Duration of ‘Phase I’ VO₂p: a comparison of methods used in its estimation and the effects of varying moderate-intensity work rate

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Running head: Phase I VO₂p is unaffected by moderate-intensity work rate

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Abstract

The present study was designed to investigate whether absolute work rate (WR) affects Phase I pulmonary oxygen uptake (VO₂p) duration during moderate-intensity (Mod) exercise, and to compare two methods for estimating Phase I VO₂p duration (P₁-Dur). Fourteen males (24±5 yrs) each completed 4-8 repetitions of Mod transitions from 20W to 50, 70, 90, 110 and 130W. P₁-Dur was identified by: (1) a marked decrease in both respiratory exchange ratio and end-tidal partial pressure of O₂ following exercise onset (i.e., visual inspection of 3 independent reviewers, and the average (Avg) of the two most similar values); or (2) the intersection (TD) of the first and second components in a bi-exponential non-linear regression of the entire VO₂p response from exercise onset. P₁-Dur did not differ amongst WRs (p>0.05), regardless of the estimation method used. No differences were detected between Avg and TD (time in s) at any of the five WRs (50W, 21±6 vs. 23±10; 70W, 23±9 vs. 23±7; 90W, 24±3 vs. 22±5; 110W, 23±6 vs. 22±6; 130W, 21±6 vs. 21±7 s; p>0.05 for Avg and TD, respectively). Broad limits of agreement within Bland-Altman plots revealed relatively weak agreement amongst reviewers for individual estimation of P₁-Dur. A non-significant correlation coefficient (r=0.13) and broad limits of agreement suggest disparity between individual Avg and TD estimates of P₁-Dur. The present data do not support a role for Mod WR in determining P₁-Dur per se. Further, this study illustrated a poor agreement of P₁-Dur estimates derived from two different, but accepted methods.
**Introduction**

Upon examination of the pulmonary oxygen uptake (VO_{2p}) response immediately following an abrupt (i.e., “step”) increase in work rate (WR), three distinct phases can be observed (43, 47). The duration of Phase I, or the so-called “cardiodynamic” phase, is generally accepted to coincide with the circulatory transit delay from contracting skeletal muscles to the lungs (2, 12, 16, 17, 19, 43, 44); as such, the appearance of deoxygenated blood from the exercising muscles predicts decreases in both the respiratory exchange ratio (RER; given as the ratio of carbon dioxide output (VCO_{2p}) and VO_{2p}) and end-tidal O₂ partial pressure (P_{ET}O₂). Thus, recent studies seeking to describe this transition from Phase I to Phase II (21, 23, 27) have primarily relied on these indices as proposed by Whipp et al. (44). Although Phase I duration is thought to represent this circulatory transit delay, its precise determination as described immediately above is hampered both by rapid decreases in pulmonary artery (i.e., mixed venous) O₂ saturation (S_{V}O₂) and increases in the partial pressure of mixed venous CO₂ (P_{V}CO₂) as reported by Casaburi et al. (9). As an alternative approach to detecting Phase I duration, some have characterized the on-transient VO_{2p} response (i.e., Phases I, II and III) from exercise onset with a two-component exponential model, where the intersection of the two components (most often termed a time delay (TD)) is accepted as the Phase I duration (10, 16, 17). However, the assumption that changes in VO_{2p} during Phase I are truly exponential is questionable (15, 45).

Theoretically, the profile of Phase I VO_{2p} is determined, or at least influenced by the venous blood volume (i.e., between the exercising muscle and the lung) and blood flow dynamics (32). Although relatively little is known about the dynamics of venous blood volume at exercise onset (32), both the dynamics of cardiac output (hence “cardiodynamic” phase) following exercise onset, as well as the activity of the muscle pump (and its contribution to an
accelerated venous return to the right atria) could be expected to contribute to the determination of Phase I duration. Importantly, both the Q kinetics and the muscle pump activity are influenced by WR (30); specifically, a lower absolute WR could be expected to elicit both a reduced (i.e., amplitude) cardiac output (Q), as well as a reduced contribution of the muscle pump (30). In support of this suggestion, McNarry et al. (21) recently reported that Phase I duration was shorter during transitions to the heavy- (i.e., supra-GET) compared to the moderate-intensity exercise domain in young as well as older middle-aged subjects; this finding was similar to that reported by Sietsema et al. (36).

At present, however, it remains unclear whether Phase I duration is mediated by absolute WR when exercise is limited to the moderate-intensity exercise domain (i.e., below the gas exchange threshold; GET). A number of previous studies have reported longer Phase I durations in older adults, that is, a population predisposed to lower absolute moderate-intensity WRs compared to younger adults (21, 23, 27). Furthermore, in light of the relationship between absolute WR and Phase I duration (r=-0.65) described by Murias et al. (27), it is reasonable to suspect that lower absolute moderate-intensity WR will be associated with prolonged Phase I durations in younger adults; however, this hypothesis has not yet been tested. Thus, the purpose of the present study was to systematically examine the effect of WR increment on Phase I duration in a group of young men performing exercise transitions within the moderate-intensity domain. Further, two methods for assessing Phase I duration were compared. It was hypothesized that Phase I would be shorter during transitions to greater WRs, and that the two methods for detecting Phase I duration would yield similar estimates.
Methods

Subjects: Fourteen young men (24 ± 5 yr; 80 ± 12 kg; 180 ± 6 cm; mean ± SD) volunteered and gave written informed consent to participate in the study. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All subjects were non-smokers and were physically active. Additionally, no subjects were taking medications that are known to affect the cardiorespiratory, metabolic or hemodynamic responses to exercise.

