Influence of Increment Magnitude and Exercise Intensity on VO₂ Kinetics, Time to Steady State, and Muscle Oxygenation

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ABSTRACT

McNulty CR, Robergs RA, Morris D. Influence of Increment Magnitude and Exercise Intensity on VO₂ Kinetics, Time to Steady State, and Muscle Oxygenation. JEPonline 2015;18(5):37-58. The purpose of this study was to quantify the oxygen uptake (VO₂) kinetics to steady state across the full range of sub-ventilatory threshold (VT) work rates. Twelve trained males participated in two separate series of five bouts of cycling. One trial (DM) involved 10 min at a percentage of their VT. The second trial involved five bouts at an increasing baseline intensity for 5 min (SM1), followed by an increase of 30% of VT for 10 min (SM2). The VO₂ kinetics was quantified by the mono-exponential time constant (τ) as well as a new method for time to steady state (TTSS). For DM, τ increased significantly from 30% and 45%VT (31 ± 22 and 33 ± 15 sec, respectively) between 60% to 90%VT (42 ± 16, 53 ± 29, 74 ± 25 sec for 60%, 75%, and 90%VT, respectively). For SM1, τ increased significantly from 40% (41 ± 16 sec) to 60%VT (74 ± 25 sec). For SM2, τ increased significantly between 60% (44 ± 11 sec) to 80%, and 90%VT (92 ± 41 and 151 ± 83 sec, respectively), and from 70% (54 ± 38 sec) to 90%VT (151 ± 83 sec). The data revealed a clear increase in τ as intensity increased, revealing a more complex VO₂ response than previously documented.

Key Words: VO₂ kinetics, Near-infrared spectroscopy, Steady state VO₂, Mono-exponential
INTRODUCTION

The kinetic response of VO\textsubscript{2} to increments to steady state has been proposed to consist of three phases (20,32,33). Phase I involves a rapid increase in VO\textsubscript{2}, lasting 15 to 30 sec, which has been explained to be a result of an increase in ventilation at the onset of work. As this near immediate hyperventilation is not concomitant to a similar increase in muscle VO\textsubscript{2}, the phase I VO\textsubscript{2} is believed to be caused by an increased lung oxygen store secondary to an increase in alveolar ventilation (33). The phase I VO\textsubscript{2} response is eventually exceeded by a delayed kinetic function (phase II) that has been argued to be more directly linked to the internal respiration of the working muscles (32). Phase III is the steady state VO\textsubscript{2} phase of the exercise increment (33).

The mono-exponential time constant (tau, $\tau$) is routinely used as the standard measure of VO\textsubscript{2} kinetics during low to moderate intensity exercise increments to steady state (34). Historically, $\tau$ was initially believed to be invariant across the low to moderate intensity exercise increment range to steady state, and was claimed to therefore adhere to first order linear kinetics (6,13,31). However, early research existed to oppose this interpretation (11-14,17,21), and such work has been thoroughly reviewed by Robergs (30). More recent inquiry has confirmed this work, revealing a slowing of VO\textsubscript{2} kinetics with an increased baseline intensity to steady state VO\textsubscript{2} at the higher end of the steady state range (8,20,22).

As phase II is primarily a function of oxidative metabolism within the working muscle, it is necessary to examine the kinetics of muscle oxygenation and/or deoxygenation to get a more complete picture of VO\textsubscript{2} kinetics based on pulmonary gas exchange. Near-infrared spectroscopy (NIRS) uses electromagnetic waves to observe tissue (in this case, skeletal muscle) oxygenation using absorptiometry (15,26). Previous research using NIRS in lower limb exercise assessment has demonstrated a similar mono-exponential response in the deoxyhemoglobin (HHb) NIRS signal to that of pulmonary VO\textsubscript{2} (23). Furthermore, Murias et al. (23) and MacPhee et al. (22) have shown a positive association between the magnitude of change in the relative HHb signal and $\tau$. As such, there is a relationship between slower VO\textsubscript{2} kinetics and the inability to increase or sustain muscle oxygenation. This has been interpreted as evidence for the importance of blood flow and oxygen delivery to contracting muscle to the pulmonary VO\textsubscript{2} kinetic response to exercise transitions within the steady state (<VT intensity) range (22,23).

