RELATIONSHIP BETWEEN (NON)LINEAR PHASE II PULMONARY OXYGEN UPTAKE KINETICS WITH SKELETAL MUSCLE OXYGENATION AND AGE IN 11 TO 15 Y OLDS

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Running Head: Oxygen uptake kinetics in youth

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New Findings

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

To investigate if the phase II parameters of pulmonary oxygen uptake ($\dot{V}O_2$) kinetics display linear, first-order behavior in association with alterations in skeletal muscle oxygenation during step cycling of different intensities or when exercise is initiated from an elevated work rate in youth.

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

We demonstrate how both linear and non-linear features of phase II $\dot{V}O_2$ kinetics may be determined by alterations in the dynamic balance between microvascular O₂ delivery/utilization in 11 to 15 y olds. We further implicate how the recruitment of higher-order (i.e. type II) muscle fibers during “work-to-work” cycling might be responsible for modulating $\dot{V}O_2$ kinetics with chronological age.
This study investigated in nineteen male youth (mean age: 13.6 ± 1.1 y, range: 11.7 – 15.7 y) the relationship between pulmonary oxygen uptake ($\dot{V}O_2$) and muscle deoxygenation kinetics during moderate- and very heavy-intensity ‘step’ cycling initiated from unloaded pedaling (i.e. U→M and U→VH) and moderate-to-very heavy-intensity step cycling (i.e. M→VH). Pulmonary $\dot{V}O_2$ was measured breath-by-breath and tissue oxygenation index (TOI) of the vastus lateralis using near-infrared spectroscopy.

There were no significant differences in the phase II time constant ($\tau_{\dot{V}O_2p}$) between U→M and U→VH (23 ± 6 s vs. 25 ± 7 s; $P = 0.36$); however, the $\tau_{\dot{V}O_2p}$ was slower during M→VH (42 ± 16 s) compared to other conditions ($P < 0.001$). Quadriceps TOI decreased with a faster ($P < 0.01$) mean response time (MRT; i.e. time delay + $\tau$) during U→VH (14 ± 2 s) compared to U→M (22 ± 4 s) and M→VH (20 ± 6 s). The difference ($\Delta$) between the $\tau_{\dot{V}O_2p}$ and MRT-TOI was greater during U→VH compared to U→M (12 ± 7 vs. 2 ± 7 s, $P < 0.001$) and during M→VH (23 ± 15 s) compared to other conditions ($P < 0.02$), suggesting an increased proportional speeding of fractional O$_2$ extraction. The slowing of the $\tau_{\dot{V}O_2p}$ during M→VH relative to U→M and U→VH correlated positively with chronological age ($r = 0.68$ and 0.57, respectively, $P < 0.01$). In youth, “work-to-work” transitions slowed microvascular O$_2$ delivery-to-O$_2$ utilization with alterations in phase II $\dot{V}O_2$ dynamics accentuated between the ages of 11 to 15 y.

**Keywords**: oxygen uptake time constant, microvascular blood flow, oxygen utilization, near-infrared spectroscopy, muscle fiber recruitment, youth
INTRODUCTION

Following the onset of step exercise, the time constant of phase II pulmonary oxygen uptake (i.e. $\tau_{O_2p}$) coheres with that observed for muscle $\dot{V}O_2$ kinetics (Grassi et al., 1996; Krustrup et al., 2009; Benson et al., 2013), or, as its surrogate, phosphocreatine (PCr) breakdown in adults (Rossiter et al., 1999) and children (Barker et al., 2008). However, whilst a progressive slowing of the $\tau_{O_2p}$ in older adults (Babcock et al., 1994; DeLorey et al., 2005) has been reported to originate during childhood (see McNarry, 2019 for a recent review), the physiological factors limiting $\dot{V}O_2$ kinetics remain less well understood in youth.

A first-order rate reaction controlling $\dot{V}O_2$ kinetics mandates that the response parameters obey the law of superimposition (Fujihara et al., 1973a; Fujihara et al., 1973b). That is, the $\tau_{O_2p}$ and primary gain ($G_p$; expressed as the $\dot{V}O_2$ per unit increment in work rate) remain constant following the onset of exercise of different intensities. In adults, whilst a slower $\tau_{O_2p}$ has been reported during step exercise above the lactate threshold (>LT) compared to <LT (Paterson & Whipp, 1991; Koppo et al., 2004; McNarry et al., 2012), other studies have reported no significant differences (Ozyener et al., 2001; Wilkerson et al., 2004). A slower $\tau_{O_2p}$ has been interpreted by some authors to reflect slower $O_2$ transport during supra-LT transitions (Hughson et al., 2001; McNarry et al., 2012). Conversely, in children, an invariant $\tau_{O_2p}$ during step exercise at progressively higher work rates (Hebestreit et al., 1998; Williams et al., 2001) or following “priming” exercise (Barker et al., 2010; Barker et al., 2014) suggests their phase II $\dot{V}O_2$ kinetics are principally limited by intracellular metabolic factors. However, in youth, the possibility that $O_2$ delivery might constrain the $\tau_{O_2p}$ in an exercise intensity dependent manner…
has previously relied on measures such as heart rate dynamics (Hebestreit et al., 1998; Breese et al., 2012), which, are removed from peripheral sites of O₂ exchange between the capillary and muscle.

The τ of muscle deoxyhemoglobin/myoglobin (deoxy[Hb+Mb]) measured by near-infrared spectroscopy (NIRS) has been reported to cohere with that of fractional O₂ extraction (Koga et al., 2012), hence, has been used to reflect the dynamic matching between O₂ delivery- (\(\dot{Q}_{\text{o2}}\)) to- O₂ utilization (\(\dot{V}'\text{o2}\)) during exercise (DeLorey et al., 2003; Grassi et al., 2003). Accordingly, for the same \(\dot{V}'\text{o2}\) kinetics, an enhanced \(\dot{Q}_{\text{o2}}/\dot{V}'\text{o2}\) response would be expected to slow deoxy[Hb+Mb] dynamics, whereas, slower \(\dot{V}'\text{o2}\) kinetics alongside a faster deoxy[Hb+Mb] mean response time (MRT; i.e. time delay + τ) has been interpreted to reflect limited microvascular O₂ delivery during the on-transition of exercise (Murias et al., 2011; Spencer et al., 2012; Murias et al., 2014). Therefore, if, based on adults studies, the kinetics of bulk O₂ delivery were slower during heavy- (>LT) compared to moderate-intensity (<LT) step transitions (Koga et al., 2005; McNarry et al., 2012), an enhanced muscle oxidative capacity in children (Ratel et al., 2008; Tonson et al., 2010) may serve to maintain linearity of their τ\(\dot{V}'\text{o2p}\) by speeding fractional O₂ extraction during supra-LT transitions in youth.

