

CENTRO
DE INVESTIGAÇÃO
FORMAÇÃO
INOVAÇÃO
E INTERVENÇÃO
EM DESPORTO
CIFI2D



UNIVERSITY OF PORTO

Faculty of Sport

Centre of Research, Education, Innovation and Intervention in Sport

Oxygen Uptake Kinetics and Bioenergetic Characterization around VO_{2max} intensity in Cyclic Individual Sports

Academic dissertation submitted with the purpose of obtaining a doctoral degree in Sport Sciences according to the Decree-Law 74/2006 from March 24th.

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Porto, December 2014

Sousa, A. (2014). Oxygen Uptake Kinetics and Bioenergetic Characterization around VO_{2max} intensity in Cyclic Individual Sports. Doctoral Thesis in Sport Sciences. Centre of Research, Education, Innovation and Intervention in Sport. Faculty of Sport, University of Porto. KEY WORDS: BIOENERGETICS, VO2 KINETICS, VO2MAX, TIME TO EXHAUSTION, CYCLIC SPORTS

Funding

This Doctoral Thesis was supported by the Portuguese Science and Technology Foundation (FCT) grants SFRH/BD/72610/2010 and PTDC/DES/101224/2008 (FCOMP -01-0124-FEDER-009577).















Acknowledgments

To my supervisor Prof. Dr. Ricardo Fernandes for the guidance in all moments of this journey and for the constant stimulus in the pursuit of my goals.

To Prof. Dr. João Paulo Vilas Boas for having always the door open and for that last word that was missing.

To Prof. Dr.^a Paola Zamparo for receiving me in her laboratory and for refreshing my ideas.

To Prof. Dr. Andrew Jones for receiving me in his laboratory and allowing me to go deeper into the VO₂ kinetics thematic.

To Prof. Dr. David Pendergast, Prof. Dr. David Pyne and Prof. Dr. Per-Ludvik Kjendlie for their co-authorships and revising my English.

To Prof. Dr. Pedro Figueiredo, my earlier fellow of graduate studies, for his intellectual participation, technical support and friendship.

To Prof. Dr. Leandro Machado for the help in math issues.

To all those that were directly and indirectly involved in the data collection, namely swimmers, rowers, runners, cyclists and their coaches, professors, fellow researchers and faculty staff.

To my true friends (they know who they are) for the support, friendship and academic debates.

To my family and especially Filipe, without whom, would have been impossible to achieve this goal.

This Doctoral Thesis is based on the following scientific papers, which are referred in the text by their Arabic and Roman numerals, respectively:

- **1**. Sousa, A., Figueiredo, P., Pendergast, D., Kjendlie, P.L., Vilas-Boas, J.P., Fernandes, R. (2014). Critical evaluation of oxygen uptake assessment in swimming. **International Journal of Sports Physiology and Performance**, 9, 190-202. DOI: 10.1123/ijspp.2013-0374.
- 2. Sousa, A., Figueiredo, P., Zamparo, P., Vilas-Boas, J.P., Fernandes, R. J. (2013). Anaerobic alactic energy assessment in middle distance swimming. European Journal of Applied Physiology, 113 (8), 2153-2158. DOI: 10.1007/s00421-013-2646-3.
- **3**. Sousa, A., Figueiredo, P., Zamparo, P., Pyne, D. B., Vilas-Boas, J.P., Fernandes, R. J. (2014). Exercise modality effect on bioenergetical performance at VO_{2max} intensity. **Medicine & Science in Sports & Exercise** (Epub ahead of print). DOI: 10.1249/MSS.0000000000000580.
- **4**. Sousa, A., Rodríguez, F., Machado, L., Vilas-Boas, J.P., Fernandes, R. J. Exercise modality effect on VO_2 off-transient kinetics at VO_{2max} intensity. Submitted for publication to **Experimental Physiology**.
- **5**. Sousa, A., Vilas-Boas, J.P., Fernandes, R. J. (2014). VO_2 kinetics and metabolic contributions whilst swimming at 95, 100 and 105% of VO_{2max} . **Biomed Research International**, 2014. DOI: 10.1155/2014/675363.
- **6**. Sousa, A., Figueiredo, P., Ribeiro, J., Silva, A., Cascais, C., Pelarigo, J., Vilas-Boas, J.P., Fernandes, R. J. (2014). Oxygen uptake kinetics and biomechanical behaviour at different percentages of VO_{2max}. In: Mason, B. (Ed.), *Proceedings of the* **XII International Symposium for Biomechanics and Medicine in Swimming** (pp. 521-525). Australian Institute of Sport. Canberra, Australia.

7. Sousa, A., Ribeiro, J., Sousa, M., Vilas-Boas, J.P., Fernandes, R. J. (2014). Influence of prior exercise on VO₂ kinetics subsequent exhaustive rowing performance. **PLoS ONE**, 9 (1), e84208. DOI: 10.1371/journal.pone.0084208.

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- I. Sousa, A., de Jesus, K., Figueiredo, P., Sousa, M., Vilas-Boas, J.P., Fernandes, R. J. (2013). Comparison between aerobic power parameters and different time-averaging intervals in swimming: an update. **Open Sports Science Journal**, 6, 22-25.
- II. Sousa, A., de Jesus, K., Figueiredo, P., Vilas-Boas, J.P., Fernandes, R. J. (2013). Oxygen uptake kinetics at moderate and extreme swimming intensities. **Brazilian Journal of Sporting Medicine**, 19 (3), 186-190. DOI: 10.1590/S1517-86922013000300008.

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 XLVI

Abstract

The oxygen uptake (VO₂) kinetics involves the study of the physiological mechanisms responsible for the dynamic VO₂ response to exercise and recovery. Its profile varies with the exercise intensity, but may be also influenced by the muscle contraction regimen and the ensuing muscle fibre recruitment profile itself, suggesting differences in-between exercise modes. The general purpose of this Thesis is to compare the VO₂ kinetics and the bioenergetic profile in-between different exercise modes - swimming, rowing, running and cycling – at 100% of maximal oxygen uptake (VO_{2max}) intensity time sustained exercise. The experiments contained two distinct protocols: (i) an incremental test to assess the velocity (vVO_{2max}) or power (wVO_{2max}) associated with VO_{2max} and (ii) a square wave transition exercise from rest to vVO_{2max}/wVO_{2max} to assess the time it could be sustained (Tlim-100%VO_{2max}). The VO₂ was continuously measured using a telemetric portable gas analyser (K4b², Cosmed, Rome, Italy) and VO₂ kinetics analysed using a double exponential curve fit. Bioenergetic profile was computed as the sum of its three components: aerobic, anaerobic lactic and anaerobic alactic (Ana_{alac}). Results pointed out that Tlim-100%VO_{2max} ranged between 187 to 245 s, being similar in-between exercise modes. Swimmers exhibited a slower response (21 ± 3 s) compared with rowers (12 \pm 3 s), runners (10 \pm 3 s) and cyclists (16 \pm 4 s), and the latter compared with runners, in the VO₂ on-kinetics. The amplitude of the fast-component VO₂ off-kinetics was higher in running compared with cycling (48±5 and 36±7 ml.kg⁻¹.min⁻¹, respectively) and the time constant of the same phase was higher in swimming compared with rowing and cycling (63±5, 56±5) and 55±3 s, respectively). The bioenergetic profile was similar between exercise modes with the exception of Analac contribution which was smaller in swimming (15.4±4.8 %) compared with the other sports (10.2±2.6, 7.6±1.2 and 10.4±3.9 %, for rowing, running and cycling, respectively). It is suggested that the mechanical differences between exercise modes influenced both the VO2 on and off-kinetics responses and bioenergetic profile at 100% of VO_{2max} intensity although similar Tlim-100%VO_{2max} was found. This analysis allowed providing new insights in the selection of the duration/ recovery of training sets at $VO_{2\text{max}}$ intensity in cyclic sports.

Key words: VO_2 kinetics, energy contribution, $VO_{2\text{max}}$, cyclic sports.

Resumo

A cinética do consumo de oxigénio (VO₂) consubstancia-se no estudo dos mecanismos fisiológicos responsáveis pela resposta dinâmica do VO2 ao exercício e recuperação. O seu perfil altera-se com a intensidade do esforço, mas também pode ser influenciado pelo regime de contracção muscular e consequente perfil de recrutamento muscular, sugerindo a existência de diferenças entre diferentes modos de exercício. O objectivo geral desta Tese é a comparação da cinética do VO2 e do perfil bioenergético entre diferentes modos de exercício – natação, remo, corrida e ciclismo – durante um esforço sustentado até à exaustão a 100% do consumo máximo de oxigénio (VO_{2max}). A parte experimental conteve dois protocolos distintos: (i) um teste incremental para avaliar a velocidade (vVO_{2max}) ou a potência (wVO_{2max}) associada ao VO_{2max} e (ii) um exercício rectangular de transição entre o repouso para a vVO_{2max}/ VO_{2max} para determinar o tempo que poderia ser sustentado (Tlim-100%VO_{2max}). O VO₂ foi medido continuamente por meio de um analisador de gás portátil telemétrico (K4b2, Cosmed, Rome, Italy) e a cinética do VO2 analisada através de um ajuste bi-exponencial. O perfil bioenergético foi avaliado como a soma das suas três componentes: aeróbica, anaeróbia láctica e anaeróbia aláctica (Ana_{alac}). Os resultados evidenciaram que o Tlim-100%VO_{2max} variou entre 187 e 245 s, sendo similar entre os modos de exercício. Os nadadores apresentaram uma resposta mais lenta (21 ± 3 s) em comparação aos remadores (12 ± 3 s), corredores (10 ± 3 s) e ciclistas (16 ± 4 s), e estes últimos em comparação com os corredores, na cinética on do VO₂. A amplitude da componente rápida da cinética off do VO₂ foi maior na corrida em comparação com o ciclismo (48±5 and 36±7 ml.kg⁻¹.min⁻¹, respectivamente) e a constante temporal desta mesma fase foi maior na natação em comparação com o remo e o ciclismo (63±5, 56±5 and 55±3 s, respectivamente). O perfil bioenergético foi similar entre os modos de exercício com a excepção da contribuição Ana_{alac} que foi inferior na natação (15.4±4.8 %)) em comparação com os outros desportos (10.2±2.6, 7.6±1.2 and 10.4±3.9 %, no remo, corrida e ciclismo, respectivamente). É sugerido que as diferenças mecânicas entre os

modos de exercício influenciaram as respostas na cinética on e off do VO_2 e o perfil bioenergético a 100% da intensidade do VO_{2max} apesar dos valores similares de Tlim-100% VO_{2max} encontrados. Esta análise permitiu fornecer novas percepções na selecção do tempo de duração/ recuperação das séries de treino à intensidade do VO_{2max} em desportos cíclicos.

Palavras chave: Cinética do VO₂, contribuição energética, VO_{2max}, desportos cíclicos.

Résumé

List of Abbreviations

τ Time constant of the PCr

 au_1/ au_{1on} Time constant of the fast component in the VO₂ on-kinetics response au_{1off} Time constant of the fast component in the VO₂ off-kinetics response au_2/ au_{2on} Time constant of the slow component in the VO₂ on-kinetics response

 τ_{200} Time constant of the 200 m at the intensity corresponding to maximum velocity

 au_{2off} Time constant of the slow component in the VO₂ off-kinetics response au_{lan} Time constant of the 200 m at the intensity corresponding to Lan_{ind}

[La⁻] Blood lactate concentrations

[La⁻]_{max} Maximal [La⁻]

 A_{1}/A_{1on} Amplitude of the fast component in the VO₂ on-kinetics response A_{1off} Amplitude of the fast component in the VO₂ off-kinetics response A_{2}/A_{2on} Amplitude of the slow component in the VO₂ on-kinetics response

Applitude of the 200 m at the intensity corresponding to maximum velocity

A_{20ff} Amplitude of the slow component in the VO₂ off-kinetics response

Aer Aerobic energy contribution

A_{lan} Amplitude of the 200 m at the intensity corresponding to Lan_{ind}

Ana_{alac} Anaerobic alactic energy contribution

Ana_{lac} Anaerobic lactic energy contribution

Ana $_{pcr}$ Ana $_{alac}$ determined from the PCr splitting in the contracting muscle Ana $_{recovery}$ Ana $_{alac}$ determined from the fast component of the VO $_2$ off-kinetics

ANOVA Analysis of variance

ATP Adenosine Triphosphate

C Energy cost of locomotion

CO₂ Carbon dioxide
DefO₂ Oxygen deficit

E_{tot} Total energy expenditure

E_{tot-inc} E_{tot} for each exercise step during the incremental protocols

E_{tot-max} Maximal metabolic expenditure

 $\mathsf{E}_{\mathsf{tot} ext{-tlim}}$ $\mathsf{E}_{\mathsf{tot}}$ during the square wave transition exercises

F F-Statistics

f Cohen effect size for F statistics

 $\begin{array}{ll} \mbox{HR} & \mbox{Heart rate} \\ \mbox{HR}_{\mbox{\scriptsize max}} & \mbox{Maximal HR} \end{array}$

HR_{peak} Peak HR

Lan_{ind} Intensity corresponding to the individual anaerobic threshold

MRT Mean response time
N Number of subjects

 O_2 Oxygen P Probability

PCr Phosphocreatine

R Correlation coefficient

R Respiratory exchange ratio r² Determination coefficient

SD Standard deviation

SI Stoke index SL Stroke length

SPSS Statistical package for the social sciences

SR Stroke rate

 TD_1/TD_{1on} Time delay of the fast component in the VO_2 on-kinetics response TD_{1off} Time delay of the fast component in the VO_2 off-kinetics response TD_2/TD_{2on} Time delay of the slow component in the VO_2 on-kinetics response

TD₂₀₀ Time delay of the 200 m at the intensity corresponding to maximum velocity

 TD_{2off} Time delay of the slow component in the VO_2 off-kinetics response TD_{lan} Time delay of the 200 m at the intensity corresponding to Lan_{ind}

Tlim-100%VO_{2max} Time to exhaustion at 100% of VO_{2max} intensity

VCO₂ Volume of CO₂ expired

V_F Ventilation

v_{max} Maximal velocity VO₂ Oxygen uptake

 VO_{2max} Maximal oxygen uptake

VO_{2peak} Peak VO₂

 vVO_{2max} Minimum velocity that elicits VO_{2max}

W_d Hydrodynamic resistance

wVO_{2max} Minimum power that elicits VO_{2max}

 η_m Mechanical efficiency η_p Propelling efficiency

Chapter 1. General Introduction

When a car starts moving, it performs mechanical work that is used to move along a specific road. In that moment, the energy injected into the car, coming from the transformation of chemical energy into mechanical energy via fuel oxidation into the engine, changes rapidly. The human motion can be compared to a car driven on a road. Whenever we got up from a chair, run to catch the bus, or even participate in any athletic event, the energy demands of our muscles change rapidly. In fact, in the transition from rest to movement, the chemical energy input increases allowing any physical activity.

The rate at which a subject can "switch on" its aerobic energy system to fulfil the requirements for the execution of exercise can determine if the subject will fatigue less rapidly and be better able to sustain the demands of that particular movement. Thus, the oxygen uptake (VO₂) kinetics can be considered to involve the study of the physiological mechanisms responsible for the dynamic VO₂ response to exercise (Jones & Poole, 2005a; Jones & Burnley, 2009), determining the instantaneous of aerobic and anaerobic energy transfer, the mixture and amount of substrate utilized and the tolerable duration of exercise (Burnley & Jones, 2007).

The investigation in this field relies in two possible VO₂ kinetics measurement approaches: (i) on the mouth, the most commonly used in the mid-1990s, and (ii) directly at the muscle level, the most challenging one. Although with the advent of a number of "new" technologies has meant that VO₂ kinetics and its putative control determinants can now be studied from a wider range of perspectives (although invasive and technically challenging), nowadays the pulmonary VO₂ kinetics technique remains important both for describing the response of the "whole" organism as well as in gaining insight into muscle VO₂ kinetics (Jones & Poole, 2005a). Inevitably, there is a degree of some contamination in measuring VO₂ at the mouth but the measurement of

pulmonary VO₂ is consider to broadly reflect muscle VO₂ during exercise (Barstow et al., 1990; Grassi et al., 1996) (Figure 1).

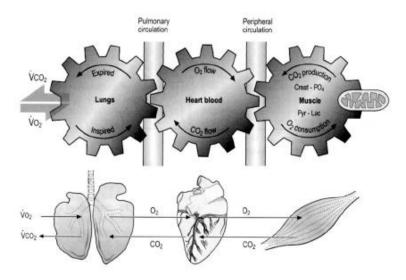


Figure 1. Schematic to illustrate the relationship between muscle VO_2 consumption and pulmonary VO_2 . O_2 is inspired from the atmosphere and delivered by the cardiovascular system to the working muscles, where it is consumed in the mitochondria to produce ATP. The deoxygenated blood is then transported back to the pulmonary circulation enabling muscle VO_2 to be estimated from pulmonary O_2 exchange. Adapted from (Jones & Poole, 2005a).

The pulmonary VO₂ following the onset of exercise has been well developed since the second half of the previous century (Whipp & Wasserman, 1972; Whipp et al., 1982) (Figure 2). The specialized literature describes that at the onset of constant work there is an early fast increase in VO₂, which is usually completed within the 15-25 s of exercise (Phase I – cardio-dynamic phase). This early response is attributed to the increase in cardiac output and thus pulmonary blood (Linnarsson, 1974; Whipp, 1987). After this initial phase, a rapid and exponential increase in VO₂, with a time constant between 20-45 s, occurs (Phase II – fast component/ fundamental phase). In this phase, pulmonary VO₂ kinetics largely reflects the kinetics of O₂ consumption in the exercising muscles (Barstow & Mole, 1987; Grassi et al., 1996; Rossiter et al., 1999). The last phase (Phase III) can vary with the exercise intensity domain in which the effort is taking place: (i) in the moderate intensity (encompasses all work rates that are below the gas exchange threshold, designated by some authors as anaerobic threshold), it is embodied by a steady state in VO₂ which

is achieved within 2-3 min in healthy subjects (Fawkner & Armstrong, 2003; Xu & Rhodes, 1999); (ii) in the heavy (comprises those work rates lying between the gas exchange threshold and the asymptote of the power-duration curve – critical power) and in the severe (comprises those work rates lying between the critical power and maximal oxygen uptake - VO_{2max} intensities) intensity domains this steady state phase III is exceeded due to the emergence of an additional slow component (Gaesser & Poole, 1996). However, the existence of this slow component has been evident for at least 40 years (Åstrand & Saltin, 1961), its source remains somewhat equivocal (Jones & Poole, 2005b). Recently, a fourth exercise domain was described for power outputs that lead to exhaustion before VO_{2max} is attained – extreme intensity -, where the VO₂ kinetics is characterized only by the development of an evident fast component and the slow component phenomenon is not observed (Hill et al., 2002).

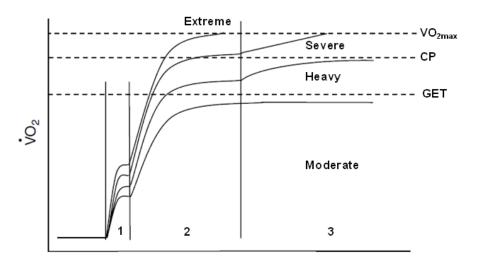


Figure 2. The three phases of the kinetic rise in VO_2 in response to a step change in exercise in four different exercise intensity domains: VO_{2max} – maximal oxygen uptake; CP – critical power; GET – gas exchange threshold. (Adapted from (Fawkner & Armstrong, 2003).

Among the many assumptions of the VO_2 kinetics thematic is that the VO_2 response to exercise can be altered considerably when different ergometers are used, suggesting that may be influenced not only be central and peripheral factors (cf. Fick equation – VO_2 =Q*(a - vO_2 difference), but also by the muscle contraction regimen and the ensuing muscle fibre recruitment profile itself (Jones & Poole, 2005b). The first study that compared the VO_2 kinetics in two

different exercise modes demonstrated that the half time (time necessary to achieve 50% of the total VO₂ response) for the on-transient phase was longer at the same absolute metabolic requirement for arm cranking compared to cycling, but was similar at the same relative exercise intensity (Cerretelli et al., 1977). Since this first pilot study, the vast majority of researches have been conducted using cycle ergometry and treadmill running and within the heavy and severe intensity domains (c.f. (Billat et al., 1998; Carter et al., 2000; Hill et al., 2003; Jones & McConnell, 1999; Roberts et al., 2005).

Considering all exercise intensities, VO_{2max} reveals itself as an important physiological parameter, expressing the subjects' maximal metabolic aerobic performance and being one of the primary areas of interest in training and performance diagnosis (Fernandes & Vilas Boas, 2012). In fact, the VO_{2max} assessment is the aim of many studies, but the capacity to sustain the minimum velocity that elicits VO_{2max} (vVO_{2max}) in time is a recent topic of research and has received little attention in cyclic sports (Fernandes et al., 2008). This capacity - Time limit (Tlim-100% VO_{2max}) - expresses the maintenance of that specific constant velocity to the point of the inability to maintain it, being accepted as a new complementary criterion to VO_{2max} and providing new insights in the selection of the intensity/duration of training sets (Billat & Koralsztein, 1996; Billat et al., 1994).

If using different ergometers and/or muscle contraction modes implies distinct VO₂ kinetic responses to exercise, it would be expectable that the time sustained at a particular velocity would diverge among different exercise modes. However, studies conducting comparisons between cycling, running, kayaking and swimming at 100% of VO_{2max} intensity (Billat et al., 1996), cycling, kayaking and swimming at 100% of VO_{2max} intensity (Faina, 1997) and comparing cycling and running at the severe intensity domain (Billat et al., 1998; Hill et al., 2003), have shown that the time sustained between these exercise modes is not different near and at 100% VO_{2max} intensity.

It has been widely appreciated that sustaining exercise beyond a few seconds depends on the appropriate supply and utilization of oxygen (Jones & Poole, 2005b). At submaximal intensities, the measure of VO₂ is sufficient to provide an overall measure of the total energy expenditure (E_{tot}) of the effort (Ferretti et al., 2011; Zamparo et al., 2011). However, at higher intensities (e.g. 100% of VO_{2max}), the determination of E_{tot} is more challenging, once the anaerobic contributions cannot be neglected (Figure 3). In fact, close to, or above VO_{2max}, a true steady state of VO₂ cannot be attained and the energy contributions other than the aerobic play a major influence role. Not taking into account these anaerobic contributions, results in an underestimation of E_{tot} affecting negatively the understanding of performance in maximal all out efforts (competitive speeds) of different durations (Zamparo et al., 2011).

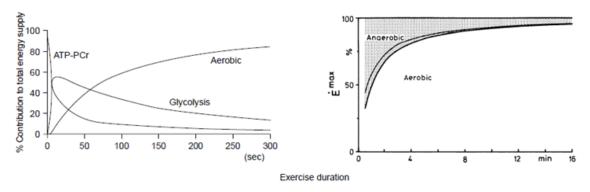


Figure 3. Left panel: Relative energy system contribution to the total energy supply for any given duration of maximal exercise (adapted from (Gastin, 2001). Right panel: maximal metabolic power is partitioned into aerobic and anaerobic components (adapted from (Di Prampero, 1986).

Although the above referred approach has been debated due to the many assumptions in the calculation of some of the parameters of interest (e.g. anaerobic lactic contribution), it has proven to be useful to estimate the energy demands during maximal and supra-maximal exercise in different exercise modes, particularly cycling (Capelli et al., 1998b), running (Di Prampero et al., 1993), kayaking (Zamparo et al., 1999; Zamparo et al., 2006) and swimming (Capelli et al., 1998a; Figueiredo et al., 2011; Zamparo et al., 2000). Nevertheless, no study aimed to compare the E_{tot} in-between different exercise modes.

The current Thesis addresses the comparison of VO_2 kinetics and the bioenergetic characterization in-between different exercise modes at 100% of VO_{2max} intensity time sustained exercise, with the experimental accomplishments presented in Chapters 2 to 8. Additionally, a general discussion was elaborated upon the results obtained from studies and with the reports of the specialized literature (Chapter 9). The main conclusions, suggestions for future research and references are presented in Chapters 10, 11 and 12, respectively.

From all exercise modes studied in this Thesis, swimming was the most challenging to explore, once the VO₂ assessment has been traditionally mainly conducted in laboratory-based treadmill running and cycle ergometry exercise. With the appearance of portable automated and lighter breath-by-breath gasanalysis systems, a more sustained and reliable bioenergetic research emerged. However, swimming never assumed before a key role in the energetics of human locomotion specialized literature. In this sense, it was our first purpose to provide a critical evaluation of the literature concerning VO₂ assessment in swimming, by describing the equipment and methods used and emphasizing the recent works conducted in ecological conditions - **Chapter 2**.

Essential to the utilization and interpretation of breath-by-breath technology in VO_2 related studies is the consideration of substantial inter-breath fluctuations of gas exchange during rest and exercise periods (Midgley et al., 2007). In fact, when studying the VO_2 response to a specific effort, it is essential to analyse the variability on the VO_2 imposed by the chosen sampling interval. Thus, the selection of optimal sampling intervals strategy is fundamental to the validation of the research findings, as well as to the correct training diagnosis and posterior prescription of the intensity of the training series. To guide subsequent work, it was our purpose to investigate the influence of different time averaging intervals on aerobic power related parameters, namely peak oxygen uptake (VO_{2peak}) and VO_{2max} - **Appendix I**. In this study we compared 10 subjects performing 200 m front crawl effort at supra-maximal intensities (VO_{2peak}) and

other 10 subjects performing the same event at maximal aerobic intensities (VO_{2max}), being hypothesized that the different intensities would significantly affect the maximal VO₂ values obtained for each averaging interval.

The magnitude and nature of the adjustment of VO₂ at the beginning of any physical exercise strongly depends on the intensity at which it is performed (Jones & Burnley, 2009). In **Appendix II**, it is presented a pilot study which aimed to analyse and compare the VO₂ kinetics at two considered very important intensities in swimming training, providing a better understanding on the aerobic and anaerobic capacities. It was conducted with 10 male swimmers of international level performing two tests: (i) 200 m front crawl at the intensity corresponding to the individual anaerobic threshold (Lan_{ind}) – moderate intensity, and (ii) an all-out 200 m front crawl corresponding to the extreme intensity. It was suggested that the VO₂ kinetics would be distinct in-between intensities.

As an important goal of this Thesis, the bioenergetic characterization of any exercise mode is ultimately determined by the ability of the muscle cells to provide energy by two distinct but integrated metabolic processes: the anaerobic and the aerobic pathways (Gastin, 2001). From the anaerobic contribution, the alactic system (Ana_{alac}) is less investigated, comparing to the lactic one, since its estimation is often based in the transition from rest to exhaustion phosphocreatine concentration decreasing in a particular active muscle mass (Ana_{pcr}) (Zamparo et al., 2011). Being often criticized by relying on some questionable assumptions, another approach has also been reported, based on the analysis of the fast component of the VO₂ off-kinetics curve (Ana_{recovery}). The study of **Chapter 3** proposed to determine and compare the contribution of this specific energy pathway, using 10 elite male swimmers performing an all-out 200 m front crawl. The comparison between the above referred methods (Ana_{pcr} vs. Ana_{recovery}) was conducted, being suggested that the Ana_{alac} would not be distinct in-between methods.

Nevertheless, analysis of different exercise modes near VO_{2max} intensity is scarce; the mechanical differences (e.g. muscular contraction regimen, active muscle mass, body position) could have a potential effect in the overall bioenergetics responses. Trying to gather the knowledge regarding VO_2 kinetics and E_{tot} , and applying it to different exercise modes, we purposed to assess Tlim-100% VO_{2max} in swimmers, rowers, runners and cyclists - **Chapter 4**. Being conducted with 40 subjects (10 swimmers, 10 runners, 10 cyclists and 10 rowers), who performed each a square wave transition from rest to 100% of VO_{2max} intensity, this study hypothesized that the performance at Tlim-100% VO_{2max} would not differ among exercise modes, but their different mechanical demands could elicit different VO_2 kinetics and energy expenditure patterns.

The study of VO₂ kinetics is not based solely in the on-transient phase, i.e., during effort, but the VO₂ response in the subsequent recovery (off-transient). This phase can provide additional information on gas exchange dynamics and aid the interpretation of the physiological events underpinning the VO₂ response in the on-transient response (Cleuziou et al., 2004). There are a reduced number of studies that have investigated the relationship between training and VO₂ off-transient kinetics performance and no study has compared yet the physiological response after exercise within different exercise modes. In fact, and similar to what was described for VO₂ on-kinetics, it is unknown whether the mechanical differences between exercise modes have a potential effect on the VO₂ off-kinetics. In **Chapter 5** of this Thesis will be presented a study with the purpose to compare the VO₂ off-transient kinetics response between swimmers, rowers, runners and cyclists, examining also the on/off symmetry in a time to exhaustion at 100% of VO_{2max} intensity. It was hypothesized that the type of exercise mode would contribute to distinct VO₂ off-transient kinetic patterns at 100% of VO_{2max} intensity, albeit the on- and off-transient kinetics will be symmetrical in shape.

Given the current level of interest in Tlim-100%VO_{2max} and VO₂ kinetics in cyclic individual sports, we found surprising that few studies have examined the time sustained at intensities around the VO_{2max} intensity, especially in swimming. In fact, being these intensities very close to the ones adopted in competitive events (within 3-5 min duration), we intended to study them more closely. In this sense, the **Chapter 6** aim was to compare the VO₂ kinetic responses and E_{tot} at different velocities around the VO_{2max} intensity. Trying to disseminate knowledge to other exercise modes rather than cycling and running, this study was conducted with 12 trained male swimmers who performed each 3 square wave transitions from rest to 95, 100 and 105% of VO_{2max} intensity. It was hypothesized that 5% of variability in swimming velocity would be insufficient to promote relevant changes in the VO₂ fast component kinetics, but would be sufficient to promote modifications in the VO₂ slow component phase.

After observing the variability induced in VO_2 kinetics behavior with only 5% change in swimming velocity, it was our aim to check if this variability would retain for the general biomechanical parameters. In this sense, the purpose of the study presented in **Chapter 7** was to compare, not only the VO_2 kinetics behavior, but also the biomechanical responses in 5 male national swimmers performing three square wave transition exercises from rest to different percentages of VO_{2max} intensity – 95, 100 and 105%. It was suggested that the different intensities performed would be sufficient to promote modifications in the VO_2 kinetics and biomechanical behaviour.