Due to the continued use of VO\textsubscript{2} kinetics research across a broad range of the physiological sciences, it is of physiological importance that the conflicting reports of linear versus non-linear VO\textsubscript{2} kinetics across the low to moderate intensity steady state range be thoroughly assessed in one study. In addition, given the noted increase in $\tau$ during increments in intensity at the higher end of the steady state range, the added exercise conditions within this range require further investigation. Thus, the purpose of this research was to:

- Determine whether there is a different VO\textsubscript{2} kinetic response for exercise increments to steady state for different incremental magnitudes, and the same magnitude from different baseline intensities;
Quantify time to steady state using a new method involving back extrapolation of steady state and application of a second order polynomial function to the initial non-linear VO₂ response; and

Determine whether the kinetics of muscle deoxygenation change in proportion to τ and time to steady state.

METHODS

Subjects

Twelve male subjects (mean age = 26.8 ± 9 yrs; height = 179.6 ± 6.5 cm; mass = 78.8 ± 9 kg) were recruited from a regional university and local gymnasiums. All subjects were in good physical health with no musculoskeletal disorders. Each subject was recruited on the basis of self-reported physical fitness of at least 30 min of moderate to vigorous exercise at least 3 times wk⁻¹). Written informed consent was obtained from each subject prior to data collection and all methods were approved by the institution’s Human Research Ethics Committee.

Procedures

All subjects underwent a familiarization session and a VO₂ maximum ramp protocol cycle ergometer test before any trial sessions were conducted. During the familiarization session each subject’s height and weight were recorded, and the cycle ergometer was adjusted for each subject’s preference and measures were recorded for future trials. Before conducting the VO₂ ramp test, the subjects were fitted with a multiple one-way 'T' valve mouthpiece system supported by an acrylic head unit (29). The mouthpiece and head support unit were securely fastened to the head of each subject as to not interfere with movement during cycling.

Electrocardiography (ECG) was also performed to acquire heart rate using a 5-lead ECG configuration (CASE, GE Healthcare, Waukesha, WI). The ECG leads were attached using gel electrodes placed over the spine of both scapulae, the iliac crest of both ilia, and between the 4th and 5th intercostal space along the mid-axillary line of the left side of the torso.

For the VO₂ ramp test, each subject was instructed to cycle at his comfortable cycling cadence, maintain cadence during the ramp test, and the proceeding trials. The ramp function for each subject was based on his prior exercise training, the need to constrain test duration to <10 min and, consequently, it varied between subjects from 25 to 35 W·min⁻¹. The ramp protocol consisted of a 2-min rest period followed by 2 min at double the ramp function Watts for that subject, then, by the near continuous ramp function (increment at 0.5 Hz). The subject was also instructed to continue cycling until absolute exhaustion (2). The test ended when the subject could no longer maintain a pedaling cadence >40 rev·min⁻¹ (2).

For indirect calorimetry, expired gas analysis was acquired using a 3 L latex compliant and elastic mixing bag placed on the expired port of the mouthpiece. Mixed expired air was sampled continuously and pumped to rapid response oxygen and carbon dioxide gas analyzers (AEI Technologies, Pittsburgh, PA). During and following each breath, the elastic recoil of the mixing bag caused air to be vented through a 1 cm diameter hole in the inferior end of the mixing bag. Expired gas
signals were acquired for 100 ms at the start of each breath and aligned to the timing of end expiration based on a pre-determined measured time-delay. Ventilation was measured by a flow turbine (UVM, VacuMed, Ventura, CA) connected to the inspired side of the mouthpiece. All data were acquired using custom developed software (LabVIEW, National Instruments, Austin, TX) and commercial electronic acquisition devices (National Instruments, Austin, TX). The breath-by-breath system was calibrated before the ramp test and before each bout in both trials using a 3 L syringe and commercial medical grade calibration gas (16% O₂ and 5% CO₂). The methods used in this study have been validated and described in more detail elsewhere (18).

Using the breath-by-breath VO₂ data collected from the ramp test, the ventilatory threshold (VT) of each subject was determined visually by the excess carbon dioxide method (16) using a custom designed computer program (LabVIEW, National Instruments, Austin, TX). The VT was used to determine the power output required for the five cycling bouts in each trial.

Exercise Trials
Research on phase II VO₂ kinetics has only used a minimal number of magnitude increases, and no multiple bouts from a baseline to increased intensity (5,31-33). Therefore, two multiple-bout cycling trials were developed. The different-magnitude trial (DM) involved five separate bouts of unloaded cycling to different increments, and the same-magnitude trials (SM) involved another five separate bouts of cycling, but from rest to five different baseline intensities (SM1) followed by a constant relative (30 %VT) magnitude increment (SM2).