Dynamic non-linearity with respect to an increased τ\(\dot{V}'\text{o2p}\) and Gₚ has also been reported when initiating cycling transitions from an elevated work rate (Hughson & Morrissey, 1982; Brittain et al., 2001; Wilkerson & Jones, 2006, 2007), with these effects suggested to reflect the recruitment of higher-order (i.e. type II) muscle fibers (Brittain et al., 2001; Wilkerson & Jones, 2006, 2007); however, other factors have been implicated (DiMenna et al., 2010a; Bowen et al., 2011; Wust et al., 2014). In vitro, type
II muscle fibers display slower $\dot{V}O_2$ kinetics and an increased ATP cost of force production compared to type I muscle fibers (Crow & Kushmerick, 1982). In this regard, a previous study has reported conversion of type I-to-II muscle fibers within the vastus lateralis between the ages of 5 to 20 y (Lexell et al., 1992) with longitudinal alterations in children's $\dot{V}O_2$ kinetics (Fawkner & Armstrong, 2004; Breese et al., 2010) showing commonality with the $\dot{V}O_2$ profiles previously reported in adults with an increased distribution of type II muscle fibers (Barstow et al., 1996; Pringle et al., 2003). Therefore, whilst a slower $\tau$ has been reported during “work-to-work” cycling in 11 to 13 y olds (Breese et al., 2012), whether effects on phase II $\dot{V}O_2$ kinetics might be amplified with increased chronological age is unclear. Additionally, whilst, previous reports of a slower $\tau$ during work-to-work exercise supports an intrinsic slowness of $O_2$ utilization in adults (Jones et al., 2008; DiMenna et al., 2010b), this proposal has not been investigated in youth in whom measurement of deoxy[Hb+Mb] responses would provide mechanistic insight by serving as a proxy for muscle fractional $O_2$ extraction.

Therefore, the primary purpose of this study was to investigate whether phase II $\dot{V}O_2$ kinetics display first-order, linear behavior in association with alterations in deoxy[Hb+Mb] kinetics in 11 to 15 y old boys. We hypothesized that a constant $\tau$ during very heavy- compared to moderate-intensity cycling transitions elicited from unloaded pedaling (i.e. $U \rightarrow VH$ vs. $U \rightarrow M$) would coincide with a faster deoxy[Hb+Mb] MRT, whereas, moderate-to-very heavy-intensity cycling transitions (i.e. $M \rightarrow VH$) would slow the $\tau$ alongside a slower deoxy[Hb+Mb] MRT compared to other conditions. Finally, we hypothesized that an increased $\tau$ and $G_p$ following the onset of $M \rightarrow VH$ would correlate positively with chronological age.
METHODS

Ethical Approval

Prior to participation, rights to confidentiality, withdrawal and benefits/risks of the study were explained with fully informed written assent and consent obtained from each participant and their parent(s) / guardian(s), respectively. All experimental procedures were approved by the Sport and Health Sciences research ethics committee at the University of Exeter (7-5-08#4) and conform to the standards set forth by the Declaration of Helsinki, except for registration in a database.

Participants

Nineteen boys (mean ± SD age: 13.6 ± 1.1 y, range: 11.7 – 15.7 y; stature: 160 ± 13 cm; and body mass: 47.9 ± 11.3 kg) volunteered to participate in this study. The data for 8/19 children were included from a previous investigation (Breese et al., 2012) using the same experimental procedures described below. The participants y from peak height velocity (PHV) was used as a descriptor of somatic maturity level using age and sitting height in a validated algorithm in male youth (Moore et al., 2015). This analysis revealed that ten participants were less than or equal to – 1 y from (i.e., pre-) PHV, with five at PHV, and four greater than 1 y from (i.e. post-) PHV, respectively.

Experimental protocol

Participants attended the laboratory on five to nine occasions over a two to four week period with each visit separated by ≥48 h. All cycling tests were performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the
Netherlands) with the seat, handlebar height, and crank length adjusted for each participant and subsequently maintained for all visits. All participants were asked to arrive at the laboratory at least 2 h postprandial and having refrained from caffeine for >2 h.

On their first visit, each participant performed a ramp incremental cycle test until task failure for determination of their peak \(\dot{V}O_2\) and the gas exchange threshold (GET). Following 3-min baseline cycling at 15 W, the work rate increased continuously by 15 W/min in 11 to 13 y olds and 25 W/min in all other participants based on the ramp rates previously estimated to attain a test duration of ~8 – 12 min across similar age categories (Fawkner & Armstrong, 2004; Breese et al., 2010). Participants were instructed to maintain a pedal rate of 70-80 rpm throughout the test with exhaustion defined as a ≥ 10 rpm drop in cadence for five consecutive seconds despite strong verbal encouragement. The peak \(\dot{V}O_2\) was taken as the highest 10-s stationary average value during the ramp test which has been shown previously to reflect a maximum \(\dot{V}O_2\) in ~93% of youth performing ramp cycling (Barker et al., 2011; Sansum et al., 2019). The GET was determined using the V-slope method (Beaver et al., 1986) as the first disproportionate increase in CO₂ production (\(\dot{V}CO_2\)) relative to the increase in \(\dot{V}O_2\), and subsequently verified from visual inspection of the increase in the ventilatory equivalent for \(\dot{V}O_2\) (\(V_{E}/\dot{V}O_2\)) with no increase in \(V_{E}/\dot{V}CO_2\).

The cycling work rates corresponding to 90% GET and 60% of the difference (Δ) between the GET and peak \(\dot{V}O_2\) were estimated using the “linear” portion of the ramp test by removing the initial 2 and final 3 min of test data and following adjustment of the \(\dot{V}O_2\) “lag time” during ramp exercise (Whipp et al., 1981). This yielded mean cycling
work rates of 72 ± 22 W equivalent to 90% GET (i.e. moderate-intensity cycling) and 163 ± 38 W equivalent to Δ60% (i.e. very heavy-intensity cycling). Each participant then returned to the laboratory to perform 1 of 2 step exercise protocols consisting of: 1) 3-min cycling at 15 W followed by 6-min of very heavy-intensity cycling (U→VH); or, 2) 3-min cycling at 15 W, followed by 4-min of moderate-intensity cycling (U→M), and then 6-min of very heavy-intensity cycling (M→VH). Each participant completed a minimum of two transitions within each step condition presented in random order.

Experimental measures

Pulmonary gas exchange and ventilation were measured and displayed breath-by-breath during each cycling trial (Metalyser 3B Cortex, Biophysik, Leipzig, Germany). Expiratory and inspiratory flows and volumes were measured via a pediatric facemask with low dead space (~ 45 ml) connected to a low-resistance (≤ 0.1 kPa/l/s at 20 l/s) digital turbine volume transducer which was manually calibrated using a 3-liter syringe (Hans Rudolph, Kansas City, MO) before each exercise test. Respired gases were continuously sampled from the facemask and analyzed for relative concentrations using an electrochemical oxygen sensor with a response time of < 100 ms. The delay in the capillary gas transit and analyzer rise time were accounted for relative to the volume signal, thereby time aligning the concentration and volume signals. Heart rate (HR) was recorded every breath during all cycling tests using short-range telemetry (Polar S610, Polar Electro Oy, Kempele, Finland).

