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Title: Test-retest reliability of pulmonary oxygen uptake and muscle deoxygenation during moderate- and heavy-intensity cycling in youth elite-cyclists

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Abstract

To establish the test-retest reliability of pulmonary oxygen uptake ($\dot{V}O_2$), muscle deoxygenation (deoxy[heme]) and tissue oxygen saturation (StO_2) kinetics in youth elite-cyclists. From baseline pedaling, 15 youth cyclists completed 6-min step transitions to a moderate- and heavy-intensity work rate separated by 8 min of baseline cycling. The protocol was repeated after 1 h of passive rest. $\dot{V}O_2$ was measured breath-by-breath alongside deoxy[heme] and StO_2 of the vastus lateralis by near-infrared spectroscopy. Reliability was assessed using 95% limits of agreement (LoA), the typical error (TE) and the intraclass correlation coefficient (ICC). During moderate- and heavy-intensity step cycling, TEs for the amplitude, time delay and time constant ranged between 3.5-21.9% and 3.9-12.1% for $\dot{V}O_2$ and between 6.6-13.7% and 3.5-10.4% for deoxy[heme], respectively. The 95% confidence interval for estimating the kinetic parameters significantly improved for ensemble-averaged transitions of $\dot{V}O_2$ ($p<0.01$) but not for deoxy[heme]. For StO_2, the TEs for the baseline, end-exercise and the rate of deoxygenation were 1.0-42.5% and 1.1-5.5% during moderate- and heavy-intensity exercise, respectively. The ICC ranged from 0.81-0.99 for all measures. Test-retest reliability data provides limits within which changes in $\dot{V}O_2$, deoxy[heme] and StO_2 kinetics may be interpreted with confidence in youth athletes.

Keywords: oxygen utilization, oxidative metabolism, microvascular blood flow, near-infrared spectroscopy, reproducibility

Introduction

Following the onset of step exercise transitions, there is an immediate increase in metabolic demand. The kinetics of pulmonary oxygen uptake ($\dot{V}O_2$), however, rise exponentially to meet this demand via oxidative phosphorylation. The time constant (τ) of the exponential rise describes the rate of adjustment of the fundamental (i.e. phase II) $\dot{V}O_2$ response that has been reported to closely reflect the kinetics of skeletal muscle O_2 consumption (Grassi et al. 2003). Faster $\dot{V}O_2$-kinetics have been demonstrated in trained compared to untrained adolescents (Breese et al. 2011; Marwood et al. 2010) and in boys compared to men (Fawkner et al. 2002; Williams et al. 2001). In addition, endurance training has been reported to speed $\dot{V}O_2$-kinetics in young and older adults (McLay et al. 2017; Murias et al. 2010).

Near-infrared spectroscopy (NIRS) is a well-established technique to measure tissue oxygenation based on the light absorption characteristics of hemoglobin and myoglobin depending on O_2 saturation (Barstow 2019). Although the signals from myoglobin and hemoglobin cannot be separated because the absorption spectrum overlaps, it has been suggested that myoglobin levels are small compared to those of hemoglobin and contribute <10% to the NIRS signal (Grassi and Quaresima 2016). Beer-Lambert law derived changes in muscle deoxygenated heme concentration (i.e. Δdeoxy[heme]) have been reported to cohere with that of fractional O_2 extraction (Koga et al. 2012), whereas spatially resolved spectroscopy (SRS) measures of tissue oxygen saturation (i.e. StO_2) are thought to reflect the dynamic balance between O_2 delivery-to-utilization (Boushel et al. 2001). It has been shown that Δdeoxy[heme] increases faster than $\dot{V}O_2$-kinetics indicating a mismatch of O_2 delivery to O_2 utilization (DeLorey et al. 2003), which is reflected by a greater ratio of Δdeoxy[heme]/$\Delta\dot{V}O_2$ at the onset of exercise in adults (Murias et al. 2011) with this finding also reported during heavy step cycling transitions in boys (Barker et al. 2014; Breese et al. 2019). However, it was found that this initial "overshoot" was abolished when O_2 delivery was altered with priming exercise (Barker et al. 2014) or after a training intervention (McKay et al. 2009; Murias et al. 2010). The latter studies found no significant changes in the Δdeoxy[heme]-kinetics, indicating that O_2 utilization increased rapidly to support the metabolic demand, whereas $\dot{V}O_2$-kinetics were significantly faster, suggesting an increased microvascular O_2 delivery in response to a training intervention.

Despite the fact that $\dot{V}O_2$ and NIRS-derived muscle deoxygenation kinetics have been used to quantify acute or training-induced alterations in O_2 delivery and O_2 utilization (McKay et al.

2009; McLay et al. 2017; Murias et al. 2010), few studies have addressed the test-retest reliability of these data, even though reliability is mandatory to interpret training-induced changes with confidence. A high test-retest reliability (i.e. a small random variation) reduce the uncertainty on whether a change is considered as real change or as unclear. Hopkins (2000) suggested a typical error (TE), expressed as the coefficient of variation, of ~10% as a threshold for acceptable reliability. A previous study in patients with chronic heart failure reported reliability of numerous NIRS-derived variables (Niemeijer et al. 2017). The best reliability given as TE and intraclass correlation coefficient (ICC) was found for StO_2 amplitudes (TE: 4.7-7.1%; ICC: 0.74-0.9). Spencer et al. (2011) investigated day-to-day reliability of $\dot{V}O_2$-kinetics and the effects of modelling single- and ensemble averaged data on the 95% confidence interval (CI95) of the τ of $\dot{V}O_2$-kinetics and Δdeoxy[heme]-kinetics. The authors reported ICCs from 0.87-0.89 and reduced CI95 when three or more averaged- rather than a single-transition was modelled.

In populations such as youth elite-athletes, current literature is especially scarce regarding reliability measures, which should be established to interpret, with confidence, maturational and longitudinal exercise-induced changes. Although, a multi-day test protocol with several transitions is optimal to increase the signal-to-noise ratio, as shown by Spencer et al. (2011), this is not a typical scenario used by sports science support staff and cycling coaches when testing youth elite-athletes. Typically, such athletes visit the laboratory on a single occasion performing a limited number of transitions. Therefore, the aim of this study was to assess the within-day test-retest reliability of $\dot{V}O_2$-, Δdeoxy[heme]- and StO_2 kinetics following the onset of moderate- and heavy-intensity step transitions in youth elite-cyclists. The magnitude of differences between two individual transitions and the impact of averaging these transitions on the confidence in parameter estimates was addressed. It was hypothesized that the test-retest reliability would be high (TE <10% and ICC >0.75) and that the CI95 would be improved when ensemble averaging two transitions of data.

Methods

Participants

Fifteen youth cyclists (2 females, 13 males) with mean ± SD age 13.9 ± 1.7 y, stature 163.2 ± 12.3 cm and body mass 51.3 ± 12.4 kg volunteered to participate in this study. The cyclists were members of a National Cycling Team, had a training history of 2-5 years and trained between 10-15 $h \cdot week^{-1}$ at the time of the study. After explanation of the procedures and

possible risks and benefits associated with the experiments, each participant and their legal guardians signed an informed consent form. All procedures performed were approved by the institutional review board and were in accordance with the 1964 Helsinki declaration and its later amendments or comparable standards.