Protocol: Initially, subjects reported to the laboratory to perform a ramp incremental test (25 W/min) to the limit of tolerance on a cycle ergometer (model: H-300-R Lode; Lode B.V., Groningen, Holland) for determination of peak VO2 (VO2peak) and GET. GET was defined as the VO2 at which CO2 output (VCO2) began to increase out of proportion to VO2 with a systematic rise in minute ventilation-to-VO2 ratio and end-tidal PO2 whereas minute ventilation-to-VCO2 ratio and end-tidal PCO2 were stable (5, 46). Within 2-3 weeks subsequent to the incremental test, subjects returned to the laboratory on 6 occasions, each separated by at least 24 hours. On each visit, subjects performed one set of square-wave leg-cycling exercises (6 min baseline at 20 W, 6 min moderate-intensity exercise, 6 min recovery at 20 W, and 6 min moderate-intensity) and, following 20 min of seated recovery, a second identical set. Thus, a total of 4 repetitions of “step” changes in WR were completed per visit, and each of the repetitions during any given visit were to the same absolute WR. The WR for the moderate-intensity transitions corresponded to one of 50 W, 70 W, 90 W, 110 W or 130 W. For each individual, the step transitions from 20 W to 130 W were performed first to verify that this WR was within the moderate-intensity domain (with the absence of a VO2p slow component); after this visit, the order of the WR transitions was randomized. For the 20 W to 50 W transitions, a total of 8 repetitions (i.e., 2
visits) were performed, as smaller WR transitions have been associated with reduced signal-to-noise ratios and thus less confidence in parameter estimates; for all other WRs, 4 transitions (i.e., 1 visit) were performed. The number of transitions required for each WR was determined in part by our previous work (41), showing that a minimum of 3 transitions were required in order to maximize the reliability of VO\textsubscript{2p} kinetics parameter estimates. We also followed the general conclusions of Lamarra et al. (18), which reinforced the importance of averaging the responses from multiple transitions in order to improve the signal-to-noise ratio for physiological variables sampled at inherently low frequency (i.e., breath-by-breath VO\textsubscript{2p}). We have also previously shown that the effect of previous moderate-intensity transitions on the VO\textsubscript{2p} kinetics response of subsequent moderate-intensity transitions is negligible (41). Subjects were instructed to maintain a consistent pedal cadence between 60-70 revolutions per minute (rpm) throughout 20 W baseline cycling and during each of the moderate-intensity cycling WRs. Cycle cadence was displayed continuously and the investigator ensured pedal cadence was in the 60-70 rpm range.

**Measurements:** Gas-exchange measurements were similar to those previously described (1). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test using a syringe of known volume. Inspired and expired gases were continuously sampled (50 Hz) at the mouth and analyzed for concentrations of O\textsubscript{2}, CO\textsubscript{2}, and N\textsubscript{2} by mass spectrometry (Innovision, AMIS 2000, Lindvedvej, Denmark) after calibration with precision-analyzed gas mixtures. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data were transferred to a computer,
which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (4).

Heart rate (HR) was monitored continuously by electrocardiogram (three-lead arrangement) using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO). Data were recorded using LabChart v6.1 (ADInstruments, Colorado Springs, CO) on a separate computer.

Data analysis: VO₂p data were filtered by removing aberrant data points that lay outside 4 SD of the local mean; the justification for this filtering process was provided by Lamarra et al. (18), who demonstrated that “noise” observed within the VO₂p signal conformed to a predictable Gaussian distribution, independent of WR. The data for each transition were linearly-interpolated to 1 s intervals and time-aligned such that time zero represented the onset of exercise. Data from all same-WR transitions were ensemble-averaged to yield five averaged responses for each subject (i.e., one for each WR); this data analysis process is consistent with previous studies from our laboratory (14, 25-29, 38-41), as well as other recent studies investigating the Phase I VO₂p response (21, 23).

Phase I determination: The Phase I-Phase II transition was determined by visual inspection of the second-by-second data by three independent reviewers; in addition, data were displayed as 5 s average “bins” to assist when examining “noisy” data (27). Before providing these data to the reviewers, data were coded such that reviewers were “blinded” to both the subject and the constant load WR condition (to prevent any possible bias arising from knowing the experimental condition). This Phase I-Phase II transition was taken as the point at which there was a sharp decrease from baseline values (20 W cycling) in both RER and PETO₂, as previously proposed (44). The results reported are from the three individual reviewers (R1, R2, R3); however, an
“average” value \((Avg)\) was also taken as the mean from the two independent investigators whose Phase I duration estimates were most similar (i.e., the disparate estimate was discarded; see Assumptions and Limitations section in Discussion).

A second approach for estimating the Phase I duration was also employed; this involved fitting the time-aligned, ensemble-averaged second-by-second \(\text{VO}_2p\) data with a two-component exponential model described by Lador et al. (16). Here, the first component corresponds to the so-called “cardiodynamic phase” (i.e., phase I, which Barstow and Molé (3) treated as exponential) and the second component to the “fundamental phase” (i.e., phase II), where the two components are separated by the time delay (TD), which has been used as an estimate of Phase I duration (10, 16). The two-component exponential model is given as:

\[
Y(t) = Y_{BSLN} + [\text{Amp}_1 (1 - e^{-t/\tau_1})] + H(t - TD) \times [\text{Amp}_2 (1 - e^{-(t-TD)/\tau_2})]; \text{[Equation 1]}
\]

where \(Y(t)\) represents the \(\text{VO}_2p\) for any given time; \(Y_{BSLN}\) is the \(\text{VO}_2p\) at baseline (fixed value calculated as the average \(\text{VO}_2p\) collected in the 2 minutes before an increase in WR); \(\text{Amp}_1\) and \(\text{Amp}_2\) are the amplitudes for first and second components, respectively; \(t\) is a given amount of time; \(\tau_1\) and \(\tau_2\) represent the time constants (i.e., time required to attain 63% of the steady-state amplitude) for the first and second component, respectively; and TD represents the time delay. \(H(t - TD)\) is the Heaviside function defined as:

\[
H(t - TD) = \begin{cases} 
0 & \text{if } t < TD \\
1 & \text{if } t \geq TD
\end{cases}
\]