A portable continuous wave (CW-) NIRS device (Portamon, Artnis Medical Systems, the Netherlands) was used to assess skeletal muscle oxygenation of the vastus lateralis
by emitting photons at two separate wavelengths (760 and 850 nm). The sampling frequency was set at 10 Hz. The spacing between the photon emitter and detector was 3.5 cm, corresponding to a depth resolution of 1.5 – 2 cm. The NIRS probe was affixed midway between the greater trochanter and lateral epicondyle of the femur using physiotherapists tape (Kinesio Tex Gold), and secured by an elastic bandage to ensure the device remained stationary and to eliminate contamination from ambient light, thereby, improving the signal-to-noise ratio.

The instrument employed a modified Beer Lambert law to estimate in micromolar (µM) concentration changes in oxygenated and deoxygenated hemoglobin and myoglobin (i.e. Δoxy[Hb+Mb] and Δdeoxy[Hb+Mb]) with respect to an initial resting value arbitrarily set equal to zero. A differential path-length factor (DPF) of 4 cm was employed to account for tissue scattering. Since assuming a constant DPF using CW-NIRS cannot resolve absolute [Hb+Mb] concentrations (Barstow, 2019), the Δdeoxy[Hb+Mb] amplitude was normalized relative to the end-exercise value prior to kinetic analysis in each condition. The tissue oxygenation index (TOI; oxy[Hb+Mb]/oxy[Hb+Mb] + deoxy[Hb+Mb], expressed as a percentage) was also calculated by spatially resolved spectroscopy as the TOI is thought to be less sensitive to changes in microvascular volume than deoxy[Hb+Mb] data (Quaresima & Ferrari, 2009).

**Data analysis and kinetic modeling**

The breath-by-breath \( \dot{\text{V}} \text{O}_2 \) data from each step transition were initially edited to exclude errant breaths by removing values lying more than four standard deviations from the local mean determined using a 5-breath rolling average. The filtered \( \dot{\text{V}} \text{O}_2 \) and
deoxy[Hb+Mb] responses were subsequently linearly interpolated with identical repetitions of each step condition time aligned to the start of exercise and ensemble averaged to improve the signal-to-noise ratio.

The first 15 s of \( \dot{V}o_2 \) data after the onset of exercise was deleted to remove the phase I (cardio-dynamic) response, and a mono-exponential model with time delay was then fitted to the averaged \( \dot{V}o_2 \) data of the following form:

\[
\Delta Y(t) = \Delta Y_p \cdot (1 - e^{-(t - TD)/\tau_p})
\]  

(1)

where \( \Delta Y(t) \) indicates the value at a given time (t) minus the baseline value (60-s average) before exercise onset, \( \Delta Y_p \) indicates the amplitude change of the primary component from baseline to its asymptote, TD and \( \tau_p \) represent the time delay and time constant of the phase II exponential function, respectively. For \( U \rightarrow M \), the model in Equation (1) was fitted to end-exercise (i.e. 4-min), whereas, for \( U \rightarrow VH \) and \( M \rightarrow VH \), the model fitting window was constrained to exclude the A_{Sc} and hence isolate the phase II component.

The onset of the A_{Sc} was determined using software (LabView, v 6.1, National Instruments, Newbury, UK) which initially fitted a mono-exponential function up to the first 60-s of \( \dot{V}o_2 \) data and then increased iteratively by 5-s until end-exercise. The estimated \( \tau \) for each fitting window was then plotted against time with the phase II portion of the response determined as the point at which the influence of the A_{Sc} lengthened the estimated \( \tau \) following an initial plateau (Rossiter et al., 2001). The parameter estimates from Equation (1) and their 95% confidence intervals (CI_{95}) were then resolved by least-squares non-linear regression (GraphPad Prism, GraphPad...
The \( A_{sc} \) was subsequently determined by calculating the difference between the end-exercise \( \dot{V}'o_2 \) and the sum of the primary amplitude and baseline \( \dot{V}'o_2 \). For all conditions, the ‘gain’ of the phase II response (\( G_p \)) was calculated by dividing the asymptotic phase II amplitude minus the baseline \( \dot{V}'o_2 \) by the increment in work rate (\( \Delta \dot{V}'o_2/\Delta W \)). Likewise, the total \( \dot{V}'o_2 \) gain (\( G_{tot} \)) at end-exercise was calculated in a similar manner.

The NIRS-derived deoxy[Hb+Mb] and TOI response were also modelled to provide information on the kinetic adjustment of fractional \( O_2 \) extraction. The TD for an exponential-like rise in muscle deoxygenation was defined as the first datum lying > 1 SD above the mean value during baseline cycling as previously described (DeLorey et al., 2003). Subsequently, following removal of data points preceding the TD, the model in Equation (1) was fitted to the initial 90 – 120 s of data to resolve the \( \tau \Delta \text{deoxy[Hb+Mb]} \) and \( \tau \text{TOI} \), or, in cases where visual inspection revealed an early ‘overshoot’ in muscle deoxygenation relative to end-exercise, to the peak value attained during the transient phase. Finally, the TD and \( \tau \) were summed to reflect the overall mean response time (MRT) of \( \Delta \text{deoxy[Hb+Mb]} \) and TOI within each step condition.

The ratio of \( \Delta \text{deoxy[Hb+Mb]} \) to \( \dot{V}'o_2 \) was also calculated using the methods originally described in adults (Murias et al., 2010) and subsequently in children (Barker et al., 2014), to infer the dynamic matching of \( \dot{Q}'o_2 \)-to-\( \dot{V}'o_2 \) during step cycling. Briefly, the \( \Delta \text{deoxy[Hb+Mb]} \) and \( \dot{V}'o_2 \) profiles were normalized such that 0% and 100% represented the values corresponding to baseline and at end-exercise, respectively. Subsequently, the \( \Delta \text{deoxy[Hb+Mb]} \) and \( \Delta \dot{V}'o_2 \) data were averaged into 5 s bins and time aligned by left shifting the \( \dot{V}'o_2 \) data by 15 s to account for the duration of phase I estimated previously.
in children (Springer et al., 1991; Hebestreit et al., 1998). The magnitude of the Δdeoxy[Hb+Mb]/Δ\(\dot{V}O_2\) “overshoot” was calculated by integrating the area under curve from the first datum lying above 1.0 or ‘unity’ to 180-s of exercise in all participants in each condition.