Protocol

Participants were asked to visit the laboratory on two separate occasions within one week. Participants were instructed to avoid strenuous exercise for at least 24 h preceding each visit and to arrive at the laboratory in a fully rested and hydrated state 3 h after a light meal. For all tests, participants used their own racing bike, which was mounted to a Cyclus2 Ergometer (RBM Electronics, Leipzig, Germany). The first visit was used to measure body mass and stature using an electronic scale and stadiometer (Seca 813 and 213, Seca, Germany) before a graded ramp-exercise test was performed. During the second visit, the participants completed two step-transitions of 6-min duration from baseline pedaling to a moderate- and heavy-intensity cycling work rate. The two transitions were interspersed by 8 min of baseline pedaling and repeated after a 1-h passive rest.

Experimental procedures

Graded ramp-exercise test

The participants completed the test at a cadence of 90 ± 2 $rev \cdot min^{-1}$ to assess peak values of oxygen uptake ($\dot{V}O_{2peak}$), defined as the highest $\dot{V}O_2$ achieved over a 15-s interval before volitional exhaustion, power output (P_{peak}) and maximal heart rate (HR_{max}) achieved at the termination of the test. In addition, the ventilatory threshold (VT) was determined by visual inspection using ventilatory and gas exchange indexes as described previously (Beaver et al. 1986). After 3 min baseline cycling at 40 W, for all participants the work rate was progressively increased by 20 $W \cdot min^{-1}$ until the limit of tolerance. Pulmonary ventilation and gas exchange were measured continuously throughout the test via breath-by-breath open circuit spirometry (MetaMax 3B, Cortex Biophysik, Leipzig, Germany). The gas analyzers were calibrated before each test with gases of known concentrations (4.99 Vol% CO_2, 15.99 Vol% O_2, Cortex Biophysik, Leipzig, Germany). Volume and flow were calibrated with a 3-L syringe (Type M 9474-C; Cortex Biophysik, Leipzig, Germany). The participants wore a facemask and breathed through a low-resistance impeller turbine. Heart rate was measured continuously throughout the test using short-range radio-telemetry.

Cycling step transitions

The work rates required for the step transitions were subsequently calculated after the graded ramp-exercise test. The moderate-intensity exercise was calculated as the power at 90%VT and the heavy-intensity exercise as the power at 50% between VT and P_{peak} (Δ50%). The step transitions started with a 3-min period of baseline cycling at 40 W, followed by an immediate increase to moderate intensity for 6min, followed by 8 min at 40 W before another 6-min at heavy intensity and a 3-min cool down. The protocol was repeated after a passive rest of 1 h to ensure full recovery, which was satisfied when the $\dot{V}O_2$ at baseline cycling was within 100 $mL \cdot min^{-1} \cdot kg^{-1}$ compared to the first transition. The participants were asked to remain the same trunk position throughout the tests and to pedal at a cadence of 90 ± 2 $rev \cdot min^{-1}$. Gas exchange and pulmonary ventilation were collected continuously as described above.

In addition, muscle oxygenation status of the right-leg vastus lateralis was measured with a portable continuous-wave NIRS device at a sampling rate of 5 Hz (PortaMon, Artinis, The Netherlands). The instrument consisted of three transmitters, emitting photons at two wavelengths (762 and 850 nm) and a photon detector, detecting the light returning from the tissue. The distances between the emitter and the detector were 30, 35 and 40 mm allowing a penetration depth of 15-20 mm. The instrument utilized simultaneously the modified Beer-Lambert law to estimate oxygenation changes in micromolar units (μM) and SRS to estimate the StO_2 (see data analysis below).

The skinfold thickness under the probe was measured using a skinfold caliper (Harpenden, Baty International, UK) and the thickness of the double skinfold was divided by 2 to obtain an estimate of the adipose tissue thickness. The adipose tissue thickness in our participants was 4.6 ± 0.8 mm (range = 3.4-6.1 mm), thereby minimizing the contribution of the overlying adipose tissue layer to light scattering contaminating the NIRS signal. After cleaning and shaving the leg around the belly of the muscle, the device was covered in a translucent household plastic and fixated longitudinally to the muscle, midway between the greater trochanter and the lateral epicondyle of the femur using tape. The probe area was marked on the skin with permanent ink to check for any movement of the probe during exercise. To minimize the intrusion of extraneous light, an elastic bandage was wrapped around the thigh and then covered with an optically dense black hose.

Data analysis and kinetic modeling

The breath-by-breath $\dot{V}O_2$ data were examined and errant breaths lying more than four standard deviations from the local mean of five data points were removed. The filtered data were linearly interpolated to provide second-by-second values for the single transitions. In addition, the two transitions were ensemble-averaged and subsequently time aligned to the onset of the exercise to yield three responses for each subject. Data obtained during the first 15 s of the transition were excluded from the analysis to most likely exclude the cardio-dynamic or phase I response (Hebestreit et al. 1998), and a single-exponential algorithm was used to model the kinetics of the fundamental (phase II) response of $\dot{V}O_2$ by the following equation:

$$\dot{V}O_2\,(t) = BL + Amp\,(1 - e^{-(t-TD)/\tau}) \qquad \text{Eq.1}$$

where $\dot{V}O_2$ (t), BL, Amp, TD and τ represent the $\dot{V}O_2$ at any given time (t), the $\dot{V}O_2$ at baseline exercise (60-10 s average before the transition), the amplitude from baseline to its asymptote, the time delay and the time constant of the response, respectively. The mean response time (MRT) was calculated as the sum of the TD and τ. For moderate-intensity exercise the model was fitted to end-exercise, whereas for heavy-intensity exercise the optimal fitting window was identified with a purpose-designed software (LabView 6.1, National Instruments, Newbury, UK), following the methods of Rossiter et al. (2002) to check the presence of a slow component (SC). Starting from the initial 60 s of exercise, the fitting window was increased iteratively by 5 s to end-exercise. For each fitting window, the estimated τ was plotted against time and the onset of the SC was determined through visual inspection as the point at which the estimated τ progressively increased following an initial plateau. The parameter estimates were then resolved by least-squares non-linear regression (GraphPad Prism 8, GraphPad Software Inc., San Diego, USA). The amplitude of the SC was calculated as the difference between the mean of the final 30 s at end-exercise and the asymptote of the fundamental response.

The NIRS-derived signals were averaged to 1-s bins and the baseline was defined as mean signal between -60 and -10 s of baseline cycling. From the signals derived using the modified Beer-Lambert law, each data point was corrected for blood volume changes (Ryan et al. 2012), such as the Δoxy[heme] and the Δdeoxy[heme] reflect a symmetrical change without a change in total[heme]. Consequently, the Δdeoxy[heme] was used for further analysis. The baseline was arbitrarily set to zero before applying Equation 1 and the amplitude derived reflect changes in μM units. The time at which the exponential increase commenced was identified as the point at which the Δdeoxy[heme] signal started to increase by 1SD above baseline (DeLorey et al. 2003). In the present data, the exponential increase in Δdeoxy[heme] was well characterized

by a fit to 60 s for moderate- and to 60-90 s for heavy-intensity exercise. In addition, the StO_2 derived from the SRS method was calculated as oxy[heme]/(oxy+deoxy[heme]) and expressed as a percentage. However, StO_2 did not approximate a clear exponential response for the majority of our data and therefore could not be modelled utilizing Equation 1. Instead, the difference between the baseline and the end-exercise value, defined as StO_2 from the last 30 s was calculated (ΔStO_2). In addition, the rate of deoxygenation (StO_2-slope; $\%\cdot s^{-1}$) was determined as the slope of linear regression modelling of StO_2 against time (Ihsan et al. 2013). The data were modelled from the corresponding time where a sustained decrease of StO_2 was observed (0-3 s) to the time where a clear levelling-off occurred (20-25 s).

