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 O_2 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.

ABSTRACT

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 + τ) during U→VH (14 ± 2 s) compared to U→M (22 ± 4 s) and M→VH (20 ± 6 s). The difference (Δ) 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. $\tau\dot{V}o_{2p}$) 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 $\tau\dot{V}o_{2p}$ 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 $\tau\dot{V}o_{2p}$ 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 $\tau\dot{V}o_{2p}$ 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 $\tau\dot{V}o_{2p}$ 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 $\tau\dot{V}o_{2p}$ 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 $\tau\dot{V}o_{2p}$ in an exercise intensity dependent manner

93 has previously relied on measures such as heart rate dynamics (Hebestreit *et al.*, 1998;
94 Breese *et al.*, 2012), which, are removed from peripheral sites of O_2 exchange between
95 the capillary and muscle.

96 The τ of muscle deoxyhemoglobin/myoglobin (deoxy[Hb+Mb]) measured by near-
97 infrared spectroscopy (NIRS) has been reported to cohere with that of fractional O_2
98 extraction (Koga *et al.*, 2012), hence, has been used to reflect the dynamic matching
99 between O_2 delivery- ($\dot{Q}o_2$) to- O_2 utilization ($\dot{V}o_2$) during exercise (DeLorey *et al.*, 2003;
100 Grassi *et al.*, 2003). Accordingly, for the same $\dot{V}o_2$ kinetics, an enhanced $\dot{Q}o_2/\dot{V}o_2$
101 response would be expected to slow deoxy[Hb+Mb] dynamics, whereas, slower $\dot{V}o_2$
102 kinetics alongside a faster deoxy[Hb+Mb] mean response time (MRT; i.e. time delay + τ)
103 has been interpreted to reflect limited microvascular O_2 delivery during the on-
104 transition of exercise (Murias *et al.*, 2011; Spencer *et al.*, 2012; Murias *et al.*, 2014).
105 Therefore, if, based on adults studies, the kinetics of bulk O_2 delivery were slower during
106 heavy- (>LT) compared to moderate-intensity (<LT) step transitions (Koga *et al.*, 2005;
107 McNarry *et al.*, 2012), an enhanced muscle oxidative capacity in children (Ratel *et al.*,
108 2008; Tonson *et al.*, 2010) may serve to maintain linearity of their $\tau\dot{V}o_{2p}$ by speeding
109 fractional O_2 extraction during supra-LT transitions in youth.

110 Dynamic non-linearity with respect to an increased $\tau\dot{V}o_{2p}$ and G_p has also been
111 reported when initiating cycling transitions from an elevated work rate (Hughson &
112 Morrissey, 1982; Brittain *et al.*, 2001; Wilkerson & Jones, 2006, 2007), with these effects
113 suggested to reflect the recruitment of higher-order (i.e. type II) muscle fibers (Brittain
114 *et al.*, 2001; Wilkerson & Jones, 2006, 2007); however, other factors have been
115 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 $\tau\dot{V}o_{2p}$ 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 τPCr 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 $\tau\dot{V}o_{2p}$ during very heavy- compared to moderate-intensity cycling transitions elicited from unloaded pedaling (i.e. U→VH *vs*. U→M) would coincide with a faster deoxy[Hb+Mb] MRT, whereas, moderate-to-very heavy-intensity cycling transitions (i.e. M→VH) would slow the $\tau\dot{V}o_{2p}$ alongside a slower deoxy[Hb+Mb] MRT compared to other conditions. Finally, we hypothesized that an increased $\tau\dot{V}o_{2p}$ and G_p following the onset of M→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 CO_2 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 $\dot{V}o_2$ ($\dot{V}_E/\dot{V}o_2$) with no increase in $\dot{V}_E/\dot{V}co_2$.

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 $\dot{V}o_2$ 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 $\dot{V}o_2$ and

231 deoxy[Hb+Mb] responses were subsequently linearly interpolated with identical
232 repetitions of each step condition time aligned to the start of exercise and ensemble
233 averaged to improve the signal-to-noise ratio.

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

237

238 $$\Delta Y_{(t)} = \Delta Y_p \cdot (1 - e^{-(t - TD)/\tau p}) \quad (1)$$

239

240 where $\Delta Y_{(t)}$ indicates the value at a given time (t) minus the baseline value (60-s average)
241 before exercise onset, ΔY_p inidicates the amplitude change of the primary component
242 from baseline to its asymptote, TD and τ_p represent the time delay and time constant of
243 the phase II exponential function, respectively. For U→M, the model in Equation (1) was
244 fitted to end-exercise (i.e. 4-min), whereas, for U→VH and M→VH, the model fitting
245 window was constrained to exclude the A_{Sc} and hence isolate the phase II component.
246 The onset of the A_{Sc} was determined using software (LabView, v 6.1, National
247 Instruments, Newbury, UK) which initially fitted a mono-exponential function up to the
248 first 60-s of $\dot{V}o_2$ data and then increased iteratively by 5-s until end-exercise. The
249 estimated τ for each fitting window was then plotted against time with the phase II
250 portion of the response determined as the point at which the influence of the A_{Sc}
251 lengthened the estimated τ following an initial plateau (Rossiter *et al.*, 2001). The
252 parameter estimates from Equation (1) and their 95% confidence intervals (CI_{95}) were
253 then resolved by least-squares non-linear regression (GraphPad Prism, GraphPad

Software, San Diego, CA). 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 τΔdeoxy[Hb+Mb] and τTOI, 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 τ were summed to reflect the overall mean response time (MRT) of Δdeoxy[Hb+Mb] and TOI within each step condition.

The ratio of Δdeoxy[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 Δdeoxy[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 Δdeoxy[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

277 in children (Springer *et al.*, 1991; Hebestreit *et al.*, 1998). The magnitude of the
278 Δdeoxy[Hb+Mb]/Δ$\dot{V}o_2$ "overshoot" was calculated by integrating the area under curve
279 from the first datum lying above 1.0 or 'unity' to 180-s of exercise in all participants in
280 each condition.
281
282 *Statistical Analysis*
283 Gaussian distribution was assessed by the Shapiro-Wilk test and subsequently
284 verified by calculating standardized scores for skewness and kurtosis for each variable.
285 A standardized value < 2 was deemed acceptably normally distributed. All pulmonary $\dot{V}$
286 o_2 and NIRS-derived variables were analyzed using one-way repeated measures ANOVA
287 with Bonferroni adjusted post hoc tests used to locate statistically significant differences
288 between step conditions. In addition, effect size (ES; using Cohen's *d*) was also calculated
289 to judge the magnitude of the observed effect, using the following thresholds: Trivial (<
290 0.2), Small (0.2), Medium (0.5), and Large (0.8). Pearson product moment correlations
291 (*r*) were used to assess the bivariate relationship between alterations in phase II $\dot{V}o_2$
292 kinetics with muscle oxygenation and chronological age. All statistical analyses were
293 conducted using PASW Statistics 18 (SPSS, Chicago, IL). Data are presented as means ±
294 SD. Statistical significance was accepted if $P < 0.05$.
295
296 **RESULTS**
297 The group mean ± SD values for peak $\dot{V}o_2$ and end HR during the initial ramp
298 incremental cycle test were 2.37 ± 0.60 l/min and 192 ± 9 bpm, respectively. The group

299 mean ± SD values for end HR during U→M, U→VH and M→VH step cycling were 129 ±
300 16, 178 ± 11, and 179 ± 12 bpm, respectively.

