Comparison between different methods of analysis of slow component of oxygen uptake: a view in severe exercise domain

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ABSTRACT

The objective of the present study was to compare in severe exercise domain, different techniques used for measuring the amplitude of the slow component (SC) of oxygen uptake kinetics. Ten trained cyclists, male (age: 25 ± 3.6 years, body mass: 67.2 ± 4.5 kg, height: 174.8 ± 6.5 cm and VO₂max: 62.4 ± 3.1 mL.kg⁻¹.min⁻¹), performed two identical bouts transitions at constant load (mean ± SD (intensity 75%Δ: 75% of the difference between the VO₂ lactate threshold and the VO₂max)) in different days. The SC was calculated from different methods: (1) bi-exponential model \( VO₂(t) = VO₂_{base} + A₁(1 - e^{-\frac{t}{TA₁}}) + A₂(1 - e^{-\frac{t}{TA₂}}) \), (2) predetermined intervals \( \Delta VO₂_{6-2}; \) difference between the second min VO₂ and the end VO₂; \( \Delta VO₂_{5-3}; \) difference between the third min VO₂ and the end VO₂; and (3) difference between the end VO₂ and the value obtained from a mono-exponential adjustment of the "primary component" (predetermined time of 120 s) \( SC_{6-PC} \). All the methods were compared among themselves. The results showed a significant underestimation of the SC obtained by method of predetermined intervals \( \Delta VO₂_{6-2}; \) difference between the second min VO₂ and SC when compared with bi-exponential model and \( SC_{6-PC} \).

INTRODUCTION

The response of the oxygen uptake (\( VO₂ \)) in the transition from rest or mild exercise to the exercising condition (moderate, intense, very intense and supramaximal) at steady load has been described in the literature¹⁻¹³. In the beginning of the exercise (after phase I "cardiodynamic" – 15 to 20 initial seconds) performed at moderate intensity (i.e., below the lactate threshold – LT), the VO₂ increases according to a mono-exponential model (Phase 2) in order to reach a new steady state (Phase 3) within 2 to 3 min. In the other hand, during intense and very intense exercises (above the LT), the VO₂ kinetics primary component is supplemented by an additional slow component (SC), which causes an increase in the VO₂ and, consequently, a delayed and increased steady state, being able to reach values of oxygen maximal uptake (\( VO₂_{max} \)). During exercises of steady load at intense domain (intensities between the LT and the Lactate Maximal Steady State (LMSS)) the SC estabilizes during exercise, but during exercises called very intense (intensities between the LMSS and the \( VO₂ \)) the SC progressively increases and can reach the \( VO₂_{max} \).

The SC may be calculated through mathematical models, which allow the identification and measurement of the breadth magnitude²⁻⁶; or through pre-set intervals ex.: \( \Delta VO₂_{5-3}; \) difference of the \( VO₂ \) between the third and sixth minute of exercise¹⁻¹¹ or \( \Delta VO₂_{6-2}; \) difference of the \( VO₂ \) between the second and sixth minute of exercise¹². These last kinds of analysis simplify the procedures; however, they can generate data which do not reflect the suitable behavior of the studied phenomenon. Within this context, Bearden and Moffatt¹³ in a study with cyclists have observed that SC values obtained from pre-set intervals \( \Delta VO₂_{6-3} \), during intense exercise (Δ30%: 30% of the difference between the \( VO₂ \) in the LT and the \( VO₂_{max} \)), presented lower breadths when compared with data obtained by the adjustment mathematical model. Nevertheless, analyses similar to the one by Bearden and Moffatt¹³ have not been performed yet in a domain of very intense intensity (intensities where there is not a SC stabilization).

Recently, Burnley et al.¹⁴ have used another mathematical model to calculate the SC. Such model was adopted from the study by Rossiter et al.¹⁵ and had as proposal the adjustment of the primary component from an interval of steady time (120 s). In that analysis, the SC was calculated by the difference between the mean value of the last seconds of exercise and the adjusted value of the "primary component" \( SC_{6-PC} \). The possible differences between this model and the remaining have been investigated. According to Bearden and Moffatt¹³, different methods of SC analyses may result in different breadths. Therefore, the aim of this study was to compare different methods of SC analysis during very intense exercise (75%Δ-75% of the difference between the \( VO₂ \) in the LT and the \( VO₂_{max} \)).