Prior exercise has been used extensively as an intervention to investigate the limitations of VO₂ following the onset of a subsequent exercise bout. In fact, it has been shown that the magnitude and nature of VO₂ responses are profoundly altered by prior exercise, by enhancing the cardiorespiratory and neuromuscular systems (Bailey et al., 2009). Given the widespread interest in the use of prior exercise, both for training and scientific purposes, it is surprising that research, once again, have focused mainly on the VO₂ kinetics response in cycling exercise. In this sense, in **Chapter 8** it will be presented a study

conducted in rowing with the purpose to examine the influence of prior exercise on subsequent VO_2 kinetics. Hence, 6 subjects performed 3 square wave transitions from rest to 100% of VO_{2max} intensity: (i) without prior exercise, (ii) with prior moderate exercise, and (iii) with prior heavy exercise, being suggested that the prior heavy exercise, but not the moderate condition, would turn VO_2 kinetics faster and the VO_2 primary amplitude higher, leading to longer exercise time at VO_{2max} .

Chapter 2

Critical Evaluation of Oxygen Uptake Assessment in Swimming

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Published on International Journal of Sports Physiology and Performance (2014), 9,190-202.

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Abstract

Swimming has become one important area of sport science research since the 1970s, with the bioenergetical factors assuming a fundamental performanceinfluencing role. The purpose of this study is to conduct a critical evaluation of the literature concerning the oxygen uptake (VO₂) assessment in swimming, by describing the equipment and methods used and emphasizing the recent works conducted in ecological conditions. Particularly in swimming, due to the inherent technical constraints imposed by swimming in a water environment, assessment of VO_{2max} was accomplished only in the 1960s. Later, the development of automated portable measurement devices allowed VO_{2max} to be assessed more effortless, even in ecological swimming conditions, but few studies have been conducted in swimming pool conditions with portable breathby-breath telemetric systems. An inverse relationship exists between the velocity corresponding to VO_{2max} and the time a swimmer can sustain it at this velocity. The energy cost of swimming varies according to its association with velocity variability. As, in the end, the supply of oxygen (which limitation may be due to central - O₂ delivery and transportation to the working muscles - or peripheral factors - O₂ diffusion and utilization in the muscles) is one of the critical factors that determine swimming performance, VO2 kinetics and its maximal values are critical in understanding swimmers' behaviour in competition and for develop efficient training programs.

Key words: Oxygen uptake assessment, direct VO₂ measurement, free swimming

Introduction

In the 1920s a sustained period of research in human exercise physiology emerged, and since then, one of the major topics has been the energetics of human locomotion and its contribution to athletic performance. Among other limits, the assessment of oxygen uptake (VO₂) for a better understanding of human bioenergetics is a key point focus of contemporary research in sport science. To better understand the basis of exercise physiology underpinning sports performance some historical details will follow.

In the late 1700s, Priestly and Scheele, independently, discovered the O_2 , and Lavoisier measured VO_2 during exercise by quantifying the decrease in O_2 in a chamber when a living animal was sealed within (DiMenna & Jones, 2009). Several decades later, in 1913, Amar assessed the effect of cycling ergometer exercise by analysing samples of expired air. Hill and Meyerhof, in 1922, discovered that the contracting muscle of a frog yielded a fast production of heat on the initial contraction, and a slow production later. Concurrently, Hill and Lupton in 1922 proposed the concept of maximal oxygen uptake (VO_{2max}) during exercise in humans (Hale, 2008). Since then, VO_{2max} assessment has been conducted primarily on laboratory-based treadmill running and cycle ergometry (Hale, 2008), but there has been a growing interest in its assessment using a variety of portable and laboratory equipment in other sports.

VO₂ uptake research in swimming was very scarce during the first half of the 20th century. (Liljestrand & Lindhard, 1920) collected expired air and other physiological parameters (e.g. blood pressure and cardiac output) in a subject swimming freely in a lake. (Karpovich & Le Maistre, 1940) studied the breaststroke, and later, (Karpovich & Millman, 1944) investigated five swimming techniques (front crawl, inverted crawl, side, breaststroke and butterfly), in an indoor swimming pool; however, none of these early studies were conducted in ecological/ real swimming conditions and/or used trained swimmers performing at (or near) competition paces. As there are some differences in swimmers'

bioenergetic and biomechanical characteristics when comparing swimming pool conditions and swimming flume (or other non-conventional methodologies as tethered swimming (Karpovich & Millman, 1944), different swimming levels (Libicz et al., 2005; Thompson et al., 2004), and sub-maximal and maximal velocities (Fernandes et al., 2006) it was expected that an underestimation of certain physiological limits, such as VO_{2max}.

The goal of competitive swimming is to obtain the fastest velocity during a race (v_{max}) , it depends on the swimmers maximal metabolic expenditure $(E_{tot-max})$, and their energy cost of locomotion (C):

$$v_{\text{max}} = E_{\text{tot-max}} / C \tag{1}$$

where, $E_{tot\text{-max}}$ can be computed based on measures/estimates of the aerobic and anaerobic energy contributions, and C is the amount of metabolic energy spent to cover one unit of distance. This metabolic energy depends on the mechanical efficiency (η_m) , the propelling efficiency (η_p) and the mechanical work to overcome hydrodynamic resistance (W_d) :

$$C = W_d / (\eta_p x \eta_m) \quad (2)$$

Some studies have examined W_d by towing a passive swimmer (Capelli et al., 1998) as well during swimming (Di Prampero et al., 1974). Similarly, methods have been developed to determine the ηm and ηp (Toussaint et al., 1988), but these methodologies have known technical limitations and are controversial. Thus, to understand the energetics of swimming, measurements of the $E_{tot\text{-max}}$ and C are the primary variables of interest. However, swimming measurements of aerobic and anaerobic pathways during swimming also have limitations imposed by the aquatic environment.

The aim of the review current study is to conduct a systematic review of the VO_2 assessment in swimming, including historic methods, but also evidencing and detailing studies that conducted VO_2 measurements in ecologic conditions. Complementarily, new perspectives and areas of study will be addressed. For this purpose, relevant literature on VO_2 consumption in swimming was located

via computer-generated citations: during December 2012, two online computer searches on PubMedTM and ScopusTM databases, and on the books of the International Symposiums on Biomechanics and Medicine in Swimming, were conducted to locate published research on VO₂ consumption. The key words used to locate relevant studies were "oxygen consumption", "maximal oxygen uptake", "aerobic capacity" and "swimming". Initially, all the articles obtained were selected by title; then, some of them were discarded after analyzing the abstract (excluding studies conducted exclusively on thriathletes, open water swimmers, water polo players, fin swimmers, divers and animals). Finally, an integral reading of the remaining studies was conducted, and those who were deemed not within the scope of the present review were also excluded.

Methods of VO₂ assessment in swimming

Cardio-respiratory limits have been traditionally assessed to study the energetics of many individual sports including swimming. However, VO₂ is difficult to measure due to technical constrains imposed by the swimming pool and the aquatic environment (Toussaint et al., 1988). Until the early 1960s, swimming research was limited by the availability of technology, particularly the inability to follow the swimmer along the pool, the tightness of the equipment, and the drag associated with the respiratory valve system used to collect expired gas. In more recent years, research has progressed as technology has envolved, and new methods have been used to assess VO₂ in ecologic/ real swimming conditions, allowing more reliable and valid results.

Standard Open Circuit Methods: the Douglas Bag

In 1911, Douglas invented the rubber-lined canvas bags for collecting expired air that allowed assessing VO₂ and CO₂ at rest and during running and cycling exercise (DiMenna & Jones, 2009; Hale, 2008). Nowadays, the Douglas bag gas exchange analysis is still considered the gold standard for VO₂ assessment (DiMenna & Jones, 2009), but in swimming this method has several limitations,

particularly on handling the bags, its permeability to the external air, and its posterior retrospective analysis determination of the relative CO_2 and O_2 concentrations (Bassett et al., 2001). In addition, to improve accuracy, some full breathing cycles are preferred, but, in a swimming pool setting, breathing cycle phases are not often counted. Hence, this method only allows determining the average VO_2 values during the period of collection chosen.

Furthermore, the Douglas bag method is difficult to conduct when swimming up and down the pool and turning at each end, as the hoses and valves pose limitations to the swimmer's technique and collection times. Thus, to overcome this difficulty, many investigators preferentially applied by collecting consecutive samples of expired air at the end of the swim (for the first 8, 20 or 40 s of the recovery period), with the VO₂ recovery onset obtain by backward extrapolating the O₂ recovery curve. It is assumed that these values represent the VO₂ of actually preceding swimming.

The 20 s recovery gas sample was firstly used by (Di Prampero et al., 1976) in speed skating, but only for two subjects performing under steady state intensity. Later, this method was shown to be valid and reproducible in treadmill cycling, treadmill testing and indoor track running (Léger et al., 1980). In addition, (Montpetit et al., 1981) used the backward extrapolation method to compare VO₂ values during free swimming and uphill treadmill running. Moreover, (Lavoie et al., 1983) and (Costill et al., 1985) showed that VO₂ measures obtained during maximal and sub-maximal tethered and free swimming could be predicted accurately from a 20 s recovery gas sample, providing and easy and reliable in-water VO₂ assessment. In fact, a high correlation between VO₂ values collected during swimming with those estimated through backward extrapolation was observed (r=0.92). These investigators lead to the conclusion that one single sample of expired air in the first 20 s of recovery period might was needed for VO₂ prediction during a maximal or sub-maximal effort (Costill et al., 1985). Conversely, it was reported that backward extrapolation method overestimates swimming VO₂, and, although being fairly relatively easy to apply in swimming, it has several sources of errors (Lavoie et al., 1983): (i) the time necessary for the swimmer to take out the mouth piece; (ii) the high possibility of leaks; (iii) the breath-by-breath analysis required has many potential errors; and (iv) the logarithmic back extrapolation requires that the VO₂ vs. time curve fits the logarithmic model, which is often not the case.

Measuring Devices

Although the Douglas bag method has been as the "gold standard" for gas exchange measurements for over a century, the need for faster and more efficient techniques that could be used during actual swimming lead to the development of fully-automated gas analysis systems. These apparatus accurately determine CO₂ and O₂ concentrations, and are used together in combination with gas flow meter recording in real time, allowing the calculation of VCO₂ and VO₂ using standard equations. The type of gas analysers vary in different laboratories and investigations, depending on size, price and principle of measurement.

Initially, gas analysing systems used a computerized metabolic system fitted with a mixing chamber (e.g. Sensormedics 2900 oxymeter, USA) measuring mixed dead space and alveolar gases (representative of the mixed expired gas) and giving time averaged values for respiratory variables (Bassett et al., 2001). This system was not originally used in a swimming pool, under ecologic/ real swimming conditions, but in a swimming flume (Holmér & Haglund, 1978), or in a circular pool (Pendergast et al., 2003). Although a flume allows setting the swim pace, the hydrodynamic resistance is probably not the same as in free swimming (Holmér & Haglund, 1978), as there is turbulent water flow (and not laminar) that likely affects how swimmers apply their force, which consequently, will influence their technique and VO₂. The VO_{2max} during free and flume swimming was seen to be highly correlated (Holmer et al., 1974), but it was also reported that swimming flumes may influence the VO_{2max}, the corresponding velocity at VO_{2max} (vVO_{2max}) and the time to exhaustion at vVO_{2max} (Fernandes et al., 2003).

Another limitation associated with the gas exchange assessment in a swimming flume deals with the instrumentation used as the valve and the connecting tube usually increase drag, and possibly leading to a change of body position during swimming may occur. Nonetheless, it use of the connecting tube allow standardization of procedures, and the evaluation of a swimmer's energetics more continuously for a long time (Bonen et al., 1980). Complementarily, determinations of VO_{2max} have also been performed during tethered swimming (Dixon & Faulkner, 1971; Magel & Faulkner, 1967), and although comparisons between this method and free swimming are difficult (due to the differences in body position and hydrodynamics), high correlations (r=0.90) were reported in college swimmers (Dixon & Faulkner, 1971; Magel & Faulkner, 1967).

In the first initial attempts to implement VO₂ measurement in ecologic/ real swimming conditions, systems were adapted from swimming flume and tethered swimming, and the apparatus were carried on a chariot along the side of the pool, accompanying the swimmer (Vilas-Boas, 1993). Over recent years, to overcome the weight of the oxymeter and the requirement of the research to push this apparatus, technological advances have resulted in portable, lightweight and automated metabolic gas analysis systems, which are widespread internationally, mostly using breath-by-breath analysis (e.g. K4b², Cosmed, Italy). The main advantage of these systems is the rapid sampling frequency, enabling the monitoring of changes in VO2 and VCO2 in short time intervals, and allowing breath-by-breath data collection. Furthermore, a more comprehensive examination of changes in VO₂ is possible, comparing to measurements systems with lower sampling frequencies (Astorino, 2009). However, the breath-by-breath gas acquisition can induce a significant variability of the VO₂ values acquired, not being clear what the optimal sampling frequency to use when assessing respiratory limits (Sousa et al., 2010).

Swimming valves and respiratory systems

In the early 1930s, Hans Rudolph designed and built respiratory valves specifically for use in pulmonary function studies with humans and animals.

These were later adapted for using during free swimming; however, by increasing the external power required for the swimmer (due to the additional hydrodynamic drag), they compromised the validity of the velocity and VO₂ measurements. To overcome this constraint, (Toussaint et al., 1987) developed a low-drag respiratory valve specifically designed for VO₂ measurements during swimming, designed so that the inspiration and expiration tubes were mounted in line and passed vertically over the subject's head; wit forward extended head area, this equipment did not add significantly to the total drag of the swimmer, enabling valid measurements of VO₂ and metabolic power output. Later, (Dal Monte et al., 1994) proposed a new respiratory valve system, in which gas collection tubes were aerodynamically designed, reducing even more the swimmer's drag.

The Toussaint's valve was initially adapted to Douglas bags, and later was used for direct VO₂ measurements (Vilas-Boas, 1993). At a later stage, the valve was rebuilt enabling breath-by-breath data collection with a portable system in laboratory conditions (Keskinen et al., 2000, 2003). Although its validation was conducted in dry land conditions (by comparing it values with derived with a standard face breathing mask), some systematic differences were reported and remained unclear (Keskinen et al., 2003). This system was also used in swimming pool conditions by (Rodríguez et al., 2001), proving to be a feasible method for measuring respiratory exchange responses at increasing speeds during free swimming. By assessing the validity of two models of a modified swimming snorkel of a small and large volume, another investigation indicated that both small and large volumes snorkels were valid devices for measuring breath-by-breath gas exchange limits across a wide physiological range (Rodríguez et al., 2008).

Despite producing a closer approach to training and competition conditions, the gas analysis measurements in free swimming conditions, still has some technical challenges, particularly the impossibility of implementation with water starts and allowing turns, and the inexistence of a proper underwater gliding

phase, as normally used; however, and despite these limitations, enables gas analysis which can provide a more reliable and representative assessment of the cardio respiratory limits during swimming. To provide a better understanding of the studies conducted in this thematic, an overview of the different methods used is presented in Table 1.

VO₂ assessment in free swimming

Initially, the main modes of exercise for establishing VO_{2max} were laboratory-based, using treadmill and cycle ergometers (Hale, 2008). These studies were fundamental in understanding basic physiological regulations, but an approach closer to competition conditions was needed. The appearance of automated portable devices for VO_2 kinetics allowed the assessment of VO_{2max} also in field conditions. This chapter describes these studies (see Table 2), aimed to assess C, VO_2 kinetics, biophysical parameters, time to exhaustion and training factors.

Energy Cost

To quantify swimming economy, one of the most relevant performance influencing factors is the assessment of C, usually determined in non ecological (not in swimming pool) conditions. (Vilas-Boas & Santos, 1994; Vilas-Boas, 1996) were pioneers in VO₂ measurement in free swimming, studying high-level breaststroke swimmers during a simulated swimming event, analysing and quantifying the relationship between speed fluctuations and C in three variants in breaststroke technique. They concluded that the undulating variant with overwater recovery of the arms was less economical than the underlying variant due to the higher intra-cyclic speed fluctuations. (Barbosa et al., 2005a; Barbosa et al., 2005b) reported that C increased significantly with increasing stroke rate (SR) and stroke index (SI), and that C tended to decrease with increasing stroke length (SL).

 $\textbf{Table 1.} \ Literature \ review \ of \ the \ different \ studies \ conducted \ in \ VO_2 \ assessment \ in \ swimming.$

Method	Authors
Studies conducted without direct VO ₂ assessment in specific ergometers or in free swimming conditions	Liljestrand and Lindhard (1920), Karpovich and Le Maistre (1940), Karpovich and Millman (1944), Van Huss and Cureton (1955), Andersen (1960), Astrand and Saltin (1961), Adrian et al. (1966), Costill (1966), Costill et al. (1967), Magel and Faulkner (1967), Dixon and Faulkner (1971), Holmér (1971, 1974a, 1974b, 1974c, 1975), McCardle et al. (1971), Holmér and Astrand (1972), di Prampero et al. (1974), Nadel et al. (1974), von Dobeln and Holmér (1974), Hay et al. (1975), Magel et al. (1967), Miyashita (1975), Kemper et al. (1976), Pendergast et al. (1977), Eriksson et al. (1978), Houston et al. (1978), Kasch (1978), Kipke (1978), Klissouras and Sinning (1978), Nomura (1979), Bonen et al. (1980), Holmér and Gullstrand (1980), Montpetit et al. (1981, 1982), Cazorla and Montpetit (1983), Kemper et al. (1982), Lavoie et al. (1983), Nomura (1982), Toussaint et al. (1982), Chatard (1985), Costill et al. (1985), Lavoie et al. (1985), Stallman et al. (1986), Bouzou et al. (1987), Kohrt et al (1987), Jang et al. (1987), Montpetit et al. (1987), Toussaint et al. (1987), Cazorla and Montpetit (1988), Montpetit et al. (1988), Toussaint et al. (1988), Smith et al. (1988), Beltz et al. (1988), Toussaint (1990), van Handel et al. (1988a, 1988b), Sharp and Costill (1990), Chatard et al. (1990), Bassett et al. (1991), Ribeiro et al. (1990), Chatard et al. (1991), Ripeiro et al. (1992), Chatard et al. (1992), Cappaert et al. (1991), Takahashi et al. (1992a, 1992b), Troup et al. (1992a, 1992b), Pakahashi et al. (1992a, 1992b), Toup et al. (1995), Wakayoshi et al. (1995), Capelli et al. (1998), Sardella et al. (1999), Cappaert (1999), Ondera et al. (1999), Termin et al. (2000), Zamparo et al. (2000), Rodriguez (2000), Demarie et al. (2001), Poujade et al. (2002), Zamparo et al. (2002), Rodríguez and Mader (2003), Kjendlie et al. (2004a, 2004b), Lafitte et al. (2000), Pendergast et al. (2005), Zamparo et al. (2006, 2008), Unnithan et al. (2009), Ratel and Poujade (2009), Mclean et al. (2010).
Studies conducted with direct VO ₂ measurement in free swimming conditions	Vilas-Boas e Santos (1994), Vilas-Boas (1996), Rodríguez et al. (2003), Fernandes et al. (2003), Cardoso et al. (2003), Millet et al. (2004), Fernandes et al. (2005), Libicz et al. (2005), Bentley et al. (2005), Barbosa et al. (2005a, 2005b), Fernandes et al. (2006a, 2006b, 2006c), Morais et al. (2006), Querido et al. (2006), Barbosa et al. (2006), Ramos et al. (2006), Balonas et al. (2006), Fernandes et al. (2008), Barbosa et al. (2008), Aspenes et al. (2009), Reis et al. (2010a, 2010b), Latt et al. (2010), Seifert et al. (2010), Figueiredo et al. (2011), Sousa et al. (2011a, 2011b), Reis et al. (2011)

Table 2. Oxygen consumption assessment studies conducted in free swimming with direct VO₂ measurements.

Study	Sample	Competitive Level	Protocol	Snorkel and Valve System	Oxymeter	VO₂ Sampling Interval	VO _{2max} / VO _{2peak} (ml.kg ⁻¹ .min ⁻¹)
Vilas-Boas and Santos (1994)	3M, 6F swimmers	National	3x200 Br 2 Submax, 1 Max	Toussaint et al. (1987)	Sensormedics 2900	20s	69.7±4.8, M 66.3±4.7, F
Vilas-Boas (1996)	13 F swimmers	National	3x200 Br 2 Submax, 1 Max	Toussaint et al. (1987)	Sensormedics 2900	20s	?
Rodríguez et al. (2003)	10M, 4F swimmers	High Level	100, 400-m Fc Max	Keskinen et al. (2003)	K4b²	5s	47.9±2.1and 53.3±3.3,M; 43.6±4.1 and 46.2±3.5,,F, for 100-m and 400-m, respectively
Fernandes et al. (2003)	15 M swimmers	High Level	Fc Inc Inter, Cont (@ vVO _{2max})	Toussaint et al. (1987)	Sensormedics 2900	20s	76.81±6.54 Inc Inter, 79.93 ± 6.39 Cont
Cardoso et al. (2003)	6F, 5M water polo players, triathletes, swimmers, students		Fc Inc Inter, Inc Cont	Toussaint et al. (1987)	Sensormedics 2900	20s	52.5±9.44 53.4±8.74, Inc Inter and Cont, all sample; 48.4±7.3, 49.0±6.5, Cont and Inc Inter, F; 56.2±9.6 59.0±5.5, Cont and Inc Inter, M
Millet et al. (2004)	10 M triathletes	High level	Fc Inc	Aquatrainer (Cosmed)	K4b ²	?	53.0±6.7
Fernandes et al. (2005)	11M, 12F swimmers	Experienced	Fc Inc Inter, Cont (@ vVO _{2max})	Toussaint et al. (1987)	Sensormedics 2900	20s	75.07±8.65, M 62.67±5.80, F
Libicz et al. (2005)	10 M triathletes	Trained	Fc Inc	?	K4b ²	20s	53.01±6.74, all sample
Bentley et al. (2005)	5M, 3F swimmers	National	Fc Inc 4x400m, 16x100m	Aquatrainer (Cosmed)	K4b ²	30s	55.7±5.8, 5x200; 51.2 ± 5.8, 4x400m; 52.0±8.4, 16x100m

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Barbosa et al. (2005a)	3M, 1F swimmers	International	Bt Inc	Keskinen et al. (2003)	K4b ²	?	?
Barbosa et al. (2005b)	3M, 2F swimmers 10 M	National	3x200m Bt 2 Submax , 1 Max	Keskinen et al. (2003)	K4b ²	?	?
Fernandes et al. (2006a)	swimmers, triathletes and physical education students 20 M swimmers	Low Level Highly Trained	Fc Inc Inter Cont (@ vVO _{2max})	Toussaint et al. (1987)	Sensormedics 2900	20s	52.1±6.5, low level swimmers; 69.9±9.3, highly trained swimmers
Fernandes et al. (2006b)	13M, 10F swimmers	High Level	Fc Inc Inter Cont (@ vVO _{2max})	Toussaint et al. (1987)	Sensormedics 2900	20s	75.14±8.20, M; 63.94±5.49, F
Fernandes et al. (2006c)	15M, 8F swimmers	Elite	Bt, Ba, Br and Fc Inc, Cont (@ vVO _{2max})	Keskinen et al. (2003)	K4b ²	5s	64.28±10.27, Fc; 66.78±11.40, Ba; 53.95±4.82, Bt; 63.21±8.14, Br
Morais et al. (2006)	15M, 14F swimmers	Trained	Fc Inc Inter	Toussaint et al. (1987)	Sensormedics 2900	20s	70.9±10.2, M ; 59.8±8.0, F
Querido et al. (2006)	5F, 2M swimmers	Elite	Fc Inc Inter, Cont (@ vVO _{2max})	Toussaint et al. (1987)	K4b ²	5s	?
Barbosa et al. (2006)	8F, 18M swimmers	International	5 Br(1F, 4M), 4 Bt (1F, 3M), 5 Ba (5M) and 12 Fc (6F, 6M) Inc	Keskinen et al. (2003)	K4b ²	?	?
Ramos et al. (2006)	7M, 3F swimmers	Elite	4 Bt (3M, 1F), 6 Br (4M, 2F) Inc, Cont (@ vVO _{2max})	Keskinen et al. (2003)	K4b ²	?	?
Balonas et al. (2006)	12 M swimmers	Elite	Fc Inc, Cont (@vVO _{2max})	Keskinen et al. (2003)	K4b²	5s	?

Fernandes et al. (2008)	3M, 5F swimmers	Elite	Fc Inc, Cont (@ vVO _{2max})	Keskinen et al. (2003)	K4b ²	5s	71.74±6.09, M; 59.80±9.97, F
Barbosa et al. (2008)	13M, 5F swimmers	Elite	Bt, Ba, Br and Fc Inc	Keskinen et al. (2003)	K4b ²	?	?
Aspenes et al. (2009)	8M, 12F swimmers (intervention and control group)		Fc (4 Submax), 4-6 min Inc	Toussaint et al. (1987)	Corta Metamax II	10s	55.00±5.80 (intervention group) 50.00±6.20 (control group)
Reis et al. (2010a)	29 M swimmers		Fc Inter, 3 all-out tests (100, 200, 400-m)	Aquatrainer (Cosmed)	K4b ²	20s	59.46±7.00, all sample
Reis et al. (2010b)	22 M swimmers		Br Inter, all-out test (100m, 200m)	Aquatrainer (Cosmed)	K4b ²	20s	63.87±12.28, 100m; 66.07±13.85, 200m
Latt et al. (2010)	25 M swimmers	Adolescents	Fc 100m all-out	Rodriguez et al. (2008)	K4b ²	10s	55.2±5.9
Seifert et al. (2010)	12 M swimmers	Elite	Fc Inc	Rodriguez et al. (2003)	K4b ²	5s	?
Figueiredo et al. (2011)	10 M swimmers	International	Fc 200m Max	Keskinen et al. (2003)	K4b ²	5s	?
Sousa ´ et al. (2011a)	10 M swimmers	International	Fc 200m Max	Keskinen et al. (2003)	K4b ²	5s	68.58±5.79
Sousa et al. (2011b)	8 M swimmers	International	Fc 200m Max	Keskinen et al. (2003)	K4b ²	5s	69±6.3
Reis et al. (2011)	21 M swimmers	Well trained	Fc Inc Inter Fc 2x7min Max	Aquatrainer (Cosmed)	K4b ²	30s	56±6.0

F=Female; M=Male; Max=Maximal; Submax=Submaximal; Inc=Incremental; Con=Continuous; Inter=Intermittent; Bt=Butterfly; Ba=Backstroke; Br=Breaststroke; Fc=Front Crawl; VO_{2max}= maximal oxygen uptake; VO_{2peak}=peak oxygen uptake; ?=Unknown data

Also, (Barbosa et al., 2005b) showed that energy expenditure increased linearly with increasing velocity, and that the increase in C was significantly associated with the increase in intra-cycle speed variation, leading to a less efficient swimming action. Afterwards, enlarging the field of evaluation, the four competitive swimming techniques were studied by this Portuguese research group, observing the primary outcomes: (i) significant relationships between energy expenditure and intra-cycle variation, C and velocity (Barbosa et al., 2005a); (ii) that front crawl was the most economic technique, followed by backstroke, butterfly, and breaststroke (Barbosa et al., 2006); and (iii) the manipulation of the SR and SL might be one of the factors through which energy cost can be altered for a given velocity (Barbosa et al., 2008). As competitive distances vary in swimming, (Seifert et al., 2010) studied the effect of swimming speciality (sprinters vs. long distance swimmers) in C (and in motor organization), observing that both groups had an increase in C of swimming with increasing velocity. For the same relative intensity, sprinters swam slower, showed a greater change in the arm coordination, and their swimming economy was lower compared to the long distance swimmers.

VO₂ Kinetics

The study of VO₂ kinetics is the study of the physiological mechanisms responsible for the dynamic VO₂ response to exercise and its subsequent recovery. Several studies have explored VO₂ swimming kinetics in laboratory and field settings, (Rodríguez et al., 2003) by connecting the swimming snorkel to a telemetric portable gas analyser, were the first to investigate VO₂ kinetics during 100 m and 400 m maximal swims. VO_{2peak} was significantly correlated with speed, in both distances, proving to be a good predictor of swimming performance. The VO₂ kinetics was faster during the 100 m compared to the 400 m, demonstrating the relationship between VO₂ kinetics and swimming intensity. The result is contrary to studies reported in other cyclic sports (running and cycling) who stated that VO₂ kinetics remains remarkably constant as exercise intensity increases. (Millet et al., 2004) compared VO₂ kinetics in cycling, arm cracking and swimming. The VO_{2peak} was higher in cycling,

followed by arm cracking and swimming. However, VO2 kinetics was slower in swimming, but there was similar amplitude of the VO2 slow component in all three exercise modes. Being this the first study to compare the VO₂ kinetics within different exercise modes where swimming was one of the sports considered, comparisons with previous literature is scarce. Comparing different methods of assessment of the VO₂ slow component, (Querido et al., 2006) reported that the utilization of the second minute of exercise for the estimation of its amplitude seemed to be a reasonable compromise when testing at vVO_{2max}, contrary to previous reports in swimming where the third minute of exercise was used. (Reis et al., 2010b) investigated this relationship in front crawl swimming, concluding that the absolute accumulated oxygen deficit error in the all-out bouts increased concomitantly with the distance. The relative error for its estimation was much lower in the 100 m event compared to the 200 m and 400 m. Later, the relationships between physiological limits and swimming performance in breaststroke were established (Reis et al., 2010a), concluding that testing with direct VO₂ measurement and blood lactate assessment clearly provides insights into the performance ability of breaststroke swimmers, using peak VO₂ and sub-maximal and supra-maximal blood lactate measurements. (Sousa et al., 2011b) characterized the VO₂ kinetics in the extreme intensity domain, during a maximal 200 m front crawl event, showing that the VO₂ kinetics response started with a sudden and exponential increase in VO2, with no slow component. (Sousa et al., 2011a) also studied the VO2 off-kinetics in the same intensity, reporting an asymmetry between the on- and off kinetic limits, although both periods were best characterized by a single exponential regression model. Also (Reis et al., 2011) characterized VO₂ kinetics, but in the heavy intensity domain, reporting that a faster VO2 kinetics allowed higher aerobic power outputs, and that the slow component is lower in swimmers with higher ventilatory thresholds.