301 *Pulmonary $\dot{V}o_2$ kinetics*

302 Table 1 presents the group mean ± SD parameter estimates for $\dot{V}o_2$ kinetics with their
303 corresponding profiles in a representative participant shown in Figure 1. There was no
304 significant difference in the $\tau\dot{V}o_{2p}$ between U→M and U→VH (P = 0.31, ES = 0.4);
305 however, the $\tau\dot{V}o_{2p}$ was slower during M→VH compared to other conditions (P < 0.001,
306 ES > 1.2). There was a significant main effect for step cycling on the G_p, which, relative
307 to U→M, decreased during U→VH (P = 0.01, ES = 0.7); however, there were no
308 significant differences during M→VH compared to other conditions (P > 0.2). The A_{Sc}
309 decreased during M→VH compared to U→VH (P = 0.01, ES = 0.8) with this difference
310 removed when normalizing A_{Sc} relative to the total $\Delta\dot{V}o_2$ above baseline pedaling
311 (U→VH: 14 ± 6 *vs*. M→VH: 13 ± 7 %, P = 0.37). Relative to U→M, the G_{tot} was greater
312 during U→VH (P = 0.045, ES = 0.6) and M→VH (P = 0.03, ES = 0.9).

313

314 *NIRS-derived variables*

315 Table 2 presents the group mean ± SD parameter estimates for NIRS-derived
316 deoxy[Hb+Mb] and TOI kinetics with their corresponding profiles in a representative
317 participant shown in Figures 2 and 3, respectively. Relative to U→M, the
318 Δdeoxy[Hb+Mb]-*TD* following exercise onset decreased in the other conditions (P <
319 0.001, ES > 1.9) with a further reduction during M→VH compared to U→VH (P = 0.03,
320 ES = 0.8). There were no significant differences (P > 0.40) between U→M and U→VH in
321 the τΔdeoxy[Hb+Mb] or τTOI; however, both were slowed during M→VH compared to

322 other conditions (P < 0.03, ES > 1.2). Accordingly, the overall MRT (i.e. TD + τ) of muscle
323 deoxygenation kinetics was faster during U→VH compared to U→M and M→VH (P <
324 0.001, ES > 1.2).
325
326 *Matching of deoxy[Hb+Mb] to $\dot{V}o_2$*
327 Comparison of the group mean ± SD kinetic parameters for $\dot{V}o_2$ and muscle
328 deoxygenation are presented in Figure 4. There were no significant differences between
329 the $\tau\dot{V}o_{2p}$ and muscle deoxygenation kinetics during U→M (P > 0.15), whereas, the MRT
330 of Δdeoxy[Hb+Mb] and TOI was speeded relative to the $\tau\dot{V}o_{2p}$ during U→VH and M→VH
331 (P < 0.001). The difference between the $\tau\dot{V}o_{2p}$ and *MRT*-Δdeoxy[Hb+Mb] increased by a
332 large effect size during M→VH compared to U→VH (18 ± 15 *vs*. 9 ± 7 s, P = 0.07, ES = 0.8,
333 Figure 4C), with a significantly greater difference between the $\tau\dot{V}o_{2p}$ and *MRT*-TOI during
334 work-to-work exercise (23 ± 15 *vs*. 12 ± 7 s, respectively, P = 0.014, ES = 1.0, Figure 4D).
335 During U→M, the normalized Δdeoxy[Hb+Mb]/Δ$\dot{V}o_2$ overshoot area yielded non-
336 normally distributed data; therefore, were not reported. As shown in Figure 5, the
337 overshoot area above unity in the normalized Δdeoxy[Hb+Mb]/Δ$\dot{V}o_2$ ratio was
338 significantly greater during the on-transition of M→VH compared to U→VH exercise
339 (17.3 ± 13.2 *vs*. 8.5 ± 7.0 %/s, P = 0.01, ES = 0.9, respectively).
340
341 *Relationship between $\dot{V}o_2$ and deoxy[Hb+Mb] kinetics*
342 The reduction of the G_p correlated positively with the speeding of the *MRT*-
343 Δdeoxy[Hb+Mb] during U→VH compared to U→M (r = 0.67; P = 0.005). During M→VH,
344 there was no significant relationship (P > 0.5) between the slowing of the $\tau\dot{V}o_{2p}$ with

alterations in the τΔdeoxy[Hb+Mb] compared to U→M or U→VH (r = 0.15 and -0.06, respectively).

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

There was no significant relationship between the $\tau\dot{V}o_{2p}$ with chronological age during U→M (r = 0.40, P = 0.09); however, both variables correlated positively during U→VH (r = 0.48, P = 0.04) with a stronger relationship observed during M→VH (r = 0.78, P < 0.001). An increased (Δ) $\tau\dot{V}o_{2p}$ and ΔG_p during M→VH relative to U→M and U→VH correlated positively with chronological age (P < 0.01, Figure 6 A-D). During M→VH, the baseline $\dot{V}o_2$ in l/min correlated positively with the ΔG_p relative to U→M (r = 0.59, P = 0.008) and U→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→M cycling, an invariant $\tau\dot{V}o_{2p}$ during U→VH was accompanied by a faster *MRT*-Δdeoxy[Hb+Mb] and *MRT*-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→VH compared U→M, the G_p decreased suggesting that