Following a 5-min warm-up at 50 Watts on the cycle ergometer, each subject was again fitted for indirect calorimetry, ECG, and NIRS (Niro-200NX, Hamamatsu Photonics, Hamamatsu City, Japan). The NIRS used two channels of recording, with channel 1 and channel 2 attached to the belly of the vastus lateralis and vastus medialis of the right quadriceps, respectively (22,23).

The DM trial involved five cycling bouts of differing magnitudes, with 15 min of seated rest between each bout to limit trial-to-trial variability due to post-exercise VO₂ (7). The magnitudes for DM in order of intensity were: 30%, 45%, 60%, 75%, and 90% of VT. This trial required the subject cycle at an unloaded 0 Watts magnitude for 2 min before completing 10 min at the increased magnitude. The SM trial had the subject cycle for 5 min at a baseline percentage of their VT followed by 10 min of cycling at a 30% of VT higher intensity. The magnitudes for SM in order of intensity were: 20-50%, 30-60%, 40-70%, 50-80%, and 60-90%VT.

Thus, the SM trials consisted of two increment conditions per trial. SM1 involved an increment from rest to the baseline intensity (20%, 30%, 40%, 50%, and 60%VT), and SM2 involved an increment from baseline to the 30%VT higher intensity (50%, 60%, 70%, 80%, and 90%VT). The subjects were instructed to maintain the same comfortable cycling cadence for each bout in both trials despite the electronic ergometer adjusting resistance with changed cadence to ensure a stable power output. The order of administration and between subject sequence of DM and SM
were determined by a Latin Squares design (19). A minimum time-frame of 48 hrs separated the completion of the VO₂ ramp test and each subsequent trial.

**Data Reduction and Analysis**

The raw breath-by-breath data that included absolute and relative VO₂, respiratory exchange ratio (RER), and the ventilatory equivalent ratios for oxygen (O₂) and carbon dioxide (Ve/VO₂ and VE/VCO₂, respectively) were processed using a 7-breath average from custom designed software (LabVIEW, National Instruments, Austin, TX). The data were then imported into a commercial graphics and curve fitting program for subsequent non-linear modelling (GraphPad, Prism, San Diego, CA), which were then further processed using the mono-exponential equation: 

\[ \text{VO}₂(t) = \text{VO}₂(1-e^{-t/\tau}) \]  

(31). The physiological time delay, phase-I (3), was not included in the derivation of \( \tau \) for each bout (33), and was eliminated from the data by visual analysis.

Time to steady state (TTSS) was quantified using custom software (LabVIEW, National Instruments, Austin, TX). Breath averaged data for each exercise increment transition were first fit with linear regression over the last 5 min of data for each 10-min phase (DM). For SM1, the steady state phase was the final 2 min of the 5-min exercise phase. A 2nd order polynomial function was then applied iteratively to the initial nonlinear phase of the VO₂ response. The program allowed a user controlled continuous data point increment for this data phase and the intersection of the nonlinear function and the linear regression of steady state was detected (time with the lowest residual for VO₂ nonlinear – VO₂ linear) as the TTSS.

The collected NIRS data were first screened and processed using custom developed software (LabVIEW, National Instruments, Austin, TX). This was necessary to remove irrelevant data file content, to screen data for quality, to rearrange data to ease subsequent data processing, and to include marker times used to identify times at which specific cycling bouts commenced. The marker times ensured accuracy when coordinating NIRS data to data from indirect calorimetry. The processed data was then imported into a commercial graphics and curve fitting program for subsequent non-linear modelling (GraphPad, Prism, San Diego, USA). When possible, the deoxygenated hemoglobin (HHb) data were processed using the mono-exponential equation: 

\[ \text{HHb}_2(t) = \text{HHb}(1-e^{-t/\tau}) \]  

(31). Where such mono-exponential models did not suit the NIRS responses, such deviance was noted and mono-exponential data were not acquired for these subject trials.