Relationship of Metabolic and other Biophysical Parameters

As described in the introduction, other factors influence swimming performance and VO₂. Despite the fact that the importance of the study of Biophysics in

sports is nowadays well accepted, there is yet a lack of research trying to understand the relationships established between the bioenergetical and biomechanical variables in swimming. In this sense, (Lätt et al., 2010) analysed the relationships between the 100 m front crawl swimming event and biomechanical, anthropometrical and physiological limits. Results indicated that biomechanical factors (90.3%) explained most of the performance variability, followed by anthropometrical (45.8%) and physiological (45.2%) ones. Using also an integrative approach (joining biomechanical and physiological variables), and despite not having given percentages relating to any of the factors, (Figueiredo et al., 2011) investigated the 200 m front crawl. It was stated that the physiological partial contributions were 65.9%, 13.6% and 20.4%, for the aerobic, anaerobic lactic and anaerobic alactic systems, respectively. Moreover, and as it could be expected on biomechanical theoretical basis, fatigue developed along the 200 m since SR increased and SL and efficiency decreased.

Time Limit

The time to exhaustion at vVO_{2max} (Tlim-100% VO_{2max}) has been considered a relevant parameter as important as the VO_{2max} . Although its study only started in the last decade, investigations already were made on elite swimmers analyzing differences (Fernandes et al., 2003); in both genders (Fernandes et al., 2005); in two performance levels (Libicz et al., 2005); with biomechanical factors in the front crawl technique (Fernandes et al., 2006b); in all four competitive strokes (Fernandes et al., 2006a); with biomechanical factors in breaststroke and butterfly techniques (Ramos et al., 2006); regarding intra cycle variation of velocity in all competitive strokes (Balonas et al., 2006) and regarding ventilatory threshold (Morais et al., 2006). The primary outcomes showed an inverse relationship between: Tlim-100% VO_{2max} and vVO_{2max} (Fernandes et al., 2003; Fernandes et al., 2006a; Fernandes et al., 2008; Libicz et al., 2005); Tlim-100% VO_{2max} and energy expenditure for the entire group and for each gender (Fernandes et al., 2005); Tlim-100% VO_{2max} and SR in front crawl, butterfly and breaststroke techniques (Fernandes et al., 2006b; Ramos et

al., 2006); Tlim-100%VO $_{2max}$ and the velocity of anaerobic threshold (Fernandes et al., 2006a; Fernandes et al., 2008); Tlim-100%VO $_{2max}$ and intra cycle variation of velocity in the front crawl and backstroke techniques (Balonas et al., 2006) and Tlim-100%VO $_{2max}$ and body surface area and lactate production (Fernandes et al., 2008). Also the results of the studies reported direct relationships between: Tlim-100%VO $_{2max}$ and the VO $_{2}$ slow component (Fernandes et al., 2003; Fernandes et al., 2008); vVO $_{2max}$ and C (Fernandes et al., 2008; Libicz et al., 2005); Tlim-100%VO $_{2max}$ and SL and SI in front crawl, butterfly and breaststroke techniques (Fernandes et al., 2006b; Ramos et al., 2006) and Tlim-100%VO $_{2max}$ and intra cycle variation of velocity in the butterfly and breaststroke techniques (Balonas et al., 2006). Despite the previously described results, this thematic is still scarcely studied, and as a take-home message, it has not yet been related with the intra-cyclic variation of the horizontal velocity of the centre of mass and with the distribution of the percentage of energy contribution from each energy system.

Training factors

The VO_{2max} thematic has also been studied in order to improve some practical issues in swimming training. In 2003, (Cardoso et al., 2003) compared two incremental protocols (continuous and intermittent) for VO_{2max} and vVO_{2max} assessment. Both protocols were suitable for its assessment, since no significant differences regarding ventilatory parameters existed between each. (Libicz et al., 2005) examined two different types of interval training sets and reported that swimming sets of the same overall time duration at vVO_{2max} , but with different work-interval durations, leads to the same VO_{2peak} values.

Continuing this thematic array, (Bentley et al., 2005) conducted a study with the purpose to determine the time sustained near VO_{2max} in two interval training swimming sessions: 4x400 m and 16x100 m, concluding that the different work interval duration led to similar VO₂ and heart rate response (Aspenes et al., 2009) went on to study the impact of a combined intervention (maximal strength and high aerobic intensity interval endurance training) in competitive swimmers.

Dealing with two different groups, the authors concluded that the strength training group improved land strength, tethered swimming force and 400 m freestyle performance more than the control group. The progress in the 400 m performance was correlated (r=-0.97) with the improvement of tethered swimming force, but no change occurred in SL, SR, performance in 50 m or 100 m, swimming economy or VO_{2peak} during swimming.

Conclusions

The measurement of VO₂ during sporting activities goes back to the late 19th century, and was driven by simple curiosity and the desire to advance knowledge. Over time, it VO₂ measurement has progressed to the point where it has become more effortless practical and relevant to real swimming conditions. However, there are few studies attempting to assess VO_{2max} on elite swimmers in real swimming pool conditions (and not in treadmill running or ergometer cycling) and through direct measurements of VO₂. So, more research to be conducted in the future in ecological competition conditions is needed in the future, to achieve better advices guidelines for coaches and swimmers, regarding correct training diagnosis and training intensities prescription.

Acknowledgments

Portuguese Science and Technology Foundation PTDC/DES/101224/2008 (FCOMP-01-0124-FEDER-009577) and SFRH/BD/72610/2010.

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Chapter 3

Anaerobic alactic energy assessment in middle distance swimming

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Published on European Journal of Applied Physiology (2013), 113 (8), 2153-2158.

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Abstract

To estimate the anaerobic alactic contribution in a 200 m middle distance swimming trial by means of two different methods based: 1 - on the fast component of the VO₂ off-kinetics (Ana_{recovery}) and 2 - on the kinetics of maximal phosphocreatine splitting in the contracting muscle (Anaper). Ten elite male swimmers performed a 200 m front crawl trial at maximal velocity during which VO₂ was directly measured using a telemetric portable gas analyser; during the recovery period data were collected until baseline values were reached. No significant differences between the two methods were observed: mean ± SD values were 31.7 \pm 2.5 kJ and 32.6 \pm 2.8 kJ, for Ana_{per} and Ana_{recovery}, respectively. Despite the existence of some caveats regarding both methods for estimation of the anaerobic alactic contribution, data reported in this study indicate that both yield similar results and both allow estimate this contribution in supra-maximal swimming trials. This has important implications on swimming energetics since the non inclusion of the anaerobic alactic contribution to total metabolic energy expenditure leads to an underestimation of the energy cost at supra maximal speeds.

Key words: Swimming, anaerobic alactic contribution, recovery, phosphocreatine

Introduction

The capability to produce mechanical work in swimming, as in other forms of human locomotion, is ultimately determined by the ability of the muscle cells to provide energy by means of two distinct but integrated metabolic processes: the anaerobic and the aerobic pathways (Gastin, 2001). Compared to the number of studies regarding the aerobic contribution to total metabolic energy expenditure, research focusing on the assessment of the anaerobic contribution is scarce, with the energy cost of swimming being generally assessed at submaximal velocities by measuring only the oxygen consumption (VO₂). At maximal and supra-maximal speeds, not considering the anaerobic contribution leads to an underestimation of the total energy requirement, which is more evident at shorter exercise duration (Di Prampero, 1986; Di Prampero et al., 2011; Zamparo et al., 2011). Of the two components of anaerobic energy contribution, the lactic system is more often investigated, as it can be estimated based on the net increase of blood lactate concentration at the end of exercise by knowing/assuming a given energy equivalent for lactate (Capelli et al., 1998a; Capelli, 1999; Di Prampero, 1986; Di Prampero et al., 2011; Fernandes et al., 2006a; Figueiredo et al., 2011; Zamparo et al., 2000; Zamparo et al., 2011).

In the literature, the anaerobic alactic contribution (Ana_{alac}) for human locomotion was estimated by assuming that, in the transition from rest to exhaustion, phosphocreatine (PCr) concentration decreases by a known amount, and with a given kinetics, in the active muscle mass (Di Prampero et al., 2011; Zamparo et al., 2011). This method was applied before to running (Di Prampero et al., 1993), cycling (Capelli et al., 1998b), kayaking (Zamparo et al., 1999) and swimming (Capelli et al., 1998a; Figueiredo et al., 2011; Zamparo et al., 2000) but, since it is based on some questionable assumptions (the value of phosphocreatine concentration at rest, the time constant of the VO₂ on-response at the muscular level and the percentage of active muscular mass), there is still need to further investigate this topic. Another reported method to

estimate the contribution of the alactic energy sources is based on the analysis of the fast component of the VO₂ off-kinetics (Beneke et al., 2002; Di Prampero et al., 1970) since, in the post-exercise period, the greatest part of the "gross" O₂ debt has been interpreted as the energy necessary to rebuild the high energy phosphate compounds splitted at the beginning of exercise (Di Prampero & Margaria, 1968; di Prampero & Ferretti, 1999; Margaria et al., 1933). However, the fact that the VO₂ fast component during recovery is independent of lactic acid removal from blood has also been questioned.

The purpose of this study was to determine (and compare) the Ana_{alac} in a middle distance swimming effort (200 m front crawl maximal trial) by means of two different methodologies: based on the fast component of the VO₂ off-kinetics and based on the maximal PCr splitting in the contracting muscle.

Methods

Experimental Procedure

Ten front crawl elite male swimmers (20.8 ± 2.3 years old, 75.9 ± 6.2 kg, 1.84 ± 0.06 m, 10.5 ± 1.6 % of fat mass, 62.3 ± 4.2 kg of lean body mass, 112.7 ± 1.9 s of mean performance for long course 200 m freestyle, and a training experience > 10 years) volunteered to participate. In a 25 m indoor swimming pool (water temperature of 27° C), subjects performed, after a standard warmup, a 200 m front crawl bout at maximal velocity, starting in-water and using open turns without gliding. During the test, expired air was continuously measured through a telemetric portable gas analyser (K4b², Cosmed, Italy), connected to the swimmers by a low hydrodynamic resistance breathing snorkel and valve system (Baldari et al., 2013). During the recovery period, expired air was continuously measured until VO₂ basal values were reached. Expired gas concentrations were collected breath-by-breath, and afterwards, to omit errant breaths (e.g. swallowing and coughing) and to reduce the noise typical from this acquisition, data were edited according to previously described procedures (Fernandes et al., 2012).

Data Analysis

The Ana_{alac} was determined by means of two methods. Firstly, from the fast component of the VO_2 off-kinetics of the post-200 m maximal effort ($Ana_{recovery}$) (Beneke et al., 2002; Di Prampero et al., 1970). To determine the kinetics of the fast and slow components, a double-exponential model (equation 1) was used. For this purpose, a nonlinear least squares method was implemented in a MatLab Software for the adjustment of this model to VO_2 data (equation 1):

$$VO_{2}(t) = A_{0 \text{off}} + A_{1 \text{off}} * exp^{(-t-TD_{1 \text{off}})/\tau_{1 \text{off}}} + A_{2 \text{off}} * exp^{(-t-TD_{2 \text{off}})/\tau_{2 \text{off}}}$$
 (1

Where VO₂ (t) is the oxygen uptake at the time t; A_{0off} is the VO₂ at rest (ml.kg⁻¹.min⁻¹); A_{1off} and A_{2off} (ml.kg⁻¹.min⁻¹), TD_{1off} and TD_{2off} (s), and, τ_{1off} and τ_{2off} (s) are the amplitudes, the corresponding time delays and time constants of the fast and slow VO₂ off-components, respectively (for further details see Ozyener et al., 2001). The $Ana_{recovery}$ was then determined as the time integral of the VO₂ values derived from the off-fast component only, and expressed in kJ by assuming an energy equivalent of 20.9 kJ.lO₂⁻¹ (Di Prampero et al., 2011; Zamparo et al., 2011). An example of a VO₂ curve during (and after) the 200 m front crawl maximal effort is presented in Figure 1.

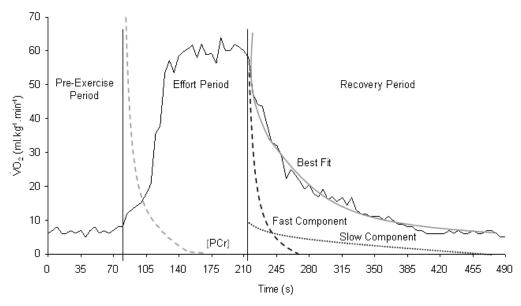


Figure 1. Individual oxygen consumption as a function of time curve, identifying pre-exercise, exercise and recovery phases. In the exercise phase, the phosphocreatine kinetics is represented as grey dotted line. In the recovery period, VO₂ fast and slow components of the recovery curve are represented as black continuous and dotted lines, respectively; the best fitting line during recovery is represented by a grey continuous line.

Secondly, the Ana_{alac} was assessed from the maximal PCr splitting in the contracting muscle (Ana_{pcr}) (Di Prampero et al., 2011; Zamparo et al., 2011) (equation 2):

$$Ana_{pcr} = PCr.(1 - e^{-t/\tau}) M \qquad (2)$$

where Ana_{pcr} is the anaerobic alactic contribution, t is the exercise time, τ is time constant of the PCr splitting at the onset of exhausting exercise (23.4 s, according to Binzoni et al. 1992), M is the body mass, PCr is the phosphocreatine concentration at rest. This latter was estimated assuming that, in transition from rest to exhaustion, its concentration decreases by 18.55 m-mole.kg⁻¹ muscle wet weight (in a maximally working muscle mass equal to 30% of the overall body mass). Ana_{pcr} was, thus, expressed in kJ by assuming an energy equivalent of 0.468 kJ.mole⁻¹ and a P/O₂ ratio of 6.25 (for further details see (Di Prampero et al., 2011; Figueiredo et al., 2011; Zamparo et al., 2011).

Statistical Analysis

Individual, mean and standard deviations (SD) computations for descriptive analysis were obtained for all studied variables. Measures of skewness, kurtosis and the Shapiro-Wilk test were used to assess the normality and homogeneity of the data. The non-parametric Mann-Whitney test was used to compare the anaerobic alactic contribution values obtained by the two above described methods. The efficiency method agreement was assessed also by linear regression analysis and the Bland–Altman plot. All statistical procedures were conducted with SPSS 10.05 and the significance level was set at 5%.

Results

The mean \pm SD values of A_{0off}, A_{1off}, A_{2off}, TD_{1off}, TD_{2off}, τ_{1off} and τ_{2off} were, respectively: 8.32 \pm 2.07, 43.90 \pm 3.15 and 4.74 \pm 2.97 ml.kg⁻¹.min-¹, and, 10.33 \pm 3.81, 57.44 \pm 10.75, 27.03 \pm 5.43 and 89.78 \pm 45.08 s, resulting in a post Ana_{alac} (calculated from the fast component of the VO₂ data) of 1.55 \pm 0.13 IO₂.

Table 1 shows individual and mean \pm SD values of the Ana_{alac} (kJ), as assessed by means of the Ana_{pcr} and Ana_{recovery}, with no differences observed between methods (p=0.48).

Table 1. Individual and mean \pm SD values of the anaerobic alactic contribution, considering the fast component of the post maximal exercise method (Ana_{recovery}), and the maximal phosphocreatine splitting in the contracting muscle method (Ana_{per}).

Swimmer	Ana _{recovery} (kJ)	Ana _{pcr} (kJ)
#1	30.5	30.9
#2	29.6	33.5
#3	35.7	28.2
#4	27.6	33.7
#5	34.6	35.1
#6	34.1	29.2
#7	33.2	28.8
#8	36.3	30.2
#9	32.0	33.7
#10	31.9	33.6
Mean ± SD	32.6 ± 2.8	31.7 ± 2.5

The Bland-Altman plot of the difference in Ana_{alac} values against its average value is reported in Figure 2. The average of the differences was low and close to zero (0.86), indicating that the two methods produced similar results. The corresponding limits of agreement (average \pm 1.96 SD) ranged between -9.86 and 8.13, indicating a small difference, in 95% of the subjects, between the two methods.

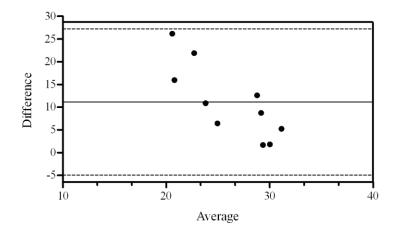


Figure 2. Bland-Altman plot of comparison between the two methods for assessing the Ana_{alac}. Average difference line (solid line) and 95% CI (dashed lines) are identified.

The influence of the Ana_{alac} to total metabolic energy expenditure (E_{tot} , kJ) and to the energy cost of swimming (C, kJ.m⁻¹) can be appreciated by utilizing the Ana_{alac} values assessed in this study to their estimation in a 200 m front crawl race. As shown by Table 2 (aerobic and anaerobic lactic contributions taken from Figueiredo et al. 2011), not taking into consideration the Ana_{alac} contribution in a 200 m front crawl race results in an 11% underestimation of both E_{tot} and C.

Table 2. Total metabolic energy expenditure (E_{tot} , anaerobic alactic contribution + anaerobic lactic contribution + aerobic contribution) and energy cost of swimming (C, as E_{tot} /velocity) when considering the anaerobic alactic contribution ($Ana_{recovery}$ and Ana_{pcr} methods) and when not considering it. Data of aerobic and anaerobic lactic contributions are taken from (Figueiredo et al., 2011).

oution Contribut J) (kJ)	ion Contribution (kJ)	E _{tot} (kJ)	(kJ.m ⁻¹)
6		286.6	1.43
7 43.4	210.6	285.7	1.43
		254.0	1.27
	(kJ)	(kJ) (kJ)	(kJ) (kJ) (RJ) 286.6 7 43.4 210.6 285.7

Discussion

The energy expenditure over short (50 and 100 m) and middle (200 m) competitive swimming distances necessarily implies the contribution of the anaerobic alactic energy source. However, the direct assessment of the Ana_{alac} through invasive methods (e. g. muscle extracts) is rarely done and non invasive alternatives have been used. In the current study two methods of determining the Ana_{alac} in a 200 m swimming maximal trial were used and compared, evidencing similar estimates of the anaerobic alactic component. Although none of these methods can be considered as a "gold standard" approach, and considering the inevitable limitations of both, the present study underlines the importance to estimate the Ana_{alac}, in order to assess energy expenditure and C at supra maximal speeds.

In the present study, the VO₂ off-fast component amplitude was found to be larger than that reported in the literature for other sports (Beneke et al., 2002; Capelli et al., 1998b; Di Prampero et al., 1993; Gastin, 2001; Guidetti et al., 2008; Roberts & Morton, 1978), which may be a result of the larger exercise intensity in our study, but also may be related to a sport-specific VO₂ answer. This higher VO₂ amplitude is justified by the high VO₂ values observed at the end of the effort (cf. Figure 1) after which VO₂ remains elevated to further decrease quickly (the VO₂ off-fast component). Indeed, the time delay value reported in this study is lower than that presented for exercises of lower intensity (Cleuziou et al., 2004; Dupont et al., 2010). Regarding the time constant, our results corroborate the recent literature conducted in other intensities domains (Cleuziou et al., 2004; Dupont et al., 2010; Özyener et al., 2001).

The Anarecovery method was first applied by (Margaria et al., 1933), analyzing the post excess VO₂ in the recovery period, being designated as alactic O₂ debt. In this post-exercise period, which is a measure of the "gross" O2 debt, a substantial fraction of the O₂ debt payment is independent of lactic acid removal from blood (di Prampero & Ferretti, 1999). So, the greatest part of this debt has been interpreted as the energy necessary to rebuild the high energy phosphate compounds (ATP and PCr) (Di Prampero & Margaria, 1968). The Anarecovery values reported in this study (~30 kJ) are lower than those reported for treadmill running (~50 and 42 kJ) (Di Prampero et al., 1970; Roberts & Morton, 1978) and cycling exercise (~40 kJ) (Beneke et al., 2002), but higher than those reported in ballet exercise (~22 kJ) (Guidetti et al., 2007; Guidetti et al., 2008). These differences could be attributed to different time durations of exercise to different exercise intensities and to different muscle masses activation patterns. In fact, in the present study, the % Ana_{alac} (~12%, considering the data of the aerobic and anaerobic lactic contributions taken from (Figueiredo et al., 2011), is similar to that reported in swimming for the same exercise duration and intensity (~14%) (Capelli et al., 1998a).

The other non-invasive approach (Ana_{pcr}) takes into account that the energy derived from complete utilization of PCr stores (during all out efforts) can be estimated assuming that, in the transition from rest to exhaustion, its concentration decreases by a known amount and with a given kinetic, in the active muscle mass (Di Prampero et al., 2011; Zamparo et al., 2011). This method has been utilized in several forms of human locomotion and in the majority of these studies it was assumed that all PCr stores were completely utilized at the beginning of supra-maximal exercise (Capelli et al., 1998a; Capelli et al., 1998b; Di Prampero, 1986; Di Prampero et al., 1993; Zamparo et al., 1999); in others studies, the kinetics of PCr breakdown was also taken into account (Capelli, 1999; Figueiredo et al., 2011; Zamparo et al., 2011). Data of Ana_{pcr} reported in this study are similar to those reported for swimming events of the same duration and intensity (Capelli et al., 1998a; Figueiredo et al., 2011; Zamparo et al., 2000).

Despite the similar values obtained by means of the two approaches, several limitations must be considered in both and these could change the estimation of Anaper. Regarding the Anarecovery method, the fact that the O₂ debt payment is independent of lactic acid removal from blood, is still a matter of debate. This could lead to some difficulties in interpreting the real alactic component of the O₂ debt, and consequently, this could affect the estimation of Ana_{pcr}. In this study it was assumed that, in the post-exercise period, the greatest part of this debt corresponds to the energy necessary to rebuild the high energy phosphate compounds (ATP and PCr), the time integral of the fast component being defined as the "alactic O₂ debt" (Margaria et al., 1933). This interpretation has been confirmed by experiments on man and on isolated muscle showing that PCr is rapidly resynthesized in the first 2 min after exercise. In fact, the analysis of the time course of the VO2 off-response allows the identification of an exponential component with a time constant of 30 s (Margaria et al., 1933; Roberts & Morton, 1978). The τ_{1off} values reported in this study (27 s) are in agreement with this assumption. However, not assuming that energy pathways

are sealed and work independently, has contributed to the alactic payment thematic still shaky.

In the Ana_{per} method, the assumption of given values in (i) the concentration of phosphocreatine per kilogram of wet muscle, (ii) the percentage of active muscular mass, and (iii) the associated time constant of the VO₂ on-response at the muscle level, can of course change the estimates of Ana_{per}. In this study, it was assumed that the muscle mass involved to perform the 200 m front crawl maximal trial was 30% of the total mass of the subject (i.e. 70% of muscle mass, about 23 kg) and that PCr concentration decreased, from rest to exhaustion, of 18.5 m-mole.kg⁻¹ of wet muscle mass. These values are close to the decline of PCr that has been measured immediately after exhausting exercise (Bangsbo et al., 1990) and thus these seem reasonable estimates of the maximal amount of energy that can be derived from maximal PCr splitting. Moreover, it was assumed that the amount of PCr used at the onset of exhausting exercise increases with the exercise duration following a first order kinetic function with a time constant of 23.4 s, as previously reported by (Binzoni et al., 1992).

Conclusions

Acording to our knowledge, there are no studies in the literature that attempted to compare different methods to assess the Ana_{alac}. In the present study, no significant differences in Ana_{alac} were observed between the two investigated methods, suggesting that both methods can be utilized to estimate Ana_{alac} at supra maximal competitive swimming speeds. The inclusion of the Ana_{alac} is necessary to adequately assess the total energy cost of swimming and our results suggest that C is underestimated (of about 10%) when the Ana_{alac} is not taken into account in a 200 m front crawl race. As a take-home message, it is important to attempt to estimate this parameter it in this type of efforts, despite

the existence of some caveats regarding both methods, and despite the absence of other (non invasive) approaches.

Acknowledgements

The authors acknowledge the Portuguese Science and Technology Foundation (FCT): PTDC/DES/101224/2008 grant. Ana Sousa acknowledges the Portuguese Science and Technology Foundation (FCT) for the PhD grant (SFRH/BD/72610/2010).

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Chapter 4

Exercise modality effect on bioenergetical performance at VO_{2max} intensity

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Published on Medicine & Science in Sports & Exercise (Epub ahead of print).

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Abstract

Purpose: A bioenergetical analysis of different exercise modes near maximal oxygen consumption (VO_{2max}) intensity is scarce, hampering the prescription of training to enhance performance. We assessed the time sustained in swimming, rowing, running and cycling at an intensity eliciting VO_{2max} and determined the specific oxygen uptake (VO₂) kinetics and total energy expenditure (E_{tot-tlim}). Methods: Four sub-groups of 10 swimmers, 10 rowers, 10 runners and 10 cyclists performed: (i) an incremental protocol to assess the velocity (vVO_{2max}) or power (wVO_{2max}) associated with VO_{2max} and (ii) a square wave transition exercise from rest to vVO_{2max}/wVO_{2max} to assess the time to voluntary exhaustion (Tlim-100%VO_{2max}). The VO₂ was measured using a telemetric portable gas analyser (K4b², Cosmed, Rome, Italy) and VO₂ kinetics analysed using a double exponential curve fit. E_{tot-tlim} was computed as the sum of its three components: aerobic (Aer), anaerobic lactic (Analac) and anaerobic alactic (Ana_{alac}) contributions. Results: No differences were evident in Tlim- $100\%VO_{2max}$ between exercise modes (swimming 187 ± 25, rowing 199 ± 52, running 245 ± 46 and cycling 227 ± 48 s; mean ± SD). In contrast, the VO₂ kinetics profile exhibited a slower response in swimming (21 ± 3 s) compared with the other three modes of exercise (rowing 12 ± 3, running 10 ± 3 and cycling 16 ± 4 s) (p<0.001). E_{tot-tlim} was similar between exercise modes even if the Analac contribution was smaller in swimming compared with the other sports (p<0.001). Conclusion: Although there were different VO₂ kinetics and ventilatory patterns, the Tlim-100%VO_{2max} was similar between exercise modes most likely related to the common central and peripheral level of fitness in our athletes.

Key words: exercise modes; time limit; oxygen uptake kinetics; energy expenditure

Introduction

The bioenergetics of cyclic sports have been studied since the 1920s, with a focus on locomotion and its contribution to athletic performance (Zamparo et al., 2011). The level of maximal oxygen uptake (VO_{2max}) as a marker of exercise intensity is considered one of the primary areas of interest in training and performance diagnosis, but the capacity to sustain it as a function of time has received little attention in cyclic sports (Fernandes et al., 2008). This capacity, usually expressed as a time limit (in this case, Tlim-100%VO_{2max}), quantifies the ability to maintain that specific constant velocity (or power output) (Billat et al., 1994). Most of the studies conducted reported similar values of Tlim-100%VO_{2max} between several exercise modes, such as cycling, kayaking, swimming, and running (Billat et al., 1996b) although kayakers performed longer than cyclists (Faina, 1997). However, no studies have analysed other physiological variables in parallel, and factors involved in determining Tlim-100%VO_{2max} across a range of sports remain unclear.

It is widely appreciated that sustaining exercise beyond a few seconds depends on the appropriate supply and utilization of oxygen (Jones & Burnley, 2005). Thus, differences in Tlim-100%VO $_{2max}$ between exercise modes could be explained by specific oxygen uptake (VO $_{2}$) responses to exercise. Most of the studies have compared only the physiological responses of different exercise modes, and very few have also addressed the kinetic parameters of the underlying (transient) VO $_{2}$ kinetic response. Studies have almost exclusively compared running with cycling (Carter et al., 2000; Hill et al., 2003; Jones & McConnell, 1999), suggesting that the time constant and VO $_{2}$ slow component in running is shorter compared with cycling. However, no studies have compared directly the VO $_{2}$ kinetics within other exercise modes.

Different performances in Tlim-100%VO_{2max} can also be derived from distinct energetic inputs at this intensity. At sub-maximal (moderate) exercise intensity, VO_2 is sufficient to provide the total energy expenditure (E_{tot}) after steady state

is achieved. However, at higher exercise intensities, not accounting for the anaerobic contribution, results in an underestimation of E_{tot} , with a negative effect on understanding the performance at this intensity. In fact, at short competitive distances where VO_2 steady-state cannot be attained, the determination of E_{tot} is scarce, with some research in swimming (Capelli et al., 1998a), running (Di Prampero et al., 1993), cycling (Capelli et al., 1998b) and rowing (Capelli et al., 1990), but no studies have compared directly E_{tot} between different exercise modes.

The mechanical differences between running and cycling have been attributed to the muscular contraction regimen (Millet et al., 2009). Although both activities are performed by muscle contraction of the lower limbs, the concentric work of cycling has a lower locomotion efficiency than running, which relies on a stretch-shortening cycle (Billat et al., 1998). On the other hand, the recruitment of a greater muscle mass could potentially compromise muscle perfusion (Saltin et al., 1998). Rowing engages most of the major muscle groups of the upper and lower body, such that performance, especially during heavy exercise, could be compromised compared with other exercise modes where a lower fraction of the total muscle mass is recruited (Secher, 1983). Of all the exercise modes, swimming requires larger energy expenditure, and thus, a lower overall efficiency of progression occurs (Di Prampero, 1986a). In addition, the horizontal position adopted by swimmers, with lower muscle perfusion pressure, may be a key difference between swimming and the other exercise modes (Koga et al., 1999).

Whether these different mechanical factors between exercise modes influence the overall bioenergetic responses is unknown. This analysis is needed to understand the physiological mechanisms that underpin performance at an intensity eliciting VO_{2max} . The purpose of this study was to compare the Tlim-100% VO_{2max} , VO_2 kinetics response and E_{tot} in swimmers, rowers, runners and cyclists. We hypothesized that the performance at Tlim-100% VO_{2max} would not differ among the exercise modes, but their different mechanical demands might

elicit different VO_2 kinetics and energy expenditure patterns. This analysis will provide new insights in the selection of the intensity/duration of training sets near the VO_{2max} intensity.