368 this parameter may be limited by decreased microvascular O_2 delivery in boys.
369 Conversely, relative to U→VH cycling, M→VH decreased the rate of fractional O_2
370 extraction (i.e. increased MRT of Δdeoxy[Hb+Mb] and TOI kinetics) in a manner that was
371 disproportionally less than the slowing of the $\tau\dot{V}o_{2p}$, thereby, eliciting a greater
372 Δdeoxy[Hb+Mb]/$\Delta\dot{V}o_2$ "overshoot" in the transition from a raised baseline work rate.
373 Finally, relative to U→M and U→VH, an increased $\tau\dot{V}o_{2p}$ and G_p during M→VH correlated
374 positively with boys' chronological age. These findings lend support to the notion that
375 developmental effects on $\dot{V}o_2$ kinetics might be linked to the recruitment of higher-
376 order (i.e. type II) muscle fibers with slower microvascular blood flow dynamics and
377 poorer efficiency in older youth.
378
379 *Comparison of $\dot{V}o_2$ and muscle deoxygenation kinetics between U→M and U→VH*
380 In the present study, we observed no significant differences in the $\tau\dot{V}o_{2p}$ between
381 U→M and U→VH; however, the G_p decreased during U→VH reflecting both linear and
382 non-linear control features of $\dot{V}o_2$ kinetics following the onset of step cycling elicited
383 from unloaded pedaling in 11 to 15 y olds. An invariant $\tau\dot{V}o_{2p}$ during different intensities
384 of step exercise is consistent with previous reports in youth (Hebestreit *et al.*, 1998;
385 Williams *et al.*, 2001; Lai *et al.*, 2008); however, these studies employed relatively low
386 sample sizes (i.e. *n* = 8), or, in the case of Hebestreit *et al*. (1998) the work rate was
387 arbitrarily normalized as a fraction of peak $\dot{V}o_2$ in children. Therefore, our findings
388 extend those previously reported by revealing an invariant $\tau\dot{V}o_{2p}$ relative to work rate
389 using procedures for resolving the kinetic parameters within carefully prescribed

390 intensity domains among a larger youth cohort (i.e. *n* = 19), hence, reducing the
391 potential for type II statistical error.

392 Following the onset of U→M and U→VH, there was a pronounced TD before muscle
393 deoxy[Hb+Mb] increased, suggesting that the hyperemic effect of skeletal muscle
394 contractions sufficiently matched the requirement for O_2 utilization within active
395 regions of vastus lateralis muscle. However, during U→VH compared to U→M, the *MRT*-
396 Δdeoxy[Hb+Mb] and *MRT*-TOI were reduced (i.e. decreased TD + τ) by a large effect size,
397 hence, suggesting that an increased rate of fractional O_2 extraction was required to
398 maintain an invariant $\tau\dot{V}o_{2p}$ between both conditions. Therefore, these findings, in line
399 with "priming" exercise studies in children (Barker *et al.*, 2010; Barker *et al.*, 2014),
400 support the notion that the $\tau\dot{V}o_{2p}$ is principally limited by intracellular metabolic factors
401 rather than the dynamic relationship between $\dot{Q}o_2$-to-$\dot{V}o_2$ during supra-LT transitions in
402 youth.

403 In the present study, we did observe a significant association between a decreased
404 G_p with the relative speeding of the *MRT*-Δdeoxy[Hb+Mb] following the onset of U→VH
405 compared to U→M. It has been reported that the τ of deoxy[Hb+Mb] kinetics coheres
406 with that observed for the reduction in microvascular O_2 partial pressure (PmvO_2)
407 following the onset of skeletal muscle contractions (Koga *et al.*, 2012). Accordingly, it is
408 conceivable that those participants evincing a greater $\dot{Q}o_2$-to-$\dot{V}o_2$ mismatch (i.e. faster
409 *MRT*-Δdeoxy[Hb+Mb]) might have accelerated the fall in PmvO_2 such that the $\dot{V}o_2$
410 increment per unit of work rate was limited consequent to a decreased O_2 flux between
411 the capillary and muscle. Therefore, in youth, our findings are consistent with the notion

412 that the G_p might be sensitive to a decreased $\dot{Q}o_2/\dot{V}o_2$ response as previously reported
413 in adults (Koga *et al.*, 1999; Jones *et al.*, 2006).
414
415 *Comparison of $\dot{V}o_2$ and muscle deoxygenation kinetics during M→VH relative to other*
416 *step conditions*
417 Whereas U→VH sped muscle deoxy[Hb+Mb] kinetics, to maintain a constant $\tau\dot{V}o_{2p}$
418 compared to U→M, there was a concomitant slowing of the $\tau\dot{V}o_{2p}$, τTOI and
419 τΔdeoxy[Hb+Hb] during M→VH compared to other step conditions. Whilst these
420 findings during M→VH are consistent with a decreased rate of O_2 extraction, it is
421 important to consider that U→VH and M→VH sped the MRT of Δdeoxy[Hb+Mb] and TOI
422 responses relative to the $\tau\dot{V}o_{2p}$ with the difference between these signals increased
423 following the onset of work-to-work transitions (Figure 4). In other words, the slowing
424 of muscle deoxygenation did not match proportionally the slowing of the $\tau\dot{V}o_{2p}$, thereby,
425 increasing the normalized Δdeoxy[Hb+Mb]/$\Delta\dot{V}o_2$ overshoot area above unity within the
426 initial few minutes of M→VH compared to U→VH (Figure 5). Collectively, these
427 responses during M→VH are consistent with an increased proportional reliance on
428 fractional O_2 extraction; hence, our results suggest for the first time in youth that slower
429 phase II $\dot{V}o_2$ kinetics coincided with a slower rate of adjustment in $\dot{Q}o_2$-to-$\dot{V}o_2$ in the
430 transition from a raised baseline work rate.
431 In boys, it had been previously suggested that eliciting step transitions from a raised
432 level of electromyogram activity increased proportionally the recruitment of type II
433 muscle fibers for power production (Breese *et al.*, 2012). This supposition was based on
434 an orderly 'size' principle of motor unit recruitment (Henneman & Mendell, 1981), which,

435 in adults, has received support with previous studies reporting a progressive reduction
436 in the glycogen content within type I followed by type IIa and IIx muscle fibers from low
437 to high force requirements (Essen, 1978; Green, 1978; Krustrup *et al.*, 2004). 'Higher-
438 order' type II muscle fibers have been reported to possess slower microvascular O_2
439 delivery (i.e. decreased $P\text{mv}O_2$ across the on-exercise transition) (Behnke *et al.*, 2003)
440 and slower $\dot{V}o_2$ kinetics *in vitro* compared with 'lower-order' type I muscle fibers (Crow
441 & Kushmerick, 1982). Therefore, during M→VH, it is conceivable that the $\dot{V}o_2$ and
442 deoxy[Hb+Mb] profiles (and their kinetic relationship) reflected the intrinsic properties
443 of a population of skeletal muscle fibers positioned higher in the recruitment hierarchy
444 in boys.