Statistical Analysis

Gaussian distribution was assessed by the Shapiro-Wilk test and subsequently verified by calculating standardized scores for skewness and kurtosis for each variable. A standardized value < 2 was deemed acceptably normally distributed. All pulmonary \(\dot{V}O_2\) and NIRS-derived variables were analyzed using one-way repeated measures ANOVA with Bonferroni adjusted post hoc tests used to locate statistically significant differences between step conditions. In addition, effect size (ES; using Cohen’s \(d\)) was also calculated to judge the magnitude of the observed effect, using the following thresholds: Trivial (< 0.2), Small (0.2), Medium (0.5), and Large (0.8). Pearson product moment correlations (\(r\)) were used to assess the bivariate relationship between alterations in phase II \(\dot{V}O_2\) kinetics with muscle oxygenation and chronological age. All statistical analyses were conducted using PASW Statistics 18 (SPSS, Chicago, IL). Data are presented as means ± SD. Statistical significance was accepted if \(P < 0.05\).

RESULTS

The group mean ± SD values for peak \(\dot{V}O_2\) and end HR during the initial ramp incremental cycle test were 2.37 ± 0.60 l/min and 192 ± 9 bpm, respectively. The group
mean ± SD values for end HR during U→M, U→VH and M→VH step cycling were 129 ± 16, 178 ± 11, and 179 ± 12 bpm, respectively.

Pulmonary $\dot{V}O_2$ kinetics

Table 1 presents the group mean ± SD parameter estimates for $\dot{V}O_2$ kinetics with their corresponding profiles in a representative participant shown in Figure 1. There was no significant difference in the $\tau_{\dot{V}O_2p}$ between U→M and U→VH ($P = 0.31, ES = 0.4$); however, the $\tau_{\dot{V}O_2p}$ was slower during M→VH compared to other conditions ($P < 0.001, ES > 1.2$). There was a significant main effect for step cycling on the $G_p$, which, relative to U→M, decreased during U→VH ($P = 0.01, ES = 0.7$); however, there were no significant differences during M→VH compared to other conditions ($P > 0.2$). The $A_{Sc}$ decreased during M→VH compared to U→VH ($P = 0.01, ES = 0.8$) with this difference removed when normalizing $A_{Sc}$ relative to the total $\Delta\dot{V}O_2$ above baseline pedaling (U→VH: 14 ± 6 vs. M→VH: 13 ± 7 %, $P = 0.37$). Relative to U→M, the $G_{tot}$ was greater during U→VH ($P = 0.045, ES = 0.6$) and M→VH ($P = 0.03, ES = 0.9$).

NIRS-derived variables

Table 2 presents the group mean ± SD parameter estimates for NIRS-derived deoxy[Hb+Mb] and TOI kinetics with their corresponding profiles in a representative participant shown in Figures 2 and 3, respectively. Relative to U→M, the $\Delta$deoxy[Hb+Mb]-TD following exercise onset decreased in the other conditions ($P < 0.001, ES > 1.9$) with a further reduction during M→VH compared to U→VH ($P = 0.03, ES = 0.8$). There were no significant differences ($P > 0.40$) between U→M and U→VH in the $\tau\Delta$deoxy[Hb+Mb] or $\tau$TOI; however, both were slowed during M→VH compared to
other conditions ($P < 0.03$, ES > 1.2). Accordingly, the overall MRT (i.e. $TD + \tau$) of muscle deoxygenation kinetics was faster during $U \rightarrow VH$ compared to $U \rightarrow M$ and $M \rightarrow VH$ ($P < 0.001$, ES > 1.2).

Matching of $\Delta \text{deoxygenation}$ kinetics to $V'\dot{o}_2$

Comparison of the group mean $\pm$ SD kinetic parameters for $\dot{V}'\dot{o}_2$ and muscle deoxygenation are presented in Figure 4. There were no significant differences between the $\tau \dot{V}'\dot{o}_2$ and muscle deoxygenation kinetics during $U \rightarrow M$ ($P > 0.15$), whereas, the MRT of $\Delta \text{deoxygenation}$ and TOI was speeded relative to the $\tau \dot{V}'\dot{o}_2$ during $U \rightarrow VH$ and $M \rightarrow VH$ ($P < 0.001$). The difference between the $\tau \dot{V}'\dot{o}_2$ and $MRT-\Delta \text{deoxygenation}$ increased by a large effect size during $M \rightarrow VH$ compared to $U \rightarrow VH$ ($18 \pm 15$ vs. $9 \pm 7$ s, $P = 0.07$, ES = 0.8, Figure 4C), with a significantly greater difference between the $\tau \dot{V}'\dot{o}_2$ and $MRT-\text{TOI}$ during work-to-work exercise ($23 \pm 15$ vs. $12 \pm 7$ s, respectively, $P = 0.014$, ES = 1.0, Figure 4D).

During $U \rightarrow M$, the normalized $\Delta \text{deoxygenation}/\Delta \dot{V}'\dot{o}_2$ overshoot area yielded non-normally distributed data; therefore, were not reported. As shown in Figure 5, the overshoot area above unity in the normalized $\Delta \text{deoxygenation}/\Delta \dot{V}'\dot{o}_2$ ratio was significantly greater during the on-transition of $M \rightarrow VH$ compared to $U \rightarrow VH$ exercise ($17.3 \pm 13.2$ vs. $8.5 \pm 7.0$ %/s, $P = 0.01$, ES = 0.9, respectively).

Relationship between $\dot{V}'\dot{o}_2$ and $\Delta \text{deoxygenation}$ kinetics

The reduction of the $G_p$ correlated positively with the speeding of the $MRT-\Delta \text{deoxygenation}$ during $U \rightarrow VH$ compared to $U \rightarrow M$ ($r = 0.67$; $P = 0.005$). During $M \rightarrow VH$, there was no significant relationship ($P > 0.5$) between the slowing of the $\tau \dot{V}'\dot{o}_2$ with
alterations in the $\tau\Delta\text{deoxy[Hb+Mb]}$ compared to $U\rightarrow M$ or $U\rightarrow VH$ ($r = 0.15$ and $-0.06$, respectively).

Relationship between phase II $\dot{\text{V}}o_2$ with chronological age and baseline $\dot{\text{V}}o_2$

There was no significant relationship between the $\tau\dot{\text{V}}o_2p$ with chronological age during $U\rightarrow M$ ($r = 0.40$, $P = 0.09$); however, both variables correlated positively during $U\rightarrow VH$ ($r = 0.48$, $P = 0.04$) with a stronger relationship observed during $M\rightarrow VH$ ($r = 0.78$, $P < 0.001$). An increased ($\Delta$) $\tau\dot{\text{V}}o_2p$ and $\Delta G_p$ during $M\rightarrow VH$ relative to $U\rightarrow M$ and $U\rightarrow VH$ correlated positively with chronological age ($P < 0.01$, Figure 6 A-D). During $M\rightarrow VH$, the baseline $\dot{V}o_2$ in l/min correlated positively with the $\Delta G_p$ relative to $U\rightarrow M$ ($r = 0.59$, $P = 0.008$) and $U\rightarrow VH$ ($r = 0.71$, $P = 0.001$); however, there was no significant relationship with the $\Delta\tau\dot{V}o_2p$ relative to other conditions ($r = 0.44$ and $0.39$, $P = 0.07$ and $0.11$, respectively).