Methods

Subjects

Forty male subjects, highly trained (≥ 6 times per week) and regularly involved in competitive sports, participated in this study. The sample consisted of four groups of 10 x 400 m swimmers; 10 x 2000 m rowers; 10 x 1500-3000 m runners, and 10 x middle-distance road cyclists - their physical characteristics are presented in Table 1. All subjects signed an informed consent form, avoided strenuous exercise in the 24 h before each testing session and were well hydrated and abstained from food, caffeine and alcohol in the 3 h before testing. The Institutional Review Board of the University of Porto, Faculty of Sport, approved the study design.

Table 1. Physical characteristics for highly-trained male swimmers, rowers, runners and cyclists (Mean \pm SD). Significant differences between the other groups are shown by * ($p \le 0.05$).

Group	Swimmers	Rowers	Runners	Cyclists
Age (years)	17.0 ± 0.9*	26.4 ± 5.2	28.7 ± 4.4	23.3 ± 1.9
Height (m)	1.80 ± 0.06	1.80 ± 0.06	1.75 ± 0.06	1.78 ± 0.06
Body mass (kg)	70.3 ± 6.2	75.6 ± 4.0	61.9 ± 6.4*	70.1 ± 4.3

Experimental Design

Subjects were tested on two occasions. In the first session, VO_{2max} and the velocity (vVO_{2max}) or the power (wVO_{2max}) associated with VO_{2max} intensity were determined with a progressive incremental protocol until exhaustion. In the second session, all subjects completed a square wave transition exercise from rest to vVO_{2max} or wVO_{2max} until exhaustion to assess the Tlim-100% VO_{2max} . Verbal encouragement was given to motivate the subjects to perform their best

effort in the incremental protocols and for as long as possible during the square wave exercises.

Incremental Protocols and Square Wave Exercises

The incremental protocols varied according to the specificity of each sport: (i) swimmers performed an intermittent protocol using the front crawl technique in a 25 m swimming pool, with initial velocity set at the individuals' performance on the 400-m freestyle followed by seven increments of velocity (Fernandes et al., 2003). In-between 200-m steps, velocity was incremented by 0.05 m.s⁻¹ with a 30 s interval until exhaustion, controlled by a visual pacer with flashing lights at the bottom of the swimming pool (TAR.1.1, GBK-electronics, Aveiro, Portugal); (ii) runners performed an intermittent protocol on a 400-m outdoor track field, with the initial velocity set according to the individuals' performance on previous similar tests. The velocity was then increased by 1 km.h⁻¹ for each 800 m step with a 30 s interval until exhaustion, controlled by audio feedback emitted in markers placed at 100 m intervals; (iii) cyclists performed a continuous protocol with 2 min step duration, increments of 40 W between steps and a self-selected cadence between 70 and 90 rpm on a Power Tap trainer (CycleOps, Madison, USA). The initial power was set according to the subject's fitness level and performance in previous tests, and (iv) rowers performed an intermittent protocol of 2 min step duration, increments of 40 W and a 30 s interval between each step and a self-selected cadence ranging between 30 and 40 rpm on a rowing ergometer (Concept II, Model D, CTS, Inc.). Similar to cyclists, the initial power was set according to the subject's fitness level and previous testing performance. During the cycling and rowing protocols, the pre-defined power was controlled by visual feedback.

Approximately 24 to 48 h after, all subjects performed a square wave transition exercise from rest to their previously determined vVO_{2max} or wVO_{2max} until exhaustion to assess Tlim-100% VO_{2max} , using the same feedback stimulus as in the incremental protocol. In all exercise modes this test consisted of three distinct phases: (i) 10min warm-up exercise at 50% of the vVO_{2max}/wVO_{2max} ; (ii)

5 min recovery, and (iii) the maintenance of the previously determined vVO_{2max}/wVO_{2max} until exhaustion. The square wave transition exercise ended when the subject could no longer follow, for three consecutive occasions, the velocity/ power feedback stimulus.

Experimental Measurements

Respiratory and pulmonary gas-exchange variables were measured using a telemetric portable gas analyser (K4b², Cosmed, Rome, Italy). In swimming, this apparatus was suspended over the water (at a 2 m height) in a steel cable following the swimmer along the pool to minimize disturbance of the normal swimming movements. This equipment was connected to the swimmer by a low hydrodynamic resistance respiratory snorkel and valve system (Baldari et al., 2013). In-water starts and open turns, without underwater gliding, were used. In rowing, running and cycling exercises, subjects breathed through a traditional facemask (K4b², Cosmed, Rome, Italy). The measurement device was placed near the center of mass of the body adding only 800 g to the total mass of the subject. The gas analyzers in the system were calibrated before each test with gases of known concentration (16% O₂ and 5% CO₂) and the turbine volume transducer calibrated with a 3 L syringe. Heart rate (HR) was monitored continuously by a Polar Vantage NV (Polar electro Oy, Kempele, Finland) that telemetrically emitted the data to the K4b2 portable unit. Capillary blood samples (5 µl) for lactate concentrations ([La-]) were collected from the earlobe before the exercise, during the 30 s intervals (in the incremental protocols) and immediately at the end of exercise during the 1st, 3rd, 5th and 7th min of the recovery period (in both protocols), until maximal values ([La]max) were reached (Lactate Pro, Arkay, Inc, Kyoto, Japan).

Data Analysis

Errant breaths (e.g. caused by swallowing, coughing and signal interruptions) were omitted from the VO_2 analysis by including only those that were between VO_2 mean \pm 4 standard deviations. After this process, individual breath-by-breath VO_2 responses were smoothed using a 3 breath moving average and

time-average every 5 s. VO_{2max} was defined as a plateau in VO_2 despite an increase in velocity/power, [La⁻] \geq 8 mmol.l⁻¹, respiratory exchange ratio R \geq 1.0, heart rate >90% of [220 – age] and a volitional exhaustion (controlled visually and case-by-case) (Howley et al., 1995).

 vVO_{2max} and wVO_{2max} were estimated as the velocity or power corresponding to the first stage of the test that elicited VO_{2max} . If a plateau of less than 2.1 ml.min⁻¹.kg⁻¹ could not be observed, the vVO_{2max} and wVO_{2max} was calculated as previously described (Kuipers et al., 1985). Physiological measures of VO_{2max} , maximal heart rate (HR_{max}), respiratory quotient (R) and minute ventilation (V_E) were measured over the last 60 s of the exercise in the incremental protocol and square wave transition exercise.

The total energy expenditure for each exercise step during the incremental protocols ($E_{tot\text{-inc}}$) was determined via the addition of the net VO_2 and O_2 equivalents of the net [La $^-$] values, using the proportionality constant of 2.7 ml.kg $^{-1}$.mM $^{-1}$ for swimming (Capelli et al., 1998a) and 3 ml.kg $^{-1}$.mM $^{-1}$ for rowing, running and cycling (Capelli et al., 1998b). The estimated total energy expenditure during the square wave transition exercises ($E_{tot\text{-tlim}}$) was assumed to be the sum of the aerobic (Aer), anaerobic lactic (Ana_{lac}) and anaerobic alactic (Ana_{alac}) energies (Zamparo et al., 2011).

The Aer was calculated from the time integral of the net VO_2 . This energy contribution (ml O_2) was then expressed in kJ assuming an energy equivalent of 20.9 kJ.l⁻¹ (Zamparo et al., 2011). The Ana_{lac} was assessed through the energy derived from lactic acid production (equation 1):

$$Ana_{lac} = b.[la]_{bnet}.M \qquad (1)$$

where [La]_{bnet} is the net accumulation of lactate after exercise, b is the energy equivalent for [La] accumulation in blood (as described above) and M (kg) is the mass of the subject. This energy contribution (ml O₂) was then expressed in kJ assuming an energy equivalent of 20.9 kJ.l⁻¹ (Zamparo et al., 2011). The

Ana_{alac} was assessed from the maximal PCr splitting in the contracting muscle (equation 2):

Ana_{alac} = PCr.
$$(1 - e^{-t/\tau})$$
.M (2)

where Ana_{alac} is the anaerobic alactic contribution, t (s) is the exercise time, τ is time constant of the PCr splitting at the onset of exhausting exercise - 23.4 s (Binzoni et al., 1992), M (kg) is the body mass and PCr is the phosphocreatine concentration at rest. This latter value was estimated assuming that, in transition from rest to exhaustion, its concentration decreases by 18.55 mM⁻¹.kg⁻¹ muscle wet weight (in a maximally working muscle mass equal to 30% of the overall body mass). Ana_{alac} was thus expressed in kJ by assuming an energy equivalent of 0.468 kJ. mM⁻¹ and a P/O₂ ratio of 6.25 (Zamparo et al., 2011).

For the VO_2 kinetic analysis, the first 20 s of data after the onset of exercise (cardio-dynamic phase) was not considered for model analysis. To allow the comparison of the VO_2 response, data were modeled using a double exponential approach to isolate the VO_2 fast component response. A non-linear least squares method was implemented in MatLab Software (Mathworks, USA) to fit the VO_2 data with each model (equation 3):

$$VO_{2}(t) = V_{b} + A_{1} * (1 - e^{-(t-TD_{1}/\tau_{1})}) + A_{2} * (1 - e^{-(t-TD_{2}/\tau_{2})})$$
 (3)

where VO_2 (t) represents the relative VO_2 at the time t, A_0 is the VO_2 at rest (ml.kg⁻¹.min⁻¹) and A_1 and A_2 (ml.kg⁻¹.min⁻¹), TD_1 and TD_2 (s), and τ_1 and τ_2 (s) are the amplitudes, corresponding time delays and time constants of the fast and slow VO_2 components, respectively.

Statistical Analysis

Individual, mean and standard deviation (SD) computations for descriptive analysis were obtained and reported for all variables. Measures of skewness, kurtosis and the Shapiro-Wilk test were used to assess the normality and homogeneity of the data. The differences between pulmonary, metabolic, performance and kinetic variables in-between exercise modes were tested using a one-way ANOVA. To test the differences between the incremental

protocol and the square wave transition exercises in each exercise mode, a paired T-test was conducted. Simple linear regression and Pearson's correlation and partial correlation coefficients were also used to characterize the degree of association between the studied variables. All statistical procedures were conducted with SPSS 10.05 and the significance level was set at 5%. Magnitudes of standardized effects |f| where determined against the following criteria: small 0.2-0.5, moderate 0.5-0.8 and large >0.8.

Results

Pulmonary and metabolic parameters assessed during the incremental protocols and the square wave transition exercises are reported in Table 2 for each exercise mode. With the exception of cyclists who exhibited higher values of V_{E} in the incremental protocol compared to the square wave transition exercise, no substantial differences were observed between the final values in both tests.

Table 2. VO_{2max} (absolute and relative), HR_{max}, R, V_E and [Lā]_{max} obtained at the end of the incremental protocols and square wave transition exercises for swimmers, rowers, runners and cyclists (Mean \pm SD). Significant differences between both tests are shown by * and between groups by ^{Ro} (rowers), ^{Ru} (runners) and ^{Cy} (cyclists) (p<0.05).

		Swimmers	Rowers	Runners	Cyclists
VO _{2max}	Inc	4.24 ± 0.60^{Ro}	5.02 ± 0.28	4.33 ± 0.45^{Ro}	4.48 ± 0.59
(l.min ⁻¹)	Tlim	4.26 ± 0.61	4.89 ± 0.26	4.52 ± 0.68	4.35 ± 0.71
VO _{2max}	Inc	61.11 ± 5.24 ^{Ru}	66.84 ± 3.88	71.38 ± 4.15	64.53 ± 5.00^{Ru}
(ml.kg ⁻¹ .min ⁻¹)	Tlim	60.92 ± 5.46 ^{Ru}	64.95 ± 4.18 ^{Ru}	73.08 ± 5.14 ^{Cy}	63.04 ± 8.35
HR _{max}	Inc	183± 8	188 ± 10	185±8	182± 10
(beats. min ⁻¹)	Tlim	181± 5	187 ± 10	184± 7	178± 6
, D	Inc	$0.94 \pm 0.07^{Ro, Ru, Cy}$	1.05 ± 0.03^{Cy}	1.06 ± 0.06^{Cy}	1.18 ± 0.06
R	Tlim	$0.99 \pm 0.09^{Ro, Cy}$	1.09 ± 0.04 ^{Cy}	1.07 ± 0.07	1.20 ± 0.07^{Ru}
V_{E}	Inc	111.85 ± 23.10 ^{Ro, Ru, Cy}	177.58 ± 18.17	156.79 ± 18.23	166.12 ± 16.12*
(l.min ⁻¹)	Tlim	117.47 ± 18.38 Ro, Ru, Cy	172.24 ± 18.76 ^{Ru}	149.44 ± 17.14	153.23 ± 13.98
[La] _{max}	Inc	8.37 ± 1.07 ^{Cy}	10.64 ± 2.44	9.77 ± 2.09	11.33 ± 1.68
(mmol.l ⁻¹)	Tlim	$8.66 \pm 0.69^{Ro, Ru, Cy}$	11.03 ± 1.01	11.58 ± 2.02	11.19 ± 2.79

VO_{2max}: absolute and relative maximal oxygen consumption; HR_{max}: maximal heart rate; R: respiratory quotient; V_E: minute ventilation; [La⁻]_{max}: maximal blood lactic acid concentrations; Inc: incremental protocols; Tlim: square wave transition exercises.

Apart from HR_{max}, a comparison between exercise modes showed that swimmers often presented lower ventilatory and metabolic mean values (and a small to moderate standardized effect, f) in the (i) incremental protocol – absolute VO_{2max} (p<0.001, f=0.28), relative VO_{2max} (p<0.001, f=0.42), R (p<0.001, f=0.67), V_E (p<0.001, f=0.65), [La $^{-1}$]_{max} (p<0.05, f=0.27), and the (ii) square wave transition exercise - relative VO_{2max} (p<0.001, f=0.39), R (p<0.001, f=0.55), V_E (p<0.001, f=0.59), [La $^{-1}$]_{max} (p<0.05, f=0.29) than other sports. The relationship between E_{tot-inc} and the correspondent steps of the incremental protocol for all exercise modes is shown in Figure 1.

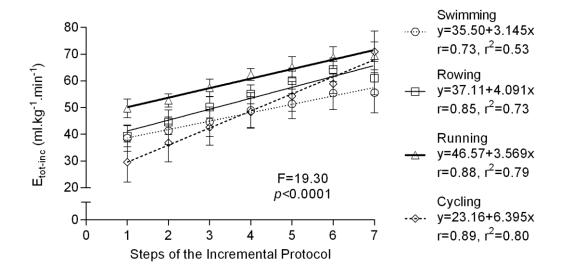


Figure 1. Relationship between $E_{tot-inc}$ and the correspondent incremental protocol steps for VO_{2max} and vVO_{2max}/WVO_{2max} assessment in all exercise modes. The regression equations, correlation and determination coefficients and the F test results are presented.

The mean value of $E_{tot-inc}$ ranged between swimmers 40-55, rowers 40-65, runners 50-70 and cyclists 30-70 ml.kg⁻¹.min⁻¹. Significant differences in the slopes of the relationship between $E_{tot-inc}$ and the corresponding steps of the incremental protocol were evident between all groups (Figure 1). The cyclists had the steepest slope of variation in $E_{tot-inc}$ as a function of power output in the incremental protocol, and swimmers, by having a slope less than 50% than cyclists, exhibited the shallowest variation.

The vVO_{2max} was 76% slower in swimming comparing to running (1.41 and 5.45 m.s⁻¹, respectively) while vVO_{2max} was very similar between rowing and cycling (402 and 392 W, respectively). The times sustained at 100%- vVO_{2max} and energy contributions (absolute - kJ and relative - %) obtained during the square wave transition exercises for all exercise modes are shown in Figure 2.

Although $E_{tot-tlim}$ mean values were similar between exercise modes, both the absolute (kJ) Ana_{lac} (p<0.001, f=0.43) contributions in swimming and the Ana_{alac} (p<0.001, f=0.49) contributions in running were lower compared with the other exercise modes. Moreover, the relative (%) Ana_{alac} contribution was higher in swimming compared with the other exercise modes (p<0.05, f=0.18); no substantial differences were observed regarding the time sustained at 100% of VO_{2max} in-between groups.

The VO₂ kinetic parameters obtained during the square wave transition exercises for swimmers, rowers, runners and cyclists are shown in Table 3. The A₀ was higher in swimming compared to running and cycling, and lower in running compared to rowing and cycling (p<0.05, f=0.29). In contrast, A₁ was lower in swimming compared to the other modes of exercise and lower in rowing and cycling compared to running (p<0.001, f=0.64). Swimmers exhibited slower VO₂ kinetics compared to the other exercise modes and cyclists a faster VO₂ kinetics compared to runners (p<0.001, f=0.67). The A₂ values (absolute and relative) were not substantially different between exercise modes.

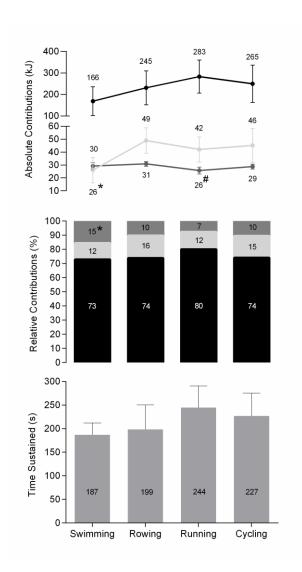


Figure 2. Upper panel: mean and SD aerobic (black), anaerobic lactic (light grey) and anaerobic alactic (dark grey) absolute contributions values (rounded to the closest unit - kJ) obtained during the square wave transition exercises for all exercise modes. Significant differences between swimming and the other groups shown by * (anaerobic lactic contributions) and between running and the other groups shown by # (anaerobic alactic contributions); middle panel: mean aerobic (black), anaerobic lactic (light grey) and anaerobic alactic (dark grey) relative contributions values (rounded to the closest unit - %) obtained during the square wave transition exercises for all exercise modes. Significant differences between swimming and the other groups shown by * (anaerobic alactic contributions); lower panel: mean and SD values of time sustained at 100%-VO_{2max} values (rounded to the closest unit) obtained during the square wave transition exercises for all exercise modes.

Table 3. Values for the VO₂ kinetic parameters in the square wave transition exercises for swimmers, rowers, runners and cyclists (Mean \pm SD). Significant differences between groups are shown by ^{Ro} (rowers), ^{Ru} (runners) and ^{Cy} (cyclists) ($p \le 0.05$).

Kinetic Parameters	Swimmers	Rowers	Runners	Cyclists
$A_0 (ml.kg^{-1}.min^{-1})$	23.6±3.1 ^{Ru,Cy}	20.6±3.6	18.5±4.4 ^{Ro,Cy}	18.4±2.1
$A_1 (ml.kg^{-1}.min^{-1})$	33.2±4.0 ^{Ro,Ru,Cy}	40.5±4.8	48.3±3.4 ^{Ro,Cy}	38.7±4.6
A ₁ - 95% confidence limits	(30.4-36.2)	(37.1-43.9)	(45.8-50.7)	(35.4-42.0)
TD ₁ (s)	9.6±2.8	6.9±3.4	9.3±4.1	12.4±4.6
$\tau_1(s)$	20.7±2.9 ^{Ro,Ru,Cy}	11.9±2.6	9.6±2.7 ^{Cy}	15.5±3.9
τ_1 - 95% confidence limits	(18.6-22.8)	(9.9-13.8)	(7.6-11.6)	(12.7-18.2)
A_2 (ml.kg ⁻¹ .min ⁻¹)	7.2±3.1	5.3±2.3	6.0±2.0	6.9±1.9
%A ₂ (%)	16.3±9.7	11.5±4.9	11.1±3.8	15.1±4.0
$TD_2(s)$	71.8±26.2	67.9±9.8	88.8±24.6	94.3±28.2
$\tau_2(s)$	125.8±27.9 ^{Ro,Ru,Cy}	57.9±40.7	60.5±43.4	65.9±45.5

 $A_0 = VO_2$ at rest; A_1 and A_2 = amplitudes of the fast and slow components, respectively; TD_1 and TD_2 = time delays of the fast and slow components, respectively; τ_1 and τ_2 = time constants of the fast and slow components, respectively; $%A_2$ = relative contribution of slow component to net increase in VO_2 .

Correlations between Tlim-100%VO_{2max} and VO_{2max} and E_{tot-tlim} and VO_{2max} in the square wave transition exercises are shown in Figure 3 for all groups combined. A moderate relationship was observed between the VO_{2max} reached during the square wave transition exercises and time sustained and between VO_{2max} and E_{tot-tlim}. No significant relationships were evident between VO_{2max} reached during the incremental protocols and the time sustained in the square wave transition exercises, when considering all subjects and within each exercise mode. Regarding the VO₂ kinetic parameters, and considering all subjects, moderate to large relationships were observed (τ_1 with A₁: r=-0.60, p<0.001; τ_1 with A₂: r=0.42, p<0.001), which lost their significance when the time sustained variable was controlled for partial correlation.

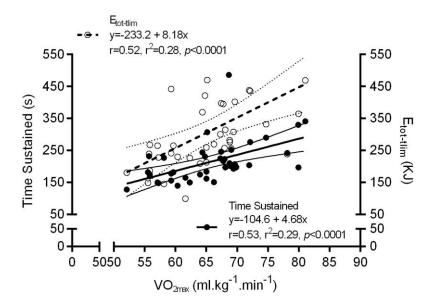


Figure 3. Relationships between VO_{2max} and time sustained (filled circles) and between VO_{2max} and $E_{tot-tlim}$ (unfilled circles) during the square wave transition exercises considering all subjects. The regression equations, determination and regression coefficients ($\pm 95\%$ confidence limits) and significance level values are identified.

Discussion

The purpose of this study was to compare the Tlim-100%VO_{2max} in swimmers, rowers, runners and cyclists and to determine their VO₂ kinetics response and maximal metabolic expenditure. The performance of the subjects at 100%-VO_{2max} intensity regarding the time sustained was similar corroborating the hypothesis that the Tlim-100%VO_{2max} would not differ among the exercise modes. Moreover, the hypothesis that their different mechanical factors would contribute to different VO₂ kinetics and metabolic patterns was also confirmed, with substantial differences observed in VO₂ kinetics (A₁ and τ_1) and metabolic profiles (Ana_{alac}) between exercise modes at 100%-VO_{2max} intensity.

 VO_{2max} is one of the most commonly measured parameters in the applied physiological sciences, and a variety of incremental exercise protocols are

used. However, no significant differences were reported between laboratory and field conditions (Berthoin et al., 1996) and between 1 to 4 min step durations (Kuipers et al., 2003). The primary criteria for evaluating the quality of an incremental test is the occurrence of a VO_{2max} plateau (Duncan et al., 1997), which usually occurs only for 50% of subjects (Doherty et al., 2003), a value closed to that observed in the current study (~40%). So, the achievement of secondary objective criteria (R, HR_{max} and [La⁻]) increased the likelihood that the highest VO₂ value achieved was the VO_{2max}.

The mean VO_{2max} values observed at the end of the incremental protocols are in accordance with data reported previously for competitive-level swimmers (Billat et al., 1996b; Faina, 1997) rowers (Sousa et al., 2014), runners (Billat et al., 1995; Renoux et al., 1999) and cyclists (Chavarren & Calbet, 1999). The VO_{2max} values were higher in running compared with cycling and swimming, with no other substantial differences between the other exercise modes, probably due to the use of larger muscle mass (Gleser et al., 1974). In addition, during running the movement of the arms and trunk demands a significant O2 requirement compared with cycling, where they have a lower contribution to the total exercise VO₂ (Hill et al., 2003). In the current study, lower values of [La]_{max} and V_E were reported in swimming compared with the other exercise modes (which can also explain the differences reported in the R mean values). Collectively these data indicate that a lower metabolic acidosis occurs in swimming, or that swimmers are less sensitive to it (Billat et al., 1996b). As expected, pulmonary and metabolic values obtained at the end of the incremental protocols were similar to those obtained at the end of the square wave transition exercises, with the exception of V_E for cyclists. This lack of differences between both protocols is reported in literature for the majority of sports, evidence of similar intensity in both tests (Billat et al., 1996b; Fernandes et al., 2003).

The relationship between E_{tot-inc} and the corresponding steps of the incremental protocol indicated that cyclists had a greater rise per step, followed by rowers,

runners and swimmers. The mean value of E_{tot-inc} ranged between 30 to 70 ml.kg⁻¹.min⁻¹ in cyclists and for each increment of 40 W in power, the metabolic expenditure increased by ~6 ml.kg⁻¹.min⁻¹, which was higher than rowing and running (~4 ml.kg⁻¹.min⁻¹) and swimming (~3 ml.kg⁻¹.min⁻¹). Thus, one notable difference between these exercise modes is the cost of exercise, suggesting that this measure depends not only of the aerobic and anaerobic contributions, but also the interval range of these contributions during incremental intensities. Although the pedaling frequency in cycling was controlled along the incremental protocol (70 to 90 rpm), it could have influenced the performance since the "energetically optimal cadence" (50-75 rpm) could not match the "freely chosen cadence" (80-100 rpm) (Millet et al., 2009). In this sense, during cycling exercise, and contrarily to rowing and running were the cadence has been described as having a lower effect on the exercise economy, the pedaling frequency should be strictly controlled.

In the current study, wVO_{2max} was not substantially different between rowing and cycling, suggesting that the higher active muscular mass attributed to rowing exercise (compared with cycling) did not influence the performance at 100% of VO_{2max}, as previously shown (Secher, 1983).. Since water is denser than the air, swimming requires a large energy expenditure to overcome the drag forces and has a lower overall efficiency of progression compared with running (Di Prampero, 1986b). Collectively, these factors lead to a large energy cost of transport, explaining the significant lower vVO_{2max} in swimming compared with running. Both wVO_{2max} and vVO_{2max} observed in the current study are in agreement with previous studies conducted in swimming (Fernandes et al., 2003) rowing (Sousa et al., 2014), running (Renoux et al., 1999) and cycling (Billat et al., 1996a; Chavarren & Calbet, 1999).

The lack of a substantial difference in Tlim-100%VO_{2max} between exercise modes is consistent with previous reports for other forms of locomotion (Billat et al., 1996b; Faina, 1997). Collectively, these studies demonstrate that the Tlim- $100\%VO_{2max}$ is independent of the exercise mode performed, as previously

suggested for the critical power/ velocity intensity (Carter & Dekerle, 2014). Although no substantial differences were observed in Tlim-100%VO_{2max} between exercise modes, we recommend total exercise duration of ~200 (for swimming and rowing) and ~250 s (for running and cycling) whenever VO_{2max} training intensity is to be enhanced. However, the mean time sustained values in the present study are lower than reported previously for swimming (Fernandes et al., 2003; Fernandes et al., 2008), running (Billat et al., 1995; Blondel et al., 2001) and cycling (Billat et al., 1996b; Faina, 1997). These differences are most likely explained by the innate ability and training status of the subjects among these studies. We are unaware of similar data for rowing exercise.

In relation to E_{tot-tlim}, the absolute Aer contribution (kJ) was similar between exercise modes; however, the Analac and Analac contributions (kJ) were found to be lower in swimming and running (respectively), compared with the other exercise modes. It should be pointed out that, whereas the lower Analac contribution (kJ) in swimming has to be attributed to the lower net accumulation of lactate after exercise (see Equation 1), the lower Ana_{alac} contribution (kJ) in running can be simply attributed to the lower runners' body mass, since no differences were found in Tlim-100%VO_{2max} (the exercise time, t) and since the same % of the overall body mass was assumed to correspond to the maximally working muscle mass (30%) in all exercise modes (see Equation 2). Despite the existence of some caveats regarding the Ana_{alac} assessment method used, it is important to attempt to estimate this energy pathway in maximal (or near maximal) efforts, as proven previously for swimming exercise (Sousa et al., 2013). With the exception of Ana_{alac} (%), which was higher in swimming compared with the other exercise modes, no substantial differences were observed in relative (%) contributions. In contrast to Tlim-100%VO_{2max}, it appears that the specific mechanical factors (e.g. muscle contraction regimen and the ensuing muscle fibre recruitment profile itself) might have had an impact on the exercise energy contribution, which in turn, depends essentially upon the type of exercise performed. In fact, it is well reported that the

proportion of type I muscle fibres is substantially lower in the muscles of the upper body compared to those of the lower body (Johnson et al., 1973). Knowing that arm stroke generates the most propulsive force in swimming (Deschodt et al., 1999), the higher Ana_{alac} found in swimming suggests a greater recruitment of type II muscle fibres, as reported during arm-crank exercise compared with cycling (Koppo et al., 2002).

Although VO₂ kinetics' is well described in the literature, especially in running and cycling exercises, some kinetics parameters are influenced by the training level of the subjects (Carter et al., 2000; Jones & McConnell, 1999). Therefore, the level of physical activity and performance of the subjects are likely to explain some inconsistencies between the current data and the literature, namely in estimates of swimming A_2 (Demarie et al., 2001; Reis et al., 2011), running τ_1 (Millet et al., 2003) and cycling A₁ and A₂ (Burnley et al., 2000; Pringle et al., 2003). A₁ mean values were lower in swimming compared with the other exercise modes. No comparisons are reported in literature between swimming and other forms of locomotion, however, these differences could be explained by the higher A₀ values observed in swimming. In fact, the normal constraints in the beginning of the exercise (entering the pool with the gas measurement apparatus) may confound the achievement of a baseline as low as those typically reported for laboratory testing. In addition, immersion in water induces a translocation of blood to the upper part of the body and a slower auto transfusion of fluid from cells to the vascular compartment, increasing stroke volume and cardiac output (Pendergast & Lundgren, 2009). These factors may also have contributed to higher A₀ values. The higher A₁ values in running compared with the other exercise modes can be explained by the higher VO_{2max} (Carter et al., 2000; Hill et al., 2003). In the present study, the absence of difference in A₁ values between rowing and cycling is also in agreement with previous reports (Roberts et al., 2005).