\(\text{VO}_2p\) data were modeled from exercise onset to 4 min (240 s) of the step-transition; this ensured that each subject had attained a \(\text{VO}_2p\) steady-state, yet did not bias the model fit during the on-transient (6, 27). The effect of WR increment on the Phase II \(\text{VO}_2p\) kinetics response has been reported elsewhere (39), and is therefore not presented nor discussed herein. Since initial parameter estimates can influence the modeling outcome, initial guesses were entered after
visual inspection of the data. The model parameters were estimated by least-squares nonlinear regression (Origin, OriginLab Corp., Northampton, MA, USA) in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the Y-axis (Y = 0). The 95% confidence interval of for the estimated TD was determined after preliminary fit of the data with all other parameters constrained to the best-fit values and the TD allowed to vary.

HR data were processed in a similar manner to that described for VO₂p (i.e., errant data points removed, linearly interpolated, time-aligned, and ensemble averaged). The WR-specific, second-by-second HR responses for each individual were then modeled using either [Equation 1] or a mono-exponential function (without a TD) given as:

\[ Y(t) = Y_{BSLN} + [Amp \times (1 - e^{-t/\tau_1})] \]  

[Equation 2]

where \( Y(t) \) represents the HR for any given time; \( Y_{BSLN} \) is the HR at baseline (fixed value calculated as the average HR collected in the 60 s before an increase in WR (60 s average HR used in Equation 1 as well); Amp is the amplitude; \( t \) is a given amount of time; and \( \tau_1 \) represents the HR time constant, as well as the mean response time (\( \tau' \)HR). For responses characterized by the bi-exponential model described in [Equation 1], the \( \tau' \)HR was calculated from the weighted average (weighting factor was the relative contributions of the two amplitudes to the overall response) of \( \tau \) values from the first and second components as described by DeRoia et al. (10). In addition, the absolute HR (HR@TD) and the change in HR (\( \Delta \)HR@TD) from the onset of moderate-intensity exercise to the end of Phase I VO₂p was calculated from the raw HR data (i.e., not modeled from the HR kinetics parameters described above); for these calculations, the VO₂p TD was used as the estimate of Phase I duration, and the absolute HR@TD was given as a 5 s average (i.e., including the 2 s before and 2 s after the TD).
Statistics: Data are presented as means ± SD. One-way repeated measures (i.e., WR) analyses of variance (ANOVA) were used to determine statistical significance for most dependent variables. Two-way repeated measures ANOVA was used to assess: i) possible differences in independent reviewers’ (Avg) and non-linear regression derived (TD) estimates of Phase I duration, and ii) possible differences in WR-specific Phase I estimates amongst R1, R2 and R3. Coefficients of variation (CV) were calculated as the quotient of the SD of the difference (SD_{Diff}) between Phase I estimates and the mean estimates, and expressed as a percent (i.e. [SD_{Diff} / mean]*100). Pearson’s product-moment correlation coefficients were used to quantify the strength of relationships amongst variables. All statistical analyses were performed using SPSS Version 19.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when p<0.05.

Results

Subject characteristics are presented in Table 1. The steady-state VO_{2p} responses elicited by transitions to 50, 70, 90, 110, and 130 W corresponded to 49 ± 6, 57 ± 7, 66 ± 7, 75 ± 7, and 85 ± 8 % of GET (as reported elsewhere; (39)), and thus, were all within the bounds of the moderate-intensity domain.

No effect (p>0.05) of moderate-intensity WR on Phase I duration was observed, regardless of the method used (i.e., Avg and TD) to describe Phase I duration (Figure 1). The Avg, along with those from R1, R2 and R3, Phase I duration estimates for each of the five WRs are present in Table 2. Importantly, no effect of WR on Phase I was identified for any of the three independent reviewers (p>0.05); yet, a main effect for differences among reviewers was detected such that Phase I duration estimates were greater (p<0.05) from R1 compared to R3. Whereas there were no differences among the three reviewers’ WR-specific Phase I duration
estimates (p>0.05 for main effect of WR and WR by Reviewer interaction), and despite the significant Pearson product-moment correlation coefficients among reviewers (R1-R2, r=0.52, p<0.05; R1-R3, r=0.35, p<0.05; R2-R3, r=0.40, p<0.05), Figure 2A-C (7) illustrates the relatively weak agreement amongst reviewers (R1-R2: range of differences = -21 – 27 s, CV = 29%; R1-R3: range = -17 – 31 s, CV = 34%; R2-R3: range = -28 – 30 s, CV = 37%) for individual estimation of Phase I duration.