**Statistical Analyses**

There were numerous independent and dependent variables involved in this study. The subjects completed two trials (DM and SM) involving a combined total of 15 (13 that were different) levels of cycle ergometer exercise tests from a baseline to steady state value. The two trials actually consisted of three conditions of exercise transitions: (a) DM for the different increment magnitudes from unloaded cycling; (b) SM1 involved in the increase in VO₂ from rest to different baseline intensities; and (c) SM2, which involved an increase from the SM1 intensity to the higher SM2 intensity. These responses were analyzed using a two-way repeated measures ANOVA (TRIAL [3] vs. INTENSITY [5]) for the dependent variables steady state VO₂, phase II \( \tau \), VO₂ increment magnitude, time delay and TTSS. If a significant
interaction effect occurred, the main ANOVA was followed by simple effects analysis, followed again if significant by specific mean contrasts. The relationship between $\tau$ and time to steady state was quantified by simple linear correlation. All data are presented as mean ± SD.

RESULTS

The subjects were healthy, active males of varied training status, physical fitness, and age. Results from the statistical analyses for the main variables of this study are presented structured by the type of variable.

VO$_2$ Kinetics

An example of the 7-breath averaged VO$_2$ data and mono-exponential curve fitting from the DM trial for a representative subject is presented in Figure 1. The 7-breath averaged VO$_2$ data and curve fitting for another representative subjects for SM1 and SM2 trials are presented in Figure 2.

![Figure 1. Representative Data from One Subject for the DM Trial.](image)

These data reveal the inherent variability of the VO$_2$ response to exercise transitions to steady state, the variability at steady state, and the need for sufficient durations of exercise testing to establish a clear steady state condition. It is also important to note the errors in curve fitting at the initial shoulder of the non-linear response in most data sets of Figures 1 and 2. We expand on this finding in the Discussion as it is important to recognize this error from the mono-exponential curve fitting prior to the presentation of the data for TTSS.
Figure 2. Representative Data from One Subject for Trials SM1 and SM2.

Inconsistency in Phase I to II Kinetics
The data revealed considerable between subjects and within subjects inconsistency in the presence of a detectable phase I VO₂ response and accompanied time delay. Such inconsistencies are demonstrated in Figures 3a-f for two different subjects for specific intensities of the DM and SM1 and SM2 trials.

For example, Figures 3a-c is for one subject revealing the presence and absence of a detectable phase I for three different trials, and Figures 3d-f present data for another subject for three different trials. For the mid-range lower intensity increments, phase I was more readily observable, but for the higher intensity increments it became more difficult to detect phase I within the rapidly increasing VO₂ data.

It was also difficult to apply phase I curve fitting to very low intensity increments. Although phase I kinetics were readily apparent, the magnitude and the duration of the phase I response encompassed the total VO₂ response to steady state, which prevented curve fitting to the remaining data. When this occurred, we were forced not to remove the phase I response.
Figures 3a-f. Representative Data Sets from Two Subjects for the Presence and Absence of a Phase I Response.

**Time Constant**
For the data from Figure 4, there was a significant main effect for trial (P<0.001) and intensity (P<0.001) and a significant trial x intensity interaction (P=0.001). For DM, $\tau$ was significantly increased between 60% to 90%VT. For SM1, $\tau$ was significantly increased between 40% to 60%VT. For SM2, $\tau$ was significantly increased between 60% to 80%VT and between 70% to 90%VT. Clearly, $\tau$ was only invariant for the low exercise transitions in each of the DM and SM conditions. For small increments to the higher end of steady state, and for the larger increments from unloaded cycling to the higher end of steady state, $\tau$ displayed considerable non-linear, complex kinetics.
Figures 4a-b. Mean ± SD Data for Tau (τ) for (a) the DM Trial and (b) the SM Trials.

The unusually long values for τ in the SM2 trial for 80% and 90% VT were not due to a slow component and failure to meet steady state conditions. Note that this research is the first to thoroughly research the VO₂ kinetics of exercise increments at the high end of steady state. The latter data points for Figure 4b SM2 trial were for 30% VT increments commencing at 50% and 60% VT, respectively. Such narrow increment, but moderately high baseline intensities have not been included in prior research and as such this study is the first to report this response. As an example, Figure 5 presents select examples from individual subjects of an extremely slow VO₂ kinetic response to these steady state conditions. The slow kinetics differed tremendously to the previously established profile of VO₂ kinetics to steady state based on lower baseline intensity and larger increment magnitudes.
Figure 5. Representative Data for Select Subjects from the SM2 Trial that Demonstrated the Unusually Slow VO\textsubscript{2} Kinetic Response to Small Magnitude Increments at the Higher End of the Steady State Range.