The τ_1 was longer in swimming compared with other sports, even though the swimmers were younger. Since τ_1 reflects the rate at which the VO₂ response

achieves the steady state, swimmers had a slower response towards the steady state VO₂. A key postural difference between swimming and the other exercise modes is that swimmers are in a horizontal position. It is known that in the supine position muscle perfusion pressure is lower, resulting in a longer τ₁ (Koga et al., 1999). The supine position also induces an increased venous blood return but reduces blood hydrostatic pressure in the legs (Libicz et al., 2005). Moreover, the inability to produce maximal muscle contractions (due to environment constraints) could limit a faster increase in VO2 kinetics. This finding suggests that swimmers, compared with athletes in the other exercise modes, would benefit more from a longer duration (~90 s) of exercise or training intervals whenever VO_{2max} training intensity is to be enhanced. In the current study, runners had a faster VO₂ kinetics compared with cyclists, a fact already reported during an exercise intensity which resulted in exhaustion in ~5 min (Hill et al., 2003). Although the explanation for this difference is not entirely clear, it may reflect differences in the type of muscle actions involved. In contrast to running, cycling involves high levels of muscular tension, which could lead to occlusion of vessels, and consequently, impede blood flow and oxygen delivery, delaying the VO₂ response. Running, on the other hand, has periods of low force production (e.g. when body is airborne), which should facilitate muscle blood flow and oxygen delivery, and consequently, speed the VO₂ response (Clarys et al., 1988). However, if muscle O₂ availability was reduced during running compared with cycling, because of greater recruitment of muscle mass, this did not impact significantly upon τ_1 . This outcome suggests that τ_1 is not altered significantly by the recruitment of a greater muscle mass, in contrast to VO_{2max}. Runners would benefit more from a shorter duration of training intervals (~50 s), compared with cyclists (~70 s), whenever VO_{2max} training intensity is to be enhanced.

The absence of differences in absolute A₂ in-between exercise modes highlights some inconsistencies in the running and cycling literature (Carter et al., 2000; Hill et al., 2003; Jones & McConnell, 1999), although no differences were reported between rowing and cycling (Roberts et al., 2005). Regarding the

relative A_2 (% A_2) our results do not support the literature where higher relative percentages of A_2 in cycling compared with running are well described (Carter et al., 2000; Hill et al., 2003; Roberts et al., 2005). The explanation for the VO_2 slow component is still a matter of debate and possibly influenced by muscle perfusion pressure and O_2 availability (Carter et al., 2000). In fact, the VO_2 slow component is positively related to the amount of work that can be performed above the critical power intensity, and therefore, with the anaerobic energy contribution to exercise (Jones et al., 2010). The fact that our subjects, independently of the exercise mode performed, have a similar training background (in their respective speciality in which they compete), is suggestive of an equivalent anaerobic energy profile, depending similarly on this energy pathway at 100% of VO_{2max} intensity. We interpret our results to indicate either that muscle O_2 availability to active muscle was well preserved in all exercise modes, or that any reduction in O_2 availability did not measurably impact the amplitude of the VO_2 slow component.

The positive relationship between VO_{2max} and Tlim-100% VO_{2max} in the square wave transition exercises reflects the dependency that the time sustained has on the underlying VO_{2max} irrespective of the mode of exercise. Although mechanical differences between exercise modes had a potential effect on the VO_2 kinetics response, the same physiological response (VO_{2max}) was observed at Tlim-100% VO_{2max} . In fact, the subjects who reached higher VO_{2max} values where the ones that reached exhaustion at a later time. Thus, the Tlim-100% VO_{2max} does not depend solely on the VO_{2max} reached during the incremental protocol, but instead, is linked to the VO_{2max} reached in the square wave transition exercises. Previous studies reported a negative correlation between both parameters in running (Billat et al., 1994), swimming (Billat et al., 1996a; Fernandes et al., 2008) and other exercise modes (Billat et al., 1996b; Faina, 1997). However, the poor correlation between VO_{2max} and Tlim-100% VO_{2max} was previously reported for running and swimming (Blondel et al., 2001; Fernandes et al., 2003).

In conclusion, when comparing the pulmonary and metabolic responses between the different exercise modes, no substantial differences were observed between the incremental and square wave protocols at an intensity requiring 100% of VO_{2max} intensity. However, swimmers exhibited lower pulmonary and metabolic values compared with the other exercise modes, at both sub-maximal and maximal intensities. For the incremental protocol, the slopes of the regression lines of Etot-inc and workload steps showed that cyclists had the greater rise in the slope and swimmers a lower slope. The maximal exercise time at 100%-VO_{2max} intensity as well as E_{tot-tlim} of all four exercise modes was similar, although swimmers had a higher Anaalac energy contribution compared with the other exercise modes. The kinetics profile was characterized by lower A₁ values in swimmers and a slower VO₂ response compared with the other exercise modes. Moreover, runners exhibited a faster VO₂ response compared with cyclists although the VO₂ slow component was observed in all exercise modes, no substantial differences in A2 values among them was evident. Swimmers presenting a slower VO₂ kinetics compared with the other exercise modes, would benefit more from a longer duration (~90 s) of each set of exercise or intervals in the intermittent VO_{2max} training work, as well as cyclists (~70 s), in comparison with runners (~50 s) In addition, coaches should consider the time sustained at each exercise mode (200 s and 250 s, for swimming and rowing, and, running and cycling, respectively) to specify the duration of work intervals at 100%-VO_{2max} intensity.

Acknowledgements

Ana Sousa acknowledges the Portuguese Science and Technology Foundation (FCT) for the PhD grant (SFRH/BD/72610/2010) and Prof. Leandro Machado for his contribution on the VO₂ kinetics modelling. The results of the present study do not constitute endorsement by ACSM.

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Chapter 5

Exercise modality effect on VO₂ off-transient kinetics at VO_{2max} intensity

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Submitted for publication on Experimental Physiology.

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Abstract

The kinetics of oxygen uptake (VO₂) during recovery (off-transient kinetics) to different exercise modes is largely unexplored, hampering the prescription of training/ recovery to enhance performance. The purpose of this study was to compare the VO₂ off-transient kinetics response between swimmers, rowers, runners and cyclists, during their specific mode of exercise at 100% of maximal oxygen uptake (VO_{2max}) intensity examining also the on/off symmetry. Four groups of 8 swimmers, 8 rowers, 8 runners and 8 cyclists performed (i) an incremental protocol exercise to assess the velocity or power associated to VO_{2max} (vVO_{2max} or wVO_{2max}, respectively) and (ii) a square-wave transition exercise from rest to vVO_{2max}/wVO_{2max} until volitional exhaustion. Pulmonary exchange parameters were measured using a telemetric portable gas analyser (K4b², Cosmed, Rome, Italy) and the on- and off-transient kinetics were analysed through a double exponential approach. For all exercise modes both transient periods were symmetrical in shape once they were both adequately fitted by a double exponential model. However, differences were found in the off-kinetic parameters between exercise modes: the amplitude of the fastcomponent VO₂ off-response was higher in running compared with cycling $(48\pm5 \text{ and } 36\pm7 \text{ ml.kg}^{-1}.\text{min}^{-1}, p<0.001, respectively)$ and the time constant of the same phase was higher in swimming compared with rowing and cycling (63±5, 56±5 and 55±3 s, p<0.001, respectively). Although both phases were well described by a double exponential model, the differences between exercise modes had a potential effect and contributed to distinct VO₂ off-transient kinetic patterns at 100% of VO_{2max} intensity.

Introduction

Oxygen uptake (VO₂) kinetics has been analyzed through mathematical modeling of the constant work rate exercise, both in on- and off-transient VO₂ responses (Whipp & Rossiter, 2005). The exponential nature of the response could indicate first or second order kinetics profiles (DiMenna & Jones, 2009), but first-order kinetics mandates on-off symmetry, which means that the change in VO₂ that occurs when the contractile activity is ceased must be a mirror image of that which occurred when it was commenced (Rossiter et al., 2005). In fact, this analysis has shown symmetry during moderate intensity exercise (under the lactate threshold, LT) since VO₂ exponentially increases at the onset of exercise (on-fast component) towards a steady state, decreasing rapidly at the offset of exercise (off-fast component) (Paterson & Whipp, 1991; Özyener et al., 2001; Scheuermann et al., 2001; Kilding et al., 2005). For heavy exercise (above the LT), the VO₂ on-dynamics is more complex and requires a secondorder model, since VO₂ is additionally increased (on-slow component) after the on-fast component (Burnley & Jones, 2007; Jones & Burnley, 2009). However, VO₂ at the offset of exercise shows only an off-fast component (Paterson & Whipp, 1991; Özyener et al., 2001; Scheuermann et al., 2001), allowing to conclude that VO₂ at this exercise intensity evidences an asymmetry between on- and off-kinetics phases. Although at the severe intensity domain (substantially above the LT) the VO₂ on- and off-kinetics both retain a twocomponent form (Özyener et al., 2001), evidencing a symmetry in second order kinetic profiles, the VO₂ kinetics has been less studied.

In recent years, research on VO_2 off-kinetics has focused mainly on the relationship with training and along lower intensities than VO_{2max} . Aiming to determine the influence of aerobic fitness level in excess post-exercise VO_2 , it was reported that trained subjects had faster relative decline during the fast-recovery phase compared with untrained subjects after heavy cycling exercise (Short & Sedlock, 1997). Studying the influence of heavy cycling exercise duration in VO_2 off-kinetics it was concluded that the off-transient kinetics was

not related to the exercise time and, therefore, was independent of the magnitude of contribution of the slow component of the on-transient kinetics (Cunningham et al., 2000). Conducted at the severe intensity (still at lower intensities than VO_{2max}), the effect of 4 weeks of intense interval-training running VO_2 off-transient kinetics was also examined, being concluded that this latter was accelerated with this type of intervention (Billat et al., 2002). The performance in repeated sprint tests was also related to the VO_2 off-kinetics at severe running exercise (120% of VO_{2max} intensity), thus strengthening the link found between VO_2 off-transient kinetics and the ability to maintain the performance during repeated sprints (Dupont et al., 2010).

The VO_{2max} intensity to have never been studied in VO₂ off-kinetics in any exercise mode may have compromised the recovery in these, since it is considered as one of the primary areas of interest in training and performance diagnosis. Moreover, the VO₂ off-kinetics study across different exercises modes is scarce and compared only upper body (arm cranking) and leg cycling exercise (McNarry et al., 2012), still being unanswered if different exercise modes could have distinct VO₂-off kinetic profiles. In fact, it is known that cycling and running differ greatly in terms of muscular contraction regimen. The concentric work of cycling may account for a lower mechanical efficiency than running, which relies on a stretch-shortening cycle (Bosco et al., 1987), resulting in a shorter on-fast component time constant (τ_{1on}) . The muscle contraction regimen utilized during exercise, including possible differences in motor unit recruitment, has been described as an important influencing factor in the manifestation of a higher on-slow component amplitude in cycling compared with running (Billat et al., 1998; Carter et al., 2000; Hill et al., 2003). Also, it has been suggested that when engaging a larger fraction of muscle mass, muscle perfusion could potentially be compromised (Saltin et al., 1998). However, no differences were found in the VO₂ on-kinetics response between rowing and cycling (Roberts et al., 2005), although a greater muscle mass is recruited in the former. Swimming, on the other hand, has a key postural difference compared with the other exercise modes since an horizontal position is adopted, which could result in a lower muscle perfusion pressure (Koga et al., 1999), although no studies are reported considering this exercise mode.

Whether these differences between exercise modes have a potential effect on the VO_2 off-kinetics, as have had in the VO_2 on-kinetics, response is unknown. This analysis, specifically the measurement of the off-transient time constant time, could be useful to further characterize and contribute to an athlete's physiological profile, enhancing the performance in each specific mode of exercise. The purpose of this study was to compare the VO_2 off-transient kinetics response between swimmers, rowers, runners and cyclists during their specific mode of exercise at 100% of VO_{2max} intensity, examining also the on/off symmetry of the VO_2 response. It was hypothesized that the type of exercise mode would contribute to distinct VO_2 off-transient kinetic patterns at 100% of VO_{2max} intensity, albeit the on- and off-transient kinetics will be symmetrical in shape.

Methods

Subjects and Ethical Approval

Thirty two male subjects (8 swimmers, 8 rowers, 8 runners and 8 cyclists), whose main physical characteristics (mean ± SD) are presented in Table 1, participated in this study. To be included in this study subjects had to be highly trained (≥ 6 training sessions per week, 2 h each session duration) and had to be regularly involved in competitive events at national level for at least 3 years. All participants avoided strenuous exercise in the 24h before each testing session, and were well hydrated and abstained from food, caffeine and alcohol in the 3 h before testing. The protocols were conducted at the same time of the day for each subject and were separated by, at least, 24 h. The Institutional Review Board of the University of Porto, Faculty of Sport, approved the study design and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Table 1. Physical characteristics for highly-trained male swimmers, rowers, runners and cyclists (Mean \pm SD). Significant differences between each group are indicated, as compared to rowing (Ro), running (Ru) and cycling (Cy) (p<0.05).

Group	Swimmers	Rowers	Runners	Cyclists
Age (years) Height (m) Body mass (kg)	18.6 ± 3.4 Ro,Ru,Cy 1.78 ± 0.06 70.8 ± 6.4 Ru	26.4 ± 3.1 1.79 ± 0.05 74.6 ± 4.1 ^{Ru}	25.1 ± 2.5 1.75 ± 0.07 61.4 ± 7.2	24.5 ± 3.3 1.77 ± 0.05 68.9 ± 3.7

Experimental Design

Subjects were tested in two occasions within a one-week period. In the first session, VO_{2max} and the minimum velocity that elicits VO_{2max} (vVO_{2max}) or the minimum power eliciting VO_{2max} (wVO_{2max}) were determined through a maximal incremental exercise protocol. In the subsequent visit, all subjects completed a single square-wave transition exercise from rest to 100% of VO_{2max} intensity to volitional exhaustion. Encouragement was given to motivate the subjects to perform their best effort in the incremental protocols and to perform as long as possible during the square-wave transition exercise.

Incremental Exercise Test

The incremental tests were specific according to the exercise mode. Swimmers performed an intermittent protocol for front crawl vVO_{2max} assessment, with increments of 0.05 m.s⁻¹ and 30 s passive intervals between each 200m stage. The initial velocity was established according to the individual level of fitness and was set at the swimmer's individual performance on the 400 m front-crawl minus seven increments of velocity (Fernandes et al., 2008). The velocity was controlled at each stage by a visual pacer with flashing lights in the bottom of the pool (TAR.1.1, GBK-electronics, Aveiro, Portugal). Runners performed in an outdoor track field an intermittent protocol of 800 m step duration, with increments of 1 km.h⁻¹ and 30 s passive intervals between each step. The initial velocity was defined according to the individual runners' individual performance on the 800 m minus seven increments of velocity, being this controlled by an audio feedback (whistle) so the subjects adjusted their running speed to the cones placed at 100 m intervals. Cyclists performed in a Power Tap trainer

(CycleOps, Madison, USA) a continuous protocol of 2 min step durations each, with increments of 40 W between steps, and an average cadence between 70 and 90 rpm. Saddle and handlebar positions were individually adjusted by the athletes according to their own bicycle adjustments. Rowers performed in a rowing ergometer (Concept II, Model D, CTS, Inc.) an intermittent protocol of 2 min steps, with increments of 40 W and 30 s passive intervals between steps with cadence ranging between 30 and 40 rpm. Both cyclists and rowers' initial power was set according to the subject's fitness level, and during the tests, power was controlled by visual feedback. The VO_{2max} was considered to be reached according to primary and secondary criteria (VO_2 plateau despite an increase in velocity/power, [La-] \geq 8 mmol.I⁻¹, respiratory exchange ratio R \geq 1.0, heart rate >90% of [220 – age] and a volitional exhaustion) (Howley et al., 1995) and as a mean value measured over the last 60 s of exercise. If a plateau less than 2.1 ml.min⁻¹.kg⁻¹ could not be observed, vVO_{2max} was calculated as previously described (Kuipers et al., 1985).

Square-Wave Transitions Exercise

24-48 h later, all subjects performed a square-wave transition exercise from rest to 100% of VO_{2max} at their previously determined vVO_{2max} or wVO_{2max} . This test consisted in a 10 min warm-up exercise at 50% of the VO_{2max} followed by a 5 min rest period and finally, the maintenance of this specific intensity until exhaustion. In all exercise modes, the velocity/power was controlled by the same feedbacks as for the incremental protocols. All tests ended when the subjects could no longer maintain the required velocity/power dictated by the feedback for three consecutive occasions. Although direct measures were made through the exercise, the peak oxygen uptake (VO_{2peak} - maximum value reached during the square-wave transitions exercises) and all ventilatory parameters mean values were measured over the last 60 s of exercise.

Experimental Measurements

Pulmonary gas-exchange variables were directly measured at the mouth using a telemetric portable gas analyser (K4b², Cosmed, Rome, Italy). In swimming,

this apparatus was suspended over the water (at a 2 m height) in a steel cable, following the swimmer along the pool, and minimizing disturbances of the normal swimming movements. This equipment was connected to the swimmer by a low hydrodynamic resistance respiratory snorkel and valve system (Aquatrainer, Cosmed, Rome, Italy) (Baldari et al., 2012). In-water starts and open turns, without underwater gliding, were used. In rowing, running and cycling exercises, subjects breathed through a low-dead-space facemask (Cosmed, Rome, Italy) and the gas-analysis device was placed near the body's centre of mass, adding 800 g to the total weight of the subject. The gas analysers were calibrated before each test with gases of known concentration (16% O₂ and 5% CO₂) and the turbine volume transducer was calibrated by using a 3-I syringe according to the manufacturer's instructions.

Data Analysis – VO₂ Kinetics

Firstly, errant VO_2 breath values were omitted from the analysis by including only those in-between VO_2 mean \pm 4 SD. Afterwards, individual breath-by-breath VO_2 values were smoothed by using a 3-breath moving average, and 5-s time-bin average intervals were used for fitting the corresponding regression equation (Fernandes et al., 2012). In both on- and off-analyses, a nonlinear least squares method was implemented in the MatLab Software (Mathworks, Place, USA) to fit the VO_2 data with the model. The first 20 s of data after the onset of exercise was not considered for model analyses to exclude the cardiodynamic phase.

To characterize the on-transient VO₂ kinetics, a double-exponential model (Equation 1) was used:

$$VO_{2}(t) = V_{b} + A_{1} * (1 - e^{-(t-TD_{1}/\tau_{1})}) + A_{2} * (1 - e^{-(t-TD_{2}/\tau_{2})})$$
 (1)

where VO₂ (t) is the weight-related VO₂ at time t, A_{0on} is the VO₂ at rest (ml.kg⁻¹.min⁻¹), and A_{1on} and A_{2on} (ml.kg⁻¹.min⁻¹), TD_{1on} and TD_{2on} (s), and τ_{1on} and τ_{2on} (s) are the corresponding amplitudes, time delays and time constants of the fast (1) and slow (2) VO₂ components, respectively.

To characterize the off-transient VO₂ kinetics, two different double-exponential models were used: (i) with independent time delays for the fast and slow VO₂

components (Equation 2), and (ii) with a common time delay for both fast and slow components (Equation 3):

$$VO_{2}(t) = A_{0 \text{ off}} + A_{1 \text{ off}} * exp^{(-t-TD_{1 \text{ off}})/\tau_{1 \text{ off}}} + A_{2 \text{ off}} * exp^{(-t-TD_{2 \text{ off}})/\tau_{2 \text{ off}}}$$
(2)

$$VO_{2}(t) = A_{0off} + A_{1off} * exp^{(-t-TD_{1off})/\tau_{1off}} + A_{2off} * exp^{(-t-TD_{1off})/\tau_{2off}}$$
(3)

where VO₂ (t) represents the relative VO₂ at the time t, A_{0off} is the VO₂ at rest after the exercise (ml.kg⁻¹.min⁻¹) and A_{1off} and A_{2off} (ml.kg⁻¹.min⁻¹), TD_{1off} and TD_{2off} (s), and τ_{1off} and τ_{2off} (s) are the corresponding amplitudes, time delays and time constants of the fast (1) and slow (2) VO₂ components respectively, or the common time delay (TD₁) in Equation 3.

Statistical Analysis

For each exercise mode, mean and SD were computed for all variables, and the normality of their distribution was checked with the Shapiro-Wilk test. In the VO_2 off-transient kinetics analysis, an F-test was used to decide which double exponential model (with independent time delays or a common time delay for both components) led to a significant reduction in the sum of squared residuals as the criterion measure for the goodness of fit of the regression model. To test the differences between the on- and off-transient kinetics parameters within each exercise mode, a paired t-test was conducted. The differences between performance variables, off-transient parameters and time sustained between exercise modes, were tested using a one-way ANOVA (Bonferroni post-hoc test). Simple linear regression and Pearson's correlation (r) and determination (r^2) coefficients were also used to test the relationship between the studied variables. All analyses were performed using SPSS (version 10.05, SPSS, Chicago, USA). The confidence level for significance was set at p<0.05.

Results

An example of the VO₂ on- and off-transient kinetics curves for each mode of square-wave exercise is shown in Figure 1. For all exercise modes, the off-transient response was better described by a double exponential equation with

a common time delay for both the fast and slow components, as indicated by the lowest sum of squared residuals. Once they were all fitted by model, the onand off-transient periods were symmetrical in shape (mirror image).

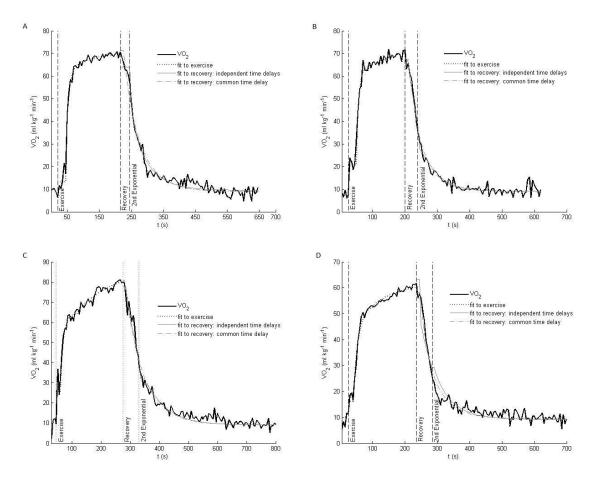


Figure 1. Examples of VO_2 to time curves during and after the square-wave exercises performed at 100% of VO_{2max} in (A) swimming, (B) rowing, (C) running, and (D) cycling. Averaged VO_2 data (thick line), and double exponential fitted equation during exercise (dotted thin line) and recovery phases with independent time delays (solid thin line) and with a common time delay (dashed thin line) are identified. Vertical lines indicate the start of the exercise and recovery, and start of the second exponential period.

The mean \pm SD values for VO_{2peak}, vVO_{2max}, wVO_{2max}, time sustained and the exercise and recovery (on- and off-transient) VO₂ kinetics estimated parameters of the square-wave exercises performed at 100% of VO_{2max} in swimming, rowing, running and cycling are presented in Table 2.

Table 2. Mean (\pm SD) values for performance and on- and off-transient VO₂ kinetics parameters in the square-wave exercises performed at 100% of VO_{2max} in swimmers, rowers, runners and cyclists. Significant differences in the off-transient kinetics between each group are indicated, as compared to rowing (Ro), running (Ro) and cycling (Cy). Differences between on- and off-transient periods are indicated by an asterisk (p<0.05).

Parameters	Swimming (n=8)	Rowing (n=8)	Running (n=8)	Cycling (n=8)
VO _{2peak} (ml·kg ⁻¹ ·min ⁻¹)	59.0±6.6 Ru	64.5±5.9	71.9±4.8 ^{Cy}	60.2±6.0
$vVO_{2max}/wVO_{2max} (m \cdot s^{-1}/W)$	1.41±0.05	394±30	5.93±0.26	388±33
Time Sustained (s)	195±23	191±26	227±27	200±24
Exercise (on-transient kinetics)				
A _{0on} (ml·kg ⁻¹ ·min ⁻¹)	17.7±2.4 *	17.7±5.0 *	16.5±5.0 *	14.6±5.6 *
A _{1on} (ml·kg ⁻¹ ·min ⁻¹)	35.6±8.9	44.3±5.7	48.4±7.0	42.4±14.0
$TD_{1on}\left(s\right)$	13.7±4.8	15.8±5.9	14.3±4.0	18.5±4.4 *
τ _{1on} (s)	20.0±3.1 *	13.6±4.7 *	10.4±4.5 *	16.6±8.8 *
A_{2on} (ml·kg ⁻¹ ·min ⁻¹)	6.5±1.9	5.5±2.8 *	7.1±1.7 *	6.8±2.0 *
TD _{2on} (s)	52.3±25.6	72.8±10.3	65.6±12.5	81.4±11.7
τ_{2on} (s)	110.9±32.9 *	48.4±26.4 *	96.5±37.3 *	20.3±12.2
Recovery (off-transient kinetics)				
A_{0off} (ml·kg ⁻¹ ·min ⁻¹)	7.5±3.3	10.7±2.3	10.0±2.9	9.4±1.2
A_{0off} (I·min ⁻¹)	0.5±0.2	0.8 ± 0.2	0.6±0.2	0.7±0.1
A _{1off} (ml·kg ⁻¹ ·min ⁻¹)	38.1±7.8	40.4±5.5	47.6±5.4 ^{Cy}	36.1±7.2
$A_{1off}(I \cdot min^{-1})$	2.7±0.7	3.0 ± 0.3	3.0±0.5	2.5±0.5
TD _{1off} (s)	10.9±6.4	10.5±4.5	10.3±2.6	10.5±5.6
τ _{1off} (s)	63.4±4.6 Ro,Cy	55.8±4.6	60.2±6.9	55.4±2.6
95% confidence intervals (s)	59.1-67.6	51.9-59.6	53.8-66.6	53.2-57.5
A _{2off} (ml·kg ⁻¹ ·min ⁻¹)	11.2±6.0	12.5±2.4	11.3±4.4	11.5±5.2
A_{2off} (I·min ⁻¹)	0.8±0.4	0.9 ± 0.2	0.7±0.3	0.8 ± 0.3
$\tau_{2off}(s)$	20.1±3.3	19.7±1.5	20.7±2.3	18.9±1.7

VO_{2peak} was higher in running compared with cycling (p=0.001), and lower in swimming compared with running (p=0.001). However, no differences were found between VO_{2max} (58.9, 65.9, 70.8 and 61.7 ml.kg⁻¹.min⁻¹, for swimming, rowing, running and cycling, respectively) reached during the incremental exercise tests and VO_{2peak} values (reached during the square-wave transition exercises) within exercise modes, and time sustained across exercise modes. Regarding the estimated parameters for the off-transient phase, no differences were found between exercise modes, with the exception of A_{1off}, which was larger in running compared with cycling (p=0.004), and τ _{1off}, which was longer in swimming compared with rowing (p=0.008) and cycling (p=0.001).

When comparing the exercise and recovery estimated parameters across exercise modes, A_{0on} was 43% larger than A_{0off} but τ_{1off} was 74% longer than τ_{1on} . Likewise, with the exception of swimming, A_{2off} was 45% larger than A_{2on} . Contrarily to the fast phase, the off-slow component phase stabilized 76% faster (shorter τ_{2off}) compared to the on-slow component, but only in swimming, rowing and running (p<0.05).

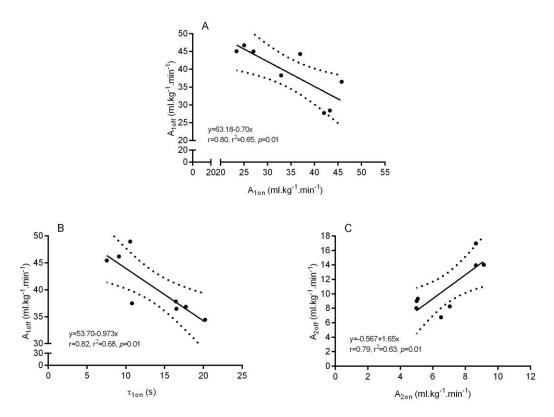


Figure 2. Significant relationships between (A) on- and off-transient fast-component amplitude (A_{1on} and A_{1off}) in swimming; (B) off-transient fast-component amplitude (A_{1off}) and on-transient fast-component time constant (τ_{1on}) in rowing; and (C) between on- and off-transient slow-component amplitude (A_{2on} and A_{2off}) in running. All parameters refer to the square-wave transition exercises performed at 100% of VO_{2max}. The regression equations (solid lines), Pearson's linear correlation (r) and determination (r^2) coefficients, p-value, and ±95% confidence limits (dotted lines) are shown.

Significant correlation results between on- and off-transient VO_2 kinetic parameters in the square-wave transition in 3 modes of exercise are shown in Figure 2: (i) in swimming, inverse relationships were found between the on- and off-transient fast component amplitudes (A_{1on} and A_{1off}), (ii) in rowing, the

subjects who stabilized the fast component earlier during exercise (shorter τ_{1on}) were the ones reaching higher amplitudes of the fast component during recovery (A_{1off}), and (iii) runners presented a direct relationship between the onand off-slow component amplitudes (A_{2on} and A_{2off}). No significant correlations were found for the cycling exercise.

Discussion

This study compared the off-transient VO_2 kinetics responses after a rest to square-wave transition exercise at the severe intensity exercise domain (i.e. 100% of VO_{2max}) in four groups of athletes (swimmers, rowers, runners and cyclists), examining also the on/off symmetry of the modelled responses. Despite the overall off- kinetics response was very similar in all four exercises, some differences were found: A_{1off} was larger in running compared to cycling – associated to larger VO_{2peak} average values – and τ_{1off} was longer in swimming comparing to rowing and cycling. These findings corroborate the hypothesis that the sport-discipline related differences would contribute to distinct off-transient VO_2 kinetic patterns at the severe-intensity exercise domain. Supporting the secondary hypothesis, the on- and off- transient periods were symmetrical in shape (mirror image) for all exercise modes, highlighting that they were both adequately fitted by a double exponential model.

The absence of differences between VO_{2max} and VO_{2peak} values across exercise modes increases the likelihood that a true VO_{2max} was measured and that a similar velocity/ power were reproduced within the 2 protocols. The VO_{2peak} values reached at the end of each square-wave transition exercise are in accordance with the specialized literature for each studied sport: rowing (Sousa et al., 2014), running (Billat et al., 1994; Renoux et al., 1999), cycling (Coyle et al., 1992; Chavarren & Calbet, 1999) and swimming (Rodríguez et al., 2007). The VO_{2peak} values were higher in the group of runners compared with cyclists and swimmers, and similar values were found between the other exercise

modes. It is possible that during running, the involvement movement of the upper limbs and trunk demands a significant oxygen requirement compared with cycling, where the arms and trunk makes a smaller contribution to the total exercise VO₂ (Hill et al., 2003). Moreover, the lower VO_{2peak} values found for swimmers compared with runners could be explained by the substantial lower body mass of this later, although no differences were found between runners and cyclists regarding body mass.