Statistical analysis

Descriptive data are presented as mean ± standard deviation (SD). The assumption of normality was verified using the Shapiro-Wilk test. Differences between variables were assessed with repeated measures ANOVA and Tukey's post-hoc test or paired t-tests where appropriate. Effect sizes are reported as Cohen's *d* with 0.2, 0.5 and 0.8 considered as small, moderate and large effect, respectively. Absolute and relative reliability was calculated with Bland-Altman statistics ± 95% limits of agreement (LoA), the typical error reported as coefficient of variation (CV; %) (Hopkins 2015) and with a two-way mixed effect intraclass correlation coefficient (ICC) (Shrout and Fleiss 1979), interpreted as excellent (> 0.9), good (0.75-0.9), moderate (0.5-0.75) and poor (< 0.5). Reliability data are reported with 90% confidence intervals (CI90). The confidence in the parameters of the kinetic and linear modeling is provided as 95% confidence interval (CI95) and the overall quality of the model as standard error of estimates (SEE). All statistical analyses were performed with the software package GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA) and the level of significance was set at $p < 0.05$.

Results

The physiological responses during the incremental cycling test are shown in Table 1, together with the work rates used for the step transitions. The group-mean, ensemble-averaged $\dot{V}O_2$, Δdeoxy[heme] and StO_2 responses during moderate- and heavy-intensity exercise are shown in Figure 1.

$\dot{V}O_2$ kinetics

The parameters for $\dot{V}O_2$ kinetics are provided in Table 2. During moderate-intensity exercise, kinetic modeling could not be applied for two subjects due to poor signal-to-noise ratios and

consequently were not included in the analyses. For the two single transitions at moderate-intensity exercise, the SEE obtained from the models were 104 ± 36 ml·min^{-1} and 106 ± 32 ml·min^{-1} (p = 0.925). There was a significant improvement to the model when the transitions were ensemble-averaged (80 ± 31 ml·min^{-1}; p < 0.001). Likewise, ANOVA revealed a significant difference (p < 0.001) between the ensemble-averaged transition from heavy-intensity exercise (102 ± 38 ml·min^{-1}) compared with the single transitions (139 ± 52 ml·min^{-1} and 140 ± 42 ml·min^{-1}; p = 0.998). There were significant differences between moderate- and heavy-intensity exercise for $\dot{V}O_{2baseline}$ (p < 0.001; *d* = -0.58), Amp (p < 0.0001; *d* = -2.40), τ (p < 0.001; *d* = -1.21) and MRT (p < 0.001; *d* = -0.94). No significant difference with a large effect size was found for the TD (p = 0.065; *d* = 0.85).

Measures of reliability are provided in supplementary Table 1. For moderate intensity exercise the TE ranged from 3.5-21.9% and the ICC from 0.87-0.91. The TE and ICC for heavy intensity exercise was 3.9-12.1% and 0.87-0.99, respectively. With the exemption of the SC (TE: 68.6%; ICC: 0.68), all parameters showed good to excellent reliability.

Bland-Altman plots of the parameters for $\dot{V}O_2$ kinetics are presented in supplementary Figure 1. During moderate-intensity exercise, the bias and LoA between the two transitions was 28 ± 57 mL·min^{-1} (-83 to 139) for $\dot{V}O_{2baseline}$, -1 ± 52 mL·min^{-1} (-103 to 101) for Amp, 0.4 ± 2.6 s (-4.8 to 5.5) for TD, 1.0 ± 4.1 s (-6.9 to 9.0) for τ, and 1.4 ± 3.6 s (-5.6 to 8.4) for MRT. The bias and LoA of the $\dot{V}O_2$ kinetics during heavy-intensity exercise transitions was -17 ± 81 mL·min^{-1} (-175 to 142) for $\dot{V}O_{2baseline}$, -25 ± 90 mL·min^{-1} (-202 to 152) for Amp, -0.6 ± 1.2 s (-3.0 to 1.8) for TD, 0.5 ± 1.8 s (-2.9 to 3.9) for τ, and -0.1 ± 2.2 s (-4.4 to 4.1) for MRT. For the SC, the bias and LoA was 13 ± 54 mL·min^{-1} (-93 to 119).

NIRS kinetics

The parameters for Δdeoxy[heme] kinetics are provided in Table 3. During moderate-intensity exercise, data from two subjects could not be used for further analyses. The SEE obtained from the models during moderate- and heavy-intensity exercise were not significantly different between the single- and ensemble-averaged transitions (p = 0.544 and p = 0.899, respectively). There were significant differences between moderate- and heavy-intensity exercise for Amp (p < 0.0001; *d* = -2.37), TD (p = 0.002; *d* = 1.28) and MRT (p = 0.012; *d* = 1.04). No significant difference was found for the τ (p = 0.126; *d* = 0.61).

Reliability was good to excellent for all parameters obtained from moderate- and heavy-intensity exercise transitions (supplementary Table 1). The TE and ICC for moderate intensity exercise were 6.6-13.7% and 0.81-0.99, respectively. For heavy intensity exercise, the TE and ICC were 3.5-22.2% and 0.92-0.98, respectively.

Bland-Altman plots of the parameters from Δdeoxy[heme] kinetics are presented in supplementary Figure 2. For moderate-intensity exercise, the bias and LoA between the two transitions were -0.3 ± 1.9 μM (-4.1 to 3.5) for Amp, 0.4 ± 1.2 s (-2.0 to 2.8) for TD, -0.7 ± 2.3 s (-5.2 to 3.8) for τ, and -0.3 ± 2.7 s (-5.6 to 5.1) for MRT. The bias and LoA of the Δdeoxy[heme] kinetics between heavy-intensity exercise transitions were 1.6 ± 2.2 μM (-2.6 to 5.9) for Amp, 0.2 ± 0.7 s (-1.2 to 1.7) for TD, 0.4 ± 1.5 s (-2.5 to 3.4) for τ, and 0.7 ± 1.5 s (-2.2 to 3.5) for MRT. For the SC, the bias and LoA was -0.3 ± 1.6 μM (-3.4 to 2.8).

Tissue oxygen saturation

The measures of StO_2 are provided in Table 4. During moderate-intensity exercise, data from two subjects could not be used for further analyses. The SEE obtained from the StO_2-slope during moderate- and heavy-intensity exercise were not significantly different between the single- and ensemble-averaged transitions (p = 0.218 and p = 0.443, respectively). There were significant differences between moderate- and heavy-intensity exercise for end-exercise StO_2 (p = 0.009; *d* = 1.14), ΔStO_2 (p = 0.0003; *d* = -1.70) and StO_2-slope (p = 0.0003; *d* = 1.72). No significant difference was found for the baseline (p = 0.219; *d* = -0.51).

Measures of reliability are provided in supplementary Table 2. For moderate intensity exercise, the TE of baseline and end-exercise StO_2 was 1.0 and 2.6%, respectively, whereas for ΔStO_2 and StO_2-slope the TE was 42.5 and 21.7%, respectively. The ICC ranged from 0.84-0.95. The TE and ICC for heavy intensity exercise was 1.1-5.5% and 0.89-0.99, respectively.

Bland-Altman plots of the measures of StO_2 are presented in supplementary Figure 3. For moderate-intensity exercise, the bias and LoA between the two transitions were -0.3 ± 1.0% (-2.2 to 1.5) for baseline, 0.2 ± 2.3% (-4.4 to 4.8) for end-exercise, -0.5 ± 1.6% (-3.7 to 2.7) for ΔStO_2, and 0.02 ± 0.05 $\%\cdot s^{-1}$ (-0.08 to 0.12) for StO_2-slope. The bias and LoA of StO_2 measures between heavy-intensity exercise transitions were 0.4 ± 1.1% (-1.7 to 2.6) for baseline, 0.4 ± 1.9% (-3.4 to 4.1) for end-exercise, 0.4 ± 0.9% (-1.3 to 2.1) for ΔStO_2, and 0.01 ± 0.03 $\%\cdot s^{-1}$ (-0.04 to 0.07) for StO_2-slope.

