



Original research article

## Could the pulmonary $\dot{V}O_2$ off-transient response to maximal short-term exercise be better characterized by a triexponential decay?



Rogério Santos de Oliveira Cruz\*, Tiago Turnes, Rafael Alves de Aguiar, Fabrizio Caputo

Human Performance Research Group, College of Health and Sport Science, Santa Catarina State University, Florianópolis, Brazil

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## ABSTRACT

The off-transient pulmonary oxygen uptake ( $\dot{V}O_2$ ) response to a single bout of intense, exhaustive exercise has been characterized over the years by a second-order exponential model. In this paper, we report the superiority of a third-order exponential decay in describing the  $\dot{V}O_2$  off-kinetics after a maximal cycling exercise lasting 60-s. Our findings are in accordance with a biphasic pattern of phosphocreatine resynthesis when muscle pH is affected.

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### 1. Introduction

Since the seminal work of Margaria et al. (1933), in which the so-called oxygen debt was partitioned in two major components, the off-transient pulmonary oxygen uptake ( $\dot{V}O_2$ ) response to a single bout of intense exercise has been characterized by a second-order exponential model. The fast, initial portion of the debt, termed by Margaria et al. (1933) as alactacid, 'is paid at a much faster rate than the lactacid oxygen debt', presenting a short time constant  $\tau_1$  of  $\approx 40$  s (di Prampero et al., 1973; di Prampero and Ferretti 1999). This early response has been interpreted over the years to be primarily driven by phosphagen resynthesis and the replenishment of body O<sub>2</sub> stores (Knuttgen and Saltin 1972; di Prampero et al., 1973). However, this notion is challenged by the fact that (i) much of the late response (i.e., the slow, second component) may also be explained by the changes in phosphocreatine concentration after exercise cessation (Gaesser and Brooks, 1984), and (ii) the time course of phosphocreatine resynthesis exhibits a biphasic pattern when intracellular pH is affected (Harris et al., 1976; McMahon and Jenkins 2002).

Although clearly ahead of their times, the findings of Margaria et al. (1933) and much of subsequent key publications in the field were prior to the common advent of automatic gas exchange measurement capabilities, which can provide a more detailed analysis of respiratory gas exchange kinetics (Macfarlane, 2001). In this report, we provide an analytical evaluation on the kinetic behaviour of breath-by-breath  $\dot{V}O_2$  during recovery from intense, maximal cycling exercise lasting 60 s. By doing so, we found that the off-transient  $\dot{V}O_2$  response follows a third-, rather than second-, order kinetics during a recovery period of 45 min. Therefore, this paper should not be regarded as a hypothesis-driven study but rather a brief report conceived to show that the  $\dot{V}O_2$  recovery kinetics following a sprint cycle exercise could be better explained by a triexponential model, which could possibly be reconciling the discrepancies described above. Therefore, the aim of this study was to compare the goodness of two non-linear curve-fitting procedures, namely the traditional biexponential versus a triexponential model, in describing the  $\dot{V}O_2$  recovery kinetics after short-term cycling performance.

### 2. Methods

#### 2.1. Ethical approval

This study was carried out in accordance with the guidelines contained in the Declaration of Helsinki and was approved by the local ethics committee. The subjects were fully informed of any

\* Corresponding author at: Laboratório de Pesquisas em Desempenho Humano CEPID/UEDESC, Rua Pascoal Simone, 358, Coqueiros, Florianópolis, SC, CEP, 88080-350, Brazil.

E-mail address: [cruz.rso@gmail.com](mailto:cruz.rso@gmail.com) (R.S.d.O. Cruz).

risks and discomforts associated with the experiments before giving their written informed consent to participate.

## 2.2. Subjects

A group of 12 recreational male cyclists (body mass:  $79 \pm 8$  kg, height:  $177 \pm 5$  cm, age:  $26 \pm 5$  years, maximal pulmonary oxygen uptake:  $53 \pm 8$  mL O<sub>2</sub> kg<sup>-1</sup> min<sup>-1</sup>, means  $\pm$  SD) volunteered for this study. All subjects were healthy with normal pulmonary function and/or cardiovascular parameters, and none of them was receiving any pharmacological or specific dietetic treatment. All participants attended properly fed and hydrated and were instructed not to perform strenuous exercise and to abstain from alcohol on the day before each session. They were also asked to maintain the same dietary pattern throughout the experiment and to refrain from consuming caffeinated beverages for at least 2 h before each trial.

## 2.3. Design

This study is part of a straightforward crossover trial looking at the effects of limb ischemic preconditioning on long sprint cycling performance, which have been published elsewhere (Cruz et al., 2016). Of relevance to this report, subjects were required to attend the laboratory on four different occasions over a two-week period. Each subject was always tested at the same time of day to minimize the effects of diurnal variation, and all tests were interspersed with approximately 48 h of recovery. In each visit, subjects were always submitted to the same exercise protocol: a light warm-up followed by a self-paced sprint performance lasting 60 s. All cycle tests were performed on an electrodynamically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) in which mechanical power output was measured at a sampling rate of 5 Hz. The ergometer seat and handlebar were adjusted for comfort, and the settings were replicated for subsequent visits. Right after the end of the test, subjects slipped into a chair positioned just behind them and at the level of the seat of the cycle ergometer, where they remained seated for a period of 45 min for the measurement of the  $\dot{V}O_2$  recovery kinetics.