METHODS

Ten trained male cyclists participated in the study (age: 25.0 ± 3.6 years, body weight: 67.2 ± 4.5 kg and height: 174.8 ± 6.5 cm). The mean weekly distance completed by the volunteers was of 378 ± 60.4 km and the experience in competitions of 4.6 ± 2.1 years. All volunteers were informed on the aim of the research, as well as its possible risks and benefits. After agreeing with the study’s proposal and all the experimental procedures having been approved by the Ethics Committee from the Federal University of São Paulo (nº 1246/03), the participants signed a Free and Clarified Consent Form. The inclusion criteria were: (a) trained male cyclists,
Experimental protocol

The participants visited the laboratory in four distinct occasions, during a period of two weeks. In the first visit, they were familiarized with the cycle-ergometer and remining experimental procedures. In the second visit, they performed a load incremental test until voluntary exhaustion, with the purpose to evaluate the LT and the VO$_2$max. In the third and fourth visits, the volunteers performed two steady load transitions [75%∆ = VO$_2$ LT + 0.75 × (VO$_2$max – VO$_2$ LT)]. The load concerning this exercise intensity was determined by linear regression, from VO$_2$ values and power, which were obtained during each stage of the incremental test.

The participants were told to arrive at the laboratory 1 h before the established time, well-hydrated and not having taken alcohol, caffeine or any kind of supplementation in the days of the test. They were also asked to avoid extenuant physical exercises in the 24 hours prior to the tests. A minimum interval of 48 h was required between each visit.

All tests were performed in a mechanical cycle ergometer (Monark 834 E, Ergomedic, Sweden). The seat’s height and handle’s position were registered in the first visit and repeated in the following tests’ sessions. The ventilatory variables were determined, breath after breath, by an open circuit computerized test system (Sensor Medicis – Vmax 29 series – Metabolic Measurement Cart, Yorba Linda, CA). The measurement of the expiratory flow was performed by a sensor of mass flow (anemometer), calibrated before each test session, by a 3-liter sereyng, in three different flow levels. A Hans Rudolph flow-by face mask (Kansas City, MO USA) was used for collection of expired gases. The analysis systems of the O$_2$ and CO$_2$ were calibrated before each session, using the room air and the concentration of known gases (16% of O$_2$, 3% of CO$_2$). The HR was registered at each 5 s, during all tests, by telemetry (Polar, Advantage NV).

In order to determine the VO$_2$max and the LT, the participants performed a progressive continuous test, with initial load of 105 W and increments of 35 W at each 3 minutes, keeping a steady cadence of 70 rpm. At the end of each stage, a blood sample from the earlobe was collected (25 μl) in order to determine the blood lactate concentration [La]. The LT was determined by the visual method ([La] x power) and defined as the exercise intensity prior to the first one and keeping increase of [La] above the rest concentrations. As analysis criterium, two independent and experienced observers were consulted. There was an agreement from the observers part on the point in which the LT for all volunteers occurred. The VO$_2$max was defined as the highest value of VO$_2$, measured in 20 s-intervals, during the incremental test. All volunteers completed at least two of the following criteria for VO$_2$max: (1) one rate of gas exchange > than 1.1, (2) one [La] peak > than 8 mmol.1$^{-1}$, and (3) one peak hear rate ≥ 90% of the maximal value expected for the age.

In the third and fourth visits, the volunteers performed steady load transitions which were initiated with a period of 4 min, pedaling in a mild load (35 watts), followed by a sudden load application, for a period of 6 min (intensity of 75%∆). The cadence was kept steady at 70 rpm. The use of two days for performance of the steady load transitions, following the recommendations by Lamarra et al., had as aim to increase data reliability.

VO$_2$ kinetics analysis

For each mild load transition for exercise, the VO$_2$ data (breath after breath) were linearly interpolated in order to obtain values with 1 s intervals. The data concerned with the two transitions were aligned, and later, the means for the VO$_2$ values were calculated. This procedure had as objective to diminish the ‘noise’ and accentuate the physiological responses characteristics. The baseline VO$_2$ (VO$_2$base) was defined as the mean value of the 4 initial minutes (pedaling at light load). The first 20 s of exercise (‘cardiodynamic’ Phase I) have not been included in the adjustment model. The VO$_2$ response, after the 20 s of exercise, was described from the two exponential components. Each mean response was described using the following equation:

$$V\cdotO_2(t) = V\cdotO_2 base + A_1 (1 - e^{-t/T\cdotA_1}) + A_2 (1 - e^{-t/T\cdotA_2})$$