Discussion

The primary aim of this study was to establish the within-day test-retest reliability of pulmonary oxygen uptake and NIRS-derived muscle deoxygenation kinetics in youth elite-cyclists following the onset of moderate- and heavy-intensity step cycling. Our study has revealed that test-retest reliability was good to excellent for the parameters of $\dot{V}O_2$, Δdeoxy[heme]- and StO_2 kinetics at both intensities. Whilst the CI95 significantly improved for all $\dot{V}O_2$-kinetic estimates when transitions were ensemble-averaged, this was not the case for Δdeoxy[heme]-kinetics or the StO_2-slope.

Following the onset of step exercise, the kinetic adjustment of $\dot{V}O_2$ alongside Δdeoxy[heme] has been used in previous cross-sectional studies to investigate the effect of age (Breese et al. 2019; Leclair et al. 2013) and training status (Marwood et al. 2010; McNarry et al. 2011) on muscle O_2 delivery/utilization relationships. However, few previous studies have addressed the test-retest reliability of the parameter estimates (i.e. TD, τ and Amp) derived from the exponential increase, even though reliable estimates are prerequisite to interpret differences with confidence.

Depending on the subjects performance level and the chosen exercise intensity, it is recommended that 2-4 similar transitions are averaged to reduce the "noise" produced by breath-to-breath fluctuations and thereby improve the signal-to-noise ratio (Lamarra et al. 1987). Whilst this does not change the overall response, the confidence in the parameter estimates is increased. In fact, it has been reported that the CI95 for estimating the $\dot{V}O_2$-τ progressively decreased when derived from one, two or averaging more than three transitions (i.e. 6, 4 and 2s, respectively), whilst the value for the $\dot{V}O_2$-τ was not significantly affected during moderate-intensity (i.e. 80% VT) exercise (Spencer et al. 2011). The authors also reported strong correlations (r = 0.87-0.89) and relatively narrow LoA (8.3-4.7 s) with respect to estimates of the $\dot{V}O_2$-τ between individual transitions. Our study confirms these observations in a cohort of trained youth participants. By comparing two step-cycling transitions, we found no significant differences in the $\dot{V}O_2$ and deoxy[heme] parameter estimates during both moderate and heavy intensities of exercise. The test-retest correlations (ICC) ranged from r = 0.87-0.99 and are therefore considered as good to excellent (Shrout and Fleiss 1979). In the present study, the ICC of 0.88 and the LoA of ± 8 s for the $\dot{V}O_2$-τ during moderate-intensity exercise are very similar to the results of Spencer et al. (2011).

The mean differences (i.e. bias) between the two transitions was close to zero for all $\dot{V}O_2$ parameters (see results and supplementary Figure 1) thereby resulting in the non-significant

differences highlighted above. The individual within-subject variations between the transitions were expressed as the SD of the bias and provided as 95% LoA (SD x 1.96) in units of measurement, or as a percentage coefficient of variation (TE) derived by analysis of the log-transformed data (supplementary Table 1) (Hopkins 2015). During moderate intensity exercise, the TE ranged from 3.5-21.9%. Considering ~10% as a general threshold for assuming acceptable reliability (Hopkins 2000), the TE for the $\dot{V}O_2$-τ (i.e. 12.8%) is slightly above this threshold, whereas the TD (21.9%) indicates unacceptably large within-subject variation and is therefore not considered as reliable. In accord with these observations, any change of less than 8 s in the $\dot{V}O_2$-τ should be considered as unclear in youth as it cannot be distinguished between a real change and random variation. During heavy-intensity exercise, we found TEs between 3.9-12.1%, hence, indicative of less within-subject variation compared with moderate-intensity exercise. Indeed, the TE was < 5% for all parameters except for the TD (12.1%) and the LoA and TE for the $\dot{V}O_2$-τ was ± 3.5 s and 4%, respectively. It should be noted that the SC, calculated as the difference between end-exercise $\dot{V}O_2$ and the asymptote of the fundamental response, displayed a very large within-subject variation (LoA: ± 106 $mL \cdot min^{-1}$; TE: 68.6%), accompanied by a moderate ICC (r = 0.68). Therefore, our findings indicate that the SC is less reliable when calculated from a single transition of $\dot{V}O_2$ data such that averaging multiple transitions may be required to improve reliability of this parameter in youth.

The improved reliability of the primary $\dot{V}O_2$ kinetics observed during heavy-intensity exercise can be explained by the superior model-quality in this condition. Although model fitting of the $\dot{V}O_2$ data in response to moderate-intensity exercise yielded a smaller SEE than for heavy-intensity exercise (i.e. 80 vs. 102 $mL \cdot min^{-1}$), the $\dot{V}O_2$ amplitude was larger in the latter and thus reflected an improved $\dot{V}O_2$ signal-to-noise ratio. In fact, in our group of highly trained youth cyclists, the magnitude of the step forcing function in work rate yielded large "signals" (i.e. amplitudes) in the $\dot{V}O_2$ responses. Although the average amplitudes were ~700 $mL \cdot min^{-1}$ during moderate-intensity exercise, we excluded the data from two subjects with amplitudes < 300 $mL \cdot min^{-1}$, which resulted in poor model-quality and confidence in the parameter estimates. However, in line with a previous study (Spencer et al. 2011), we observed significant improvements in the CI95 for resolving the $\dot{V}O_2$-τ by ensemble-averaging multiple transitions, whilst the parameter estimates remained unchanged. In that study, the amplitude and the TD were constrained after a preliminary fit of the data, allowing the τ only to vary and thus further improve the $\dot{V}O_2$-τ CI95. Whilst this approach is useful in studies where any change or

difference in $\dot{V}O_2$-τ is the main focus of interest, in the present study, we aimed to provide reliability data for the model and consequently did not constrain any of the parameter estimates. Nevertheless, the $\dot{V}O_2$-τ CI95 obtained from modelling two individual moderate-intensity step transitions (i.e. 5.9 and 4.8 s) and ensemble-averaged data (i.e. 3.9 s) are comparable to those previously reported in adults (Spencer et al. 2011).

In the present study, neither the model quality nor confidence in estimating the parameters of Δdeoxy[heme] kinetics was affected by ensemble-averaging two cycling transitions. Compared with $\dot{V}O_2$-kinetics, we found higher model qualities for Δdeoxy[heme]-kinetics during both moderate- and heavy-intensity exercise, which is most likely attributable to the smaller level of noise observed in the raw data. This observation confirms the results of Grassi et al. (2003) where, in line with the present study, the $\dot{V}O_2$-τ CI95 was ~4 s whereas the Δdeoxy[heme]-τ CI95 was ~0.3 s. For our data, reliability was good to excellent (r = 0.81-0.99) for all Δdeoxy[heme] parameters with TE values of 6.6-13.7% and 3.5-10.4% during moderate- and heavy-intensity exercise, respectively. In contrast to $\dot{V}O_2$, we observed a smaller within-subject variation of the SC (LoA: ± 3.1 μM; TE: 22.2%) and an excellent ICC (r = 0.96). At both intensities, the largest TE was found for the τ, resulting in LoA of 4.5 s and 2.9 s for moderate- and heavy-intensity exercise, respectively. However, the LoA of 5.3 s for the MRT during moderate-intensity exercise was similar to that reported by Spencer et al. (2011).