Figure 3 illustrates the VO₂p response (2 s average for illustrative purposes only) to each of the five WRs in a representative subject with the bi-exponential model and residuals superimposed. Table 3 displays the parameter estimates from “Phase I” of the two-segment exponential model. During transitions to higher WRs the absolute Amp₁ was greater (p<0.05), the proportion of the total (i.e., Amp₁/(Amp₁+Amp₂)) was unchanged (p>0.05), and the 95% confidence interval for the TD (CI₉₅ TD) was reduced (p<0.05) (Table 3). Neither TD (Figure 1) nor τ₁ VO₂p differed amongst the five WRs (p<0.05). Although the WR-specific Avg and TD did not differ (p>0.05; Figure 1) from one another, both the non-significant correlation coefficient (r=0.13; p>0.05) and the Bland-Altman (7) plot (Figure 2D) suggest disparity between individual Avg and TD estimates of Phase I duration.

Figure 4 illustrates the second-by-second HR response to each of the five WRs in a representative subject (same subject as presented in Figure 3) with the mono- or bi-exponential model superimposed. Table 4 presents the results of the HR kinetics analyses. Briefly, baseline HR (HR₉BLN) was similar across the five WRs; thus, the progressive increase in HR₉AMP (or the sum of HR₉AMP₁+HR₉AMP₂) at each WR led to greater steady-state HR (HR₉SS) responses with increasing WR. In addition to probable increases in τ₁ HR with increasing WR (p=0.053), the τ’HR increased (p<0.05) progressively with increasing WR as well. There was a weak, but
significant association between $\tau$'HR and TD ($r = -0.28$). Finally, both $\Delta$HR@TD and the absolute HR@TD progressively increased with increasing WR.

**Discussion**

The purpose of the present study was to systematically examine the effect of WR increment on Phase I duration in a group of young men performing step transitions in exercise intensity within the moderate-intensity domain. Further, two methods for assessing Phase I duration were compared. The main findings of the present study were that: i) moderate-intensity WR does not appear to mediate Phase I duration; and ii) there was a generally poor agreement amongst reviewers tasked with identifying Phase I duration using ventilatory indices (i.e., drop in RER and $P_{ETO_2}$), and also between this approach to Phase I duration detection and the two-component exponential modelling of the exercise on-transient.

*Moderate-intensity WR does not affect Phase I $VO_2p$ duration*

Prolonged Phase I durations have been reported in populations predisposed to lower absolute WR during moderate-intensity exercise, including older adults (21, 23, 27) and diseased populations (who, along with the compromised ability to perform absolute work also demonstrate blunted Q kinetics) such as heart transplant and heart failure patients (8, 20, 22, 31, 35) and pulmonary vascular disease (34); yet, the influence of WR *per se* in determining Phase I duration during moderate-intensity exercise remains unclear. Sietsema et al. (36) observed changes in Phase I duration during step transitions of varying intensity such that greater WRs elicited shorter Phase I durations; however, two important aspects should be noted. First, the protocol employed by Sietsema et al. (36) required subjects to begin exercise from rest, rather than “loadless” (i.e., 20 W) cycling. Although caution was taken to avoid any potential effect of
having to overcome the ergometer’s inertia (by using a motor to drive the flywheel prior to commencing exercise), it is known that the Phase I response comprises a greater proportion of the overall response when exercise is initiated from rest rather than light work (for example, see lower WR increments in Sietsema et al. (36)), and thus the studies may not be directly comparable. Second, it is clear from Figure 2 therein (36) that not all exercise transitions were limited to the moderate-intensity domain. In this regard, McNarry et al. (21) recently illustrated shortened Phase I responses during heavy-intensity compared to moderate-intensity transitions in a group of older middle-aged and young adults. So, whereas an effect of WR per se seems to have been established, its impact during moderate-intensity exercise remains unclear.

Data from the present study indicate that WR does not have a determining effect on Phase I duration during moderate-intensity exercise in healthy, young men. While questions about the accuracy and precision of Phase I duration persist (see below for further discussion), the fact that there was no evidence of WR-mediated differences in Phase I duration (p=0.75 for WR), regardless of the approach used in its estimation (p=0.86 for WR by Method (i.e., TD vs. Avg) interaction), strongly supports this conclusion. If, as the present data suggest, there is no intra-individual relationship between Phase I duration and WR (within the moderate-intensity domain), it could be suggested that our previous finding (27) of longer Phase I durations in older compared to younger adults (who exercised at lower absolute moderate-intensity WRs) were likely related to aging, rather than the effects of WR per se; this would suggest that the negative association (r=-0.65) we reported could have been coincidental. However, a cause-effect relationship between moderate-intensity WR and Phase I duration has not been investigated in older adults (see Assumptions and Limitations section for further discussion).
We hypothesized that Phase I would be shorter during transitions to greater WRs, primarily because of a greater activity of the muscle pump and a greater Q response (i.e., amplitude) at higher WR; yet, no relationship was observed between WR and Phase I duration. Whereas a lower WR could be expected to elicit both a reduced Q (i.e., L·min⁻¹) and a reduced contribution of the muscle pump (30), it is also possible that the rate of adjustment for Q could be faster at lower WR (i.e., where the rapid withdrawal of parasympathetic activity could be sufficient to meet the demands for greater Q). This suggestion is supported, at least in part, by the observation of increasing \( \tau_1 \) HR (\( p=0.053 \)) and \( \tau' \)HR values with increasing WRs, and by the (albeit weak) inverse relationship between \( \tau' \)HR and TD (implying slower adjustments of Q relate to longer Phase I VO₂p) in the present study. Thus, in this scenario, it is possible that the increased activity of the muscle pump at higher WRs (30) could be offset by the potentially slower adjustment of Q, resulting from the need for increased contribution of the slower activating sympathetic activity. What this analysis fails to address, however, are the potential influences of changes in stroke volume and the dynamics of intervening venous volume, which to date remains poorly understood.