DISCUSSION

This study combined simultaneous measurements of $\dot{V}o_2$ and NIRS-derived muscle deoxygenation kinetics to investigate the relationship between dynamic (non)linearity of the $\tau\dot{V}o_2p$ and $G_p$ with alterations in skeletal muscle $O_2$ delivery/utilization during step exercise in 11 to 15 y olds. In line with our study hypothesis, relative to $U\rightarrow M$ cycling, an invariant $\tau\dot{V}o_2p$ during $U\rightarrow VH$ was accompanied by a faster $MRT-\Delta\text{deoxy[Hb+Mb]}$ and $MRT-\text{TOI}$, suggesting that an increased rate of fractional $O_2$ extraction mitigated a decreased $\dot{Q}o_2/\dot{V}o_2$ response during supra-LT transitions initiated from unloaded pedaling. However, during $U\rightarrow VH$ compared $U\rightarrow M$, the $G_p$ decreased suggesting that
this parameter may be limited by decreased microvascular $O_2$ delivery in boys.

Conversely, relative to $U \rightarrow VH$ cycling, $M \rightarrow VH$ decreased the rate of fractional $O_2$
extraction (i.e. increased MRT of $\Delta deoxy[Hb+Mb]$ and TOI kinetics) in a manner that was
disproportionally less than the slowing of the $\tau \dot{V}O_2p$, thereby, eliciting a greater
$\Delta deoxy[Hb+Mb]/\Delta \dot{V}O_2$ “overshoot” in the transition from a raised baseline work rate.

Finally, relative to $U \rightarrow M$ and $U \rightarrow VH$, an increased $\tau \dot{V}O_2p$ and $G_p$ during $M \rightarrow VH$ correlated
positively with boys’ chronological age. These findings lend support to the notion that
developmental effects on $\dot{V}O_2$ kinetics might be linked to the recruitment of higher-
order (i.e. type II) muscle fibers with slower microvascular blood flow dynamics and
poorer efficiency in older youth.

Comparison of $\dot{V}O_2$ and muscle deoxygenation kinetics between $U \rightarrow M$ and $U \rightarrow VH$

In the present study, we observed no significant differences in the $\tau \dot{V}O_2p$ between
$U \rightarrow M$ and $U \rightarrow VH$; however, the $G_p$ decreased during $U \rightarrow VH$ reflecting both linear and
non-linear control features of $\dot{V}O_2$ kinetics following the onset of step cycling elicited
from unloaded pedaling in 11 to 15 y olds. An invariant $\tau \dot{V}O_2p$ during different intensities
of step exercise is consistent with previous reports in youth (Hebestreit et al., 1998;
Williams et al., 2001; Lai et al., 2008); however, these studies employed relatively low
sample sizes (i.e. $n = 8$), or, in the case of Hebestreit et al. (1998) the work rate was
arbitrarily normalized as a fraction of peak $\dot{V}O_2$ in children. Therefore, our findings
extend those previously reported by revealing an invariant $\tau \dot{V}O_2p$ relative to work rate
using procedures for resolving the kinetic parameters within carefully prescribed
intensity domains among a larger youth cohort (i.e. \( n = 19 \)), hence, reducing the potential for type II statistical error.

Following the onset of U→M and U→VH, there was a pronounced TD before muscle deoxy[Hb+Mb] increased, suggesting that the hyperemic effect of skeletal muscle contractions sufficiently matched the requirement for O\(_2\) utilization within active regions of vastus lateralis muscle. However, during U→VH compared to U→M, the \( MRT-\Delta\text{deoxy[Hb+Mb]} \) and \( MRT-\text{TOI} \) were reduced (i.e. decreased TD + \( \tau \)) by a large effect size, hence, suggesting that an increased rate of fractional O\(_2\) extraction was required to maintain an invariant \( \tau\hat{\text{O}_2p} \) between both conditions. Therefore, these findings, in line with “priming” exercise studies in children (Barker et al., 2010; Barker et al., 2014), support the notion that the \( \tau\hat{\text{O}_2p} \) is principally limited by intracellular metabolic factors rather than the dynamic relationship between \( \dot{\text{Q}}\text{o}_2\)-to-\( \dot{\text{V}}\text{O}_2 \) during supra-LT transitions in youth.

In the present study, we did observe a significant association between a decreased \( G_p \) with the relative speeding of the \( MRT-\Delta\text{deoxy[Hb+Mb]} \) following the onset of U→VH compared to U→M. It has been reported that the \( \tau \) of deoxy[Hb+Mb] kinetics coheres with that observed for the reduction in microvascular O\(_2\) partial pressure (\( P_{mvO_2} \)) following the onset of skeletal muscle contractions (Koga et al., 2012). Accordingly, it is conceivable that those participants evincing a greater \( \dot{\text{Q}}\text{o}_2\)-to-\( \dot{\text{V}}\text{O}_2 \) mismatch (i.e. faster \( MRT-\Delta\text{deoxy[Hb+Mb]} \)) might have accelerated the fall in \( P_{mvO_2} \) such that the \( \dot{\text{V}}\text{O}_2 \) increment per unit of work rate was limited consequent to a decreased O\(_2\) flux between the capillary and muscle. Therefore, in youth, our findings are consistent with the notion
that the $G_p$ might be sensitive to a decreased $\dot{Q}_{o2}$/$\dot{V}_{o2}$ response as previously reported in adults (Koga et al., 1999; Jones et al., 2006).

Comparison of $\dot{V}_{o2}$ and muscle deoxygenation kinetics during $M\rightarrow VH$ relative to other step conditions

Whereas $U\rightarrow VH$ sped muscle deoxy[Hb+Mb] kinetics, to maintain a constant $\tau_\dot{V}_{o2p}$ compared to $U\rightarrow M$, there was a concomitant slowing of the $\tau_\dot{V}_{o2p}$, $\tau_{TOI}$ and $\tau_{\Delta deoxy[Hb+Hb]}$ during $M\rightarrow VH$ compared to other step conditions. Whilst these findings during $M\rightarrow VH$ are consistent with a decreased rate of $O_2$ extraction, it is important to consider that $U\rightarrow VH$ and $M\rightarrow VH$ sped the MRT of $\Delta deoxy[Hb+Mb]$ and TOI responses relative to the $\tau_\dot{V}_{o2p}$, with the difference between these signals increased following the onset of work-to-work transitions (Figure 4). In other words, the slowing of muscle deoxygenation did not match proportionally the slowing of the $\tau_\dot{V}_{o2p}$, thereby, increasing the normalized $\Delta deoxy[Hb+Mb]/\Delta \dot{V}_{o2}$ overshoot area above unity within the initial few minutes of $M\rightarrow VH$ compared to $U\rightarrow VH$ (Figure 5). Collectively, these responses during $M\rightarrow VH$ are consistent with an increased proportional reliance on fractional $O_2$ extraction; hence, our results suggest for the first time in youth that slower phase II $\dot{V}_{o2}$ kinetics coincided with a slower rate of adjustment in $\dot{Q}_{o2}$-to-$\dot{V}_{o2}$ in the transition from a raised baseline work rate.