In addition, we analyzed the amplitudes of StO_2 (i.e. baseline, end-exercise and ΔStO_2) in conjunction with muscle deoxygenation kinetics expressed as StO_2-slope. A previous study in patients with chronic heart failure (Niemeijer et al. 2017) reported reliability of numerous NIRS-derived variables. The authors found that reliability was highest (TE: 4.7-7.1%; ICC: 0.74-0.9) for baseline, minimum, maximum, and end-exercise StO_2. In contrast, the onset- and recovery-kinetics of StO_2 and deoxy[heme] were modelled and TEs ranged between 23.5-39.8% (ICC: 0.44-0.53) for the on-kinetics of StO_2, and 27.7-60% (ICC: 0.26-0.59) for deoxy[heme]. Another study in recreational team sports players reported % StO_2 during 30 min of continuous running at 70% of the velocity corresponding to $\dot{V}O_{2max}$ and StO_2-slopes during ten bouts of 30 s intermittent running at a velocity equivalent to $\dot{V}O_{2max}$ (Ihsan et al. 2013). It was found that StO_2-baseline and StO_2-mean throughout 30 min of continuous running provided good reliability (TE: 5.2% and 3.5%; ICC: 0.87 and 0.90, respectively), whereas moderate reliability was reported for ΔStO_2 (TE: 34.7%; ICC: 0.69). For the StO_2-slope during intermittent running, the TE and ICC was 7.2% and 0.94, respectively. In accordance with these

studies, we observed good to excellent reliability for baseline and end-exercise StO_2 during moderate-intensity exercise whereas ΔStO_2 and StO_2-slope showed large within-subject variation but excellent ICC (see supplementary Table 2). During heavy-intensity exercise, all measures of StO_2 provided good to excellent reliability. It should be noted, that an exponential model could not be applied to our StO_2 data due to unsatisfactory fits for most transitions (i.e. coefficient of determination, R^2 <0.85), whilst the study of Niemeijer et al. (2017) revealed a R^2 of 0.95 ± 0.03 for their patients with only one exclusion. However, despite the good fits of their data, reliability was moderate to poor (as shown above).

Comparing the estimates of deoxy[heme]-kinetics of the present study with those of Niemeijer et al. (2017), the superior reliability observed in our study is most likely related to the differences in work rate (120 ± 26 W vs. 62 ± 23 W) that increase the amplitude of muscle deoxygenation in response to exercise in the aerobically trained state. The adipose tissue thickness under the probe was on average similar (4.6 ± 0.8 mm vs. 4.3 ± 2.4 mm in Niemeijer et al. (2017)), albeit the dispersion was much smaller in our youth cyclists. In addition, heterogeneity of muscle tissue regarding fiber type, recruitment pattern and blood flow within the vastus lateralis affect deoxygenation (Koga et al. 2011) and consequently reliability. Whilst in the present study we assessed reliability within a single visit, in which the probe remained at the same location, other studies were conducted over separate days (Niemeijer et al. 2017; Spencer et al. 2011). However, observations from our laboratory revealed that repositioning of the probe after 3-7 days using pen marks on the skin, does not affect the estimated parameters of muscle deoxygenation kinetics during the on-transient. This observation agrees with the results of Spencer et al. (2011) who did not observe significant day-to-day changes.

Conclusions

The present study quantified the within-day test-retest reliability of pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate- and heavy-intensity step transitions in youth elite-cyclists. We observed superior model quality from fitting of $\dot{V}O_2$ and Δdeoxy[heme] responses during heavy step cycling, with ensemble averaging multiple exercise transitions further improving confidence in the primary phase $\dot{V}O_2$ kinetic parameter estimates. In addition, we reported good to excellent reliability (i.e. high ICC values) in the amplitude and kinetics of muscle oxygen saturation (i.e. StO_2) thereby supporting the utility of this NIRS variable in exploring muscle O_2 delivery/utilization relationships in youth athletes. Our results have implications for coaches and those providing sport science support, since, the established

reliability data provide limits within which training induced adaptations may be interpreted with confidence.

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Author contributions

AN, BCB and CAW conceived and designed the study and drafted the manuscript. AN, BP, MZ and CR performed the experiments and data analysis. All authors revised and approved the final version of the manuscript.

Disclosure statement

No conflicts of interest, financial or otherwise, are declared by the authors.