The increase in absolute Amp₁ during transitions to greater WRs is likely to explain, at least in part, the increased confidence in TD estimates (i.e., reduced CI₉₅ TD). To this end, Lamarra et al. (18) described the effects of signal-to-noise ratio in VO₂p data and confidence in parameter estimates (albeit referring to Phase II data, primarily). So, these findings are perhaps not surprising; that the proportion of the overall VO₂p response comprised by Phase I did not vary across WRs was not necessarily expected, however. This latter finding may reflect a matching of the Q response to absolute WR, such that greater WR increments elicit proportionally greater increases in Q.
Whereas the amplitude of the phase I response may be related to the “cardiodynamic” aspect of the increase in \( \text{VO}_{2p} \) occurring primarily as a result of the increase of Q and pulmonary blood flow, the duration of the phase I is related to the time period until an increased oxygen extraction occurs in the contracting skeletal muscle plus the circulatory transit time for that deoxygenated blood to be expressed at the lungs. Near-infrared spectroscopy measures suggest that with the very rapid initial increase in blood flow at the exercise-onset (30, 42), the increasing \( \text{VO}_2 \) of the muscle is achieved by the increased \( \text{O}_2 \) delivery with “extraction” (deoxygenation) unchanged (or even reduced) for a period in the order of 10 seconds (11, 13, 26). Following this time delay for deoxygenation, the time for transport to the lungs can be estimated from studies of “recirculation” time in exercise. The lung-to-lung circulation time has been measured to be approximately 20 seconds during steady-state moderate-intensity exercise (see Figure 2 in: (24)) and at least \( \frac{1}{2} \) and likely \( \frac{2}{3} \) of this time is from capillary to lung – thus a period of 10 to 15 seconds. Sowton et al. (37) noted a recirculation time during moderate exercise of approximately 8 to 11 seconds for the earliest “reappearance” of the tracer but also noted that the peak concentration of the indicator in the “recirculation hump” (reflecting active muscle blood flow) was reached in twice the recirculation time – thus in the order of 15 to 20 seconds. Thus the delay in deoxygenation of 10 seconds and the circulatory transit time of about 15 seconds correspond to a total phase I duration for increasing \( \text{VO}_2 \) at the lung in the order of 25 seconds. The measured duration from the present data (i.e., Avg) ranged from 20 to 24 seconds for the 5 WRs.

Methodological considerations for Phase I \( \text{VO}_{2p} \) estimation

Figure 2 illustrates that whereas the group mean estimates of Phase I duration were generally similar amongst independent reviewers (i.e., the biases were 1.06 for R1-R2 (p>0.05
from 0 s), 2.36 for R1-R3 (p<0.05 from 0 s), and 1.30 s for R2-R3 (p>0.05 from 0 s); see Table 2), perhaps the more revealing information is the width of the 95% Confidence Limits. Indeed, what Figure 2 is primarily intended to illustrate is the general lack of agreement amongst reviewers in identifying Phase I duration. The criteria considered by these independent reviewers was the “sharp drop” in both RER and $P_{ET}O_2$ that is predicted by the arrival of deoxygenated blood from the exercising muscles to the lung. While this approach to Phase I detection seems to be the “traditional” option, it is clear that the method is subject to some serious limitations. The root of these limitations (for examples of technical challenges, see Figure 1 in Murias et al. (27)) seems to reside in the assumption that the O₂ and CO₂ content in mixed-venous (i.e., pulmonary artery) blood remains unchanged until such time as blood that passed through the muscle capillary bed at or after the onset of exercise returns to the lung.

Under circumstances where moderate-intensity exercise is initiated from light work (rather than rest), it is likely that the O₂ and CO₂ content of mixed-venous blood specifically between the exercising muscles and the lung will be lower and higher, respectively, than venous blood in other regions of the body (since the exercising muscles would necessarily rely at least in part on a widened arterio-venous O₂ difference (a-vO₂diff), and would “release” more CO₂ than at rest). Accordingly, upon the initiation of exercise, it is this “more deoxygenated” blood which would be accelerated by the activity of the muscle pump, while the “less deoxygenated” blood from other (inactive) regions would not likely be subject to the effects of a muscle pump (30). Indeed, Casaburi et al. (9) have demonstrated that the assumption of unchanged O₂ and CO₂ content in the pulmonary arteries during Phase I is invalid, as both rapid (i.e., within 6 s) decreases in pulmonary artery (i.e., mixed venous) S$_V$O₂ and rapid increases in the P$_V$CO₂ were
observed following exercise onset (from rest rather than light exercise, no less). As a result, the term “cardiodynamic phase” to describe Phase I is probably a misnomer.