Equation 1

The exponential model included breadths (A$_1$ and A$_2$), time constants ($\tau_1$ and $\tau_2$) and delayed times (TA$_1$ and TA$_2$). In order to determine the parameters of the best adjustments of the curves, a non-linear algorhythm of the minimum squares has been used (MatLab, version 6.5) which adopted the minimization of the sum of square errors as converging criterium. The A$_1$, to $\tau_1$ and the TA1 described the parameters concerned with the primary component, while the A$_2$, to $\tau_2$ and the TA$_2$ described the parameters concerned with the SC of the VO$_2$. Since the A$_1$ asintote value may represent a higher value than currently reached in the end of the 6 min of exercise, the SC value was defined as A$_1'$. In order to obtain the other values concerning the SC, pre-set intervals of time have been adopted, the $\Delta V\cdotO_2$: difference between the VO$_2$ values in the third (mean of the 20 initial s) and the sixth one (mean of the 20 final s) minutes of exercise and the $\Delta V\cdotO_2$: difference between the VO$_2$ values in the second (mean of the 20 initial s) and sixth (mean of the 20 final s) minutes of exercise. Besides these, the SC was obtained from the model used by Burnley et al. and adopted by Rossiter et al. In this model, the primary component was adjusted through a steady time (120 s). The first 20 s of exercise (‘cardiodynamic’ phase) have been excluded from the adjustment. The equation used was the following:

$$V\cdotO_2(t) = V\cdotO_2 base + A (1 - e^{-t/T\cdotA1})$$

Equation 2

SC was calculated as the difference between the mean value of the last 20 seconds of exercise and the value of the ‘primary’ breadth (120 initial s of exercise), obtained by the mono-exponential adjustment (SC$_{e^{-1}\cdotPC}$).

Statistical procedures

The data are presented as mean ± standard deviation (SD). The Kolmogorov-Smirnov test was adopted for the verification of the sample’s data normality and for comparison between the different methods of SC analysis, the variance analysis for repeated measurements (ANOVA) was applied. The Tukey Test was used to verify the position of the differences. The Pearson Correlation was used in order to verify the relationship between the different models. The statistical analyses were performed by the STATISTICA software (Statsoft, Inc, version 6.0). A significance level of p < 0.05 was established. The accuracy of the curve adjustments procedure was evaluated by the calculation of the determination coefficient ($r^2$).

RESULTS

The mean value of the VO$_2$max in the incremental test was of 62.4 ± 3.1 ml.kg$^{-1}$.min$^{-1}$ and the LT occurred at 66.3 ± 7.4% VO$_2$max. The maximal power reached was 339 ± 33 watts, the [La] peak was 9.3 ± 2.1 mmol.1$^{-1}$ and the maximal HR was 188 ± 6 bpm. The VO$_2$ concerning the 75%∆ in the end of the exercise was of approximately 95% of the VO$_2$max.

The SC breadths determined by the different methods and the remaining parameters obtained with the bi-exponential adjustment are presented in table 1. Figure 1 illustrates a bi-exponential adjustment of the VO$_2$ of a volunteer representative of the study.
The outcomes of this study demonstrated that the SC determined from the ΔVO₂6–2: 432 ± 126 ml.min⁻¹ and the ΔVO₂6–3: 279 ± 88 ml.min⁻¹ were significantly lower (31.8 and 57.9%, respectively) than the SC breadth obtained by the bi-exponential adjustment (A₂': 676 ± 136 ml.min⁻¹; p < 0.05). The SC₆–PC (719 ± 265 ml.min⁻¹), which used a steady time for adjustment of the primary component, was not significantly different from A₂'. There was a positive statistically significant correlation between ΔVO₂6–2 and the ΔVO₂6–3 (r = 0.79). The remaining correlations (A₁' versus ΔVO₂6–2: r = 0.07; A₂' versus ΔVO₂6–2: r = 0.11; A₂' versus SC₆–PC: r = -0.43; SC₆–PC versus ΔVO₂6–3: 0.58; SC₆–PC versus ΔVO₂6–3: r = 0.21) were not statistically significant. The mean value found for the r² was of 0.97, which showed a good adjustment of the bi-exponential model for the VO₂ data.

**DISCUSSION**

The main finding of this study was that the SC magnitude, when analyzed from pre-set time intervals ΔVO₂6–2 and ΔVO₂6–3, during exercises performed in very intense domain (75%Δ) was underestimated when compared with the bi-exponential models[20] and SC₆–PC[14]. The outcomes concerning the comparison between the bi-exponential model and the ΔVO₂6–3 are in agreement with the findings by Bearden and Moffatt[13], which found similar effects in exercises performed in intense domain (30%Δ).