In boys, it had been previously suggested that eliciting step transitions from a raised level of electromyogram activity increased proportionally the recruitment of type II muscle fibers for power production (Breese et al., 2012). This supposition was based on an orderly ‘size’ principle of motor unit recruitment (Henneman & Mendell, 1981), which,
in adults, has received support with previous studies reporting a progressive reduction in the glycogen content within type I followed by type Ila and IIX muscle fibers from low to high force requirements (Essen, 1978; Green, 1978; Krstrup et al., 2004). ‘Higher-order’ type II muscle fibers have been reported to possess slower microvascular O$_2$ delivery (i.e. decreased PmvO$_2$ across the on-exercise transition) (Behnke et al., 2003) and slower $\dot{V}$o$_2$ kinetics in vitro compared with ‘lower-order’ type I muscle fibers (Crow & Kushmerick, 1982). Therefore, during M→VH, it is conceivable that the $\dot{V}$-o$_2$ and deoxy[Hb+Mb] profiles (and their kinetic relationship) reflected the intrinsic properties of a population of skeletal muscle fibers positioned higher in the recruitment hierarchy in boys.

There was a significant main effect for step cycling on the $G_{tot}$, which, relative to U→M, was greater during U→VH and M→VH consequent to the development of the $A_{Sc}$ in these conditions. However, relative to U→VH, the $A_{Sc}$ decreased by ~ 50% during M→VH such that $\dot{V}$-o$_2$ kinetics reverted toward a mono-exponential profile. There is evidence to suggest that the development of the $A_{Sc}$ is related in some manner to the recruitment profile and metabolic features of type II muscle fibers with slower $\dot{V}$-o$_2$ kinetics and poorer efficiency [i.e. increased ATP/force output ratio (Crow & Kushmerick, 1982)] compared with type I muscle fibers (see Jones et al., 2011 for review). Therefore, in adults, an explanation for a smaller $A_{Sc}$ has considered the earlier (rather than latent) expression upon the pulmonary $\dot{V}$-o$_2$ signal of higher-order (i.e. type II) muscle fibers when supra-LT transitions are initiated from an elevated work rate (Wilkerson & Jones, 2007; DiMenna et al., 2008). However, this proposal predicts that the $G_0$ would have
been higher during M→VH relative to other conditions, which, in boys, was not present
with this effect associated with chronological age (Figure 6).

Relationship between $\dot{V}O_2$ kinetics with chronological age

A novel finding was that the $\tau_{\dot{V}O_2}$ and chronological age, whilst not significantly
associated during U→M, were both positively correlated during U→VH with this
relationship strengthened by an increased pre-transition work rate. In other words,
M→VH exercise slowed by a greater extent the $\tau_{\dot{V}O_2}$ and increased the $G_p$ within the
age range between 11 to 15 y (Figure 6). It would have been expected that U→M
transitions predominantly recruited a population of type I muscle fibers (Krustrup et al.,
2004) with the mean $\tau_{\dot{V}O_2}$ in this condition in boys (i.e. ~ 23 s) less likely to be limited
by muscle $O_2$ delivery based on a previous study in adults (Murias et al., 2011).

Conversely, a previous investigation has reported a slower $\tau_{\dot{V}O_2}$ alongside slower limb
blood flow dynamics following the onset of work-to-work exercise in adults (MacPhee
et al., 2005) with further evidence in support of a decline in the maximal rate of $O_2$
transport between the ages of 12 to 17 y (Koch, 1984) and in the proportion of type I
muscle fibers within the vastus lateralis between the ages of 5 to 20 y (Lexell et al., 1992).

Therefore, we propose indirectly that an age-related slowing of the $\tau_{\dot{V}O_2}$ during M→VH
might have reflected differences in muscle perfusion and the distribution of $O_2$ in
conjunction with alterations in muscle fiber recruitment in older youth.

Alternatively, it is important to consider that larger (older) boys produced higher
cycling power outputs corresponding to the GET and at task failure during the initial
ramp incremental test. Therefore, during M→VH, it would have been expected that
baseline pedaling equivalent to 90% GET recruited a larger muscle mass resulting in a greater pre-transition $\dot{V}\dot{O}_2$ compared to smaller (younger) children. In this regard, it has been reported that the $\tau_{\dot{V}\dot{O}_2p}$ and $G_p$ increased linearly at progressively higher baseline power outputs (hence $\dot{V}\dot{O}_2$) in adults (Keir et al., 2016), providing an additional explanation for the relationships presented in Figure 6. However, we reported no significant association between baseline $\dot{V}\dot{O}_2$ in l/min during M→VH with the $\Delta\tau_{\dot{V}\dot{O}_2p}$ relative to U→M and U→VH exercise.

Assuming that U→M immediately followed by M→VH evoked an orderly recruitment of motor units, the relationships presented in Figure 6 lend support to the notion that work-to-work cycling revealed a greater disparity in the $\tau$ and $G$ values between higher- relative to lower-order muscle fibers with increased chronological age (Figure 7). Accordingly, if the measured $\dot{V}\dot{O}_2$ profile during U→VH reflected the summed response of muscle fiber pools recruited separately during U→M and M→VH (Wilkerson & Jones, 2007), then those positioned higher in the recruitment hierarchy (i.e. type II) would be expected to elicit a net slowing of pulmonary $\dot{V}\dot{O}_2$ during the on-transition of exercise and/or extend the $A_{5c}$ in older children. This $\dot{V}\dot{O}_2$ response is characteristic of that previously observed longitudinally in youth (Fawkner & Armstrong, 2004; Breese et al., 2010); therefore, our findings shed potential novel insight into the physiological factors responsible for modulating $\dot{V}\dot{O}_2$ kinetics between the ages of 11 to 15 y.

