rat muscles of contrasting fibre types. *J Physiol* **549**, 597–605.
- 527 Benson AP, Grassi B & Rossiter HB (2013). A validated model of oxygen uptake and circulatory
528 dynamic interactions at exercise onset in humans. *J Appl Physiol* **115**, 743–755.
- 529 Bowen TS, Murgatroyd SR, Cannon DT, Cuff TJ, Lainey AF, Marjerrison AD, Spencer MD,
530 Benson AP, Paterson DH, Kowalchuk JM & Rossiter HB (2011). A raised metabolic rate
531 slows pulmonary O₂ uptake kinetics on transition to moderate-intensity exercise in humans
532 independently of work rate. *Exp Physiol* **96**, 1049–1061.
- 533 Brittain CJ, Rossiter HB, Kowalchuk JM & Whipp BJ (2001). Effect of prior metabolic rate on
534 the kinetics of oxygen uptake during moderate-intensity exercise. *Eur J Appl Physiol* **86**,
535 125–134.
- 536 Cannon DT, White AC, Andriano MF, Kolkhorst FW & Rossiter HB (2011). Skeletal muscle
537 fatigue precedes the slow component of oxygen uptake kinetics during exercise in humans.
538 *J Physiol* **589**, 727–739.
- 539 DiMenna FJ, Bailey SJ, Vanhatalo A, Chidnok W & Jones AM (2010). Elevated baseline VO₂
540 per se does not slow O₂ uptake kinetics during work-to-work exercise transitions. *J Appl*
541 *Physiol* **109**, 1148–1154.
- 542 Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK & Wagner PD (1996). Muscle O₂
543 uptake kinetics in humans: implications for metabolic control. *J Appl Physiol* **80**, 988–998.
- 544 Grassi B, Rossiter HB & Zoladz JA (2015). Skeletal muscle fatigue and decreased efficiency:
545 two sides of the same coin? *Exerc Sport Sci Rev* **43**, 75–83.
- 546 Hoffmann U, Drescher U, Benson AP, Rossiter HB & Essfeld D (2013). Skeletal muscle VO₂
547 kinetics from cardio-pulmonary measurements: assessing distortions through O₂ transport
548 by means of stochastic work-rate signals and circulatory modelling. *Eur J Appl Physiol* **113**,
549 1745–1754.

- 550 Hughson RL & Morrissey M (1982). Delayed kinetics of respiratory gas exchange in the
551 transition from prior exercise. *J Appl Physiol* **52**, 921–929.
- 552 Keir DA, Nederveen JP, Paterson DH & Kowalchuk JM (2014). Pulmonary O₂ uptake kinetics
553 during moderate-intensity exercise transitions initiated from low versus elevated metabolic
554 rates: insights from manipulations in cadence. *Eur J Appl Physiol* **114**, 2655–2665.
- 555 Koga S, Poole DC, Ferreira LF, Whipp BJ, Kondo N, Saitoh T, Ohmae E & Barstow TJ (2007).
556 Spatial heterogeneity of quadriceps muscle deoxygenation kinetics during cycle exercise. *J*
557 *Appl Physiol (Bethesda, Md 1985)* **103**, 2049–2056.
- 558 Krstrup P, Jones AM, Wilkerson DP, Calbet JAL & Bangsbo J (2009). Muscular and
559 pulmonary O₂ uptake kinetics during moderate- and high-intensity sub-maximal knee-
560 extensor exercise in humans. *J Physiol* **587**, 1843–1856.
- 561 Lamarra N, Whipp BJ, Ward SA & Wasserman K (1987). Effect of interbreath fluctuations on
562 characterizing exercise gas exchange kinetics. *J Appl Physiol* **62**, 2003–2012.
- 563 MacPhee SL, Shoemaker JK, Paterson DH & Kowalchuk JM (2005). Kinetics of O₂ uptake, leg
564 blood flow, and muscle deoxygenation are slowed in the upper compared with lower region
565 of the moderate-intensity exercise domain. *J Appl Physiol* **99**, 1822–1834.
- 566 McDonough P, Behnke BJ, Padilla DJ, Musch TI & Poole DC (2005). Control of microvascular
567 oxygen pressures in rat muscles comprised of different fibre types. *J Physiol* **563**, 903–913.
- 568 Murias JM, Spencer MD & Paterson DH (2014). The critical role of O₂ provision in the dynamic
569 adjustment of oxidative phosphorylation. *Exerc Sport Sci Rev* **42**, 4–11.
- 570 Özyener F, Rossiter HB, Ward SA & Whipp BJ (2001). Influence of exercise intensity on the on-
571 and off-transient kinetics of pulmonary oxygen uptake in humans. *J Physiol* **533**, 891–902.
- 572 Robergs RA (2014). A critical review of the history of low- to moderate-intensity steady-state
573 VO₂ kinetics. *Sport Med* **44**, 641–653.
- 574 Rossiter HB (2011). Exercise: Kinetic Considerations for Gas Exchange. *Compr Physiol* **1**, 203–
575 244.
- 576 Scheuermann BW & Barstow TJ (2003). O₂ uptake kinetics during exercise at peak O₂ uptake. *J*
577 *Appl Physiol* **95**, 2014–2022.
- 578 Sheetz MP, Chasan R & Spudich J a. (1984). ATP-dependent movement of myosin in vitro:
579 Characterization of a quantitative assay. *J Cell Biol* **99**, 1867–1871.