## 2.4. Sprint cycling performance

Before the sprint tests, subjects were submitted to a standard 12-min continuous warm-up protocol (set at 90% of the individual's first lactate threshold) and then were instructed to rest passively sitting on the bike. Five minutes after the warm-up, subjects performed a 60-s seated sprint cycle test. They were all allowed to choose their own pattern of work rate distribution (i.e. a self-paced strategy) in order to achieve their best possible performance, and instructed to reproduce the chosen pacing strategy on subsequent visits. The participants commenced tests from a stationary start after a 10-s countdown with the crank for their preferred leg positioned at 45° angle to the horizontal. During the sprint, they were verbally encouraged to give their best effort and informed of the time elapsed every 10 s, but were unable to see the display of the ergometer and were not informed of their performance at any stage until the end of the experimental protocol. The resistance applied on the pedals was that corresponding to 7.5% of the individual body weight (Wittekind and Beneke 2011). Therefore, performance (defined as the total mechanical work done:  $32 \pm 3$  kJ, mean  $\pm$  SD) was a direct reflection of the total number of revolutions attained within the fixed period of 60 s ( $87 \pm 8$  rpm, mean  $\pm$  SD). This test was found to be satisfactorily reliable: inter-trial coefficient of variation of 2% for total mechanical work.

## 2.5. $\dot{V}O_2$ off-kinetics

Throughout each testing protocol, cyclists wore a facemask, and respiratory gas exchange was measured breath-by-breath using an automated open-circuit gas analysis system (Quark CPET, Cosmed Srl, Rome, Italy). Gas analysers were always previously calibrated using ambient air and gases containing 16% oxygen and 5% carbon dioxide. The turbine flow meter used for the determination of minute ventilation was calibrated with a 3-L calibration syringe. The proper alignment between the gas flow and the associated gas fractions and the precise reconstruction of the gas signals measured at the mouth are of paramount relevance for a correct breath-by-breath approach (Capelli et al., 2011). In the Quark system, the end-expiratory point is sensed by determining when the flow signal passes below a threshold which is located a small increment above the zero-flow base line, and the expired volume is then derived by trapezoidal rule digital integration. As the O<sub>2</sub> partial pressure input signal occurs delayed in time with respect to the flow signal by (1) the sample line transport time delay and (2) the response time of the gas analyser, this total delay is determined during calibration. Importantly, the paramagnetic O<sub>2</sub> sensor contained in the Quark system is thermostated, while pressure and sampling rate are maintained constant by a feedback control system. Therefore, after the delay has been compensated, the gas concentration and the flow are appropriately in phase and can be multiplied and integrated at each sample interval to give  $\dot{V}O_2$  for each breath. It is worthy to note that the Quark CPET underwent factory validation using a metabolic calibrator capable of delivering cyclic air flows of known tidal volume, frequency, and gas makeup.

After collection, the breath-by-breath gas exchange data from each test were initially examined to exclude occasional errant breaths caused by coughing, swallowing, sighing, etc., which were considered not to be reflective of the underlying kinetics; i.e. values greater than four standard deviations from the local mean were omitted (Özyener et al., 2001). Firstly, the biexponential model was applied in order to characterize the  $\dot{V}O_2$  recovery kinetics in its traditional fast and slow components:

$$\dot{V}O_2(t) = A1e^{-t/\tau_1} + A2e^{-t/\tau_2} + \text{Rest}\dot{V}O_2$$

where A1 is the amplitude and  $\tau_1$  is the time constant of the fast component of the  $\dot{V}O_2$  recovery kinetics, and A2 and  $\tau_2$  are the amplitude and time constant of the slow component. Subsequently, an additional exponential decay term was inserted into the equation to yield a triexponential model and a new analysis was performed:

$$\dot{V}O_2(t) = B1e^{-t/\gamma_1} + B2e^{-t/\gamma_2} + B3e^{-t/\gamma_3} + \text{Rest}\dot{V}O_2$$

where 1 and 2 denote the two faster components identified in the present study, and 3 represent the slow component. The  $\gamma$  and B are the associated time constant and amplitude terms, respectively. For all analysis, a brief delay period (TD) containing 25 s (di Prampero et al., 1973) was omitted from the fitting field to obviate any distorting influence on the subsequent kinetics (Özyener et al., 2001; de Aguiar et al., 2015).

## 2.6. Blood lactate responses

Capillary blood samples (25  $\mu$ L) were taken from the hyperaemic earlobe (Finalgon, Thomae, Bieberach, Germany) and analysed for blood lactate concentration. Samples were collected immediately before the sprint test and then throughout recovery (every min from 0 to 10 min and every 2 min from 10 to 20 min) in order to determine the peak post-exercise value. All blood samples were stored in Eppendorf tubes containing 50  $\mu$ L of 1% NaF in a  $-20^\circ\text{C}$  environment. Later, samples were analysed by enzyme

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