Limitations

It is recognized that there exist limitations with CW-NIRS assuming constant tissue optical properties (i.e. path length, absorption and scattering coefficients), which, has
been reported to confound interpretation of deoxy[Hb+Mb] data (see Barstow et al., 2019 for a recent review). Moreover, we also recognize that the absorbance spectra of Hb and Mb overlap within the NIR range; therefore, the relative (%) contribution from each chromophore to the NIRS-derived signal is uncertain (Masuda et al., 2010; Davis & Barstow, 2013). Additionally, we left shifted the normalized \( \dot{\text{VO}}_2 \) by 15 s to account for the estimated phase I duration in children (Springer et al., 1991; Hebestreit et al., 1998), thereby, time aligning the start of phase II \( \dot{\text{VO}}_2 \) to the onset of exercise, which, has been reported to coincide with muscle \( \dot{\text{VO}}_2 \) within 10% (Barstow et al., 1994). Therefore, the extent to which inter- and intra-participant differences in the circulatory muscle-to-lung transit time influenced the \( \Delta \text{deoxy[Hb+Mb]}/\Delta \dot{\text{VO}}_2 \) overshoot is unclear. It should also be cautioned that the pulmonary \( \dot{\text{V}} \text{O}_2 \) amplitude during exercise includes minor contributions from cardiorespiratory support processes (Poole et al., 1991), which, has the potential to influence its ratio when expressed relative to the adjustment in deoxy[Hb+Mb] kinetics. Therefore, in the present study, we stress that precedence be given to interpreting the TD and \( \tau \) of muscle deoxygenation with these preliminary kinetic data supported by the \( \Delta \text{deoxy[Hb+Mb]}/\Delta \dot{\text{V}} \text{O}_2 \) ratio to infer the dynamic (mis)matching between \( \text{O}_2 \) delivery/utilization. Finally, it should be noted that baseline pedaling during M→VH involved simultaneously raising pre-transition \( \dot{\text{V}} \text{O}_2 \) with work rate, which, when both are dissociated, has the potential to influence the \( \tau \dot{\text{V}} \text{O}_2 \) and \( G_p \) via independent mechanisms (DiMenna et al., 2010a; Bowen et al., 2011; Wust et al., 2014). Therefore, in the present study, whether an increased baseline work rate \textit{per se} altered phase II \( \dot{\text{V}} \text{O}_2 \) kinetics cannot be established.
Conclusions

This study in 11 to 15 y olds reported dynamic non-linearity of the phase II $\dot{V}o_2$ kinetic parameters, with respect to a decreased $G_p$ during U→VH compared to U→M, whereas, a slower $\tau_{\dot{V}o_2p}$ was dependent on an increased pre-transition work rate in youth. Furthermore, whilst “work-to-work” cycling slowed the $\tau$ of muscle deoxygenation, when expressed relative to the adjustment in $\dot{V}o_2$ kinetics, the ratio between both of these signals increased, suggesting a greater proportional speeding of fractional $O_2$ extraction; hence, the slower $\tau_{\dot{V}o_2p}$ during M→VH was consequent to a slowing of microvascular blood flow relative to $O_2$ utilization. Finally, an increased $\tau_{\dot{V}o_2p}$ and $G_p$ during the transition from a raised baseline work rate correlated positively with chronological age. These novel findings further our understanding of the physiological factors modulating the $\dot{V}o_2$ kinetic response, and, thereby, oxidative metabolism, and their association with chronological age in healthy youth.
REFERENCES


Murias JM, Spencer MD, Kowalchuk JM & Paterson DH (2011). Muscle deoxygenation to \( \dot{V}\) \(_{\text{O}_2} \) relationship differs in young subjects with varying \( \tau\dot{V}\) \(_{\text{O}_2} \). *Eur J Appl Physiol* **111**, 3107-3118.


**ADDITIONAL INFORMATION**

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**AUTHOR CONTRIBUTIONS**

Conception or design of the work: B.C.B. and C.A.W. Acquisition, analysis or interpretation of data for the work and revising it critically for important intellectual content: all authors. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.
Table 1. Amplitude and kinetics of pulmonary oxygen uptake ($\dot{V}O_2$) following the onset of exercise in each step condition

<table>
<thead>
<tr>
<th></th>
<th>AVOVA</th>
<th>U→M</th>
<th>U→VH</th>
<th>M→VH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}O_2$bl (l/min)</td>
<td>&lt; .001</td>
<td>0.69 ± 0.18</td>
<td>0.72 ± 0.17</td>
<td>1.21 ± 0.34†</td>
</tr>
<tr>
<td>TDp (s)</td>
<td>.01</td>
<td>11 ± 3</td>
<td>9 ± 3</td>
<td>8 ± 7*</td>
</tr>
<tr>
<td>$\tau_{\dot{V}O_2}$ (s)</td>
<td>&lt; .001</td>
<td>23 ± 6</td>
<td>26 ± 8</td>
<td>42 ± 15†</td>
</tr>
<tr>
<td>CI95 (s)</td>
<td>.006</td>
<td>7 ± 2</td>
<td>6 ± 3</td>
<td>10 ± 4†</td>
</tr>
<tr>
<td>$A_p$ (l/min)</td>
<td>&lt; .001</td>
<td>0.53 ± 0.19</td>
<td>1.25 ± 0.30*</td>
<td>0.82 ± 0.31†</td>
</tr>
<tr>
<td>$G_p$ (ml/min/W)</td>
<td>.04</td>
<td>9.9 ± 1.3</td>
<td>9.1 ± 1.0*</td>
<td>9.6 ± 1.2</td>
</tr>
<tr>
<td>TDSc (s)</td>
<td>-</td>
<td>-</td>
<td>160 ± 33</td>
<td>184 ± 35</td>
</tr>
<tr>
<td>$A_{Sc}$ (l/min)</td>
<td>-</td>
<td>-</td>
<td>0.21 ± 0.13</td>
<td>0.11 ± 0.06†</td>
</tr>
<tr>
<td>$\dot{V}O_{2tot}$ (l/min)</td>
<td>&lt; .001</td>
<td>1.22 ± 0.36</td>
<td>2.18 ± 0.55*</td>
<td>2.14 ± 0.58*</td>
</tr>
<tr>
<td>$G_{tot}$ (ml/min/W)</td>
<td>.008</td>
<td>9.9 ± 1.3</td>
<td>10.5 ± 0.9*</td>
<td>11.0 ± 1.0*</td>
</tr>
</tbody>
</table>

Values are mean ± SD. $\dot{V}O_{2bl}$, mean $\dot{V}O_2$ during baseline cycling; TDp, phase II time delay; $\tau_{\dot{V}O_2}$, phase II time constant; CI95, 95% confidence interval for $\tau_{\dot{V}O_2}$; $A_p$, amplitude of phase I + II, excluding $\dot{V}O_{2bl}$; TDSc, slow component time delay; $A_{Sc}$, amplitude of slow component; $\dot{V}O_{2tot}$, mean $\dot{V}O_2$ during the last 30 s of cycling; $G_p$ and $G_{tot}$, ‘gain’ (i.e. $\Delta \dot{V}$ $O_2/\Delta W$) of the phase II component and at end-exercise, respectively. Significant differences ($P < 0.05$) vs. *U→M and vs. †other step conditions.
Table 2. Kinetics of NIRS-derived variables following the onset of exercise in each step condition