445 There was a significant main effect for step cycling on the G_{tot}, which, relative to
446 U→M, was greater during U→VH and M→VH consequent to the development of the A_{Sc}
447 in these conditions. However, relative to U→VH, the A_{Sc} decreased by ~ 50% during
448 M→VH such that $\dot{V}o_2$ kinetics reverted toward a mono-exponential profile. There is
449 evidence to suggest that the development of the A_{Sc} is related in some manner to the
450 recruitment profile and metabolic features of type II muscle fibers with slower $\dot{V}o_2$
451 kinetics and poorer efficiency [i.e. increased ATP/force output ratio (Crow & Kushmerick,
452 1982)] compared with type I muscle fibers (see Jones *et al.*, 2011 for review). Therefore,
453 in adults, an explanation for a smaller A_{Sc} has considered the earlier (rather than latent)
454 expression upon the pulmonary $\dot{V}o_2$ signal of higher-order (i.e. type II) muscle fibers
455 when supra-LT transitions are initiated from an elevated work rate (Wilkerson & Jones,
456 2007; DiMenna *et al.*, 2008). However, this proposal predicts that the G_p would have

457 been higher during M→VH relative to other conditions, which, in boys, was not present
458 with this effect associated with chronological age (Figure 6).
459
460 *Relationship between $\dot{V}o_2$ kinetics with chronological age*
461 A novel finding was that the $\tau\dot{V}o_{2p}$ and chronological age, whilst not significantly
462 associated during U→M, were both positively correlated during U→VH with this
463 relationship strengthened by an increased pre-transition work rate. In other words,
464 M→VH exercise slowed by a greater extent the $\tau\dot{V}o_{2p}$ and increased the G_p within the
465 age range between 11 to 15 y (Figure 6). It would have been expected that U→M
466 transitions predominantly recruited a population of type I muscle fibers (Krustrup *et al.*,
467 2004) with the mean $\tau\dot{V}o_{2p}$ in this condition in boys (i.e. ~ 23 s) less likely to be limited
468 by muscle O_2 delivery based on a previous study in adults (Murias *et al.*, 2011).
469 Conversely, a previous investigation has reported a slower $\tau\dot{V}o_{2p}$ alongside slower limb
470 blood flow dynamics following the onset of work-to-work exercise in adults (MacPhee
471 *et al.*, 2005) with further evidence in support of a decline in the maximal rate of O_2
472 transport between the ages of 12 to 17 y (Koch, 1984) and in the proportion of type I
473 muscle fibers within the vastus lateralis between the ages of 5 to 20 y (Lexell *et al.*, 1992).
474 Therefore, we propose indirectly that an age-related slowing of the $\tau\dot{V}o_{2p}$ during M→VH
475 might have reflected differences in muscle perfusion and the distribution of O_2 in
476 conjunction with alterations in muscle fiber recruitment in older youth.
477 Alternatively, it is important to consider that larger (older) boys produced higher
478 cycling power outputs corresponding to the GET and at task failure during the initial
479 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 $\dot{V}o_2$ compared to smaller (younger) children. In this regard, it has been reported that the $\tau\dot{V}o_{2p}$ and G_p increased linearly at progressively higher baseline power outputs (hence $\dot{V}o_2$) 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 $\dot{V}o_2$ in l/min during M→VH with the $\Delta\tau\dot{V}o_{2p}$ 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 τ and *G* values between higher- relative to lower-order muscle fibers with increased chronological age (Figure 7). Accordingly, if the measured $\dot{V}o_2$ 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 $\dot{V}o_2$ during the on-transition of exercise and/or extend the A_{Sc} in older children. This $\dot{V}o_2$ 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 $\dot{V}o_2$ 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

503 been reported to confound interpretation of deoxy[Hb+Mb] data (see Barstow *et al.*,
504 2019 for a recent review). Moreover, we also recognize that the absorbance spectra of
505 Hb and Mb overlap within the NIR range; therefore, the relative (%) contribution from
506 each chromophore to the NIRS-derived signal is uncertain (Masuda *et al.*, 2010; Davis &
507 Barstow, 2013). Additionally, we left shifted the normalized $\dot{V}o_2$ by 15 s to account for
508 the estimated phase I duration in children (Springer *et al.*, 1991; Hebestreit *et al.*, 1998),
509 thereby, time aligning the start of phase II $\dot{V}o_2$ to the onset of exercise, which, has been
510 reported to coincide with muscle $\dot{V}o_2$ within 10% (Barstow *et al.*, 1994). Therefore, the
511 extent to which inter- and intra-participant differences in the circulatory muscle-to-lung
512 transit time influenced the Δdeoxy[Hb+Mb]/Δ$\dot{V}o_2$ overshoot is unclear. It should also be
513 cautioned that the pulmonary $\dot{V}o_2$ amplitude during exercise includes minor
514 contributions from cardiorespiratory support processes (Poole *et al.*, 1991), which, has
515 the potential to influence its ratio when expressed relative to the adjustment in
516 deoxy[Hb+Mb] kinetics. Therefore, in the present study, we stress that precedence be
517 given to interpreting the TD and τ of muscle deoxygenation with these preliminary
518 kinetic data supported by the Δdeoxy[Hb+Mb]/Δ$\dot{V}o_2$ ratio to infer the dynamic
519 (mis)matching between O_2 delivery/utilization. Finally, it should be noted that baseline
520 pedaling during M→VH involved simultaneously raising pre-transition $\dot{V}o_2$ with work
521 rate, which, when both are dissociated, has the potential to influence the τ$\dot{V}o_{2p}$ and G_p
522 via independent mechanisms (DiMenna *et al.*, 2010a; Bowen *et al.*, 2011; Wust *et al.*,
523 2014). Therefore, in the present study, whether an increased baseline work rate *per se*
524 altered phase II $\dot{V}o_2$ kinetics cannot be established.
525

526 *Conclusions*

527 This study in 11 to 15 y olds reported dynamic non-linearity of the phase II $\dot{V}o_2$ kinetic
528 parameters, with respect to a decreased G_p during U→VH compared to U→M, whereas,
529 a slower $\tau\dot{V}o_{2p}$ was dependent on an increased pre-transition work rate in youth.
530 Furthermore, whilst "work-to-work" cycling slowed the τ of muscle deoxygenation,
531 when expressed relative to the adjustment in $\dot{V}o_2$ kinetics, the ratio between both of
532 these signals increased, suggesting a greater proportional speeding of fractional O_2
533 extraction; hence, the slower $\tau\dot{V}o_{2p}$ during M→VH was consequent to a slowing of
534 microvascular blood flow relative to O_2 utilization. Finally, an increased $\tau\dot{V}o_{2p}$ and G_p
535 during the transition from a raised baseline work rate correlated positively with
536 chronological age. These novel findings further our understanding of the physiological
537 factors modulating the $\dot{V}o_2$ kinetic response, and, thereby, oxidative metabolism, and
538 their association with chronological age in healthy youth.
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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.