Considering these limitations associated with the traditional method for Phase I detection, others have used a two-component exponential model to detect the Phase I-Phase II transition (10, 16, 17). In the present study, the correlation between this approach and the traditional approach of using ventilatory indices was not significant ($r=0.13; p>0.05$), and, more to the point, the agreement of individual estimates was quite poor (see Figure 2). Given the inherent limitations described above, it could be suggested that agreement should not be expected if the two-component model is generating valid and accurate estimates of TD; however, the basic assumption of the two-compartment model is not free from criticism. While Barstow and Molé (3) used an exponential function to characterize Phase I, others have questioned this practice, because of a lack of experimental evidence supporting its exponential nature, the limited number of data points, and uncertainty about its true asymptotic value (6, 15, 45). Indeed, a purely mono-exponential Phase I should only be expected if its adjustment is governed by the rapid changes in Q alone. As with the traditional method, this scenario ignores the fact that changes in pulmonary artery (i.e., mixed venous) $SVO_2$ and $PVCO_2$ (9) would necessarily distort a purely exponential adjustment, unless their rates of change perfectly mirrored that of Q; to our knowledge, this has never been shown. In aggregate, the lack of agreement between the two methods, and the fact that both are underpinned by potentially flawed assumptions, suggests that at least for an individual, estimates of Phase I duration represent “an illusion of Phase I” rather than a precise and accurate measurement; neither method is favoured over the other based upon the present analysis. Further studies into the profile and description of Phase I VO$_2$ seem warranted.
Assumptions and Limitations

Phase I VO$_2p$ comprises a relatively brief portion of the exercise on-transient. In light of the fact that breath-by-breath VO$_2p$ is sampled at an inherently low frequency, it is common practice to ensemble-average the responses from several repetitions of the same magnitude (18). We acknowledge that a true second-by-second VO$_2p$ response was not collected; however, the data analysis techniques employed in the present study are consistent with others in the literature. Furthermore, because of its brief nature, uncertainty persists about the true exponential nature of Phase I VO$_2p$; we acknowledge this as a limitation of the use of [Equation 1].

Given the wide variability and generally poor agreement amongst reviewers in determining Phase I duration from the gas exchange indices, we believed that inclusion of “poor” (i.e., disparate) estimates in the overall average value (i.e., $Avg$) jeopardized accuracy in this measure. In fact, post hoc regressions of the two accepted measures (i.e., ignoring the disparate measure) resulted in a much stronger correlation ($r = 0.95$) than those reported for any two reviewers. The association between the $Avg$ measure and the discarded estimate approached zero ($r = 0.08$). Our aim was to describe the Phase I duration as accurately as possible based upon visual inspection of the gas exchange data, and we felt that eliminating “poor” estimates advanced this goal.

Notwithstanding concerns about the accuracy and precision of Phase I duration estimates, the Phase I response has been shown to be prolonged in older compared to younger subjects (23, 27). Mezzani et al. (23) proposed that this prolongation was a result of aging per se; however, a significant correlation ($r = -0.65$) between Phase I duration and moderate-intensity WR (27) challenged this interpretation. Three main concerns precluded us from including an older group in the present study: 1) it did not address the study’s primary purpose (i.e., to determine the
effect of WR on phase I duration independent of aging). If the WR-related differences that we proposed in our previous study exist, then we expected to be able to detect them independently of a potential aging effect. The intent of the present study was not to attempt to identify or reinforce an effect of aging, but rather to examine a “pure” effect of WR on Phase I $\text{VO}_2\text{p}$ duration; 2) logistically, it is impractical to attempt to identify five distinct moderate-intensity WRs in older adults, as their estimated GET is generally much lower than those observed in young, healthy subjects; and 3) given the requirement for low WR increments in older adults, the resulting signal-to-noise ratio in their $\text{VO}_2\text{p}$ data is dramatically reduced in comparison to populations capable of performing larger WR increments in the moderate-intensity domain.

Despite these concerns, we remained interested in investigating the possibility that moderate-intensity WR affects Phase I duration in older adults. As a result, we re-analyzed data from a previously published study (40) by our laboratory in order to address this question. In that study, subjects performed WR transitions from 20 W to a WR corresponding to 90% of GET (Full Step; FS), as well as two equal WR increments from 20 W to 90% of GET (Lower Step; LS, Upper Step; US). So, in those older subjects ($n=7; 69\pm5$ yr; $28\pm4$ mL·kg$^{-1}$·min$^{-1}$ $\text{VO}_2\text{peak}$), comparisons between the FS and LS ($\Delta\text{WR} = \frac{1}{2}$ of FS) offer a glimpse into the effects of moderate-intensity WR. We modeled the second-by-second $\text{VO}_2\text{p}$ responses with the bi-exponential regression described in [Equation 1] and identified TD values (i.e., phase I duration estimates) of $27.5 \pm 7.9$ s (FS) and $27.8 \pm 5.9$ s (LS). These values were not significantly different ($p>0.05$) from one another, suggesting that Phase I duration is not mediated by absolute moderate-intensity WR in older adults; this is consistent with the findings in young adults.
**Perspectives and Significance**

Immediately upon exercise onset, muscle \( \text{VO}_2 \) \( (\text{VO}_2\text{m}) \) adjusts exponentially in response to an increased need for ATP resynthesis; the temporal profile of this adjustment has been used to investigate metabolic and cardiovascular control mechanisms. Given the technical challenges and expense associated with directly measuring \( \text{VO}_2\text{m} \), \( \text{VO}_2\text{p} \) is more often used. While it is generally accepted that the Phase II \( \text{VO}_2\text{p} \) profile is similar (i.e., within \(~10\%) to that of \( \text{VO}_2\text{m} \) (13, 33), the factors that influence the characteristics of Phase I are not as clearly understood. Aside from the impact of including/excluding Phase I data on parameter estimates of Phase II \( \text{VO}_2\text{p} \) (27), few have considered the Phase I \( \text{VO}_2\text{p} \) response and its determinants; additionally, controversy persists regarding the preferred method for detecting the end of the Phase I response. A previous study from our laboratory (27) identified an association between WR and Phase I; however, this had not been rigorously tested. The present study has shown that changes in moderate-intensity WR do not induce changes in Phase I duration, but do alter the response amplitude. The present study also demonstrated that in spite of similar group mean Phase I estimates, the two generally accepted methods for describing Phase I duration demonstrated poor agreement for individual subjects.