- 580 Spencer MD, Murias JM, Kowalchuk JM & Paterson DH (2011). Pulmonary O₂ uptake and
581 muscle deoxygenation kinetics are slowed in the upper compared with lower region of the
582 moderate-intensity exercise domain in older men. *Eur J Appl Physiol* **111**, 2139–2148.
- 583 Spencer MD, Murias JM, Kowalchuk JM & Paterson DH (2013). Effect of moderate-intensity
584 work rate increment on phase II tauVO₂, functional gain and Delta[HHb]. *Eur J Appl*
585 *Physiol* **113**, 545–557.
- 586 Spencer MD, Murias JM & Paterson DH (2012). Characterizing the profile of muscle
587 deoxygenation during ramp incremental exercise in young men. *Eur J Appl Physiol* **112**,
588 3349–3360.
- 589 Swanson GD (1980). Breath-to-breath considerations for gas exchange kinetics. In *Exercise*
590 *Bioenergetics and Gas Exchange*, ed. Cerretelli P & Whipp BJ, pp. 211–222. Elsevier,
591 Amsterdam.
- 592 Whipp BJ & Mahler M (1980). Dynamics of pulmonary gas exchange during exercise. In
593 *Pulmonary Gas Exchange, Vol II, Organism and Environment*, ed. West JB, pp. 33–96.
594 Academic Press, New York.
- 595 Whipp BJ, Ward SA, Lamarra N, Davis JA & Wasserman K (1982). Parameters of ventilatory
596 and gas exchange dynamics during exercise. *J Appl Physiol* **52**, 1506–1513.
- 597 Williams AM, Paterson DH & Kowalchuk JM (2013). High-intensity interval training speeds the
598 adjustment of pulmonary O₂ uptake, but not muscle deoxygenation, during moderate-
599 intensity exercise transitions initiated from low and elevated baseline metabolic rates. *J*
600 *Appl Physiol* **114**, 1550–1562.
- 601 Wüst RC, McDonald JR, Sun Y, Ferguson BS, Rogatzki MJ, Spires J, Kowalchuk JM, Gladden
602 LB & Rossiter HB (2014). Slowed muscle oxygen uptake kinetics with raised metabolism
603 are not dependent on blood flow or recruitment dynamics. *J Physiol* **592**, 1857–1871.
- 604 Wüst RCI, Grassi B, Hogan MC, Howlett R a, Gladden LB & Rossiter HB (2011). Kinetic
605 control of oxygen consumption during contractions in self-perfused skeletal muscle. *J*
606 *Physiol* **589**, 3995–4009.
- 607

608 **Table Legends**

609 **Table 1.** Mean parameter estimates and fit statistics of phase II $\dot{V}O_{2p}$ data for each 40W
610 transition from progressively increasing baseline work rates (mean \pm SD)

611 ^{a-f} indicate significant differences between conditions ($p < 0.05$). “^a” indicates difference from “**20**
612 \rightarrow **60**”, “^b” indicates difference from “**40** \rightarrow **80**”, and so forth. * indicates significant differences
613 amongst all conditions ($p < 0.05$).

614

615 **Table 2.** Mean parameter estimates and fit statistics of phase II $\dot{V}O_{2p}$ data for each Δ WR
616 transition from a constant baseline of 20W (mean \pm SD)

617 ^{a-d} indicate significant differences between conditions ($p < 0.05$). “^a” indicates difference from “**20**
618 \rightarrow **60**”, “^b” indicates difference from “**20** \rightarrow **80**”, and so forth. * indicates significant differences
619 amongst all conditions ($p < 0.05$).