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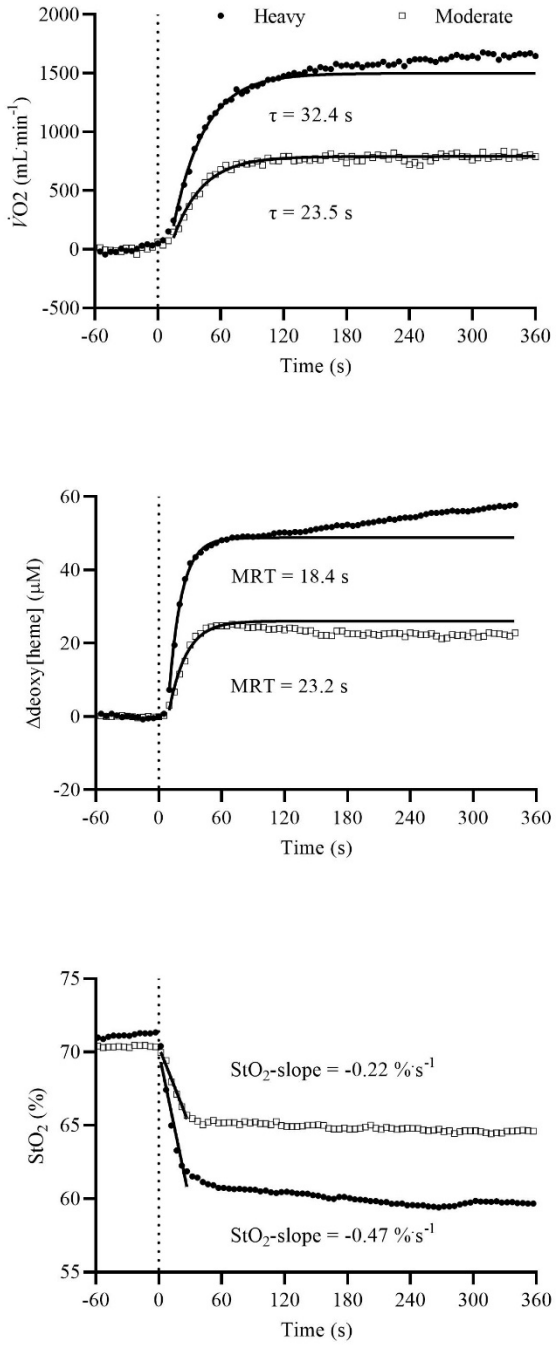
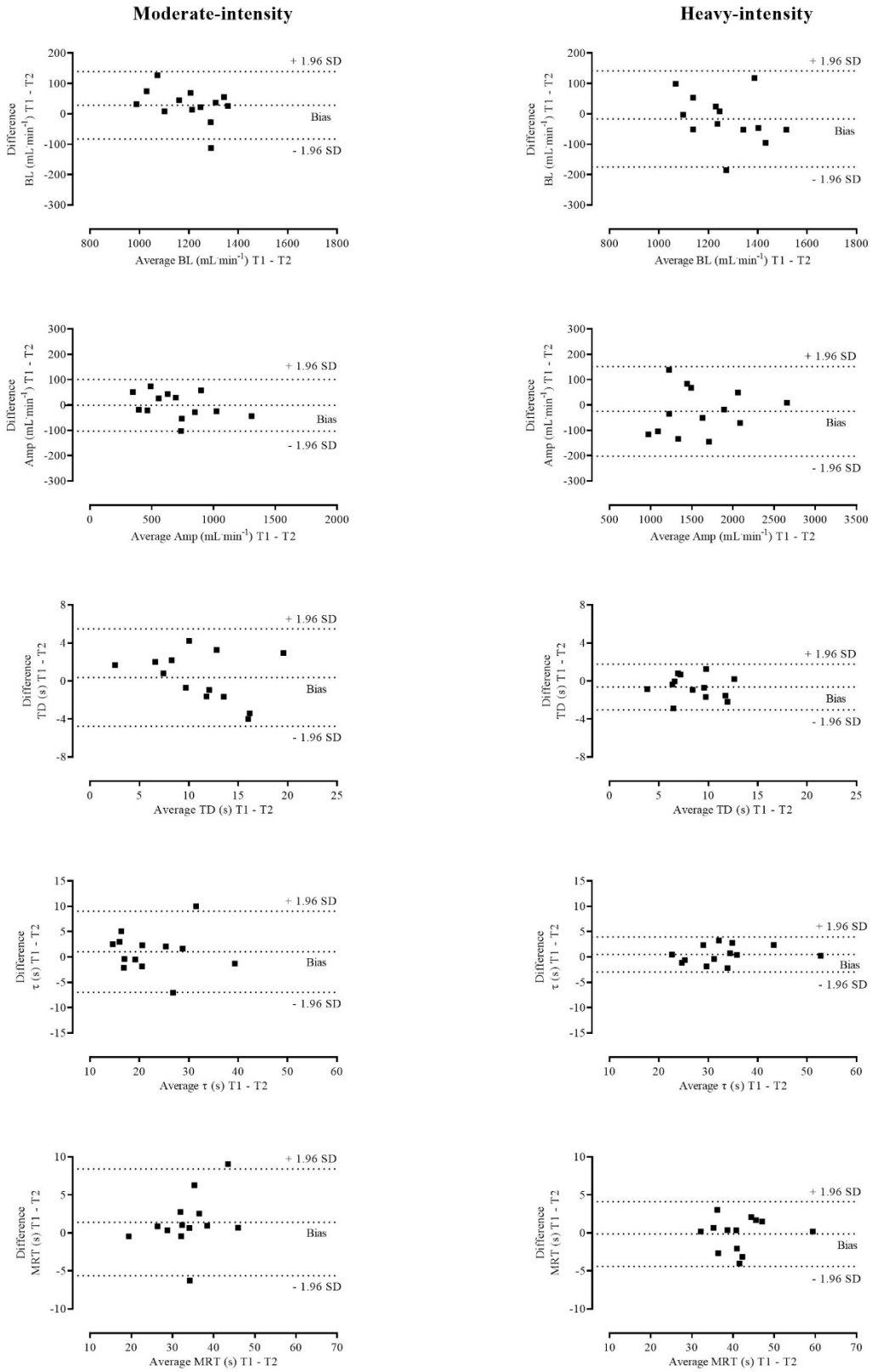
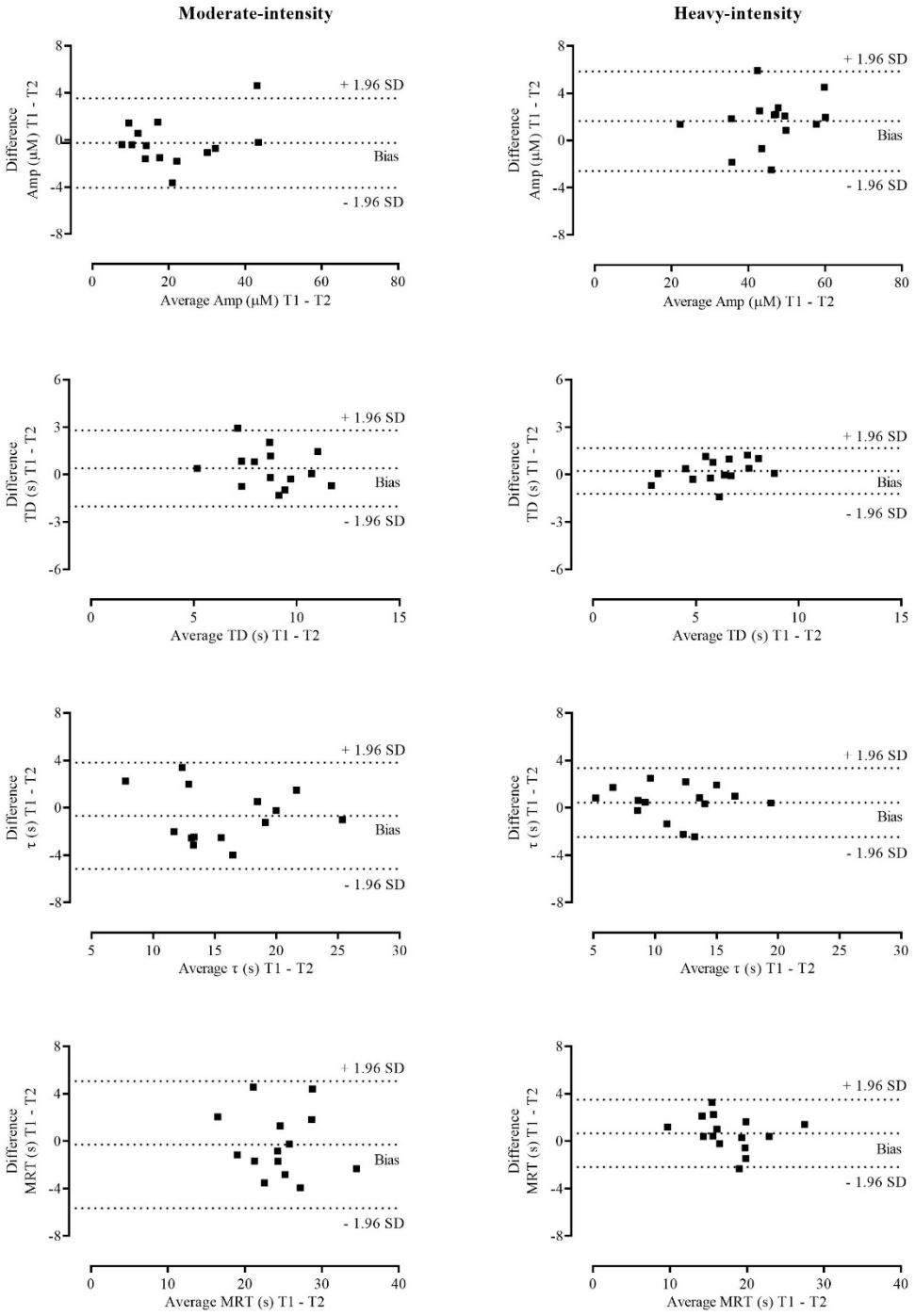