TABLES

Table 1. Amplitude and kinetics of pulmonary oxygen uptake ($\dot{V}O_2$) following the onset of exercise in each step condition

	AVOVA	U→M	U→VH	M→VH
$\dot{V}O_{2bl}$ (l/min)	< .001	0.69 ± 0.18	0.72 ± 0.17	1.21 ± 0.34†
TD_p (s)	.01	11 ± 3	9 ± 3	8 ± 7*
$\tau\dot{V}O_{2p}$ (s)	< .001	23 ± 6	26 ± 8	42 ± 15†
CI_{95} (s)	.006	7 ± 2	6 ± 3	10 ± 4†
A_p (l/min)	< .001	0.53 ± 0.19	1.25 ± 0.30*	0.82 ± 0.31†
G_p (ml/min/W)	.04	9.9 ± 1.3	9.1 ± 1.0*	9.6 ± 1.2
TD_{Sc} (s)	-	-	160 ± 33	184 ± 35
A_{Sc} (l/min)	-	-	0.21 ± 0.13	0.11 ± 0.06†
$\dot{V}O_{2tot}$ (l/min)	< .001	1.22 ± 0.36	2.18 ± 0.55*	2.14 ± 0.58*
G_{tot} (ml/min/W)	.008	9.9 ± 1.3	10.5 ± 0.9*	11.0 ± 1.0*

Values are mean ± SD. $\dot{V}O_{2bl}$, mean $\dot{V}O_2$ during baseline cycling; TD_p, phase II time delay; $\tau\dot{V}O_{2p}$, phase II time constant; CI_{95}, 95% confidence interval for $\tau\dot{V}O_{2p}$; A_p, amplitude of phase I + II, excluding $\dot{V}O_{2bl}$; TD_{Sc}, 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

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

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 ($\dot{V}o_2$) 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 $\dot{V}o_2$ kinetic response [see Equation (1)].

Figure 2. Muscle deoxy[Hb+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[Hb+Mb] kinetic response [see Equation (1)].

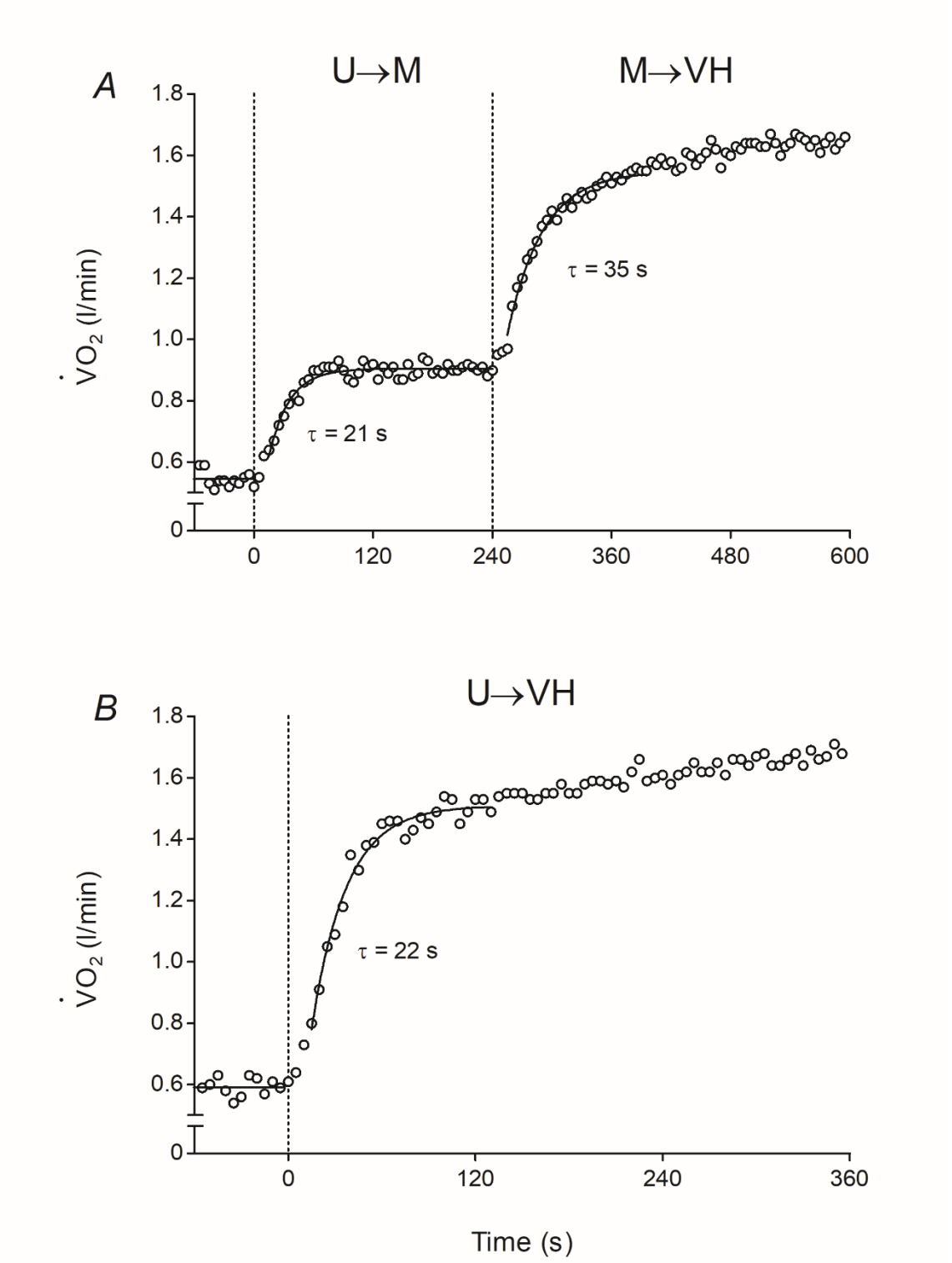
Figure 3. Tissue oxygenation index (TOI) of the vastus lateralis in a representative participant following the onset of step cycling in each condition. The vertical dashed lines indicate the onset of step exercise. The solid black lines denote the least squares regression fit of the primary TOI kinetic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

Figure 4. Comparison of $\dot{V}o_2$ and muscle deoxygenation kinetics following the onset of step cycling. Panels *A* and *B* show the group mean ± SD $\tau\dot{V}o_{2p}$ (*black bars*) and mean response time (*MRT*) of Δdeoxy[Hb+Mb] and TOI (*white bars*) within each step condition. Panels *C* and *D* present those values for $\tau\dot{V}o_{2p}$ *minus* the *MRT*-Δdeoxy[Hb+Mb] and *MRT*-TOI during U→M, U→VH and M→VH, respectively. $^{\#}P < 0.01$ relative to the $\tau\dot{V}o_{2p}$ within condition, $^{*}P < 0.01$ *vs*. U→M, and $^{\dagger}P < 0.05$ *vs*. other step conditions.