620

621 **Table 3.** Mean parameter estimates and confidence interval of mono-exponential fit of [HHb]
622 data for each 40W transition from progressively increasing baseline work rates (mean \pm SD)

623 ^{a-f} indicate significant differences between conditions ($p < 0.05$). “^a” indicates difference from “**20**
624 \rightarrow **60**”, “^b” indicates difference from “**40** \rightarrow **80**”, and so forth. * indicates significant differences
625 amongst all conditions ($p < 0.05$).

626

627 **Table 4.** Mean parameter estimates and confidence interval of mono-exponential fit of [HHb]
628 data for each Δ WR transition from a constant baseline of 20W (mean \pm SD)

629 ^{a-d} indicate significant differences between conditions ($p < 0.05$). “^a” indicates difference from “**20**
630 → **60**”, “^b” indicates difference from “**20** → **80**”, and so forth. * indicates significant differences
631 amongst all conditions ($p < 0.05$).

632

633 **Figure Captions**

634 **Figure 1.** Schematic of experimental protocols and procedures. See text for details.

635

636 **Figure 2.** Ensemble-averaged group mean responses of $\dot{V}O_{2p}$ in the variable baseline condition:
637 six 40 W transitions from different baseline work rates. Vertical dashed lines indicate the onset
638 of the transition (time = 0 s). The group mean phase II kinetic responses for each condition are
639 superimposed on the data (*dark lines*, fitted with a mono-exponential function using group mean
640 parameter estimates). $\tau\dot{V}O_{2p}$ values (\pm SD) are inset under each transition and error bars at
641 specific time points indicate SD.

642

643 **Figure 3.** A) Baseline $\dot{V}O_{2p}$ ($\dot{V}O_{2pBSL}$) as a function of baseline WR. B) $\dot{V}O_{2p}$ amplitude (A) as
644 function of $\dot{V}O_{2pBSL}$. C) $\dot{V}O_{2p}$ time constant ($\tau\dot{V}O_{2p}$) as function of $\dot{V}O_{2pBSL}$. D) Gain (G_p) as
645 function of $\dot{V}O_{2pBSL}$. Symbols represent group mean \pm SD. ^{a-f} indicate significant differences
646 between conditions ($p < 0.05$). “^a” indicates difference from “**20** \rightarrow **60**”, “^b” indicates difference
647 from “**40** \rightarrow **80**”, and so forth. * indicates significant differences amongst all transitions
648 ($p < 0.05$).

649

650 **Figure 4.** Ensemble-averaged group mean responses of $\dot{V}O_{2p}$ in the constant baseline condition:
651 four different increments in work rate (ΔWR) each from a 20 W baseline work rate. Vertical
652 dashed lines indicate the onset of the transition (time = 0 s). The group mean phase II kinetic
653 responses for each condition are superimposed over the data (*dark lines*, fitted with a mono-
654 exponential function using group mean parameter estimates). $\tau\dot{V}O_{2p}$ values (\pm SD) are inset
655 under each transition and error bars at specific time points indicate SD.

656

657 **Figure 5.** Comparison of A) baseline $\dot{V}O_{2p}$ ($\dot{V}O_{2pBSL}$); B) $\dot{V}O_{2p}$ amplitude (A); C) $\dot{V}O_{2p}$ time
658 constant ($\tau\dot{V}O_{2p}$); and D) gain (G_p) as function of as a function of WR increment (ΔWR).
659 Symbols represent group mean \pm SD. ^{a-d} indicate significant differences between conditions
660 ($p < 0.05$). “^a” indicates difference from “**20** \rightarrow **60**”, “^b” indicates difference from “**20** \rightarrow **80**”, and
661 so forth. * indicates significant differences amongst all transitions ($p < 0.05$).

662

663 **Figure 6.** Ensemble-averaged group mean responses of [HHb] in the variable baseline condition:
664 six 40 W transitions from different baseline work rates. Vertical dashed lines indicate the onset
665 of the transition (time = 0 s). The group mean phase II kinetic responses for each condition are
666 superimposed on the data (*dark lines*, fitted with a mono-exponential function using group mean
667 parameter estimates). τ [HHb] values (\pm SD) are inset under each transition and error bars at
668 specific time points indicate SD. Error bars at specific time points are not displayed due to the
669 large SD in [HHb] amongst subjects (see Table 3).