In the current study, both on- and off- transient kinetic phases were well described by a double exponential model (Özyener et al., 2001). In the specialized literature, some studies modelled the off-response by using a double exponential model with independent time delays for the fast and slow components (Dupont et al., 2010) whereas others used a common time delay for both (Özyener et al., 2001). Only one study compared both double exponential models in the off-transient kinetic response -though at the heavy intensity domain - concluding that a significant reduction on residual variance was obtained with the double exponential model with two independent time delays (Cleuziou et al., 2004). Contrarily, in the current study and for all exercise modes, the off-transient phases were better described by a double exponential model with a common fast and slow components time delay, with which a significant decrease in the sum of squared residuals occurred. In fact, the two exponential processes observed during the off-transient kinetic responses were both present at the end of exercise and would decay simultaneously during early recovery, but at different rates. Even if most studies are in agreement with the fact that two phases can be differentiated, there is no consensus in the literature as to which mathematical model should be used. since a variety of additional factors have been shown to exert an influence on this phenomenon (Cleuziou et al., 2004). In this sense, more studies are needed to clarify the physiological explanation for the presence of a second time delay, as well as for the presence of a common time delay in the recovery period.

In the current study, although the overall response profile was quite similar, differences were found in two of the off-transient kinetic parameters between exercise modes. First, A_{1off} was larger in running compared with cycling (~24% on average), perhaps simply reflecting the ~16% higher VO_{2peak} values of the group of runners. It was reported that lower body (cycle) heavy ergometer exercise would result in a higher A_{1off} compared with upper body (arm cranking) exercise in trained and untrained pubertal girls, although a mono-exponential approach was used (McNarry et al., 2012). The difference found in the current study could be explained by the "gross" O₂ debt, which has been interpreted as the energy necessary to rebuild the high energy phosphate compounds splitted at the beginning of exercise (Margaria et al., 1933). Despite this study did not focus on this phenomenon, we could hypothesise that the running exercise could induce a greater accumulation of high-energy phosphate, thus explaining higher A_{1off} values. Notwithstanding, A_{1off} in running was higher than those reported for the same exercise intensity (Billat et al., 2002; Dupont et al., 2010), which can be explained by different training level of the subjects studied. In cycling exercise, the A_{1off} values found is similar to previous reports (Cunningham et al., 2000; Cleuziou et al., 2004; Dupont et al., 2010).

Second, τ_{1off} was longer in swimming compared with rowing (~16% on average) and cycling (~13%). Since τ_{1off} reflects the rate at which the VO₂ response achieves the VO₂ steady state, swimmers evidenced a slower rate of response towards reaching that balance. Knowing that changes in body position (i.e. from supine to orthostatic) may lead to lower systolic volume and muscle perfusion pressure due to central redistribution of blood volume (Sheldahl et al., 1987), resulting in a longer τ_{1on} (Koga et al., 1999), the current findings seem to support the concept that the off-transient kinetics may be also influenced by the different body position adopted during the exercise period. In fact, pulmonary VO₂ off-kinetics has been reported as a reflexion of muscular PCr kinetics (Rossiter et al., 2002), which in turn are considered to reflect the rate of mitochondrial respiration, and consequently, skeletal muscle oxidative capacity.

Thus, the longer $\tau_{1\text{off}}$ values found for swimming may be attributable to a lower muscle oxidative capacity (due to the body position). Collectively, these suggests that swimmers, compared with rowers and cyclists, would benefit more from a longer duration of training intervals after each set of exercise whenever $VO_{2\text{max}}$ training intensity is to be enhanced. In fact, by presenting a longer $\tau_{1\text{off}}$, the findings of the current study seems to support the idea that the VO_2 off-kinetics is modality specific, at least for swimming. Notwithstanding, $\tau_{1\text{off}}$ mean value found for swimming was similar to previous reports (Sousa et al., 2011), since this kinetic parameter seems to remain constant when comparisons between different intensities are made (Cleuziou et al., 2004).

All exercise modes evidenced a VO₂ slow component during the recovery period, as previously showed for heavy (Cleuziou et al., 2004) and severeintensity exercises (Özyener et al., 2001). However, and with the exception of swimming, all exercise modes evidenced larger A_{2off} values compared with the correspondent on-transient parameter, and no differences were found between exercise modes. These findings support the concept that at the severe-intensity range (i.e. 100% of VO_{2max}), whereas the slow component cannot be discerned or cannot be expressed because of the insufficient duration of the exercise, there is a clearly distinguishable slow phase in the recovery from exercise (Özyener et al., 2001). Moreover, since the magnitude of the VO₂ slow component is dependent on the intensity and duration of exercise (Jones & Poole, 2005), considering that the square-wave transition exercises were mainly designed to assess the time they could be sustained, the on-slow component development could have been compromised by temporal issues at this particular intensity. We suspect, but cannot prove, that a longer exercise time would induce a greater VO₂ on-slow component, thus minimising the differences between the corresponding off-kinetic parameters. Thus, we suggest that the physiological process involved in both on- and off-slow component phases was similar, especially for running, as shown in the relationship found in Figure 2 (panel C).

Despite the on/off symmetry observed in the present study, differences between both phases of the response were actually found. First, A₀ was larger in the ontransient compared with the off-transient in all exercise modes. The 10-min warm-up exercise at 50% of the VO_{2max} that preceded the square-wave exercise to exhaustion could explain this difference, although it was reported that the warm-up intensity and duration of exercise had no influence on VO₂ ontransient kinetics (Bailey et al., 2009). Nevertheless, the lack of differences in all exercise modes between A_{1on} and A_{1off} is in accordance with previous results reported for heavy-intensity cycling exercise (Cleuziou et al., 2004), although an inverse relationship was observed between this parameters in swimming (Figure 2, panel A). Second, longer τ_{1off} values compared with τ_{1on} were found for all exercise modes. This parameter is a major focus of interest in the VO₂ kinetics related literature. A longer τ_{1off} value corroborates previous data obtained in cycling heavy-intensity exercise (Cleuziou et al., 2004; Yano et al., 2007), extreme-intensity swimming exercise (Sousa et al., 2011) and very heavy cycle exercise in adolescents (Lai et al., 2008), suggesting that the time needed for VO₂ steady-state achievement is longer after severe-intensity exercise, independently of the exercise mode performed. Collectively, both the amplitude and the time constant of the fast component (i.e. A_1 and τ_1) suggest that both O₂ deficit and debt during exercise at 100% of VO_{2max} intensity do not match, contrarily to what was reported for exercise performed at an intensity below the LT (Paterson & Whipp, 1991; Özyener et al., 2001), a fact that is evident in the relationship observed for rowing exercise (Figure 2, panel B).

We need to acknowledge some limitations in the present study. First, and most important, the different exercise modes studied were performed by different groups of athletes. Although this seems a good approach in terms of task, testing and training specificity, differences in somatic or physiological characteristics among groups could have influenced the results. Second, the muscle mass and muscle type involved in each exercise modality, as well as differences in muscle flood flow and body position, could have also influenced the outcomes of the kinetic analysis performed. Future studies on this topic

should involve groups of athletes capable of performing well in the different exercise modes, thus reducing inter-individual variability across exercise modalities, and allowing for better statistical models to be used (e.g. multiple repeated-measures ANOVA). The interpretation and application of the current findings are confined only to the severe-intensity exercise domain. Finally, although the measurement of pulmonary VO₂ at the mouth is accepted to reflect muscle VO₂ during exercise, literature is presently inexistent on their relationship in the off-transient kinetics response.

In conclusion, this study shows that the on and off-transient VO₂ kinetics responses from rest to a square-wave transition exercise at the severe-intensity exercise domain (i.e. 100% of VO_{2max}) in four groups of athletes (swimmers, runners, rowers and cyclists) of comparable level are well described by a double exponential model and are symmetrical in shape (mirror image). However, a larger off-transient amplitude in running compared to cycling, associated to larger VO_{2peak}, and a slower rate of VO₂ decrease during the fast phase of recovery in swimming comparing to rowing and cycling, appear to corroborate the hypothesis that sport discipline- or exercise-related differences would contribute to distinct off-transient VO₂ kinetics pattern at this particular exercise domain. Further research is needed to establish the influence of somatic, physiological and training subjects' characteristics, as well as of other variables such as muscle mass involved and body position during exercise and recovery.

Acknowledgements

This work was supported by the Portuguese Science and Technology Foundation (FCT) - PhD grant (SFRH/BD/72610/2010).

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Chapter 6

VO_2 Kinetics and Metabolic Contributions Whilst Swimming at 95, 100 and 105% of the Velocity at VO_{2max}

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Published on Biomed Research International (2014), 2014.

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Abstract

A bioenergetical analysis of swimming at intensities near competitive distances is inexistent. It was aimed to compare the transient VO₂ kinetics responses and metabolic contributions whilst swimming at different velocities around VO_{2max}. 12 trained male swimmers performed (i) an incremental protocol to determine the velocity at VO_{2max} (vVO_{2max}) and (ii) three square wave exercises from rest to 95, 100 and 105% of vVO_{2max}. VO₂ was directly measured using a telemetric portable gas analyser and its kinetics analysed through a double exponential model. Metabolic contributions were assessed through the sum of three energy components. No differences were observed in the fast component response (τ_1 - 15, 18 and 16 s, $A_1 - 36$, 34 and 37 ml.kg⁻¹.min⁻¹ and Gain - 32, 29 and 30 $ml.m^{-1}$ at 95, 100 and 105% of the vVO_{2max} , respectively) but A_2 was higher in 95 and 100% comparing to 105% intensity (480.76 ± 247.01, 452.18 ± 217.04 and 147.04 ± 60.40 ml.min⁻¹, respectively). The aerobic energy contribution increased with the time sustained (83 \pm 5, 74 \pm 6 and 59 \pm 7% for 95, 100 and 105%, respectively). The adjustment of the cardiovascular and/or pulmonary systems that determine O2 delivery and diffusion to the exercising muscles did not changed with changing intensity, with the exception of VO₂ slow component kinetics metabolic profiles.

Introduction

In the 1920s a sustained period of research in human exercise physiology emerged, with swimming (along with cycling and running) as one of the primary areas of research in Sport Sciences. In this sport, the maximal oxygen uptake (VO_{2max}) reveals itself as an important physiological parameter, expressing the swimmers' maximal metabolic aerobic performance (Renoux, 2001; Sousa et al., 2011b), that is one of the primary areas of interest in training and performance diagnosis (Fernandes & Vilas Boas, 2012). In fact, determining the VO_{2max} is the main aim of some studies, but the capacity to sustain this intensity in time is a recent topic of research. A big temporal gap exists between the pioneer study where VO_{2max} was measured in swimming (Liljestrand & Lindhard, 1920) and the first studies where time to exhaustion at the velocity corresponding to VO_{2max} (Tlim-100%VO_{2max}) was assessed (Billat et al., 1996; Demarie et al., 2001; Faina, 1997). Although conducted in non-specific swimming pool conditions (swimming flume), these studies evidenced that Tlim- $100\%VO_{2max}$ depended inversely on the vVO_{2max} , but was not related with VO_{2max}. To date, few studies have been conducted in conventional competition conditions (Alberty et al., 2008; Alberty et al., 2009; Fernandes et al., 2003; Fernandes et al., 2006a; Fernandes et al., 2008a; Fernandes et al., 2006b; Renoux, 2001), and none have proposed how VO₂ kinetics might impact concerning distinct vVO_{2max} swimming intensities.

The quantification of the dynamic characteristics of VO₂ kinetics has gained popularity in exercise physiology as a mean to unveil the mechanisms underlying the control O₂ muscular consumption in humans during exercise (Adami et al., 2011). Traditionally, the dynamic VO₂ response to exercise has been studied at three intensity domains: moderate - below the anaerobic threshold, heavy - above the anaerobic threshold and below the critical power and severe - above the critical power until the VO_{2max} boundary (Jones & Burnley, 2009; Xu & Rhodes, 1999). In the severe intensity domain, neither VO₂ nor blood lactate concentrations ([La⁻]) can be stabilized, rising inexorably until

fatigue ensues, with VO_2 achieving its maximum value (Gaesser & Poole, 1996). Here, the VO_2 slow component is more pronounced compared to during heavy exercise, with its magnitude dependent on the duration and type of exercise (Jones et al., 2011). More recently, the extreme exercise domain has been proposed for power outputs that lead to exhaustion before $VO_{2\text{max}}$ is attained (Hill et al., 2002), with the kinetics of VO_2 characterized by the development of an evident fast component with insufficient time for the appearance of a discernible VO_2 slow component (Burnley & Jones, 2007). Although the characteristics of the VO_2 kinetics in the moderate and heavy intensity domains are well described (particularly in cycle and running ergometer exercise) few studies have investigated it during swimming around the $VO_{2\text{max}}$ intensity (Demarie et al., 2001; Fernandes et al., 2003; Fernandes et al., 2008a). Moreover, these studies have only conducted a simplistic VO_2 kinetics characterization by presenting the VO_2 slow component as the unique kinetics parameter.

Given the current level of interest in exercise tolerance and VO_2 kinetics, it is surprising that no study have examined both Tlim-100% VO_{2max} and VO_2 kinetics response at intensities close to VO_{2max} . Trying to overcome this absence of data and aiming to disseminate knowledge to other forms of human locomotion, the purpose of this study is to compare the transient VO_2 kinetics responses whilst swimming until exhaustion at different velocities around the VO_{2max} intensity. In addition, the different metabolic contributions of each exhaustion exercise will also be analysed. It was hypothesized that 5% of variability in swimming velocity will not promote significant changes in the VO_2 fast component kinetics response phase, but will promote distinct VO_2 kinetics in the slow component phase.

Material and Methods

Subjects

Twelve well trained male swimmers (mean \pm SD; age: 18.2 \pm 4.1 yrs, height: 179.4 \pm 6.5 cm, body mass: 70.5 \pm 5.8 kg and mean performance for long course 200 m freestyle of 86.5 \pm 3.7% of the 2013 world record for this event), participants in national level competitions, volunteered to participate. All the swimmers, specialized in middle distance freestyle events, trained at least eight times per week and competed in National Championships for at least the 4 years. Their maturational index (according to Tanner Scale) corresponded to the 4 or 5 stages. Swimmers were familiar with the testing procedures as they were involved in previous physiological evaluations. All subjects avoided strenuous exercise in the 24 h before each testing session and were well hydrated and abstained from food, caffeine and alcohol 3 h before testing. The protocols were conducted at the same time of the day for each subject and were separated by, at least, 24 h. All participants (or parent/guardians when subjects were under 18 yrs) provided informed written consent before data collection.

Experimental Design

Subjects visited the swimming pool facilities on four different occasions over a two week period. In the first session, VO_{2max} and vVO_{2max} were determined through an intermittent incremental protocol until exhaustion. In the subsequent visits, subjects completed three square wave exercises from rest to distinct percentages of vVO_{2max} intensity until exhaustion to assess its time sustained. Encouragement was given to motivate the swimmers to perform their best effort in the incremental protocol and to perform as long as possible during the square wave exercises.

Incremental Protocol

Briefly, each subject performed an individualized intermittent incremental protocol for front crawl vVO_{2max} assessment, with increments of 0.05 m.s⁻¹ and

30 s intervals between each 200 m stage until exhaustion (Fernandes et al., 2006a; Fernandes et al., 2012). According to these studies, the initial velocity was established according to the individual level of fitness and was set at the swimmer's individual performance on the 400 m front crawl minus seven increments of velocity. The velocity was controlled at each stage by a visual pacer with flashing lights in the bottom of the pool (TAR.1.1, GBK-electronics, Aveiro, Portugal). VO_{2max} was considered to be reached according to primary and secondary criteria (Howley et al., 1995) and all ventilatory parameters mean values were measured over the last 60 s of the exercise. If a plateau less than 2.1 ml.min⁻¹.kg⁻¹ could not be observed, vVO_{2max} was calculated as previously described (Kuipers et al., 1985).

Time to Exhaustion Tests

A total of three experimental exhaustive conditions were conducted in randomized order: 95, 100 and 105% of vVO_{2max} . Each square wave exercise consisted in three distinct phases: 10 min warm-up at 50% of the VO_{2max} , a short rest period (300 s) and, the maintenance of the specific swimming intensity until exhaustion (Fernandes et al., 2003; Fernandes et al., 2008a). The square wave exercises ended when the swimmers could no longer maintain the required velocity dictated by the visual feedback. Ventilatory parameters were calculated as the average values measured over the last 60 s of each exhaustive exercise.

Experimental Measurements

Respiratory and pulmonary gas-exchange variables were directly measured using a telemetric portable gas analyzer (K4b², Cosmed, Rome, Italy), suspended over the water (at a 2 m height) in a steel cable following the swimmer along the pool and minimizing disturbances of the normal swimming movements. This apparatus was connected to the swimmer by a low hydrodynamic resistance respiratory snorkel and valve system (Aquatrainer, Cosmed, Rome, Italy; (Baldari et al., 2012)), that presents inspiratory and expiratory tubes of 86 cm length counting a volume of 847 ml from the

mouthpiece to the turbine. It contains a dead space at the valves assembly, of 11.3 ml. In-water starts and open turns, without underwater gliding, were used. Heart rate (HR) was monitored and registered continuously by a Polar Vantage NV (Polar electro Oy, Kempele, Finland) that telemetrically emitted the data to the K4b² portable unit. The gas analysers were calibrated before each test with gases of known concentration (16% O_2 and 5% CO_2) and the turbine volume transducer was calibrated by using a 3 L syringe. Capillary blood samples (25 μ l) for [La] were collected from the earlobe during the 30 s intervals (incremental protocol) and immediately at the end of exercise during the 1st, 3rd, 5th and 7th min of the recovery period, until maximal values were reached ([La] $_{max}$), in both incremental and square wave exercises (Lactate Pro, Arkay, Inc, Kyoto, Japan).

Data Analysis

Firstly, occasional VO_2 breath values were omitted from the analysis by including only those in-between VO_2 mean \pm 4 standard deviation. After verification of the data, individual breath-by-breath VO_2 responses were smoothed by using a 3-breath moving average and time-average of 5 s (Fernandes et al., 2012). For VO_2 kinetics analysis, the first 20 s of data after the onset of exercise (cardio-dynamic phase) were not considered for model analysis. For this, a double-exponential (Equation 1) equation was used where a nonlinear least squares method was implemented in the MatLab Software to fit the VO_2 data with the model.

$$VO_{2}(t) = V_{b} + A_{1} * (1 - e^{-(t - TD_{1}/\tau_{1})}) + A_{2} * (1 - e^{-(t - TD_{2}/\tau_{2})})$$
 (1)

Where VO₂ (t) represents the relative VO₂ at the time t, A₀ is the VO₂ at rest (ml.kg⁻¹.min⁻¹) and A₁ and A₂ (ml.kg⁻¹.min⁻¹), TD₁ and TD₂ (s), and τ_1 and τ_2 (s) are the amplitudes, the corresponding time delays and time constants of the fast and slow VO₂ components, respectively. The oxygen deficit (DefO₂) was calculated as the multiplication of A₁ and τ_1 . In turn, A₁ was used to determine the gain (A₁/velocity) of the primary fast component. The relative contribution of the slow component to the overall increase in VO₂ at the end-exercise was calculated as [A₂/ (A₁+A₂)]. For the primary fast component to be accurately

described by an exponential model, the duration of the VO_2 primary rise before the onset of the VO_2 slow component must be sufficiently long (\geq 4 times τ_1), in all subjects at all swimming intensities.

The maximal metabolic expenditure (E_{tot-max}) amounted during the square wave exercises was assumed to be the sum of the three components: aerobic (Aer), anaerobic lactic (Ana_{lac}) and anaerobic alactic (Ana_{alac}) energies (Capelli et al., 1998a; Figueiredo et al., 2011; Zamparo et al., 2000). The Aer contribution was calculated from the time integral of the net VO₂ versus time relationship in the appropriate time ranges (ml O₂) and then expressed in kJ assuming an energy equivalent of 20.9 kJ IO₂⁻¹ (Zamparo et al., 2011). The Ana_{lac} was estimated through the energy derived from lactic acid production (equation 2):

$$Ana_{lac} = b.[la]_{bnet}.M \qquad (2)$$

where [La]_{net} is the net accumulation of lactate after exercise, b is the energy equivalent for lactate accumulation in blood (2.7 ml O₂ mM⁻¹.kg⁻¹ (Di Prampero et al., 1978), and M (kg) is the mass of the swimmers(ml O₂). Then, it was expressed in kJ assuming an energy equivalent of 20.9 kJ. IO2⁻¹ (Zamparo et al., 2011).

The Ana_{alac} was assessed from the maximal PCr splitting in the contracting muscle (equation 3):

Ana_{alac} = PCr.
$$(1 - e^{-t/\tau})$$
.M (3)

where Ana_{alac} is the anaerobic alactic contribution, t is the exercise time, τ is time constant of the PCr splitting at the onset of exhausting exercise (23.4 s (Binzoni et al., 1992), M is the body mass and PCr is the phosphocreatine concentration at rest. This latter was estimated assuming that, in transition from rest to exhaustion, its concentration decreases by 18.55 m-mole.kg⁻¹ muscle wet weight (in a maximally working muscle mass equal to 30% of the overall body mass). Ana_{alac} was, thus, expressed in kJ by assuming an energy equivalent of 0.468 kJ.mole⁻¹ and a P/O2 ratio of 6.25 (Sousa et al., 2013).

Statistical Analysis

Individual, mean and standard deviations (SD) values were used for descriptive analysis for all studied variables and measures of skewness, kurtosis and the Shapiro-Wilk test allowed to assess the normality and homogeneity of the data. The differences in ventilatory, metabolic, VO₂ kinetics parameters and time sustained between the square wave exercises at 95, 100 and 105% of vVO_{2max} were tested for statistical significance using ANOVA for repeated measures. When a significant F value was achieved, the Bonferroni post hoc procedures were conducted to locate the pairwise differences between the averages. Simple linear regression and Pearson's correlation coefficient were also used. All statistical procedures were conducted with SPSS 10.05 and the significance level was set at 5%.

Results

Time to exhaustion decreased with increasing swimming velocity: 344.09 ± 63.64 , 194.17 ± 47.79 and 122.64 ± 20.06 s at 1.34 ± 0.05 , 1.39 ± 0.06 and 1.46 ± 0.07 m.s⁻¹ (corresponding to 95, 100 and 105% of the vVO_{2max}, respectively). Table 1 lists the mean \pm SD data regarding the ventilatory and metabolic parameters assessed during the incremental and the square wave bouts performed at different percentages of vVO_{2max}.

Table 1. Mean \pm SD values for VO_{2max}, HR_{max}, R, V_E and [La $\bar{}$]_{max} obtained at the end of the incremental protocol and the time to exhaustion tests (n=12).

Parameters	Incremental	95%	100%	105%
Parameters	Protocol	of vVO _{2max}	of vVO _{2max}	of vVO _{2max}
VO _{2max} (I.min ⁻¹)	4.21 ± 0.61	4.36 ± 0.55	4.41 ± 0.74	4.24 ± 0.64
VO _{2max} (ml.kg ⁻¹ .min ⁻¹)	60.75 ± 5.17	61.34 ± 5.58	60.05 ± 6.10	59.78 ± 6.45
HR _{max} (beats. min ⁻¹)	180.6 ± 6.96	176.75 ± 9.63	176.91 ± 8.57	177.00 ± 8.90
R	0.93 ± 0.05	0.95 ± 0.06	1.01 ± 0.09	0.97 ± 0.06
V _E (l.min ⁻¹)	112.04 ± 23.38	116.54 ± 21.94	115.75 ± 34.25	119.27 ± 17.17
[La-] _{max} (mmol.l ⁻¹)	7.18 ± 2.52	8.04 ± 1.70	8.86 ± 1.63	8.45 ± 1.91

 VO_{2max} = maximal oxygen uptake; HR_{max} = maximal heart rate; R = respiratory quotient; V_E = ventilation; $[La^{-}]_{max}$ = maximal blood lactate concentrations.

There were no differences between the determined physiologic parameters obtained in the incremental protocol and the square wave exercises, and inbetween the different time to exhaustion tests. Table 2 shows the VO₂ kinetics parameters obtained at 95, 100 and 105% of vVO_{2max} during the square wave exercises.

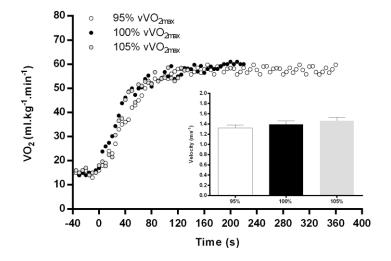
Table 2. Mean \pm SD values for the VO₂ kinetics responses during at the time to exhaustion tests (n=12).

VO ₂ kinetics parameters	95% of vVO _{2max}	100% of vVO _{2max}	105% of vVO _{2max}
A ₀ (ml.min ⁻¹)	1214.71 ± 351.79	1358.53 ± 368.71	1389.55 ± 257.32
A_1 (ml.min ⁻¹)	2568.31 ± 384.22	2402.64 ± 327.82	2628.24 ± 410.31
TD_1 (s)	11.28 ± 3.98	8.60 ± 2.49	8.05 ± 3.49
τ_1 (S)	14.82 ± 4.01	18.06 ± 3.07	16.37 ± 3.81
95% confidence intervals (s)	(12.3-17.4)	(16.1-20.1)	(13.9-18.8)
DefO ₂ (I)	0.60 ± 0.12	0.77 ± 0.24	0.74 ± 0.19
Gain (ml.m ⁻¹)	32.07 ± 4.54	29.39 ± 4.72	29.84 ± 4.43
A_2 (ml.min ⁻¹)	480.76 ± 247.01 b	452.18 ± 217.04 ^b	147.07 ± 60.40
TD_2 (s)	106.29 ± 28.67 a,b	59.99 ± 12.50	69.07 ± 5.70
τ ₂ (s)	120.23 ± 31.77 ^b	121.12 ± 31.71 ^b	61.46 ± 27.29
% A ₂	15.81 ± 7.87 ^b	16.36 ± 6.04 ^b	5.32 ± 1.99

 $A_0=VO_2$ just before the beginning of exercise; A_1 , TD_1 , τ_1 , $DefO_2$ and Gain=fast component amplitude, time delay, time constant, O2 deficit and gain, respectively; A_2 , TD_2 , τ_2 and % $A_2=1$ slow component amplitude, time delay, time constant and relative contribution of slow component in relation to the end exercise VO_2 of that bout, respectively. Differences between intensities are identified by a and b (100 and 105% of VVO_{2max}) ($p \le 0.05$).

At all intensities, the best data fit was obtained when the model incorporated a slow component (r^2 =0.94, 0.92 and 0.90 for 95, 100 and 105% of vVO_{2max} intensity) as opposed to a single exponential model, once a significant decrease in the sum of squared residuals occurred (the criterion measure used for each model). In fact, the F-test values evidenced a high heterogeneity between both models variances, also confirmed by the differences between their mean values. No differences were found in-between different time to exhaustion bouts regarding the fast component phase, but A_2 was higher at 95 and 100%

comparing to the 105% vVO_{2max} intensity and with physiological meaning (\geq 200 ml.min⁻¹) only at former intensities. In addition, the relative contribution of A₂ to total VO_2 kinetics response was similar in-between 95 and 100% and higher than 105% vVO_{2max} . Moreover, the TD_2 was higher at 95% comparing to 100 and 105% of vVO_{2max} and τ_2 was higher in 95 and 100% comparing to 105% of vVO_{2max} . An individual VO_2 kinetics response and the mean and SD values for the swimming velocity and the metabolic contributions at 95, 100 and 105% of vVO_{2max} are shown in the upper and lower panels of Figure 1.



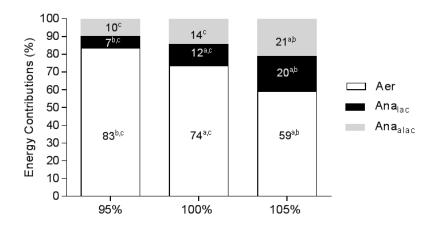


Figure 1. Upper panel: VO₂ kinetics individual response 5 s time-average at 95, 100 and 105% of vVO_{2max}. The insets represent the mean velocity values of each time to exhaustion bout; Lower panel: mean aerobic, anaerobic lactic and anaerobic alactic percentual contributions obtained during the square wave exercises at 95, 100 and 105% of vVO_{2max} (differences between intensities are identified by a , b and c for 95, 100 and 105% of vVO_{2max}, respectively; p<0.05).

Each square wave started with a sudden and exponential VO_2 increase, independently of the swimming intensity. Differences between the Aer, Ana_{lac} and Ana_{alac} contributions were found in-between time to exhaustion intensities, with exception to the Ana_{alac} that was similar between 95 and 100% of vVO_{2max} .

In Figure 2 it is possible to observe the relationships between swimming performance indicators and VO_2 kinetics parameters obtained at the different time to exhaustion bouts, with significant correlation values found between: (i) HR_{max} and τ_1 at 95% of vVO_{2max} (upper panel); (ii) vVO_{2max} and time sustained at 100% of vVO_{2max} (middle panel); and (iii) vVO_{2max} and time sustained at 105% of vVO_{2max} , and, vVO_{2max} and A_1 (bottom panel).

Discussion

Studies investigating VO_2 kinetics when performing to exhaustion have been conducted mainly in cycle ergometry and treadmill exercise, presenting a pretty simplistic approach when comparing different exercise intensities. The current study is the first attempt to examine and compare the VO_2 kinetics during swimming to exhaustion at different velocities around the VO_{2max} intensity. The exercise duration decreased when intensity increased, similarly to what was proposed for other cyclic sports. In addition, no differences were found in the VO_2 fast component related parameters (τ_1 , A_1 and Gain) between 95, 100 and 105% of vVO_{2max} , supporting our hypothesis that a 5% change in swimming velocity surrounding the VO_{2max} intensity would not be sufficient to promote changes in the primary phase of VO_2 kinetics response. However, A_2 was higher at 95 and 100% comparing to 105% of vVO_{2max} corroborating the hypothesis that different swimming intensities near vVO_{2max} would promote distinct VO_2 slow component kinetic profiles. In addition, $E_{tot-max}$ was different between the studied intensities.