Figure 5. Group mean normalized ratio between the adjustment of deoxy[Hb+Mb] relative to $\dot{V}o_2$ 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 (Δ) between baseline and end-exercise (i.e. 0 – 100%) with the $\dot{V}o_2$ 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 (Δ) in the $\tau\dot{V}o_{2p}$ 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 $\dot{V}o_2$ 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 $\dot{V}o_2$ 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.



929
930 Fig. 1
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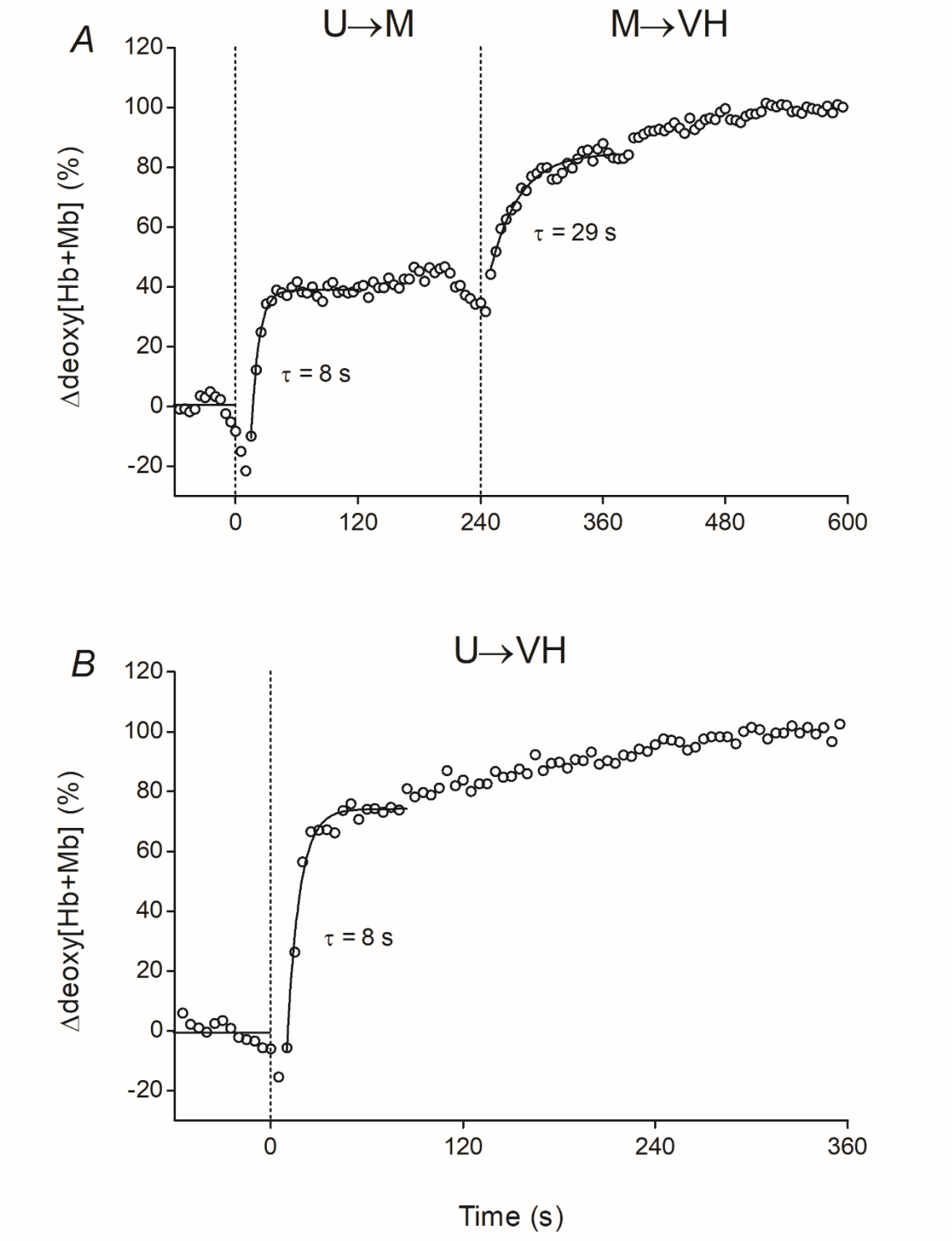
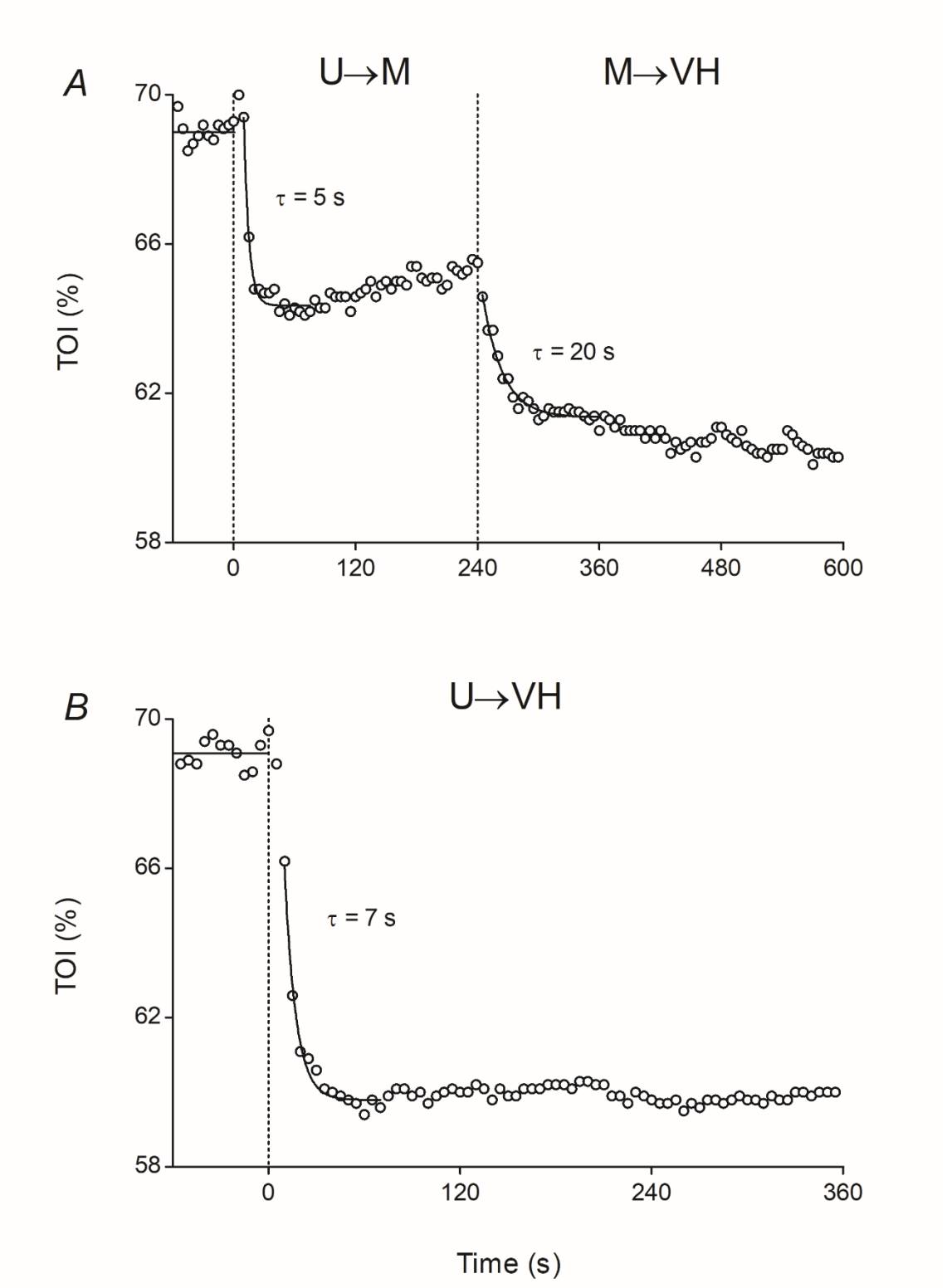