670

671 **Figure 7.** Ensemble-averaged group mean responses of [HHb] in the constant baseline condition:
672 four different increments in work rate (ΔWR) each from a 20 W baseline work rate. Vertical
673 dashed lines indicate the onset of the transition (time = 0 s). The group mean phase II kinetic
674 responses for each condition are superimposed over the data (*dark lines*, fitted with a mono-
675 exponential function using group mean parameter estimates). τ [HHb] values (\pm SD) are inset
676 under each transition. Error bars at specific time points are not displayed due to the large SD in
677 [HHb] amongst subjects (see Table 4).

678

679

680

681 **Figure 8.** Comparison of the “time to reach steady-state” between $\dot{V}O_{2p}$ and NIRS-derived
682 deoxygenated hemoglobin ([HHb]) signals following exercise onset. “Time to reach steady-
683 state” was computed as the difference between the sum of 4 time constants for phase II $\dot{V}O_{2p}$ ($4 \times$
684 $\tau\dot{V}O_{2p}$) and 4 time constants plus the time delay for [HHb] ($[\text{HHb}]\text{-TD} + (4 \times \tau[\text{HHb}])$). Panel *A*
685 shows group mean \pm SD for the variable baseline condition; 40 W transitions from different
686 baseline work rates (*black bars*). Panel *B* shows group mean \pm SD for each the constant baseline
687 condition; variable increments in work rate each from a 20 W baseline (*grey bar*). There were no
688 differences amongst transitions ($p > 0.05$).

689

690 **Figure 9.** The output of a dynamic computational simulation to illustrate the influence of
691 circulatory dynamics on the phase II pulmonary $\dot{V}O_{2p}$ during exercise transitions in both the
692 variable and constant baseline conditions. Modulation of $\tau\dot{V}O_{2p}$ at a fixed muscle $\tau\dot{V}O_{2m}$ ($\tau\dot{V}O_{2m}$;
693 26.1 s) and muscle $\tau\dot{Q}$ ($\tau\dot{Q}_m$; 28.5 s) is examined for step-transitions from a 20 W baseline to
694 increasing ΔWR increments (*panel A*) and for $\Delta 40\text{W}$ step-transitions from increasing baseline
695 WR (WR_{bsl} ; *panel B*). *Panel C* displays the effect of circulatory dynamics on $\tau\dot{V}O_{2p}$ during
696 simulated $\Delta 40\text{W}$ exercise transitions from increasing baseline WRs at various $\tau\dot{V}O_{2m}$ (*circles*, 20
697 s; *triangles*, 30 s; *squares*, 40 s). The $\tau\dot{Q}_m$ values used in the simulations are based on data from
698 MacPhee et al. (2005) who showed that \dot{Q}_m dynamics slow with transitions from elevated
699 metabolic rates. Note that as $\tau\dot{Q}_m$ increases with increasing baseline WR for each isopleth of
700 $\tau\dot{V}O_{2m}$, $\tau\dot{V}O_{2p}$ becomes smaller (faster kinetics) which is opposite to the results from the *in vivo*
701 data. See text for further explanation.

702
703
704
705
706
707
708
709
710
711
712
713
714
715

Figure 10. Ensemble-averaged group mean responses of $\dot{V}O_{2p}$ for the $\Delta 100W$ step-transition from a 20 W baseline work rate. Vertical dashed lines indicate the onset of the transition (time = 0 s). A theoretical model containing six muscle compartments of equal size but varying $\dot{V}O_{2m}$ and \dot{Q}_m kinetics ($\tau\dot{V}O_{2m} = 15, 20, 25, 30, 35, 40$ s and $\tau\dot{Q}_m = 20, 25, 30, 35, 40, 45$ s for compartments 1 – 6, respectively) was developed and computational simulations were used to investigate whether the conflation of \dot{Q}_m dynamics amongst compartments may modulate the “summed” (or homogenized) $\dot{V}O_{2m}$ kinetics towards a faster $\tau\dot{V}O_{2p}$ response in a 100W step-change from 20 W. Assuming equal contributions from each compartment, the resultant $\dot{V}O_{2p}$ response (*black line*) is overlaid on the *in vivo* group mean response (*white circles*). The phase II $\tau\dot{V}O_{2p}$ from the six compartment model was 23.5 s, which is slightly faster than the mean $\tau\dot{V}O_{2m}$ of all compartments, 27.5 s and similar to the $\tau\dot{V}O_{2p}$ measured in the study participants (21.8 ± 4.8 s).