**Conclusions**

The present study demonstrated that, in young adults, Phase I \( \text{VO}_2\text{p} \) duration is not affected by WR increment during moderate-intensity exercise initiated from light (i.e., 20 W) work. Further, this study illustrated a poor agreement of Phase I duration estimates derived from two different, but accepted methods; importantly, these data highlight the limitations of the respective methods and imply that caution should be taken when attempting to interpret the physiological implications of Phase I duration within individuals. Finally, this study
demonstrated that while the absolute amplitude of Phase I is WR-dependent, the relative contribution of Phase I to the overall VO$_2p$ response (i.e., $\text{Amp}_1+\text{Amp}_2$) is invariant during moderate-intensity step transitions.
Acknowledgments: We would like to express our gratitude to the subjects in this study and to acknowledge the technical assistance provided by Brad Hansen. We would also like to acknowledge and thank Kaitin McLay and Tatsu Amano for their assistance with blinding reviewers prior to estimation of Phase I duration.

Grants: This study was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) research and equipment grants. M.D. Spencer was supported by an Ontario Graduate Scholarship (OGS).
References

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Figure Captions

Figure 1. Comparison of Avg and TD estimates of Phase I duration for transitions performed from 20 W to 50, 70, 90, 110, and 130 W, respectively. Estimates of Phase I duration did not differ either between Avg and TD for a given WR (p>0.05), nor across WRs for either estimation method (p>0.05).

Figure 2. Bland-Altman plots illustrating the questionable agreement between: (A) R1 and R2; (B) R2 and R3; (C) R1 and R3; and (D) Avg and TD. Precision given as ± SD; Limits of Agreement given as mean ± 2SD.

Figure 3. VO₂p response to 50, 70, 90, 110, and 130 W transitions from 20 W in a representative subject. Two-component exponential models superimposed; residuals shown below varying randomly around y=0 (solid horizontal line). Dashed vertical line denotes moderate-intensity exercise onset. Note: exponential models were derived from the fit of second-by-second VO₂p data; 2 s average data are presented for illustrative purposes only.

Figure 4. HR response to 50, 70, 90, 110, and 130 W transitions from 20 W in a representative subject (same subject as in Figure 3). Mono-exponential (50-110 W) and bi-exponential (130 W) models superimposed. Dashed vertical line denotes moderate-intensity exercise onset.
Table Legend

**Table 1.** Subject characteristics
Values are mean ± SD.

**Table 2.** WR-specific Phase I duration estimates from R1, R2, R3 and Avg
Values are mean ± SD. No differences (p>0.05) were observed amongst R1, R2 and R3 for a given WR; also, no differences (p>0.05) were observed across WRs for any Reviewer or Avg.
*Significantly different from R1 (p<0.05).

**Table 3.** Parameter estimates for VO₂\(_{p}\) derived from the first component of the two-component exponential regression of the exercise on-transient.
Values are mean ± SD. Amp₁, amplitude of first component; Amp₁ (%) , proportion of overall VO₂\(_{p}\) amplitude comprised by Amp₁ (given as Amp₁/ (Amp₁ + Amp₂)*100); τ₁, time constant for the first component; TD, time delay of the second component taken as an estimate of Phase I duration; CI\(_{95}\) TD, 95% confidence interval for the time delay. *, p<0.05 from 50 W; †, p<0.05 from 70 W; ‡, p<0.05 from 90 W; §, p<0.05 from 110 W.

**Table 4.** Parameter estimates for HR derived from the mono- or bi-exponential regressions of the exercise on-transient.
Values are mean ± SD. HR\(_{BSLN}\), HR baseline; HR\(_{AMP1}\) and HR\(_{AMP2}\), HR amplitude for first and second component, respectively; τ₁ HR and τ₂ HR, time constants for the first and second components, respectively; HR\(_{SS}\), steady-state HR; τ’HR, HR mean response time calculated from the weighted average of τ₁ HR and τ₂ HR (note: τ’HR = τ₁ HR when mono-exponential
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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Mass (kg)</th>
<th>Height (m)</th>
<th>VO$_{2}\text{peak}$ (mL·kg$^{-1}$·min$^{-1}$)</th>
<th>Peak WR (W)</th>
<th>GET (L·min$^{-1}$)</th>
<th>WR at GET (W)</th>
</tr>
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<tr>
<td><strong>Mean</strong></td>
<td>24</td>
<td>80</td>
<td>1.80</td>
<td>52.3</td>
<td>361</td>
<td>2.41</td>
<td>159</td>
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<tr>
<td><strong>SD</strong></td>
<td>5</td>
<td>12</td>
<td>0.06</td>
<td>6.8</td>
<td>37</td>
<td>0.20</td>
<td>18</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
Table 2. WR-specific Phase I duration estimates from R1, R2, R3 and Avg