Delta VO\textsubscript{2}

For the data in Figures 6a-b, there was a significant main effect for trial (P = 0.001) and intensity (P<0.001) and a significant trial x intensity interaction (P<0.001). For DM, the delta VO\textsubscript{2} for all exercise conditions were significantly different (P<0.05) from each other. There were no differences in delta VO\textsubscript{2} between SM1 and SM2.
The significant interaction effect was caused by the smaller increment in delta VO$_2$ for SM2 versus SM1. This was caused by the exercise condition of SM1 involving cycling from a resting baseline; whereas, SM2 involved an increment in cycling from a lower to higher relative intensity. Such exercise condition differences between the two SM trials were intentional to document the difference in VO$_2$ kinetics when starting from rest versus prior exercise and for the different absolute increment magnitudes.

As shown in Figure 6b, the SM2 conditions extended the relative intensity range of the exercise with the increase in steady state VO$_2$ following a similar linear increase. In fact, all three trials have data that when superimposed on each other reveal near identical steady state VO$_2$ response trends. These results are to be expected given the tight causal relationship between exercise intensity and steady state VO$_2$.

**Steady State VO$_2$**

For the data from Figures 7a-b, there was a significant main effect for trial (P<0.001) and intensity (P<0.001) and a significant trial x intensity interaction (P<0.001). For DM, the steady state VO$_2$ for all exercise conditions were significantly different.
(P<0.05) from each other. The significant interaction effect was caused by the different absolute and relative intensities from the SM1 versus SM2 trials.

Figures 7a-b. Mean ± SD Data for Steady State VO$_2$ for (a) the DM Trial and (b) the SM Trials.

**Time to Steady State VO$_2$**

To demonstrate the process for quantifying TTSS VO$_2$, a representative condition from the DM trial for a subject is presented in Figure 8. Each of the mono-exponential, the 2nd order polynomial, and the final linear regression of the steady state region are presented. Note the inability of the mono-exponential fit to model the rapid initial kinetic response of VO$_2$ and the superior fit of the combined polynomial and linear regression segments.

Figure 8. Sample Data from a Test to Steady State VO$_2$ from DM for a Representative Subject.
As to the data in Figures 9a-b, there was a significant main effect for trial (P<0.001) and intensity (P<0.001) and a significant trial x intensity interaction (P<0.001). For DM, TTSS VO₂ increased significantly (P<0.05) after 30%VT. For SM1, TTSS VO₂ remained invariant from 20% to 60%VT. For SM2, time to steady state VO₂ increased significantly (P<0.05) after 70%VT. The significant interaction effect was caused by the different intensity responses between SM1 and SM2. Thus, for light intensity exercise, TTSS VO₂ occurs in less than 2 min. For higher intensity exercise, time to steady state VO₂ increases with the magnitude of the increment as well as the magnitude of the baseline intensity of the exercise transition.

The relationships between τ and TTSS for each of DM, SM1 and SM2 are presented in Figures 10a-c, respectively. This relationship was linear for DM and SM1, but mono-exponential for SM2, which revealed a methodological limitation for either τ or TTSS for exercise transitions that are preceded by increasing baseline intensities.
Figures 10a-c. Use of Individual Data to Illustrate Relationships Between $\tau$ and TTSS for (a) DM, (b) SM1, and (c) SM2 Trial Conditions.
**HHb Kinetics**

We could not perform group statistical analyses on the data for muscle deoxyhemoglobin signals due to considerable between-subjects and within-subjects variability in the complexity of the signals from the NIRS unit. For example, Figures 11a-d presents examples of the varied complexity of the HHB signals.

**Figures 11a-d. Example Data Sets for the Complexity of the NIRS Signals**

**DISCUSSION**

Despite the long history of prior research in steady state VO$_2$ kinetics, this is the first study to test subjects using multiple (>2) exercise transition conditions spanning the near complete range of steady state exercise (20% to 90%VT). Furthermore, this is the first to incorporate
such testing similarly for both single increments of varied magnitude and multiple increment conditions that vary in the baseline exercise intensity but increase by the same relative intensity (30% VT). This is also the first study to quantify time to steady state \( \text{VO}_2 \) and, therefore, effectively challenge the conventional practice of relying on the mono-exponential time constant (\( \tau \)) as a means to interpret the kinetics of the transition to steady state.