Table 1. Mean parameter estimates (\pm SD) for phase II $\dot{V}O_{2p}$ kinetics during 40 W exercise transitions from six different baseline work rates (variable baseline condition).

n = 14	Step-transition power output (W)					
	20 \rightarrow 60	40 \rightarrow 80	60 \rightarrow 100	80 \rightarrow 120	100 \rightarrow 140	120 \rightarrow 160
$\dot{V}O_{2pBSL}$ (L \cdot min $^{-1}$)*	0.75 \pm 0.07	0.93 \pm 0.06	1.10 \pm 0.07	1.30 \pm 0.06	1.48 \pm 0.06	1.71 \pm 0.08
A (L \cdot min $^{-1}$)	0.35 \pm 0.02 ^{cdef}	0.37 \pm 0.02 ^{def}	0.39 \pm 0.02 ^{af}	0.40 \pm 0.20 ^{ab}	0.41 \pm 0.03 ^{ab}	0.42 \pm 0.04 ^{abc}
$\dot{V}O_{2pSS}$ (L \cdot min $^{-1}$)*	1.10 \pm 0.07	1.30 \pm 0.06	1.49 \pm 0.07	1.70 \pm 0.07	1.90 \pm 0.07	2.12 \pm 0.08
TD (s)	12 \pm 6	10 \pm 4	7 \pm 6	6 \pm 6	6 \pm 3	7 \pm 3
$\tau\dot{V}O_{2p}$ (s)	22 \pm 5 ^{cdef}	23 \pm 5 ^{def}	28 \pm 6 ^{af}	31 \pm 7 ^{ab}	34 \pm 6 ^{ab}	35 \pm 9 ^{abc}
CI ₉₅ (s)	3 \pm 1	3 \pm 1	3 \pm 1	4 \pm 1	4 \pm 1	4 \pm 1
χ^2 (x 10 $^{-4}$)	7.6 \pm 4.2	10.2 \pm 5.8	8.9 \pm 3.8	12.2 \pm 5.7	11.3 \pm 4.5	13.6 \pm 4.4
MRT- $\dot{V}O_{2p}$ (s)	34 \pm 6 ^{ef}	32 \pm 4 ^{ef}	36 \pm 7 ^f	38 \pm 6	41 \pm 6 ^{ab}	45 \pm 13 ^{abc}
O _{2Def} (mL)	193 \pm 32 ^{def}	197 \pm 26 ^{ef}	233 \pm 43 ^f	251 \pm 47 ^{af}	285 \pm 51 ^{ab}	319 \pm 115 ^{abcd}
G _p (mL \cdot min $^{-1}$ \cdot W $^{-1}$)	8.7 \pm 0.6 ^{cdef}	9.2 \pm 0.5 ^{def}	9.7 \pm 0.6 ^{af}	10.0 \pm 0.6 ^{ab}	10.3 \pm 0.8 ^{ab}	10.5 \pm 0.9 ^{abc}

^{a-f} indicate significant differences between conditions (p<0.05). “^a” indicates difference from “20 \rightarrow 60”, “^b” indicates difference from “40 \rightarrow 80”, and so forth. * indicates significant differences amongst all conditions (p<0.05).

Table 2. Mean parameter estimates (\pm SD) for phase II $\dot{V}O_{2p}$ kinetics during exercise transitions from 20W to four different work rates (constant baseline condition).