<table>
<thead>
<tr>
<th></th>
<th>50 W</th>
<th>70 W</th>
<th>90 W</th>
<th>110 W</th>
<th>130 W</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>22.4 ± 4.3</td>
<td>24.9 ± 8.0</td>
<td>25.1 ± 4.4</td>
<td>22.0 ± 6.2</td>
<td>20.6 ± 5.6</td>
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<tr>
<td>R2</td>
<td>19.3 ± 7.7</td>
<td>22.1 ± 11.1</td>
<td>23.9 ± 3.5</td>
<td>22.9 ± 5.9</td>
<td>21.4 ± 6.5</td>
</tr>
<tr>
<td>R3*</td>
<td>18.6 ± 5.6</td>
<td>22.5 ± 8.9</td>
<td>23.1 ± 5.3</td>
<td>19.8 ± 7.1</td>
<td>19.2 ± 7.1</td>
</tr>
<tr>
<td>Avg</td>
<td>20.6 ± 5.9</td>
<td>23.2 ± 8.7</td>
<td>23.6 ± 3.4</td>
<td>22.6 ± 6.0</td>
<td>21.0 ± 5.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD. No differences (p>0.05) were observed amongst R1, R2 and R3 for a given WR; also, no differences (p>0.05) were observed across WRs for any Reviewer or Avg. *Significantly different from R1 (p<0.05).
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<tr>
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<th>110 W</th>
<th>130 W</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amp₁ (L·min⁻¹)</strong></td>
<td>0.10 ± 0.07</td>
<td>0.15 ± 0.14</td>
<td>0.24 ± 0.11*†</td>
<td>0.30 ± 0.14*†</td>
<td>0.43 ± 0.23*†§</td>
</tr>
<tr>
<td><strong>Amp₁ (%)</strong></td>
<td>37 ± 22</td>
<td>35 ± 24</td>
<td>36 ± 15</td>
<td>35 ± 14</td>
<td>38 ± 18</td>
</tr>
<tr>
<td><strong>τ₁ (s)</strong></td>
<td>19 ± 22</td>
<td>24 ± 31</td>
<td>23 ± 17</td>
<td>18 ± 17</td>
<td>24 ± 20</td>
</tr>
<tr>
<td><strong>TD (s)</strong></td>
<td>21 ± 9</td>
<td>23 ± 7</td>
<td>22 ± 5</td>
<td>22 ± 6</td>
<td>21 ± 7</td>
</tr>
<tr>
<td><strong>CI₉₅ TD (s)</strong></td>
<td>4 ± 3</td>
<td>3 ± 1</td>
<td>2 ± 1*</td>
<td>2 ± 1*</td>
<td>2 ± 1*‡</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Amp₁, amplitude of first component; Amp₁ (%), proportion of overall VO₂p amplitude comprised by Amp₁ (given as Amp₁/(Amp₁+Amp₂)*100); τ₁, time constant for the first component; TD, time delay of the second component taken as an estimate of Phase I duration; CI₉₅ TD, 95% confidence interval for the time delay. *, p<0.05 from 50 W; †, p<0.05 from 70 W; ‡, p<0.05 from 90 W; §, p<0.05 from 110 W.
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<th>110 W</th>
<th>130 W</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR_{BSLN} (bpm)</td>
<td>85 ± 6</td>
<td>86 ± 10</td>
<td>88 ± 9</td>
<td>88 ± 9</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>HR_{AMP1} (bpm)</td>
<td>8 ± 2</td>
<td>13 ± 3*</td>
<td>20 ± 5*†</td>
<td>23 ± 5*†‡</td>
<td>27 ± 8*†‡§</td>
</tr>
<tr>
<td>τ1 HR (s)</td>
<td>16 ± 11</td>
<td>17 ± 7</td>
<td>26 ± 19</td>
<td>23 ± 18</td>
<td>22 ± 24</td>
</tr>
<tr>
<td>TD HR (s)</td>
<td></td>
<td></td>
<td>40 ± 14 (n=3)</td>
<td>36 ± 18 (n=9)</td>
<td></td>
</tr>
<tr>
<td>HR_{AMP2} (bpm)</td>
<td></td>
<td></td>
<td>7 ± 4</td>
<td>11 ± 8</td>
<td></td>
</tr>
<tr>
<td>τ2 HR (s)</td>
<td></td>
<td></td>
<td>44 ± 14</td>
<td>49 ± 29</td>
<td></td>
</tr>
<tr>
<td>HR_{SS} (bpm)</td>
<td>93 ± 6</td>
<td>98 ± 9*</td>
<td>108 ± 9*†</td>
<td>113 ± 10*†‡</td>
<td>124 ± 12*†‡§</td>
</tr>
<tr>
<td>τ’HR (s)</td>
<td>16 ± 11</td>
<td>17 ± 7</td>
<td>26 ± 18*†</td>
<td>25 ± 16*</td>
<td>30 ± 21*†</td>
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<tr>
<td>ΔHR@TD (bpm)</td>
<td>6 ± 3</td>
<td>9 ± 4</td>
<td>13 ± 5*†</td>
<td>16 ± 6*†</td>
<td>19 ± 8*†‡§</td>
</tr>
<tr>
<td>HR@TD (bpm)</td>
<td>91 ± 8</td>
<td>95 ± 9</td>
<td>100 ± 8*</td>
<td>103 ± 10*</td>
<td>110 ± 9*†‡§</td>
</tr>
</tbody>
</table>

Values are mean ± SD. HR_{BSLN}, HR baseline; HR_{AMP1} and HR_{AMP2}, HR amplitude for first and second component, respectively; τ1 HR and τ2 HR, time constants for the first and second components, respectively; HR_{SS}, steady-state HR; τ’HR, HR mean response time calculated from the weighted average of τ1 HR and τ2 HR (note: τ’HR = τ1 HR when mono-exponential regression was used); ΔHR@TD, change in HR from exercise onset to end of Phase I VO_{2p} (using VO_{2p} TD estimate); HR@TD, absolute HR at end of Phase I VO_{2p} (using VO_{2p} TD estimate). *, p<0.05 from 50 W; †, p<0.05 from 70 W; ‡, p<0.05 from 90 W; §, p<0.05 from 110 W.