A
U→M
M→VH
120
100
80
60
40
20
0
-20
Δdeoxy[Hb+Mb] (%)
τ = 8 s
τ = 29 s
0
120
240
360
480
600
B
U→VH
120
100
80
60
40
20
0
-20
Δdeoxy[Hb+Mb] (%)
τ = 8 s
0
120
240
360
Time (s)

Fig. 2



940
941 Fig. 3
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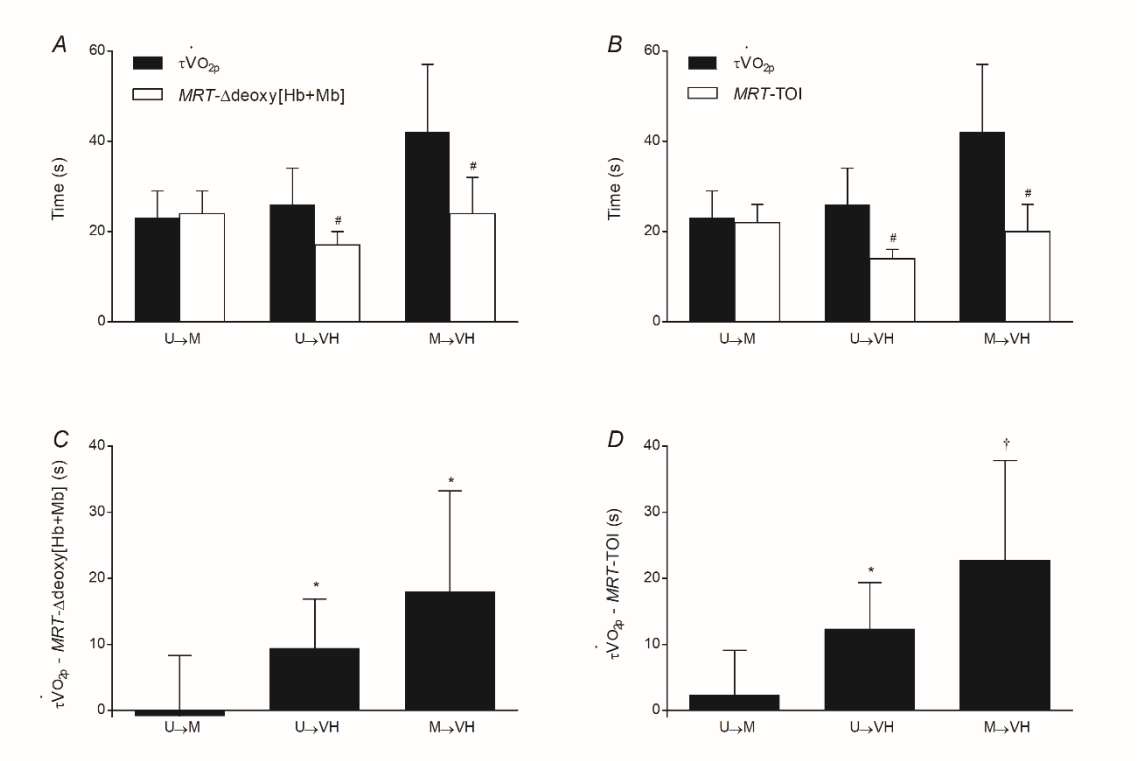
A
B
C
D
τV̇O2p
MRT-Δdeoxy[Hb+Mb]
MRT-TOI
Time (s)
τV̇O2p - MRT-Δdeoxy[Hb+Mb] (s)
τV̇O2p - MRT-TOI (s)
U→M
U→VH
M→VH

Fig. 4

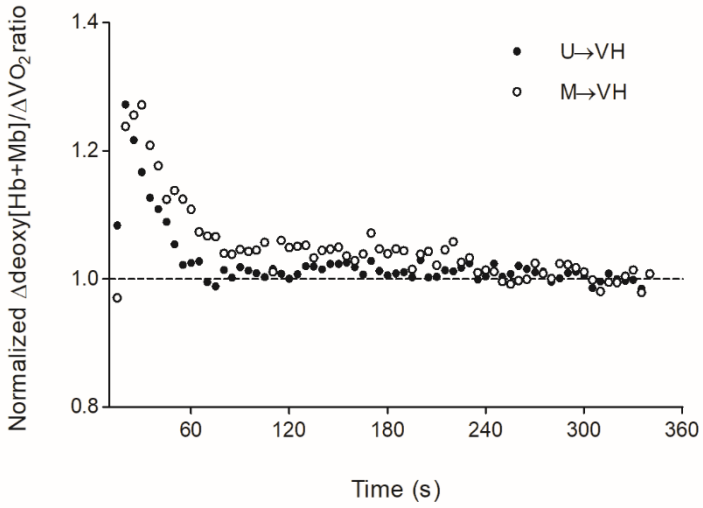
Normalized Δdeoxy[Hb+Mb]/ΔV̇O2 ratio
U→VH
M→VH
Time (s)