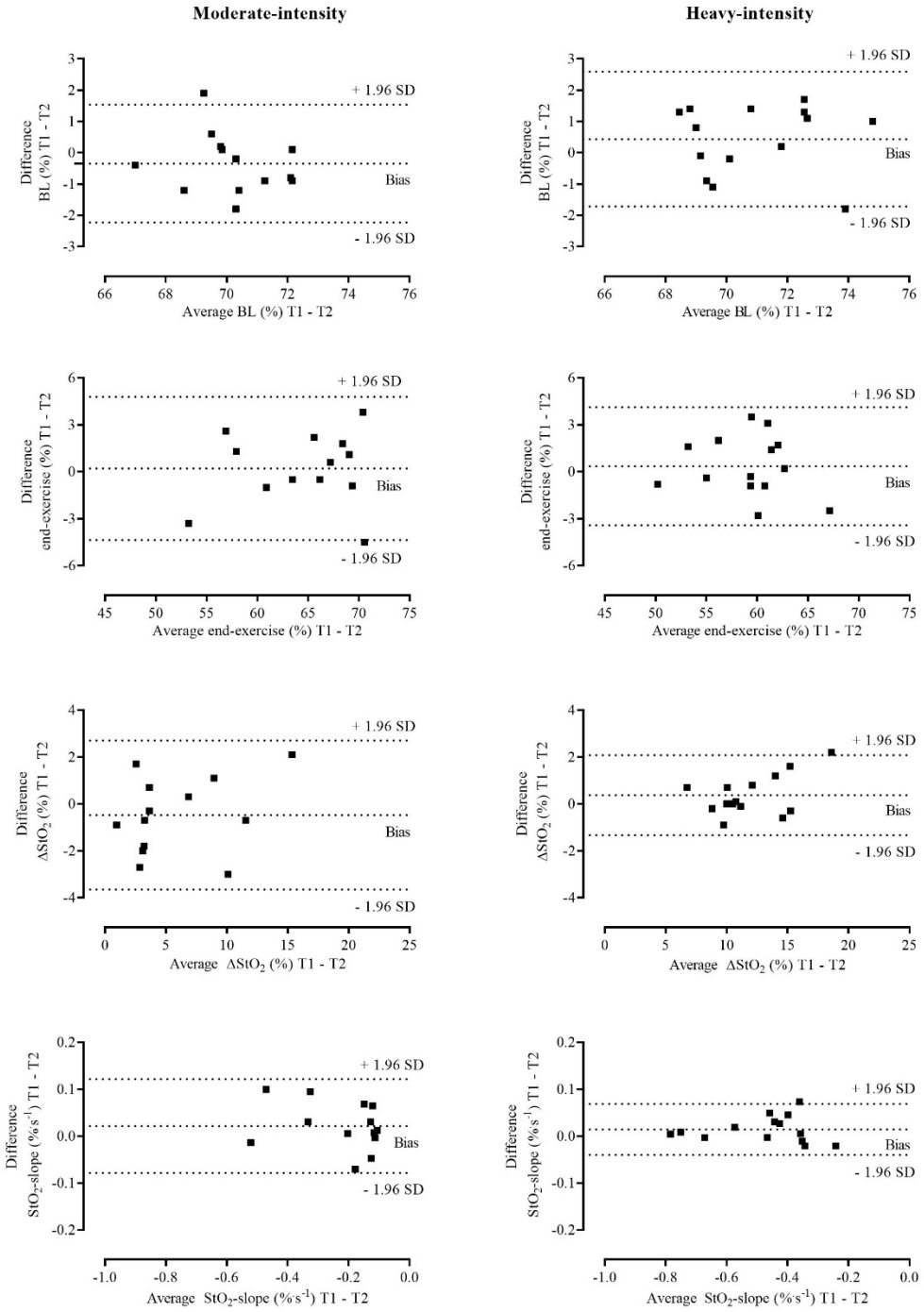
Figure 1: Group-mean, ensemble-averaged responses of $\dot{V}O_2$- (upper panel), Δdeoxy[heme] (middle panel) and StO_2 kinetics (lower panel) following the onset of moderate- and heavy-intensity step cycling. Error bars are omitted for clarity



Supplementary Figure 1: Bland-Altman plots of $\dot{V}O_2$-kinetic parameters between two identical step transitions (T1-T2) of moderate- (left panel) and heavy-intensity cycling (right panel)



Supplementary Figure 2: Bland-Altman plots of Δdeoxy[heme]-kinetic parameters between two identical step transitions (T1-T2) of moderate- (left panel) and heavy-intensity cycling (right panel)



Supplementary Figure 3: Bland-Altman plots of StO_2 measures between two identical step transitions (T1-T2) of moderate- (left panel) and heavy-intensity cycling (right panel)

Table 1: Physiological responses from the ramp incremental cycle test and target work rates used for the step transitions

Measure	**Mean ± SD**	**Range**
$\dot{V}O_{2peak}$ ($mL \cdot min^{-1}$)	3165 ± 726	2175-4743
$\dot{V}O_{2peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	62.1 ± 4.2	55.2-69.5
P_{peak} (W)	275 ± 59	182-385
P_{peak} ($W \cdot kg^{-1}$)	5.4 ± 0.5	4.6-6.2
HR_{max} ($b \cdot min^{-1}$)	195 ± 8	183-202
90%VT (W)	120 ± 26	92-188
90%VT ($W \cdot kg^{-1}$)	2.4 ± 0.3	2.0-3.1
Δ50% (W)	204 ± 43	137-279
Δ50% ($W \cdot kg^{-1}$)	4.0 ± 0.4	3.3-4.7

Δ50% = power at 50% between VT and P_{peak}; HR_{max} = maximal heart rate; P_{peak} = peak power output; $\dot{V}O_{2peak}$ = peak oxygen uptake VT = ventilatory threshold;

Table 2: Comparison of the model fitting quality and $\dot{V}O_2$ kinetic parameters between individual and averaging of two step transitions of moderate- and heavy-intensity cycling (Mean ± SD)

	Step transition			
Moderate-intensity	**1**	**2**	**Average 1-2**	**ANOVA**
SEE ($mL \cdot min^{-1}$)	104 ± 36	106 ± 32	80 ± 31**	< 0.001
BL ($mL \cdot min^{-1}$)	1214 ± 110	1186 ± 135	1212 ± 127	0.069
Amp ($mL \cdot min^{-1}$)	702 ± 262	703 ± 282	701 ± 255	0.950
CI95	16 ± 6	14 ± 4	11 ± 4**	< 0.001
TD (s)	11.4 ± 4.3	11.1 ± 5.2	10.4 ± 4.2	0.423
CI95	4.4 ± 1.8	3.6 ± 1.3	2.9 ± 1.5*	0.011
τ (s)	23.0 ± 7.7	22.0 ± 7.7	23.5 ± 6.8	0.348
CI95	5.9 ± 2.2	4.8 ± 1.7	3.9 ± 1.7**	0.002
MRT (s)	34.5 ± 7.7	33.1 ± 6.5	33.9 ± 6.3	0.305
Heavy-intensity				
SEE ($mL \cdot min^{-1}$)	139 ± 52	140 ± 42	102 ± 38***	< 0.001
BL ($mL \cdot min^{-1}$)	1262 ± 131	1278 ± 157	1285 ± 135	0.328
Amp ($mL \cdot min^{-1}$)	1588 ± 483	1613 ± 470	1615 ± 484	0.328
CI95	45 ± 18	45 ± 21	32 ± 14**	0.003
TD (s)	8.2 ± 2.6	8.9 ± 2.7	7.5 ± 2.9	0.049
CI95	3.3 ± 0.7	3.4 ± 1.3	2.4 ± 0.7**	0.003
τ (s)	33.3 ± 8.3	32.8 ± 7.9	32.4 ± 8.3	0.546
CI95	5.4 ± 1.1	5.4 ± 2.3	3.8 ± 1.2**	0.005
MRT (s)	41.5 ± 7.0	41.7 ± 6.9	39.9 ± 6.9	0.110
SC ($mL \cdot min^{-1}$)	130 ± 61	117 ± 67	127 ± 62	0.395