<table>
<thead>
<tr>
<th></th>
<th>ANOVA</th>
<th>U→M</th>
<th>U→VH</th>
<th>M→VH</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOI_{bl} (%)</td>
<td>&lt; .001</td>
<td>69 ± 1</td>
<td>69 ± 3</td>
<td>66 ± 4†</td>
</tr>
<tr>
<td>TOI_{end} (%)</td>
<td>&lt; .001</td>
<td>66 ± 3</td>
<td>58 ± 4*</td>
<td>58 ± 4*</td>
</tr>
<tr>
<td>TD-TOI (s)</td>
<td>&lt; .001</td>
<td>13 ± 3</td>
<td>7 ± 2*</td>
<td>5 ± 5*</td>
</tr>
<tr>
<td>τTOI (s)</td>
<td>&lt; .001</td>
<td>9 ± 3</td>
<td>7 ± 2</td>
<td>15 ± 5†</td>
</tr>
<tr>
<td>SEE</td>
<td></td>
<td>1 ± 1</td>
<td>1 ± 0</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>MRT-TOI (s)</td>
<td>&lt; .001</td>
<td>22 ± 4</td>
<td>14 ± 2†</td>
<td>20 ± 6</td>
</tr>
<tr>
<td>TD-Δdeoxy[Hb+Mb] (s)</td>
<td>&lt; .001</td>
<td>13 ± 3</td>
<td>8 ± 2*</td>
<td>6 ± 3†</td>
</tr>
<tr>
<td>τΔdeoxy[Hb+Mb] (s)</td>
<td>&lt; .001</td>
<td>11 ± 7</td>
<td>9 ± 3</td>
<td>18 ± 6†</td>
</tr>
<tr>
<td>SEE</td>
<td></td>
<td>1 ± 1</td>
<td>1 ± 0</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>MRT-Δdeoxy[Hb+Mb] (s)</td>
<td>&lt; .001</td>
<td>24 ± 5</td>
<td>17 ± 3†</td>
<td>24 ± 8</td>
</tr>
</tbody>
</table>

Values are mean ± SD. TOI, tissue oxygenation index; Δdeoxy[Hb+Mb], change in deoxygenated haemoglobin + myoglobin concentration; MRT, mean response time; SEE, standard error of the estimate for the τTOI and τΔdeoxy[Hb+Mb]. Significant differences (P < 0.05) vs. *U→M and vs. †other step conditions.
FIGURE LEGENDS

Figure 1. Pulmonary oxygen uptake (\(\dot{V}'\text{O}_2\)) response in a representative participant following the onset of step cycling in each condition. The vertical dashed lines indicate the onset of step exercise. The solid black lines denote the least squares regression fit of the phase II \(\dot{V}'\text{O}_2\) kinetic response [see Equation (1)].

Figure 2. Muscle deoxy[\(\text{Hb}+\text{Mb}\)] response of the vastus lateralis in a representative participant following the onset of step cycling in each condition. Data are normalized relative to the end-exercise amplitude after correcting for the mean value during unloaded (15 W) pedaling. The vertical dashed lines indicate the onset of step exercise. The solid black lines denote the least squares regression fit of the primary deoxy[\(\text{Hb}+\text{Mb}\)] kinetic response [see Equation (1)].

Figure 3. Tissue oxygenation index (TOI) of the vastus lateralis in a representative participant following the onset of step cycling in each condition. The vertical dashed lines indicate the onset of step exercise. The solid black lines denote the least squares regression fit of the primary TOI kinetic response [see Equation (1)].

Figure 4. Comparison of \(\dot{V}'\text{O}_2\) and muscle deoxygenation kinetics following the onset of step cycling. Panels A and B show the group mean ± SD \(\tau_{\dot{V}'\text{O}_2}\) (black bars) and mean response time (MRT) of \(\Delta\text{deoxy}[\text{Hb}+\text{Mb}]\) and TOI (white bars) within each step condition. Panels C and D present those values for \(\tau_{\dot{V}'\text{O}_2}\) minus the MRT-\(\Delta\text{deoxy}[\text{Hb}+\text{Mb}]\) and MRT-TOI during U→M, U→VH and M→VH, respectively. *\(P < 0.01\) relative to the \(\tau_{\dot{V}'\text{O}_2}\) within condition, *\(P < 0.01\) vs. U→M, and †\(P < 0.05\) vs. other step conditions.

Figure 5. Group mean normalized ratio between the adjustment of deoxy[\(\text{Hb}+\text{Mb}\)] relative to \(\dot{V}'\text{O}_2\) following the onset of U→VH (black circles) and M→VH (white circles) step transitions. The ratio was calculated after normalizing both signals relative to the total increase (\(\Delta\)) between baseline and end-exercise (i.e. 0 – 100%) with the \(\dot{V}'\text{O}_2\) data left shifted by 15 s to account for the muscle-to-lung transit delay. Please note error bars are excluded for clarity. Note the greater ‘overshoot’ area above unity (horizontal dashed line) within the initial few minutes of M→VH compared to U→VH exercise.

Figure 6. Relationship between alterations (\(\Delta\)) in the \(\tau_{\dot{V}'\text{O}_2}\) and \(G_p\) with chronological age following the onset of work-to-work cycling transitions. The y-axis values represent those in M→VH minus U→M (A – B) and U→VH (C – D), respectively, *\(P < 0.01\).

Figure 7. Pulmonary \(\dot{V}'\text{O}_2\) response during U→M (black circles) and M→VH (white circles) step cycling in a male youth participant aged 12 y (A – B) and 16 y (C – D) with an estimated maturity offset from PHV of −2.4 and +2.3 y, respectively. The \(\dot{V}'\text{O}_2\) data is expressed per unit change in work rate (i.e. ‘gain’). Continuous lines represent the fitted responses extrapolated backward to the pre-transition value (i.e. during the phase I region) with the model extended to 6 min during U→M (A and C). See text for further explanation.
Fig. 1

A

\[ \dot{V}O_2 \text{ (l/min)} \]

\[ \tau = 21 \text{ s} \]

\[ \tau = 35 \text{ s} \]

\[ 0 \quad 120 \quad 240 \quad 360 \quad 480 \quad 600 \quad \text{Time (s)} \]

B

\[ \dot{V}O_2 \text{ (l/min)} \]

\[ \tau = 22 \text{ s} \]

\[ 0 \quad 120 \quad 240 \quad 360 \quad \text{Time (s)} \]
Fig. 2

**A**

$\Delta \text{deoxy[Hb+Mb]}$ (%)

- $\tau = 8\, \text{s}$
- $\tau = 29\, \text{s}$

Time (s)

**B**

$\Delta \text{deoxy[Hb+Mb]}$ (%)

- $\tau = 8\, \text{s}$

Time (s)
Fig. 4

Fig. 5
Fig. 6

Fig. 7