During the last decades, mathematical models have been developed and improved with the aim to investigate the kinetics response of oxygen uptake[2,6]. Several methodologies have been used in order to determine the SC during the years[1,2,12]. Initially, Whipp and Wasserman[1] proposed the use of the ΔVO₂6–2, as a means to quantify the SC. Later, Barstow et al.[3] proposed the bi-exponential adjustment mathematical model as an important tool in the determination of the SC magnitude and it has become a widely used model over the years. More recently, in the trial to readapt the way of calculating the SC with pre-set time (ΔVO₂6–3), Koppo and Bouckaert[16] used the ΔVO₂6–3, based on previous data that the SC appearance could occur in time lower than 3 min (approximately from 70 to 120 s). Within this context, the present study demonstrated that even when using ΔVO₂6–3, the SC outcomes have been underestimated (31.8%) when compared with the bi-exponential adjustment model. The same situation occurred with the ΔVO₂6–3, which was underestimated in 57.9%. A possible justification for this result is the early appearance of SC, which in the present study had a mean of 74 s and a breadth of 58-95 s. Similar delay times for the beginning of the SC (TA₂) have been described in the literature[20]. Barstow et al.[20] in a study with trained and non-trained individuals suggest that the TA₂ does not depend on physical aptitude. Moreover, other studies demonstrate that the TA₂ also depends on the intensity of the exercise[21-26]. Thus, the results found in the present study suggest that this differentiated behavior of the SC may be present in other exercise intensities and among other populations. Another factor which could have generated the differences between the analyses is the time constant of the SC (τ), which reflects the change rate in the VO₂ concerned with the time, a factor which is not considered in the analysis with pre-set time. As suggested in the previous study by Bearden and Moffatt[13], the TA₂ and the τ responses are factors which may have significantly influenced the differences found between the analyzed models.

The SC₆–PC adapted model by Rossiter et al.[10] was recently used by Burnley et al.[14] with the purpose to detect small breadths of the SC, in a protocol used to study the SC attenuation in response to the practice of previous exercise. In the present study, the SC₆–PC values were similar to the values found by the bi-exponential adjustment model; on the other hand, they were statistically different from the analysis methods with pre-set interval (table 1). This second result may have been explained by the significant difference found between the ‘primary breadth’ obtained with the mono-exponential of the 120 initial s of exercise (3361 ± 491 ml.min⁻¹) and the breadth of the second (3592 ± 372 ml.min⁻¹) and third (3766 ± 360 ml.min⁻¹) minute of exercise (p < 0.05), values which are crucial for the SC calculation in these models. Another important point to be highlighted is the low correlation between the results obtained by the SC₆–PC models and bi-exponential adjustment (r = 0.43). This low correlation suggests that these analyses may not reflect the same behavior of the SC. Such fact also occurred with the remaining correlations between the different models, except between the ΔVO₂6–2 and the ΔVO₂6–3 (r = 0.79). In this case, there was a good correlation, suggesting that both analyses are presenting the same behavior of the SC.

There are important limitations in the use of mathematical models for the SC analysis which should be considered, especially when a steady state in the VO₂ is not reached, for instance, in exercises characterized as very intense. In these cases, a plateau in the VO₂ is not reached, what enables a totally accurate determination of the SC through the exponential adjustment. On the other hand, despite the SC determination through a pre-set interval be low reliable, since it does not consider the SC beginning, it has been suggested for the protocols of test which only performs one transition. In these cases there is a great biological variability between
breaths (‘noise’), which may make the process of interaction used by the mathematical models difficult. Further studies which search for improvement of the mathematical models are needed to minimize these limitations.

The origin of the kinetics SC of the VO₂ is not completely defined yet. However, some evidence suggests that the SC behavior may be concerned with the processes which lead to fatigue. The study of the mechanisms which involve the SC may provide important information on the tolerance to exercise in healthy and with pathologies populations. Within this context, the findings of the present study suggest that the use of different methodologies for the SC analysis (very intense domain) may generate distinct interpretations, and consequently may make the comparison of results among different studies difficult. Thus, more careful interpretation and comparison when different methodologies are used in the SC analysis are necessary in future investigations.

In conclusion, the data of the present study suggest that the SC magnitude during very intense exercise (75%Δ) may suffer influence of the method of analysis. The values of the SC obtained from the ∆VO₂6-2 and the ∆VO₂6-3 may be underestimated when compared to the values obtained by the bi-exponential adjustment models and with steady time of adjustment (120 s) of the ‘primary component’. Moreover, the present study suggests that the SC determination during very intense exercise, as well as the comparison between the studies, should be carefully analyzed, since there may be differences of results when different methodologies of analyses are used.

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