Amp = amplitude; BL = baseline; CI95 = 95% confidence interval; MRT = mean response time; SC = slow component; SEE = standard error of estimate; TD = time delay; τ = time constant; significantly different from transition 1 and 2 at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

Table 3: Comparison of the model fitting quality and Δdeoxy[heme] kinetic parameters between individual and averaging of two step transitions of moderate- and heavy-intensity cycling (Mean ± SD)

	Step transition			
Moderate-intensity	**1**	**2**	**Average 1-2**	**ANOVA**
SEE (μM)	1.2 ± 0.5	1.3 ± 0.8	1.3 ± 0.4	0.544
Amp (μM)	20.9 ± 12.2	21.2 ± 11.7	20.6 ± 12.2	0.517
CI95	1.2 ± 0.9	1.7 ± 1.3	1.2 ± 0.7	0.194
TD (s)	8.9 ± 1.7	8.6 ± 1.9	8.2 ± 1.5	0.151
CI95	0.9 ± 0.3	1.4 ± 0.7	1.2 ± 0.3	0.048
τ (s)	15.4 ± 4.7	16.1 ± 4.9	15.0 ± 4.7	0.154
CI95	2.3 ± 0.9	2.4 ± 1.0	2.4 ± 0.8	0.783
MRT (s)	24.4 ± 4.5	24.7 ± 4.9	23.2 ± 4.5	0.104
Heavy-intensity				
SEE (μM)	1.4 ± 0.5	1.4 ± 0.6	1.3 ± 0.4	0.899
Amp (μM)	46.6 ± 10.2	45.0 ± 9.7	45.6 ± 9.6	0.101
CI95	0.9 ± 0.5	0.9 ± 0.5	1.0 ± 0.9	0.837
TD (s)	6.1 ± 1.9	5.9 ± 1.6	6.1 ± 1.8	0.531
CI95	0.5 ± 0.2	0.6 ± 0.3	0.7 ± 0.3	0.049
τ (s)	11.9 ± 3.8	11.5 ± 3.9	12.3 ± 4.5	0.101
CI95	1.0 ± 0.5	1.1 ± 0.5	1.0 ± 0.4	0.829
MRT (s)	18.0 ± 4.1	17.4 ± 4.4	18.4 ± 5.0	0.057
SC (μM)	9.3 ± 5.4	9.6 ± 5.5	10.2 ± 5.6	0.079

Amp = amplitude; BL = baseline; CI95 = 95% confidence interval; MRT = mean response time; SC = slow component; SEE = standard error of estimate; TD = time delay; τ = time constant;

Table 4: Comparison of tissue oxygen saturation (StO_2) responses between individual and averaging of two step transitions of moderate- and heavy-intensity cycling (Mean ± SD)

	Step transition			
Moderate-intensity	**1**	**2**	**Average 1-2**	**ANOVA**
BL (%)	70.0 ± 1.5	70.4 ± 1.7	70.2 ± 1.5	0.218
End-exercise (%)	64.7 ± 5.9	64.5 ± 5.6	64.6 ± 5.7	0.754
ΔStO_2 (%)	5.6 ± 4.6	6.1 ± 4.2	5.8 ± 4.3	0.310
StO_2-slope ($\%\cdot s^{-1}$)	-0.21 ± 0.14	-0.23 ± 0.15	-0.22 ± 0.14	0.158
CI95	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.201
SEE (%)	0.51 ± 0.39	0.39 ± 0.15	0.36 ± 0.21	0.218
Heavy-intensity				
BL (%)	71.2 ± 2.1	70.7 ± 2.1	71.1 ± 2.1	0.162
End-exercise (%)	59.3 ± 4.3	59.0 ± 4.5	59.1 ± 4.3	0.508
ΔStO_2 (%)	12.2 ± 3.4	11.8 ± 3.0	12.0 ± 3.2	0.134
StO_2-slope ($\%\cdot s^{-1}$)	-0.47 ± 0.16	-0.48 ± 0.16	-0.47 ± 0.16	0.072
CI95	0.05 ± 0.03	0.05 ± 0.03	0.04 ± 0.03	0.361
SEE (%)	0.64 ± 0.41	0.65 ± 0.38	0.58 ± 0.37	0.443

BL = baseline; CI95 = 95% confidence interval; ΔStO_2 = difference between BL and end-exercise; SEE = standard error of estimate; StO_2-slope = linear regression analysis slope

Supplementary Table 1: Reliability measures of $\dot{V}O_2$ and Δdeoxy[heme]-kinetics between two identical step transitions of moderate- and heavy-intensity cycling

Moderate-intensity	$\dot{V}O_2$		Δdeoxy[heme]	
	TE (%)	**ICC**	**TE (%)**	**ICC**
BL	3.5	0.91	-	-
CI90	2.6-5.3	0.79-0.97		
Amp	6.2	0.99	6.6	0.99
CI90	4.7-9.6	0.96-0.99	5.0-9.9	0.97-1.00
TD	21.9	0.87	11.3	0.81
CI90	16.1-34.9	0.69-0.95	8.5-17.3	0.57-0.92
τ	12.8	0.88	13.7	0.90
CI90	9.5-20.0	0.71-0.95	10.2-20.9	0.77-0.96
MRT	7.0	0.89	8.4	0.86
CI90	5.2-10.8	0.74-0.96	6.4-12.8	0.67-0.94
Heavy-intensity				
BL	4.6	0.87	-	-
CI90	3.5-7.1	0.68-0.95		
Amp	5.0	0.99	3.5	0.98
CI90	3.8-7.7	0.96-0.99	2.7-5.2	0.95-0.99
TD	12.1	0.91	9.8	0.92
CI90	9.0-18.9	0.78-0.97	7.5-14.7	0.82-0.97
τ	4.0	0.98	10.4	0.94
CI90	3.0-6.1	0.95-0.99	7.9-15.5	0.85-0.97
MRT	3.9	0.96	6.5	0.95
CI90	2.9-5.9	0.90-0.99	4.9-9.6	0.88-0.98
SC	68.6	0.68	22.2	0.96
CI90	48.4-120.7	0.33-0.87	16.7-34.0	0.91-0.99

Amp = amplitude; BL = baseline; ICC = intraclass correlation coefficient; MRT = mean response time; SC = slow component; TD = time delay; TE = typical error; τ = time constant;

Supplementary Table 2: Reliability measures of tissue oxygen saturation (StO_2) between two identical step transitions of moderate- and heavy-intensity cycling

		StO_2
Moderate-intensity	**TE (%)**	**ICC**
BL	1.0	0.84
CI90	0.7-1.5	0.62-0.94
End-exercise	2.6	0.93
CI90	2.0-4.0	0.83-0.97
ΔStO_2	42.5	0.94
CI90	30.7-71	0.86-0.98
StO_2-slope	21.7	0.95
CI90	16.0-34.6	0.87-0.98
Heavy-intensity		
BL	1.1	0.89
CI90	0.8-1.6	0.73-0.95
End-exercise	2.3	0.92
CI90	1.7-3.4	0.80-0.97
ΔStO_2	4.7	0.97
CI90	3.5-7.0	0.92-0.99
StO_2-slope	5.5	0.99
CI90	4.2-8.3	0.97-1.00

BL = baseline; ICC = intraclass correlation coefficient; ΔStO_2 = difference between BL and end-exercise; StO_2-slope = linear regression analysis slope; TE = typical error