n = 14	Step-transition power output (W)			
	20 \rightarrow 60	20 \rightarrow 80	20 \rightarrow 100	20 \rightarrow 120
$\dot{V}O_{2pBSL}$ (L·min ⁻¹)	0.75 \pm 0.07	0.75 \pm 0.06	0.76 \pm 0.06	0.76 \pm 0.06
A (L·min ⁻¹)*	0.35 \pm 0.02	0.54 \pm 0.02	0.72 \pm 0.04	0.93 \pm 0.04
$\dot{V}O_{2pSS}$ (L·min ⁻¹)*	1.10 \pm 0.07	1.30 \pm 0.06	1.47 \pm 0.05	1.68 \pm 0.07
TD (s)	12 \pm 6	11 \pm 4	12 \pm 3	11 \pm 3
$\tau\dot{V}O_{2p}$ (s)	22 \pm 5	20 \pm 4	22 \pm 6	22 \pm 5
CI ₉₅ (s)	3 \pm 1	2 \pm 1	2 \pm 1	1 \pm 0
χ^2 (x 10 ⁻⁴)	7.6 \pm 4.2	10.7 \pm 5.3	12.0 \pm 5.5	12.5 \pm 4.5
MRT- $\dot{V}O_{2p}$ (s)	34 \pm 6	31 \pm 3	33 \pm 4	33 \pm 5
O _{2Def} (mL)*	193 \pm 32	276 \pm 26	389 \pm 47	501 \pm 73
G _p (mL·min ⁻¹ ·W ⁻¹)	8.7 \pm 0.6 ^{bd}	9.0 \pm 0.4 ^a	8.9 \pm 0.4	9.3 \pm 0.4 ^a

^{a-d} indicate significant differences between conditions (p<0.05). “^a” indicates difference from “20 \rightarrow 60”, “^b” indicates difference from “20 \rightarrow 80”, and so forth. * indicates significant differences amongst all conditions (p<0.05).

1 **Table 3.** Mean parameter estimates and confidence interval of mono-exponential fit of [HHb] data for each 40W transition from
 2 progressively increasing baseline work rates (mean \pm SD)
 3

	Step-transition power output (W)					
	20 \rightarrow 60	40 \rightarrow 80	60 \rightarrow 100	80 \rightarrow 120	100 \rightarrow 140	120 \rightarrow 160
[HHb] _{bsl} (μ M) *	19.2 \pm 7.2	21.9 \pm 8.1	22.3 \pm 8.9	24.0 \pm 9.5	24.5 \pm 10.6	27.1 \pm 11.5
Δ [HHb] _{amp} (μ M)	3.4 \pm 2.6	2.6 \pm 1.4	2.6 \pm 2.1	3.1 \pm 2.8	2.7 \pm 2.2	3.0 \pm 2.5
[HHb]-TD (s)	16 \pm 4 ^{ef}	15 \pm 3 ^e	13 \pm 4	11 \pm 5	11 \pm 4 ^a	9 \pm 5 ^{ab}
τ [HHb] (s)	9 \pm 4 ^{def}	11 \pm 3	14 \pm 7	17 \pm 6 ^a	18 \pm 11 ^a	18 \pm 6 ^a
MRT-[HHb] (s)	25 \pm 6	26 \pm 5	26 \pm 6	28 \pm 5	29 \pm 9	28 \pm 5
CI ₉₅ (s)	1 \pm 1	2 \pm 1	2 \pm 1	2 \pm 1	2 \pm 1	2 \pm 1

4

5 ^{a-f} indicate significant differences between conditions (p<0.05). “^a” indicates difference from “20 \rightarrow 60”, “^b” indicates difference from
 6 “40 \rightarrow 80”, and so forth. * indicates significant differences amongst all conditions (p<0.05).
 7

8

9

10

11

12

13 **Table 4.** Mean parameter estimates and confidence interval of mono-exponential fit of [HHb] data for each Δ WR transition from a
 14 constant baseline of 20W (mean \pm SD)
 15

	Step-transition power output (W)			
	20 \rightarrow 60	20 \rightarrow 80	20 \rightarrow 100	20 \rightarrow 120
[HHb] _{bsl} (μ M)	19.2 \pm 7.2	19.8 \pm 7.1	19.2 \pm 7.2	19.9 \pm 7.6
Δ [HHb] _{amp} (μ M)	3.4 \pm 2.6 *	4.8 \pm 3.3 *	6.3 \pm 4.6 *	9.4 \pm 2.8 *
[HHb]-TD (s)	16 \pm 4	14 \pm 3	13 \pm 2	11 \pm 3
τ [HHb] (s)	9 \pm 4	10 \pm 5	8 \pm 3	8 \pm 3
MRT-[HHb] (s)	25 \pm 6	24 \pm 7	20 \pm 4	20 \pm 4
CI ₉₅ (s)	1 \pm 1	1 \pm 1	1 \pm 1	1 \pm 0

16

17 ^{a-d} indicate significant differences between conditions (p<0.05). ^{“a”} indicates difference from “20 \rightarrow 60”, ^{“b”} indicates difference from
 18 “20 \rightarrow 80”, and so forth. * indicates significant differences amongst all conditions (p<0.05).

19



