Fig. 5

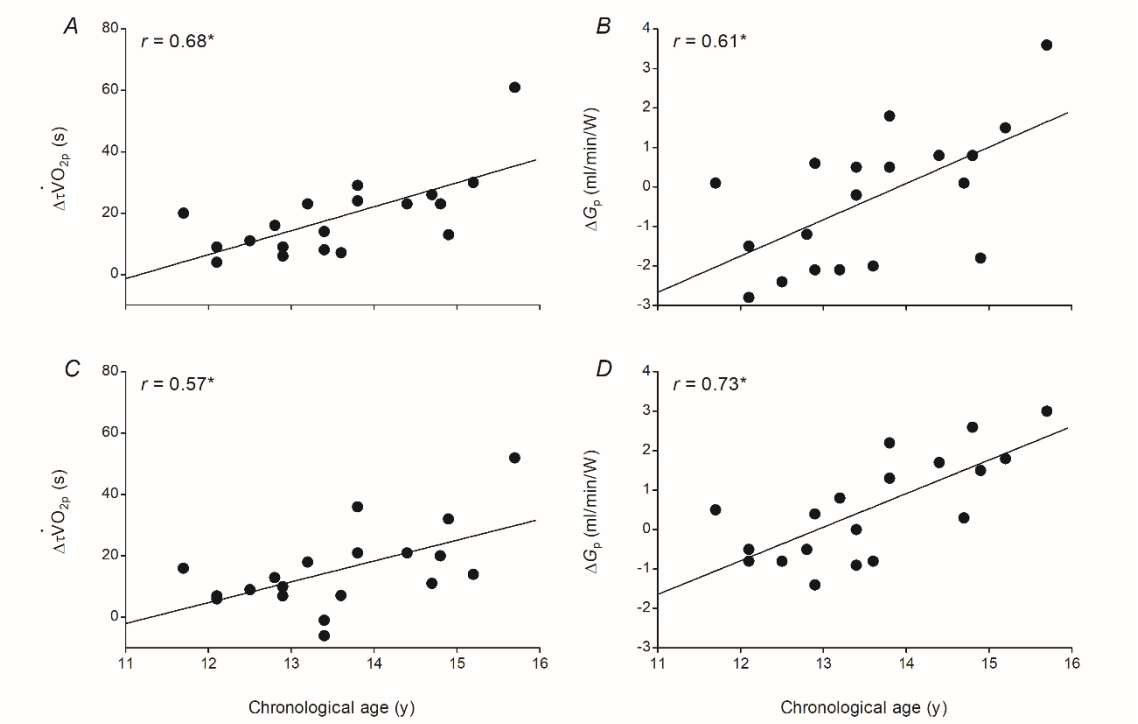
A
r = 0.68*
ΔτV̇O2p (s)
B
r = 0.61*
ΔGp (ml/min/W)
C
r = 0.57*
ΔτV̇O2p (s)
D
r = 0.73*
ΔGp (ml/min/W)
Chronological age (y)
Chronological age (y)

Fig. 6

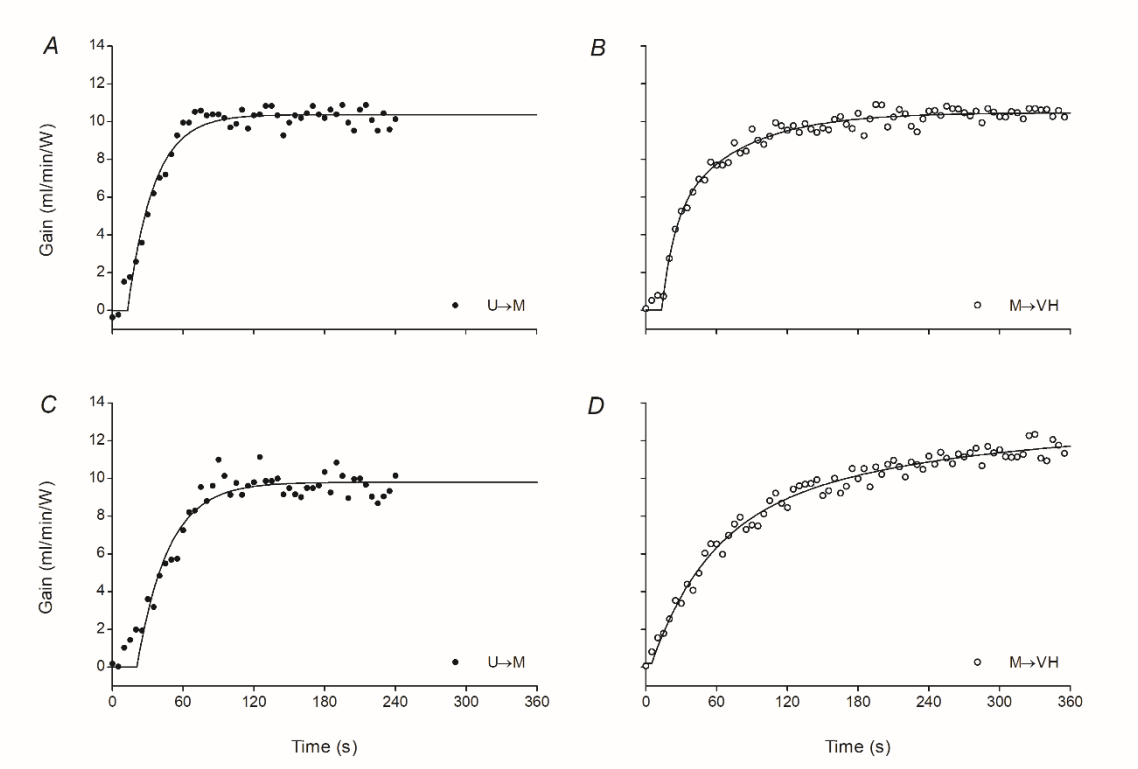
A
Gain (ml/min/W)
U→M
B
M→VH
C
Gain (ml/min/W)
U→M
D
M→VH
Time (s)
Time (s)

Fig. 7