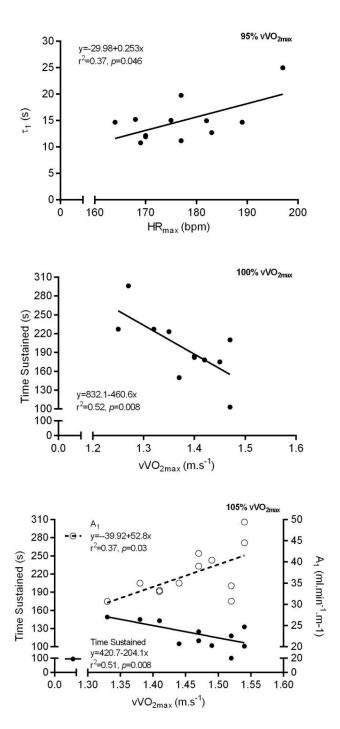


Figure 2. Relationships between maximum peak heart rate (HR_{max}) and fast component time constant (τ_1) at 95% of vVO_{2max} intensity (upper panel), velocity and time sustained at 100% of vVO_{2max} intensity (middle panel), and velocity and time sustained (filled circles) and velocity and fast component amplitude (unfilled circles) at 105% of vVO_{2max} intensity (bottom panel). The regression equations, determination coefficients and significance level values are also identified.

VO_{2max} is the most commonly measured parameter in applied physiological sciences. The mean values obtained at the end of the incremental protocol are in accordance with those presented for middle distance swimmers (Billat et al., 1996; Faina, 1997), but lower than those described for elite swimmers (Fernandes et al., 2003; Fernandes et al., 2006a; Fernandes et al., 2008a; Fernandes et al., 2006b), runners (Billat et al., 1995; Renoux et al., 1999), cyclists (Chavarren & Calbet, 1999; Coyle et al., 1992) and rowers (Jensen et al., 1996; Sousa et al., 2014), probably explained by the use of a larger muscle mass in these sports. Also, the observed [La]_{max} mean values are lower compared to other exercise modes, which can explain the lower R mean values found. This fact suggests that a lower metabolic acidosis occurs in swimming compared to other sports, or that swimmers are less sensitive to it (Billat et al., 1996). Furthermore, no differences were observed in the ventilatory and metabolic parameters between the incremental protocol and the square wave exercises, in agreement with the literature for 100% vVO_{2max} swimming exercise (Billat et al., 1996; Fernandes et al., 2008a; Renoux, 2001). In-between intensities comparison did not evidenced changes in ventilatory and metabolic mean values, , conversely to the differences found between 100 and 105% of vVO_{2max} in running (Billat et al., 1995).

Specifically in swimming, only Demarie et al. (Demarie et al., 2001) analysed a time to exhaustion at intensities different from 100% of vVO_{2max}, showing that swimmers were able to sustain ~375 s at 96% of vVO_{2max}, studying agreement with our current data (~344 s). The values reported in the current study for 100% vVO_{2max} are also similar to those presented for highly trained swimmers performing at the same intensity (Fernandes et al., 2006a; Fernandes et al., 2008a; Fernandes et al., 2006b), but are lower than others obtained in non real competition conditions (Billat et al., 1996; Faina, 1997). In addition, Alberty et al. (Alberty et al., 2008; Alberty et al., 2009) conducted studies performing at 95 and 100% of the velocity of the 400 m front crawl (not measuring ventilatory parameters), observing a longer time to exhaustion comparing to ours (~670 and 238 s). Collectively, these studies seem to evidence that time sustained at

intensities around vVO_{2max} depend also on the conditions in which they occurred. In fact, it has been reported that swimming flume might influence the VO_{2max} , vVO_{2max} and the Tlim-100% VO_{2max} assessment, as well as the swimming technique, and therefore, could explain the differences found (Fernandes et al., 2003).

Concerning the VO₂ kinetics, the observed τ_1 mean value is lower than values obtained for to 100 and 400 m front crawl all-out efforts (Reis et al., 2013: Rodríguez et al., 2003), but higher compared to the 200 m front crawl all-out effort (Sousa et al., 2011a; Sousa et al., 2011b). Conversely, the value found is in accordance with previous reports for 7 min swimming at heavy (Pessoa Filho et al., 2012; Reis et al., 2011) and severe intensities (Pessoa Filho et al., 2012) and for 400 m performed at 100% vVO_{2max} (Bentley et al., 2005). The VO_2 kinetics response was also characterized by a similar τ_1 in-between the time to exhaustion tests, in line with previous cycling and treadmill ergometer studies who have showed that it remains constant as exercise intensity increases from moderate to heavy and to severe intensity domains, despite the increasing acidosis (Barstow, 1994; Barstow et al., 1996; Pringle et al., 2003a; Scheuermann & Barstow, 2003). The current study also corroborates the absence of differences in τ_1 in-between intensities around VO_{2max} cycling exercise (90, 100 and 120%, (Scheuermann & Barstow, 2003). It has been suggested that the characteristics of VO₂ kinetics provide insights into the physiological mechanisms responsible for the control of, and the limitations to, VO₂ kinetics following the onset of exercise (Jones & Poole, 2005). Thus, similar τ₁ values observed seem to suggest that an O₂ delivery and diffusion are not influenced by a 5% external arousal in swimming at vVO_{2max} intensity. In fact, at 95% of vVO_{2max}, a positive correlation was observed between HR_{max} and τ_1 , evidencing that if a limiting factor exists, it may be related to peripheral factors (from convective O2 transport, to its diffusion and utilization in the muscles) and not to central ones (O₂ delivery and transportation to the working muscles).

Complementarily, the A₁ mean value obtained in this study is in accordance with previous reports for swimming at heavy intensity (Reis et al., 2011), but lower compared to higher intensities (Pessoa Filho et al., 2012; Sousa et al., 2011a; Sousa et al., 2011b). The similar A₁ values across conditions does not corroborate the fact that an increase in amplitude is linearly related to the increase in exercise intensity (Barstow et al., 1996; Pringle et al., 2003a; Scheuermann & Barstow, 2003). However, when comparing intensities around VO_{2max} in cycling exercise (Scheuermann & Barstow, 2003), differences have only been observed between 90% and 110% of vVO_{2max}. These results suggest that the 5% velocity change on our experimental set were not sufficient to induce modifications in A_1 , as previously noted for τ_1 . Well linked to the VO₂ first component amplitude, is the fast component Gain that evidenced a tendency to decrease with increasing intensity, in line with the decrease reported as the exercise intensity approaches the individuals' VO_{2max} (Scheuermann & Barstow, 2003). Comparisons in the VO₂ gain between intensities around VO_{2max} (90, 100 and 110% of VO_{2max}) has been reported only for cycling exercise (Scheuermann & Barstow, 2003), being this study the first attempt to assess the VO₂ gain during swimming exercise surrounding the VO_{2max} intensity. The lack of differences seems to indicate that the 5% changes in swimming velocity were insufficient to induce an adjustment of O₂ delivery and diffusion to the exercising muscles.

The magnitude of the VO₂ slow component is considered to have physiological meaning only when it is ≥200 ml.min⁻¹ (Billat et al., 2000) (although this value is still a matter of debate), occurring at 95 and 100% of vVO₂max. At these intensities (the severe intensity domain), the attainment of a VO₂ steady state is delayed due to the emergence of a supplementary slowly developing component of the VO₂ response (Jones et al., 2011), corroborating the lack of differences found in A₂ in-between 95 and 100% of vVO₂max intensities. The A₂ values found in the current study for 95 and 100% of vVO₂max intensities are in accordance with those previously reported at 96% of vVO₂max (Demarie et al., 2001), but are higher than those presented for 100% of vVO₂max (Fernandes et

al., 2003; Fernandes et al., 2008a) and for the heavy intensity exercise domain (Pessoa Filho et al., 2012; Reis et al., 2011). This lack of agreement could be explained by the method that was used to assess the VO₂ slow component–fixed interval method, that seems to result in lower values comparing to the mathematical modelling method (Reis et al., 2013), and is consider a simple rough estimate of this parameter (Jones & Poole, 2005).

At intensities higher than VO_{2max} (the extreme intensity domain), the exercise duration is so short (≤2 min) that a VO₂ slow component is not readily observed (Jones & Burnley, 2009), confirming at 105% of vVO_{2max}. The relative contribution of the VO₂ slow component to the overall increase in VO₂ at the end-exercise was higher than those presented for heavy domain (Pessoa Filho et al., 2012; Reis et al., 2011), but being A₁ and A₂ dependent variables, this was an expectable outcome. Although some explanations to better understand the VO₂ slow component phenomenon have been proposed (Fernandes & Vilas Boas, 2012), its origin in swimming is even more uncertain that in running and cycling (Demarie et al., 2001). The current study seems to indicate that possibly the type and pattern of recruitment of the available motor units was clearly modified. The lack of direct relationships between time sustained and VO₂ slow component, as found previously (Fernandes et al., 2003; Gaesser & Poole, 1996; Whipp, 1994), suggests that near the vVO_{2max} intensity the slow component is not linked with the time sustained.

Regarding $E_{tot-max}$ values, the Aer contribution was higher at 95% compared to 100 and 105% of vVO_{2max} and the anaerobic (lactic and alactic) contribution evidenced the opposite trend. These relative values(percentage of the total energy spent), show similar absolute values for the Ana_{lac} and Ana_{alac} contributions (~21, 26 and 28 kJ and 29.3, 29.2 and 29.1 kJ for 95, 100 and 105% of vVO_{2max}, respectively) but different ones for the Aer contribution (~265, 169 and 184 kJ at 95, 100 and 105% of vVO_{2max}, respectively). This fact can be explained by the time sustained at each intensity once the assessment of the Aer contribution was through the time integral from the VO₂ to time curve. To

date, this metabolic comparison has never been conducted at different intensities around vVO_{2max}. In fact, the literature regarding all energetic contributions in all sports is very scarce, and particularly in swimming, has been applied remotely (Capelli et al., 1998a; Figueiredo et al., 2011; Zamparo et al., 2000). Thus, caution must be taken when comparisons between the present results with others studies are made once the method by which the energy release was determined can have a significant influence on the calculated relative contribution of the energy systems during periods of maximal exercise (Gastin, 2001). At 95% of vVO_{2max}, the Aer contribution (~83%) was similar to the percentage proposed previously for 333 s exercise duration (Zamparo et al., 2000), but the Analac (~7%) and Analac (~10%) contributions were lower than those presented for 112 s (Capelli et al., 1998a) and 200 m all-out effort (Figueiredo et al., 2011). At 100 and 105% of vVO_{2max}, the Aer contributions (~74 and 59%) were similar to previous reports (Zamparo et al., 2000) [25, 27] but some inconsistencies were found in the remaining fractions of Analac (~12 and 20%) and Ana_{alac} (~14 and 21%) contributions (Capelli et al., 1998a) (Figueiredo et al., 2011) . The observed inverse relationship between time sustained and vVO_{2max} at 100 and 105% of vVO_{2max} (Figure 2), is in accordance with previous reports for similar swimming intensities (Billat et al., 1996; Faina, 1997; Fernandes et al., 2003; Fernandes et al., 2006a; Renoux, 2001), and could indicate a significative strenuous effort, with a more pronounced recruitment of anaerobic energy pathways (Figure 1). This fact could lead to earlier fatigue stages and, consequently, to shorter time sustained efforts. The inverse trend (greater dependency of the aerobic energy pathway) could explain the lack of an inverse relationship between time to exhaustion and vVO_{2max} at 95% of vVO_{2max} .

Further studies to compare the transient VO_2 kinetics responses and metabolic contributions whilst swimming at different velocities around maximal intensities are supported by the data from this study. However, the fact that only one transition from rest to 95, 100 and 105% of vVO_{2max} intensity was done, could lead to a low signal-to-noise-ratio. Consequently, this factor could have

influence the swimmers' performance and subsequent VO₂ kinetics, and for that, should be construed as a possible limitation of the present study.

Conclusions

This study was the first attempt to examine and compare the VO_2 kinetics and the metabolic profile during time to exhaustion exercise at different velocities around the vVO_{2max} intensity. The 5% velocity variability across conditions was not sufficient to promote changes in the kinetics of the VO_2 fast component (τ_1 , A_1 and Gain), but resulted in differences in the kinetics of the VO_2 slow component (A_2) and the corresponding metabolic profiles. Although well documented in cycling and running exercise, VO_2 kinetics has received considerably less research attention in swimming, even though providing a noninvasive into oxidative metabolism at the muscle level. Since athletes typically train at intensities surrounding the VO_{2max} , understanding how subtle variations in intensity surrounding the VO_{2max} impacts on oxidative metabolism and performance might have important implications for optimising high-intensity interval training.

Acknowledgments

The authors acknowledge the Portuguese Science and Technology Foundation (FCT): PTDC/DES/101224/2008 grant. Ana Sousa acknowledges the Portuguese Science and Technology Foundation (FCT) for the PhD grant (SFRH/BD/72610/2010).

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Chapter 7

Oxygen uptake kinetics and biomechanical behaviour at different percentages of $VO_{2\text{max}}$

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Published on XII International Symposium for Biomechanics and Medicine in Swimming (2014): 521-525.

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Abstract

The dynamic oxygen uptake (VO₂) kinetics at moderate and heavy exercise intensities are well documented in the literature, namely in treadmill running and cycle ergometer exercise. In swimming, knowing that maximal oxygen uptake (VO_{2max}) is considered one of the primary areas of interest in training and performance diagnosis, it is odd that VO₂ kinetics related studies near this intensity are almost non-existent. The purpose of this study was to compare the VO₂ kinetics during three square wave swimming transitions from rest to different percentages of VO_{2max} intensity. Five national level male swimmers $(16.8 \pm 0.8 \text{ yrs}, 72.1 \pm 6.1 \text{ kg}, 1.80 \pm 0.06 \text{ m})$ performed an incremental protocol to VO_{2max} and corresponding minimum velocity assessment (vVO_{2max}) and three square wave exercise transitions (from rest to 95%, 100% and 105% of VO_{2max} intensity) to assess its time to exhaustion. Ventilatory parameters were collected breath-by-breath (and averaged each 5 s) using a portable and telemetric gas analyzer (K4b², Cosmed, Italy). A double-exponential model – $VO_2(t) = V_b + A_1 * (1 - e^{-(t-TD_1/\tau_1)}) + A_2 * (1 - e^{-(t-TD_2/\tau_2)})$ - and a nonlinear least squares method was implemented for baseline VO₂ (A₀), amplitudes (A₁ and A₂), time delays (TD₁ and TD₂) and time constants (τ_1 and τ_2) assessment, representing the VO₂ kinetics fast (1) and slow (2) components. Biomechanical variables (stroke length - SL, stroke frequency - SF and stroke index - SI) were also assessed on all trials. Comparison between conditions was done using ANOVA repeated measures with Bonferroni post-hoc test (p≤0.05). No differences were observed for the kinetic parameters between trials, with the exception of A2 between 100% and 105% of VO_{2max} (7.4 ± 1.9 and 2.6 ± 1.5 ml.kg⁻¹.min⁻¹, respectively). SF increased as intensity raised (0.63 \pm 0.01 and 0.66 \pm 0.01 Hz, for 100 and 105% respectively) and SI was higher in 105% compared to 95 and 100% of VO_{2max} intensity (3.08 \pm 0.3, 3.16 \pm 0.3 and 3.34 \pm 0.3 m².s⁻¹, for 95, 100 and 105%, respectively). Although presently there is no consensus regarding whether or not the τ_1 is unchanged for work rates in the heavy and severe domains compared to the moderate intensity, our results show that the exercise intensities performed were not sufficient to promote significant changes in both fast and slow components, with the exception of A_2 . However, the different intensities promoted an increase in SF and SI, which reflects the mechanic adaptation of the swimmers at higher velocities.

Introduction

Sustaining exercise beyond a few seconds depends upon the appropriate supply and utilization of oxygen (Jones & Poole, 2005). However, the kinetics of oxygen uptake (VO₂) response to exercise depends on its intensity, being described three main exercise intensities - moderate, heavy and severe (Gaesser & Poole, 1996) - and more recently, a fourth much less studied one extreme (Hill et al., 2002). The VO₂ kinetics at moderate and heavy exercise intensities is well documented in the literature, namely in treadmill running and cycle ergometer exercise (Burnley & Jones, 2007; Sousa et al., 2011b). To date, the investigation of VO₂ kinetics in swimming has been limited either by the use of specific competitive distances (Rodríguez et al., 2003; Sousa et al., 2011a; Sousa et al., 2011b) or by presenting the VO₂ slow component as the only kinetic parameter of the VO₂ response (Demarie et al., 2001; Fernandes et al., 2003). The purpose of this study was to compare the VO2 kinetics and biomechanical responses in three time to exhaustion exercises from rest to different percentages of maximal oxygen uptake (VO_{2max}) intensity - 95, 100 and 105%.

Methods

Five national male swimmers (16.6 ± 2.8 yrs, 68.1 ± 3.9 kg and 1.78 ± 0.05 m) volunteered to participate in the study. All were familiar with the testing procedures, as they were involved in previous swimming evaluations. The subjects were tested in four different occasions over a two weeks period, always at the same time of the day for each subject and separated by, at least, 24 h from other test.

In the first session, VO_{2max} and the minimum velocity correspondent to VO_{2max} (vVO_{2max}) were assessed through an incremental protocol performed in the front crawl technique, with 200 m steps duration and increments of 0.05 m.s⁻¹ and 30

s intervals until exhaustion between each step. The initial velocity was set at the swimmers' individual performance on the 400 m freestyle minus seven increments of velocity, as described previously by our group in the Biomechanics and Medicine in Swimming Symposium in St. Etienne 2002 (Cardoso et al., 2003). VO_{2max} was considered to be reached according to primary and secondary criteria (Howley et al., 1995) and its mean value was measured over the last 60 s of the exercise. In the following 3 sessions, time to exhaustion exercises were performed in randomized order until voluntary exhaustion: 95, 100 and 105% of VO_{2max}. Each test was preceded by 10 min warm up at 60% of VO_{2max} followed by a short rest period. Then, each swimmer was asked to maintain the defined vVO_{2max} intensity until exhaustion. In all sessions, velocity was controlled by a visual pacer with flashing lights in the bottom of the pool (TAR.1.1, GBK-electronics, Aveiro, Portugal), and the tests ended when the subjects could no longer maintain the required velocity.

Gas-exchange parameters were directly measured using a telemetric portable gas analyzer (K4b², Cosmed, Rome, Italy), connected to the swimmer by a low hydrodynamic resistance respiratory snorkel and valve system (Baldari et al., 2012). In-water starts and open turns, without underwater gliding, were used. After including only the VO_2 values in-between VO_2 mean \pm 4 standard deviation, individual breath-by-breath VO₂ responses were smoothed (3-breath moving average) and time-averaged in 5-s. To allow the comparison of the VO₂ kinetic responses, data was modeled using a double exponential model - $VO_{_{2}}(t) = V_{_{b}} + A_{_{1}} * (1 - e^{-(t - TD_{_{1}}/\tau_{_{1}})}) + A_{_{2}} * (1 - e^{-(t - TD_{_{2}}/\tau_{_{2}})}) \quad \text{- with a nonlinear least squares}$ method implemented for baseline VO_2 (A_0), amplitudes (A_1 and A_2), time delays (TD₁ and TD₂) and time constants (τ_1 and τ_2) assessment (representing the VO₂ kinetics fast -1- and slow -2- components). Stroke rate (SR) was determined as the number of strokes per min (registered by the number of strokes in each 25 m), stroke length (SL) was calculated by dividing velocity by SR, and the product of SL to velocity allowed the assessment of stroke index (SI). Comparison between conditions was performed using ANOVA repeated measures with Bonferroni post-hoc test with the level of significance set at

 $p\le0.05$. Since a limited sample was used, effect size was computed with Cohen's f. It was considered (1) small effect size if $0\le|f|\le0.10$; (2) medium effect size if $0.10<|f|\le0.25$; and (3) large effect size if |f|>0.25 (Cohen, 1988b).

Results

Table 1 shows the mean \pm SD values for the VO₂ kinetics and biomechanical parameters obtained in all square wave transitions.

Table1. Mean \pm SD values for the VO₂ kinetics and biomechanical parameters obtained at 95, 100 and 105% of vVO_{2max} intensity. ^{1,2,3} Different from 95, 100 and 105% conditions, respectively ($p \le 0.05$).

Parameters	95%	100%	105%
A ₀ (ml.kg ⁻¹ .min ⁻¹)	17.5 ± 2.4	18.2 ± 2.4	18.9 ± 3.9
A_1 (ml.kg ⁻¹ .min ⁻¹)	37.4 ± 5.2	35.7 ± 3.1	36.7 ± 5.6
$TD_1(s)$	9.1 ± 3.7	7.4 ± 2.3	9.1 ± 4.6
$\tau_1(s)$	16.1 ± 2.1	17.4 ± 1.9	19.8 ± 11.4
A_2 (ml.kg ⁻¹ .min ⁻¹)	4.3 ± 1.8	7.4 ± 1.9^3	2.6 ± 1.5
$TD_2(s)$	120.5 ± 36.7	76.9 ± 24.4	72.5 ± 18.8
$\tau_2(s)$	87.8 ± 22.7	63.1 ± 13.1	72.6 ± 5.6
SF (Hz)	0.59 ± 0.01	0.63 ± 0.01^{1}	0.66 ± 0.01^{1}
SL (m.cycle ⁻¹)	2.29 ± 0.2	2.24 ± 0.2	2.25 ± 0.2
SI (m ² .s ⁻¹)	3.08 ± 0.3	3.16 ± 0.3	$3.34 \pm 0.3^{1,2}$
SI (m ² .s ⁻¹)	3.08 ± 0.3	3.16 ± 0.3	$3.34 \pm 0.3^{1,2}$

 A_0 = baseline VO_2 ; A_1 , TD_1 and τ_1 = fast component amplitude, time delay and time constant, respectively; A_2 , TD_2 and τ_2 = slow component amplitude, time delay and time constant, respectively; SF = stroke frequency; SL = stroke length; SL = stroke index.

There were no differences for the kinetic parameters between trials, with the exception of the A_2 between 100% and 105% of VO_{2max} (Table 1). Regarding the biomechanical parameters, SF and SI increased with increasing velocity. However, SF was significant different between 95% and the other conditions and SI was higher in 105% intensity compared to the other two conditions. The intensity at which each square wave transition was performed (95, 100 and 105% of vVO_{2max}) had a large effect on A_2 ($F_{(32.55; 5.18)}$ =6.27, p<0.02, f=0.61), SF

 $(F_{(0.008; 0.000)}$ =41.84, p<0.000, f=0.91) and SI values obtained $(F_{(0.11; 0.004)}$ =28.11, p<0.001, f=0.87).

An individual VO₂ kinetic response, the corresponding mean values of the time sustained at each studied intensities and the relationships obtained between the kinetic and biomechanical parameters are shown in Figure 1.

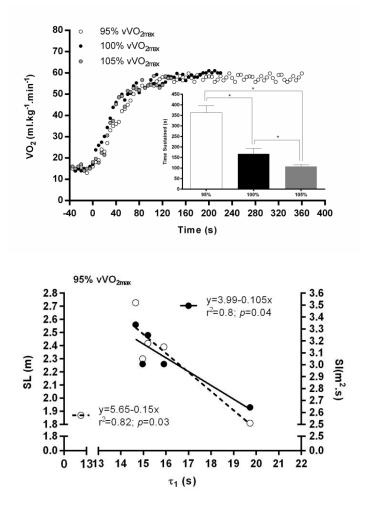


Figure 1. Left panel: VO_2 kinetic response of one subject at 95, 100 and 105% of vVO_{2max} exercise intensity. The *insets* in the respective VO_2 graph represent the mean values of the time sustained at the corresponding intensity (differences between conditions are identified by *). Right panel: relationships obtained between τ_1 and SL (closed circles) and τ_1 and SI (open circles) in the 95% vVO_{2max} exercise condition. The regression equations, determination coefficients and significance level values are also identified.

The time sustained was higher at 95% compared to 100% and 105% intensities, and lower at 105% compared to 100% vVO_{2max} swimming intensity. Inverse

relationships were obtained between τ_1 and SL and between τ_1 and SI in the 95% vVO_{2max} exercise intensity. No significant correlations were observed in the 100 and 105% exercise conditions.

Discussion

The analysis of the VO_2 kinetic response across different percentages of VO_{2max} intensities has never before been conducted in swimming. That aerobic power is one of the main physiological requirements of swimming also evidences the pertinence of its study. Its purpose was to compare the VO_2 kinetics and biomechanical responses at different percentages of VO_{2max} intensity – 95, 100 and 105%. These intensities were not sufficient to promote changes in both fast and slow components, with the exception of A_2 . However, the different intensities were responsible for an increase in SF and SI, without changing SL.

The mean values of A₁ obtained are in accordance with previous studies conducted at swimming heavy intensity (Reis et al., 2011; Reis et al., 2010), but are lower than those presented for the extreme domain (Sousa et al., 2011a; Sousa et al., 2011b). During the fast VO₂ component, the increase in amplitude is described to be related with the increase in exercise intensity (Pringle et al., 2003a; Scheuermann & Barstow, 2003). However, in the present study no differences were found between the 95, 100 and 105% of vVO_{2max} intensities. Regarding τ_1 , the present values corroborated the specialized literature (Reis et al., 2011; Reis et al., 2010) but are lower than those obtained for all-out swimming (Rodríguez et al., 2003; Sousa et al., 2011a; Sousa et al., 2011b) and cycling exercises (Scheuermann & Barstow, 2003). There is little consensus regarding whether or not the τ_1 remains constant or modifies as exercise intensity increases (Jones & Poole, 2005), however no differences were found between the distinct intensities. The mean A2 values reported at 100% vVO_{2max} were higher than those suggested previously for the same swimming intensity (Fernandes et al., 2003; Fernandes et al., 2008a), but in

accordance with those reported for lower intensity domains (Reis et al., 2011; Reis et al., 2010). In addition, A_2 was higher in the 100% vVO_{2max} comparing to 105% vVO_{2max} condition. In fact, at the severe domain the VO_2 slow component is much more developed comparing to the extreme domain, (Xu & Rhodes, 1999). Moreover, in the 95% vVO_{2max} intensity the subjects showed also a VO_2 slow component phenomenon with physiological meaning (<200 ml.min⁻¹), a fact not observed at the highest intensity studied (105% vVO_{2max}). In fact, this latter intensity as being part of the extreme intensity domain, the VO_2 slow component did not occur as it was previously reported during cycling exercise at 110% VO_{2max} intensity (Scheuermann & Barstow, 2003).

The time sustained at 95% vVO $_{2max}$ was higher compared to 100 and 105% vVO $_{2max}$, with a progressive increase of the SR and SI as intensity increased. The opposite trend was observed for SL, although without statistical significance. The increase in SR compensated the reduction in SL, which corroborates previous swimming observations (Alberty et al., 2008; Alberty et al., 2009). In fact, the swimmers of the current study showed a mechanic adaptation at higher velocities by increasing SR, with a concomitant tendency to decrease the SL, which contributed to higher SI values in 105% vVO $_{2max}$ compared to the 100% intensity condition. Moreover, inverse correlations were found between τ_1 and SL, and SI at 95% vVO $_{2max}$ swimming intensity, suggesting that swimmers with a higher SR and SI experienced more difficulties in achieving VO $_2$ steady state phase at this exercise intensity. Hence, the capacity to maintain high rates of SL and SI at 95% vVO $_{2max}$ seems to indicate an improvement in the VO $_2$ kinetic response, being technical efficiency an important factor at this swimming intensity.

Conclusions

The present results showed that the different exercise intensities performed were not sufficient to promote significant changes in both fast and slow

components, with the exception of A_2 . However, the different intensities were sufficient to promote an increase in SR and SI, without changing in SL, which reflects the mechanic adaptation of the swimmers at higher velocities.

Acknowledgments

The authors acknowledge the Portuguese Science and Technology Foundation (FCT): PTDC/DES/101224/2008 grant. Ana Sousa acknowledges the Portuguese Science and Technology Foundation (FCT) for the PhD grant (SFRH/BD/72610/2010).

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Chapter 8

Influence of prior exercise on VO₂ kinetics subsequent exhaustive rowing performance

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Published on PLoS ONE (2014), 9 (1): e84208.

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Abstract

Prior exercise has the potential to enhance subsequent performance by accelerating the oxygen uptake (VO₂) kinetics. The present study investigated the effects of two different intensities of prior exercise on pulmonary VO₂ kinetics and exercise time during subsequent exhaustive rowing exercise. It was hypothesized that in prior heavy, but not prior moderate exercise condition, overall VO₂ kinetics would be faster and the VO₂ primary amplitude would be higher, leading to longer exercise time at VO_{2max} . Six subjects (mean \pm SD; age: 22.9 ± 4.5 yrs; height: 181.2 ± 7.1 cm and body mass: 75.5 ± 3.4 kg) completed square-wave transitions to 100% of VO_{2max} from three different conditions: without prior exercise, with prior moderate and heavy exercise. VO2 was measured using a telemetric portable gas analyser (K4b², Cosmed, Rome, Italy) and the data were modelled using either mono or double exponential fittings. The use of prior moderate exercise resulted in a faster VO₂ pulmonary kinetics response ($\tau_1 = 13.41 \pm 3.96$ s), an improved performance in the time to exhaustion (238.8 ± 50.2 s) and similar blood lactate concentrations ([La]) values (11.8 \pm 1.7 mmol.L⁻¹) compared to the condition without prior exercise $(16.0 \pm 5.56 \text{ s}, 215.3 \pm 60.1 \text{ s} \text{ and } 10.7 \pm 1.2 \text{ mmol.L}^{-1}, \text{ for } \tau_1, \text{ time sustained at }$ VO_{2max} and [La⁻], respectively). Performance of prior heavy exercise, although useful in accelerating the VO2 pulmonary kinetics response during a subsequent time to exhaustion exercise ($\tau_1 = 9.18 \pm 1.60$ s), resulted in a shorter time sustained at VO_{2max} (155.5 ± 46.0 s), while [La⁻] was similar (13.5 ± 1.7 mmol.L⁻¹) compared to the other two conditions. Although both prior moderate and heavy exercise resulted in a faster pulmonary VO2 kinetics response, only prior moderate exercise lead to improved rowing performance.

Introduction

Prior exercise is traditionally accepted as indispensable before participation in a subsequent vigorous exercise (Jones et al., 2003a). Enhancing the cardiorespiratory and neuromuscular systems, "priming exercise" has been used extensively as an intervention to investigate the limitations of pulmonary oxygen consumption (VO₂) following the onset of a subsequent exercise bout (Jones et al., 2008a). These limitations may be due to central (O₂ delivery and transportation to the working muscles) or peripheral factors (from convective O₂ transport, to its diffusion and utilization in the muscles) (Jones et al., 2006; Wilkerson et al., 2004). Measurement of pulmonary VO₂ at the mouth is accepted to reflect muscle VO₂ during exercise (Grassi et al., 1996), thus studying the VO₂ kinetics at the onset of exercise may provide a valid insight into the factors that regulate oxidative metabolism at the muscle (Barker et al., 2010).