This study confirmed results from previous research (8,10-14,17,20-22) that steady state \( \text{VO}_2 \) kinetics slows with increasing steady state exercise intensities and, as well, for a constant increment magnitude that starts from a progressively increased baseline intensity. Consequently, our data further confirm that exercise transitions to steady state \( \text{VO}_2 \) do not obey linear first order kinetics as was assumed by earlier research (1,4,6,9,10,13,25,31). Our research incorporated 15 (13 that were different) steady state exercise conditions, which allowed for the first assessment of steady state \( \text{VO}_2 \) across the entire steady state domain. We not only documented the significant slowing of \( \text{VO}_2 \) kinetics with increasing absolute intensity, but were able to better profile the trend of this response with varying exercise intensity and increment magnitude.

We extended these methods to also study the NIRS response for the same conditions, revealing significant complexity in the deoxyhemoglobin response in the subjects. This study is the first to document this complexity, and it clearly shows the over-simplistic nature of the assumption of a mono-exponential model for muscle de- oxygenation kinetics during the transitions to steady state exercise (22,23).

Due to the multiple conditions and variables measured, the Discussion is based on the following variables. We also refer to differences in the responses between the exercise trial conditions (DM vs. SM1 vs. SM2).

**Time Constant**

The mono-exponential time constant, \( \tau \), has been used routinely to quantify the kinetics of exercise transitions to steady state data over the past four decades (13,34-36). Prior to 2006, consensus within the \( \text{VO}_2 \) kinetics literature was that steady state \( \text{VO}_2 \) kinetics operates as a linear first order system, which means \( \tau \) would remain constant independent of power output (1,4,6,9,10,13,25,31). However, results of this study have shown \( \tau \) to be predictably variant depending on steady state exercise intensity, as well as the baseline intensity prior to the exercise transition. Our data for both the DM trial and the SM trial have shown an obvious mean increase in \( \tau \) as exercise intensity increases (refer to Figure 4). For DM, the increase was linear, which revealed a significant increase in \( \tau \) from 30%VT at and above 60%VT. The data were even more interesting for the two SM exercise intensities. For the low (baseline) rest to exercise transition (SM1), \( \tau \) was invariant for increment intensities <50%VT. However, for SM2, \( \tau \) was significantly increased in a nonlinear profile as indicated by the more abrupt increase after 70%VT. These data may aid in explaining why earlier research, which mainly used low intensities (1,6,14,25,28,31) that did not detect an increase in \( \tau \) with the increase in exercise intensity.

It should also be noted that although the highest condition for both trials (DM vs. SM2) was 90% of the subjects VT, there is a more significant increase in \( \tau \) for the SM2 trial. This has also been described previously by Hughson and Morrissey (17). At 60%VT, the DM and SM
mean $\tau$ were similar (Figures 4a and 4b), suggesting that the more delayed increase in time to steady state VO$_2$ from a baseline intensity only occurs for relatively small increments from a relatively high (sub VT) baseline intensity. The SM2-specific data sets presented in Figure 5 also reveal the clear slow kinetics of these responses, which differ in context to the prior explained retention of a similar phase II response between steady and non-steady state exercise with an additional slow component in the latter condition (24). Our SM2 data show a much different phase II response, where the kinetics commences with a much slower and more complex profile. Such complex kinetics raise interesting questions concerning the differences in systemic circulatory versus motor unit recruitment versus intramuscular signals that influence VO$_2$ kinetics at lower versus higher baseline intensities within the low to moderate intensity steady state VO$_2$ range, as well as for small increment magnitudes. Conversely, the differences in our data between the responses of $\tau$ and time to steady state (see below) could reveal methodological limitations of the assumption of mono-exponential modeling of VO$_2$ increments to steady state.

**Time to Steady State**

This study is the first to quantify time to steady state (TTSS) using a new method involving steady state data back extrapolation to intersect with a second order polynomial mathematical function applied to the initial non-linear phase (Figure 8). This approach allowed improved modeling of the VO$_2$ response to exercise increments, and this was especially so for the initial VO$_2$ kinetics within the first 60 sec of the increment. This abrupt shift in the VO$_2$ data is not well represented and deviates from a mono-exponential model, causing $\tau$ to over-estimate (slow) the kinetic response time (see Figures 2 and 8).

There was a consistent lengthening of time to steady state with increasing intensity in both DM and SM2 trials (Figures 9a-b). In fact, the TTSS data of DM and SM2 were not different from each other. The similarity is based only on a consistent steady state intensity from completely different increments (magnitude, absolute, and relative intensity) for different trials performed on different testing days is testament to the robust internal validity of the TTSS methodology. These responses differed to that of $\tau$ (Figures 4 and 10). For example, there was no difference in TTSS between DM and SM2 for any exercise intensity. The TTSS was even more invariant to $\tau$ across the 5 intensities for SM1. However, $\tau$ data for SM2 80 and 90%VT conditions revealed a difficult to explain doubling of $\tau$ compared to the same conditions for DM.

The minor increases in $\tau$ for the higher intensities of SM1 and invariant nature of $\tau$ between 50 to 70%VT of SM2 is difficult to interpret. During data processing, it was clear that for several subjects, the assumption of a mono-exponential function for the higher intensity exercise increments was incorrect, for despite attaining steady state, the VO$_2$ responses appeared to have a rapid initial function followed by a slower function to steady state VO$_2$. This may have resulted in a disproportionally slower kinetic response based on the mono-exponential model compared to the TTSS methodology. This discrepancy is also seen in Figure 10c, where the TTSS trend approached a plateau near 360 sec (6 min) while $\tau$ revealed a continuous increase. Although no previous research has examined $\tau$ over a complete steady state exercise intensity range (and no other research has used our TTSS method), it can be postulated that the comparison of the results of both $\tau$ and absolute TTSS
VO₂ adds further evidence to question the validity of phase II \( \tau \) as an indirect reflection of time to steady state.

**Delta VO₂**
The data from the change in absolute VO₂ from the pre- and post-conditions (\( \Delta VO₂ \)) displayed an almost linear increase with increasing magnitude in DM. It is logical that the change in VO₂ should be nearly identical for given increments in power output to steady state (Figures 6a-b). Given that SM1 and SM2 involved the same relative magnitude increase from baseline VO₂ values (though started lower for SM1) for all five bouts of exercise, it could be deduced from the DM data that the mean \( \Delta VO₂ \) would remain the same for all five conditions. However, there was a trend for an increase in VO₂ as the baseline intensity increased (although not as apparent as in DM). The data may reveal a decreasing efficiency of cycling at higher steady state intensities, as has been postulated by others (27). Alternatively, as exercise intensity is increased, the non-linear increase in the work of ventilation with increased exercise intensity causes a progressively larger additional ventilatory-VO₂ component (30) that may help to explain the increased whole body VO₂ with increasing increment magnitudes.

**Steady State VO₂**
The mean data for steady state VO₂ followed an almost identical fit as intensity increased in both DM and SM1 and SM2 (Figures 7a-b). As for delta VO₂, steady state VO₂ data also revealed a slightly higher change at higher relative intensities.

**VO₂ Time Delay (Phase I)**
The phase I time delay (TD), as previously described by Auchincloss et al. (3) and Whipp et al. (33), was removed from the data prior to calculating \( \tau \). This is because phase I had been attributed to an abrupt increase in VO₂ caused by the rapid increase in ventilation and lung oxygen stores at the onset of increased workload and as such is not induced by respiration at the working muscle (33). Past research using \( \tau \) to quantify time to steady state VO₂ has consistently removed phase I prior to processing (6,9,10,21,33).

**Near-Infrared Spectroscopy**
As the phase II VO₂ is primarily a function of O₂ metabolism within the working muscle, it was necessary to examine the kinetics of muscle deoxygenation in an attempt to compare the kinetics of muscle deoxygenation to VO₂ kinetics based on pulmonary gas exchange. Murias et al. (23) demonstrated a mono-exponential response for deoxygenated hemoglobin (HHb) during knee extension exercise increments. In addition, both Murias et al. (23) and MacPhee et al. (22) demonstrated the importance of the change in HHb relative to the change in pulmonary VO₂. For example, exercise training improved the matching between \( \Delta HHb \) and \( \Delta VO₂ \) (22), and exercise transitions at the higher end of the steady state range (similar to our SM2 trial) had greater mismatch (23).

We were unable to quantify steady state responses of HHb as we revealed a more complex NIRS response in both DM and SM (1 & 2) than in any of the past research (Figures 10a-c). We interpret this to be based on our thorough assessment of the entire steady state VO₂
range, and our data revealed the need to question the assumption of a mono-exponential HHB response to all steady state exercise increments. Furthermore, our results cast concern on prior interpretations of decreases in HHB signals to directly infer changes in oxygen delivery and extraction and their influence on VO₂ kinetics. If the HHB signal varies in many subjects and exercise increment conditions (Figures 9a-b), yet VO₂ responses remain conforming to prior non-linear modeling, then, there may be no physiological connections between changes in HHB signals and VO₂ kinetics. Clearly, these responses require further research.

Apart from the hypothesis of this study, a number of other observed complexities of the data should be discussed. First, the validity of mono-exponential fitting to quantify τ in both trials is questionable as the model is erroneous in fitting initial (<60 sec) VO₂ kinetics. Figures 1 and 2 identify this for nearly all exercise increments, and which is again illustrated in Figure 8. However, based on the data presented in Figure 10c, this appears to be more relevant for exercise increments from higher intensities within the steady state VO₂ range. Obviously, this methodological limitation is prevented when using our approach at quantifying TTSS. Second, this study revealed high between and within-subjects inconsistency with the phase I time delay of the VO₂ kinetics. Figures 3a-f display six data sets where phase I is present (a,b,d,e) and not present (c, f). Consequently, it is not possible to consistently remove phase I from the data when quantifying τ. This finding introduces inconsistencies in the conditions used to derive τ to quantify VO₂ kinetics to steady state.

Interpreting Complex VO₂ Kinetics
Brittain et al. (8) suggested that mechanisms underlying the variation in the phase II VO₂ kinetic response may be due to different recruitment profiles of muscle fibers. Koppo et al. (20) also concluded that different muscle fiber recruitment patterns may be responsible for a varying τ, along with related factors such as mitochondrial density and oxidative enzyme activity, capillary density, and muscle perfusion. Recent research by Murias et al. (23) using NIRS studied the effects of the implementation of a 3-wk training intervention on τ. The study focused on microvascular O₂ delivery and extraction during cycle ergometry. The authors recorded a decrease in τ following the intervention. Similar research has also reported that O₂ delivery, tissue O₂ demand, and muscle fiber mechanisms may be responsible for variations in τ (22,27).

Given that this study shows a potential limitation of NIRS data to understanding muscle extraction and oxygen delivery components to complex VO₂ kinetics, the results of Pringle et al. (27) and Koppo et al. (20) are all the more relevant. For example, Pringle et al. (27) demonstrated significant correlations between τ and type I muscle fiber proportion of the vastus lateralis for heavy (~150%VT) to intense (~170%VT) exercise increments. Interestingly, such a relationship did not exist for their only steady state increment (80%VT) condition. Conversely, Koppo et al. (20) argued that the slowing of VO₂ kinetics towards the upper end of the steady state range, where oxygen supply and delivery were not compromised, was evidence that motor unit recruitment, not muscle fiber type proportions of peripheral or central oxygen delivery, were likely to be more influential to the changing and more complex VO₂ kinetics profile. Also, the findings in the present study support this interpretation, as there does appear to be a threshold intensity response to both τ and TTSS (Figures 4b and 9b), and use of muscle EMG data may reveal associations between root
mean square EMG activity and $\tau$ or TTSS. The between-subject differences in muscle fiber type could also provide an added explanation for variable responses (i.e., the extent of the added slowed kinetics) between subjects for the slow kinetics reported in the SM2 conditions.

**CONCLUSIONS**

For exercise increments to intensities below ~50%VT, VO$_2$ kinetics is invariant and adheres to linear first order kinetics. However, for intensities above 50%VT, whether from unloaded cycling or from a baseline intensity, the VO$_2$ kinetic response is progressively slowed in proportion to the steady state intensity and, therefore, displays complex kinetics. The threshold nature of the linear to complex VO$_2$ kinetic response supports a possible motor unit recruitment dependency to the muscle VO$_2$ kinetics response to exercise increments within the low to moderate (<VT) steady state exercise range. The variable and often complex responses for muscle deoxygenation to exercise increments to steady state casts uncertainty to the simplistic mono-exponential modeling of data from NIRS to aid in data interpretation to VO$_2$ kinetics. Similarly, varied responses to the tissue HHB signal despite stable VO$_2$ kinetics data represent evidence that muscle deoxygenation may not contribute to the muscle or pulmonary VO$_2$ kinetic response.

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