Previously, the kinetics of pulmonary VO₂ response to exercise has been studied in three different intensity domains: moderate, heavy and severe (Gaesser & Poole, 1996). For moderate exercise (at intensities below the lactate threshold), a steady-state VO₂ is normally reached within 2-3 min of exercise onset (Pringle et al., 2003b); in the heavy domain (at exercise intensities higher than the lactate threshold but below critical power), an additional complexity (VO₂ slow component) delays the achievement of a VO₂ steady-state (Barstow & Mole, 1991). During severe intensity exercise (above critical power), VO₂ does not achieve a steady state, but continues to increase until the point of exhaustion, as VO_{2max} is reached (Jones et al., 2003a).

It has previously been shown that the magnitude and nature of VO₂ responses are profoundly altered by prior exercise. The increases in bulk O₂ delivery to the exercising muscle has dramatic effects on the response to subsequence exercise (Jones et al., 2003a). In fact, the renewed interest on this VO₂ kinetics area was generated by the report of (Gerbino et al., 1996), who demonstrated

that prior heavy exercise could speed the overall VO2 kinetics during a second bout of heavy exercise performed 6 min after the first. Typically, studies conducted on VO₂ kinetics have involved different prior exercise intensities (Burnley et al., 2000; Burnley et al., 2002a; Gerbino et al., 1996), group ages (Barker et al., 2010), durations of recovery time (Bailey et al., 2009; Burnley et al., 2001), body positions (DiMenna et al., 2010a; DiMenna et al., 2010d; Jones et al., 2006), baseline pulmonary VO₂ values (Breese et al., 2012; DiMenna et al., 2008; DiMenna et al., 2010b; Wilkerson & Jones, 2007), pedal rates (Dimenna et al., 2009), type of exercises (Dimenna et al., 2010c; Jones et al., 2008a; Jones et al., 2008b; Jones et al., 2008c; Koppo et al., 2002), combinations of prior warm-up (Burnley et al., 2002b, 2005; Koppo et al., 2003; Wilkerson et al., 2004) and types of subsequent bouts of exercise (Palmer et al., 2009; Parker Simpson et al., 2012). The studies that analysed specific prior intensities have shown that the subsequent exercise performance can benefited by prior heavy exercise, as a result of an increased amplitude of the primary component and a reduced amplitude of the slow component, with no change in the primary component time constant (Burnley et al., 2000; Burnley et al., 2001; Burnley et al., 2011). While the aforementioned alterations in VO₂ kinetics might be expected to enhance exercise tolerance, the appropriate combination of prior exercise intensity and recovery time duration can be even more important than the prior exercise intensity *per se* (Bailey et al., 2009).

We are only aware of one previous study conducted at perimaximal intensities (100%, 110% and 120% of VO_{2max}) (Jones et al., 2003b), that demonstrated that the time sustained at VO_{2max} was higher when prior heavy exercise was performed. Nonetheless, the effects of prior exercise have not been addressed in rowing exercise in trained athletes. Given the widespread interest in the use of prior exercise, both for training and scientific purposes, it is surprising that research focused mainly on the VO_2 pulmonary kinetics response in cycling exercise using heavy intensity prior exercise. Thus, it is unclear whether the prior exercise regimes that are ergogenic during cycle ergometry are also ergogenic during rowing ergometry. The purpose of the present study was to

examine the influence of prior moderate and heavy intensity exercise on pulmonary VO_2 kinetics and rowing performance. On the basis of cycling data from previous studies performed at the same exercise intensity used in the present study (100% VO_{2max}) (Jones et al., 2003b; Wilkerson et al., 2004), it was hypothesized that in prior heavy, but not prior moderate exercise condition, overall VO_2 kinetics would be faster and the VO_2 primary amplitude would be higher, leading to longer exercise time at VO_{2max} .

Material and Methods

Ethics statement

The present study was approved by the Ethics Committee of Faculty of Sport from the University of Porto. All of the participants (or parent/guardian when subjects were under 18 yrs) provided informed written consent before data collection. The procedures were performed according to the Declaration of Helsinki.

Subjects

Six nationally ranked highly trained male subjects (mean \pm SD; age: 22.9 \pm 4.5 yrs, height: 181.2 \pm 7.1 cm and body mass: 75.5 \pm 3.4 kg) volunteered to participate in the current study. Subjects were familiar with the laboratory testing procedures, as they were involved in previous similar evaluations. All participants avoided strenuous exercise in the 24 h before each testing session, and were well hydrated and abstained from food, alcohol and caffeine intake. The protocols were conducted at the same time of the day for each subject and were separated by, at least, 24 h.

Experimental design

Subjects visited the laboratory on four different occasions over a two week period to perform the rowing ergometer exercises (Concept II, Model D, CTS, Inc.). In their first visit, VO_{2max} and the lactate threshold were determined.

During each of the subsequent visits, all subjects completed exhaustive exercise at 100% of VO_{2max} with prior moderate and heavy intensity exercises and without prior exercise. All exhaustive exercise bouts were performed at the same cadence on the rowing ergometer (ranging between 30 and 40 rpm) and encouragement was given to motivate the subjects to perform their best effort.

Incremental exercise and exhaustive bouts

An intermittent incremental protocol of 2 min step durations, with increments of 40 W per step and 30 s intervals between each step, until volitional exhaustion, was used to assess VO_{2max} and the corresponding minimal power that elicited VO_{2max} . VO_{2max} was considered to be reached according to primary and secondary criteria (Howley et al., 1995) and the VO_2 mean value was measured over the last 60 s of the exercise.

A total of three experimental exhaustive conditions were investigated, conducted in randomized order. In the control condition (without prior exercise), subjects performed 2 min of rowing at 20% of maximal power (previously determined in the incremental exercise), followed by 7 min of passive recovery, and an abrupt step increment to the intensity of 100% of the minimal power that elicits VO_{2max}. The subjects' then sustained their individual intensity until voluntary exhaustion. Voluntary exhaustion was defined as when the subjects' could no longer sustained the previously determined power. In the other two conditions, after the initial 2 min period of rowing at 20% of corresponding minimal power that elicits VO_{2max}, 6 min bouts of prior exercise were performed at moderate or heavy intensity. After the prior exercise, they had 7 min of passive recovery, which was followed by the abrupt step increment to the minimal power that elicits VO_{2max}, and they maintained this for as long as possible (cf. Figure 1). VO_{2peak} and HR_{peak} were deterred as the average VO₂ and HR values measured over the last 60 s of the exercise in the exhaustive exercise bouts.

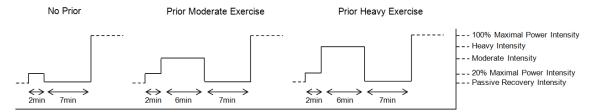


Figure 1. Schematic illustration of the experimental protocol: without prior (2 min of rowing at 20% of maximal power, 7 min of passive recovery and a transition to 100% of maximal power), prior moderate (2 min of rowing at 20% of maximal power, 6 min of rowing at the moderate intensity, 7 min of passive recovery and a transition to 100% of maximal power), prior heavy (2 min of rowing at 20% of maximal power, 6 min of rowing at the heavy intensity, 7 min of passive recovery and a transition to 100% of maximal power).

Experimental measurements

VO₂ was measured using a telemetric portable gas analyzer (K4b², Cosmed, Rome, Italy), with the subjects breathing through a facemask with a low-dead-space. The gas analysers were calibrated before each test with gases of known concentration (16% O₂ and 5% CO₂) and the turbine volume transducer was calibrated by using a 3-L syringe. Heart rate (HR) was monitored and registered continuously by a Polar Vantage NV (Polar electro Oy, Kempele, Finland) that telemetrically emitted the data to the K4b² portable unit. Capillary blood samples (25 μl) for determination of lactate concentrations ([La⁻]) were collected from the earlobe at 30 s intervals immediately at the end of exercise, and during the 1st, 3rd, 5th and 7th min of the recovery period in the intermittent incremental protocol (Lactate Pro, Arkay, Inc, Kyoto, Japan). In the exhaustive exercise bouts, capillary blood samples were collected just before the exercise, after the prior exercise (in the 6 min of the passive recovery), and during the 1st, 3rd, 5th and 7th min of recovery.

Data analysis

Firstly, occasional VO_2 breath values were omitted from the analysis by including only those in-between VO_2 mean \pm 4 standard deviation. After verification of the data, individual breath-by-breath VO_2 responses were smoothed by using a 3-breath moving average and time-average of 5-sec (Fernandes et al., 2012).

 VO_2 kinetics during exhaustive exercises was assessed using 5-sec average VO_2 data. The first 20 s of data after the onset of exercise (cardio-dynamic phase) were not considered for model analysis with both a mono-exponential (Equation 1) or double-exponential (Equation 2) equations. For both model fits, a nonlinear least squares method was implemented in the MatLab Software to fit the VO_2 data with each model. To allow the comparison of the VO_2 response, data were modeled using both mono and double exponential approaches to isolate the VO_2 fast component response. An F-Test (p>0.05) was used to evaluate whether the mono-exponential or double-exponential models provided the best fit to each data set. A T-Test (p<0.05) was employed to compare the difference between mono-exponential and double-exponential mean values.

$$\begin{split} VO_{2}(t) &= V_{b} + A * (1 - e^{-(t - TD_{1}/\tau)}) \quad \textbf{(1)} \\ VO_{2}(t) &= V_{b} + A_{1} * (1 - e^{-(t - TD_{1}/\tau_{1})}) + A_{2} * (1 - e^{-(t - TD_{2}/\tau_{2})}) \end{split} \tag{2}$$

Where VO₂ (t) represents the relative VO₂ at the time t, V_b is the VO₂ at rest (ml.kg⁻¹.min⁻¹) and A₁ and A₂ (ml.kg⁻¹.min⁻¹), TD₁ and TD₂ (s), and τ_1 and τ_2 (s) are the amplitudes, the corresponding time delays and time constants of the fast and slow VO₂ components, respectively. The mean response time (MRT) was used to represent the overall pulmonary VO₂ kinetics response and was calculated as the sum of TD₁ and τ_1 .

The lactate threshold was determined by visual inspection of the data as the disproportionate increase in [La¯] as a function of work rate. In addition, to confirm the lactate threshold, it was also determined by the [La¯]/velocity curve mathematically modelling method (least squares) (Fernandes et al., 2008b), allowing the exact point of exponential rise in [La¯] to be determined. Having determined the individual minimal power that elicits VO_{2max} and the lactate threshold, the work rates equivalent to 90% of the work rate at lactate threshold and to 50% of difference between the work rate at lactate threshold and at VO_{2max} were estimated and assumed to represent the moderate and heavy intensities, respectively.

Statistics

Individual, mean and standard deviations (SD) are used for descriptive analysis for all studied variables. Measures of skewness, kurtosis and the Shapiro-Wilk test were used to assess the normality and homogeneity of the data. The differences between [La] and HR mean values before and after performing the exhaustive bouts were tested using the unpaired T-Test. The differences in pulmonary VO₂ kinetics parameters and time sustained between the exhaustive bouts preceded by moderate intensity and heavy intensity exercise and without prior exercise were tested for statistical significance using ANOVA for repeated measures. When a significant F value was achieved, the Bonferroni post hoc procedures were performed to locate the pairwise differences between the averages. Simple linear regression and Pearson's correlation coefficient were also used. All statistical procedures were conducted with SPSS 10.05 and the significance level was set at 5%.

Results

The mean (\pm SD) VO_{2max} values of the subjects were 67.4 \pm 4.1 ml.kg⁻¹.min⁻¹, with the lactate threshold taking place at 298.3 \pm 25.6 W (corresponding to 74.9 \pm 5.7% of VO_{2max}). The work rates corresponding to moderate and heavy prior exercise intensity bouts conditions were 268.5 \pm 23.1 and 348.3 \pm 16.1 W, respectively.

The basal [La], baseline VO₂ and HR mean values, just before and after the prior exercises were: 1.1 ± 0.2 mmol.L⁻¹, 6.1 ± 1.2 ml.kg⁻¹.min⁻¹ and 74.6 ± 8.4 bpm, increasing to 1.23 ± 0.1 mmol.L⁻¹, 7.3 ± 2.2 ml.kg⁻¹.min⁻¹ and 83.3 ± 7.5 bpm (p<0.05) for the without prior exercise condition, 1.14 ± 0.3 mmol.L⁻¹, 6.7 ± 1.2 ml.kg⁻¹.min⁻¹ and 73.1 ± 6.1 bpm, increasing to 2.8 ± 0.8 mmol.L⁻¹ (p<0.01), 9.8 ± 2.7 ml.kg⁻¹.min⁻¹ (p<0.05) and to 97.1 ± 4.5 bpm (p<0.01), for the moderate prior exercise condition and 1.38 ± 0.3 mmol.L⁻¹, 6.3 ± 1.5 ml.kg⁻¹.min⁻¹ and 73.4 ± 4.1 bpm, increasing to 5.9 ± 1.2 mmol.L⁻¹ (p<0.01), 12.3 ± 2.2

ml.kg⁻¹.min⁻¹ (p<0.01) and to 114.2 \pm 5.3 bpm (p<0.01), for the heavy prior exercise condition.

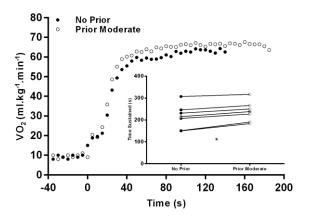
Table 1 shows the pulmonary VO₂ kinetic parameters in the exhaustive exercise bouts, without prior exercise and with prior moderate and prior heavy exercises.

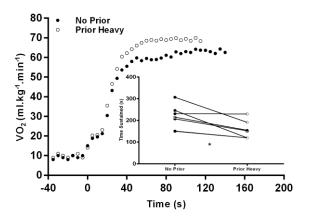
Table 1. Mean (± SD) values for the VO₂ kinetics, ventilatory and metabolic parameters in the time to exhaustion bouts performed without prior exercise, with prior moderate and with prior heavy exercises.

Parameters	Without prior exercise	Prior moderate exercise	Prior heavy exercise
A ₀ (ml.kg ⁻¹ .min ⁻¹)	20.48 ± 3.49	20.91 ± 3.48	21.61 ± 5.28
A ₁ (ml.kg ⁻¹ .min ⁻¹)	44.07 ± 2.13	43.74 ± 6.52	40.76 ± 5.83
TD ₁ (s)	4.04 ± 3.46	8.67 ± 4.66	7.49 ± 2.73
τ ₁ (s)	16.0 ± 5.56 ^a	13.41 ± 3.96 ^b	9.18 ± 1.60
MRT (s)	20.05 ± 5.44	22.08 ± 7.46	17.24 ± 3.29
Time sustained at VO _{2max} (s)	215.30 ± 60.10 a	238.83 ± 50.22 b	155.50 ± 46.05
VO _{2peak} (ml.kg ⁻¹ .min ⁻¹)	66.64 ± 1.85	66.96 ± 3.53	63.08 ± 6.02
HR _{peak} (bpm)	179.0 ± 15.12	182.80 ± 9.51	182.80 ± 14.09
[La ⁻] (mmol.L ⁻¹)	10.71 ± 1.20	11.76 ± 1.69	13.46 ± 1.72

 $A_0 = VO_2$ at rest, A_1 = amplitude of the fast component, TD_1 = time delay of the fast component, τ_1 = time constant of the fast component, MRT = mean response time ($TD_1 + \tau_1$); VO_{2peak} = peak oxygen consumption, HR_{peak} = peak heart rate, [La] = blood lactate concentrations. ^a significant different from prior moderate and prior heavy exercises; ^b significant different from prior heavy exercise.

There were no significant differences regarding A_0 , A_1 , TD_1 and VO_{2peak} values between all three studied conditions. The overall pulmonary VO_2 kinetics response in the fast phase was not significantly different when performing prior exercise, independently of its intensity, comparing to the condition where no previous exercise was conducted. However, τ_1 was higher when no prior exercise was performed, comparing to the other two conditions. No significant differences were found between MRT, HR_{peak} and $[La^{-}]$. A representative pulmonary VO_2 kinetics and the individual and mean values of the time sustained responses at each studied condition are shown in Figure 2.





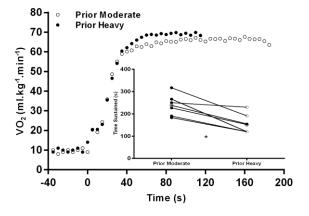


Figure 2. VO_2 dynamic response of one subject performing time to exhaustion exercise bouts after no prior exercise (closed circles) and after prior moderate exercise (open circles) (upper panel); after no prior exercise (closed circles) and after prior heavy exercise (open circles) (middle panel); after prior moderate exercise (closed circles) and after prior heavy exercise (open circles) (lower panel). The *insets* in the respective VO_2 graphs represent the individual (full black and full white) and mean (full grey) values in the time sustained at the correspondent exercise bout released. *significant differences between the two studied conditions (p<0.05).

The time sustained in the exhaustive exercise bouts was longer when prior moderate exercise was performed compared to the other two studied conditions. Moreover, when a prior heavy exercise bout was implemented, the time to exhaustion was significantly shorter when compared to the without prior exercise condition. Figure 3 shows the positive relationships between HR_{peak} and the time sustained in the exhaustive bouts (all conditions). In addition, the subjects who had higher values of HR_{peak} were the ones with higher A_1 values when no prior exercise was performed. However, in the prior heavy exercise condition, subjects who presented lower HR_{peak} values, had an enhanced fast component of VO_2 kinetics (given by the MRT value). No significant relationships were found between VO_{2peak} and VO_{2max} and all other kinetic parameters.

Discussion

Studies regarding the effect of "priming exercise" on VO₂ pulmonary kinetics have been conducted mainly in cycle ergometry and using heavy intensity prior exercise domain. Only one study examined VO₂ kinetics during rowing (Roberts et al., 2005a), but this study did not address the influence of "priming exercise". The current study is the first to examine the influence of prior moderate and heavy exercises on subsequent pulmonary exhaustive rowing exercise compare to the absence of prior exercise (warm up). The main findings were that both prior moderate and heavy exercises significantly altered the pulmonary VO₂ kinetics response to subsequent exhaustive exercise performed at 100% of VO_{2max} . In these two conditions, the τ_1 was significantly shorter compared to the condition without prior exercise, in opposition to our hypothesis that prior heavy, but not moderate exercise condition, would reduce τ₁ phase II pulmonary VO₂ kinetics. In addition, there were significant differences among all studied conditions regarding the time sustained at VO_{2max}, with higher values when prior moderate exercise was performed, again not supporting our hypothesis that time sustained at VO_{2max} would be increased when prior heavy, but not moderate, exercise would be performed

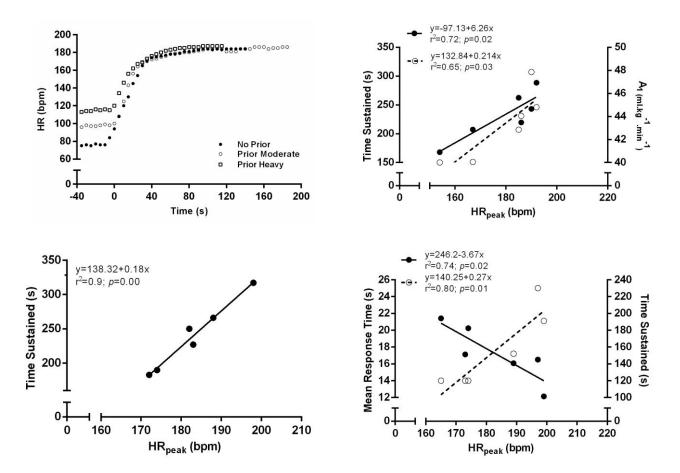


Figure 3. HR dynamic response of one subject performing time to exhaustion exercise bouts after no prior exercise (closed circles), after prior moderate exercise (open circles) and after prior heavy exercise (open squares) (upper left panel); relationships between peak heart rate and time sustained (filled circles) and between peak heart rate and amplitude of the fast component (unfilled circles) when no prior exercise was performed (upper right panel), between peak heart rate and time sustained when prior moderate exercise was performed (lower left panel) and between peak heart rate and mean response time (filled circles) and between peak heart rate and time sustained (unfilled circles) when prior heavy exercise was performed (lower right panel). The regression equations, determination coefficients and significance level values are identified.

There were significant differences in VO_2 kinetics (τ_1) between all studied conditions, with the values being 16.2% and 42.6% longer when no prior exercise was performed, compared to the conditions with prior moderate and heavy exercise conditions, respectively. These results for rowing are not consistent with previous studies conducted in cycling (Bailey et al., 2009; Burnley et al., 2000; Burnley et al., 2001; Burnley et al., 2002a; DiMenna et al.,

2010b) or running exercise (Jones et al., 2008a). These differences suggest that in rowing exercise, pulmonary VO₂ steady-state is achieved faster than in cycling. It has been suggested that similarities and differences in VO₂ kinetics between exercise sports provide insight into the physiological mechanisms responsible for the control of, and the limitations to, VO₂ kinetics following the onset of exercise (Jones & Poole, 2005).

Only one study has been conducted comparing the pulmonary VO₂ kinetic responses to step transitions to moderate and heavy intensity exercises during upright cycle and rowing ergometer exercises (Roberts et al., 2005b). These authors showed that VO₂ kinetic responses were similar between both types of exercise. This was not an expected outcome since rowing exercise engages a higher percentage of active muscular mass (Secher, 1993), potentially compromising muscle perfusion, particularly during heavy exercise where a larger fraction of the maximal cardiac output is used (Secher et al., 1977), Under this condition, a slower VO₂ kinetics might be expected in rowing compared to cycling, which was not verified. This outcome suggests that the greater active muscular mass engaged in rowing exercise is not, per se, an important explanatory factor of the differences between pulmonary VO2 kinetic rowing and cycling responses in moderate and heavy exercise intensities. This may also indicate that VO₂ kinetic responses may be strongly influenced not only by metabolic constrains, but also by the muscle contraction regimen and muscle fibre recruitment profile (Jones & Poole, 2005). Due to the higher intensity performed in our study (100% of VO_{2max}) it was expectable that bulk muscle blood flow was become even more committed compared to cycling exercise. Moreover, comparison of exercise performed with both arms and the legs reveals that muscle blood flow decreases, compared to the condition when legs or arms are exercised alone, which is explained by the sympathetic control of blood flow (muscle pressor reflex) (Secher & Volianitis, 2006). However, this possible site of control may have been attenuated by the performance of a prior exercise and eventually resulted in a faster pulmonary VO₂ kinetics response, which was not verified in cycling exercise. Moreover, the differences in training

status of the subjects, could explain the absence of agreement between our results and the data reported in the literature, particularly for other exercise modes.

In the current study, there were differences in the time exercise was sustained at VO_{2max} between the three studied conditions, with higher values when prior moderate exercise was performed. In fact, in this condition, the time sustained at VO_{2max} was increased in 10.9% and 34.9% compared to the without prior and prior heavy exercise conditions, respectively. However, exercise time was diminished 27.8% compared to the without prior exercise condition. Recent studies have shown that exercise performance could be compromised after 6 min of cycling with a severe exercise (Burnley et al., 2011; Ferguson et al., 2007), enhanced after 6 min of cycling heavy exercise (Burnley et al., 2011; Jones et al., 2003b) or even have no influence after 6 min of cycling at severe exercise (Burnley et al., 2011). It has been reported that prior exercise may predispose subjects to increase exercise tolerance in the subsequent bout of exercise, due to the sparing of anaerobic energy as a result of the increase in muscle aerobic energy turnover (Gerbino et al., 1996; Krustrup et al., 2001). This was verified in the present study by shorter τ_1 values in the prior moderate exercise condition (compared to the without prior exercise condition), although no significant differences were found in HR kinetics between each studied condition The unexpected result that the time sustained at VO_{2max} in the prior heavy exercise condition was shorter than the other two conditions may be due to the significant higher [La] values observed before the exhaustive bout was begun, compared to the prior heavy bout exercise and no prior exercise conditions.

As suggested previously, residual acidosis provides a stimulus for an increased O_2 availability through facilitation of vasodilatation and a Bohr shift in the O_2 dissociation curve (Burnley et al., 2002a; Gerbino et al., 1996; Jones et al., 2008a). However, the effects of prior heavy exercise lead also to an exaggerated accumulation of metabolites in the vascular beds in the exercised

muscles and a decrease in blood pH, although muscle oxygenation was reported to be improved (Bailey et al., 2009; Jones et al., 2008a). In order to preserve the effects of prior exercise on VO₂ kinetics and provide sufficient time for muscle homeostasis, (Bailey et al., 2009) reported that prior high intensity exercise can enhance the tolerance to subsequent high intensity exercise if it is coupled with adequate recovery duration (≥ 9 min) in-between bouts. In fact, blood [La], VO₂ baseline and HR mean values were significantly elevated in the baseline period preceding the exhaustive exercise bout (5.9 mmol.L⁻¹, 12.3 ml.kg⁻¹.min⁻¹ and 114.2 bpm), which could indicate that the recovery period may not long enough to allowed sufficient time for restoration of intramuscular high energy phosphates and/or removal of fatiguing metabolites before the beginning of the subsequent exhaustive exercise bout. The elevated [La], VO₂ baseline and HR prior to subsequent exercise could lead to a lower exercise tolerance (Jones et al., 2003b; Jones et al., 2008b; Jones et al., 2008d). This suggests that in the prior heavy exercise condition in the present study that led to the faster pulmonary VO₂ kinetics (shown by shorter τ₁ mean values in the prior heavy exercise condition compared to the non prior exercise one), was not the single determinant of the duration of the exercise. Instead, might do so through interaction with other physiological parameters, and, in contrast to our hypothesis, the time sustained at VO_{2max} in the prior heavy exercise condition was shorter.

In the current study, based on the positive relationship between HR_{peak} and exercise time at VO_{2max} , it was shown that the subjects who had higher HR_{peak} in all three studied conditions, were also the ones who sustained exhaustive exercises time longer. However, since no significant differences were found in HR_{peak} between all studied conditions, the O_2 availability during exercise was similar, and so, once again, this factor is not, *per se*, the single determinant of the tolerable duration of exercise. Moreover, the subjects who presented higher A_1 were the ones that achieved higher HR_{peak} values when no prior exercise was conducted. When prior heavy exercise was performed, negative relationships were observed between MRT and HR_{peak} , as these relationships

were influenced by significantly shorter τ_1 values. In fact, a shorter τ_1 in this condition lead to an anticipated steady-state compared to the other conditions; however, this condition has not contributed advantageous, *per si*, to a longer exercise time at VO_{2max} intensity.

Further studies to define the optimal intensity of prior exercise and subsequent recovery time required to optimise exercise performance are supported by the data from this study. However, the methodologies used to establish the intensities in both prior moderate and heavy exercise conditions (90% of anaerobic threshold and $\Delta 50\%$, respectively) may have allowed that some subjects had performed them at intensities similar to important physiological boundaries: the anaerobic threshold and the critical power in the prior moderate and heavy exercise conditions, respectively. Consequently, this could have limited the rowers' performance and influenced its VO₂ kinetics, and for that, should be construed as a possible limitation of the present study.

Conclusions

Performance of prior moderate exercise resulted in faster VO_2 pulmonary kinetics and also improved exercise time rowing at 100% of VO_{2max} . Prior heavy exercise, although effective in accelerating VO_2 kinetics in a subsequent exhaustive exercise, it resulted in a shorter exercise time at VO_{2max} compared to no prior exercise and prior moderate exercise. These results may have important implications for the preparation of athletes in training and competition suggesting the use of an optimal warm-up exercise intensity (and duration) with optimal recovery combination to improve performance.

Acknowledgments

The author would like to thank Professor David Pendergast for his revision of the manuscript.

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Chapter 9. General Discussion

The general purpose of this Thesis was to compare the VO_2 kinetics and the bioenergetic characterization in-between different exercise modes (swimming, rowing, running and cycling) at 100% of VO_{2max} intensity time sustained exercise. Likewise, it was aimed to identify some VO_2 kinetics influencing factors. Although Tlim-100% VO_{2max} has received little attention among cyclic sports, especially in swimming exercise, it begins to be considered as one of the primary areas of interest in training and performance diagnosis.

The main conclusions pointed out that swimmers, rowers, runners and cyclists were able to maintain an exercise effort intensity corresponding to VO_{2max} during a mean temporal interval ranging between 187 to 245 s, with no differences in-between exercise modes. However, (i) the fast component VO₂ on-kinetics profile showed a slower response in swimming (21±3 s s) compared with rowers (12±3 s), runners (10±3 s) and cyclists (16±4 s), and the latter compared with runners, (ii) no differences were observed in the slow component VO₂ on-kinetics phase in-between exercise modes, (iii) the amplitude of the fast-component VO₂ off-response was higher in running compared with cycling (48±5 and 36±7 ml.kg⁻¹.min⁻¹, respectively), (iv) the time constant of VO₂ off-response was higher in swimming compared with rowing and cycling (63±5, 56±5 and 55±3 s, respectively) and, (v) no differences were found in the slow off-component phase. Moreover, E_{tot} evidenced a contribution of the Ana_{alac} sources larger in swimming (~15%) compared with rowing (~10%), running (~8%) and cycling (~10%), with no differences observed in Aer and Ana_{lac} sources.

Research in human exercise physiology emerged in the 1920s, although some earlier attempts to describe the energetics of human locomotion devoted to walking started some years earlier (Di Prampero, 1986). Swimming was no exception, as its physiologic characterization was investigated by (Lijestrand & Stenstrom, 1920) and (Lijestrand & Lindhard, 1920) who determined gas

exchange, heart rate, blood pressure and cardiac output on subjects swimming the breaststroke in a lake. Notwithstanding, VO₂ research in swimming was very scarce during the first half of the 20th century, becoming an important area of sport science research only in the 1970s.

Up to today, almost all studies were conducted in none specific swimming conditions. As there are some differences in swimmers' bioenergetic and biomechanical characteristics when comparing swimming-pool conditions and swimming flume (Fernandes & Vilas Boas, 2012), the purpose of the **Chapter 2** study was to conduct a systematic review of VO₂ assessment in swimming, including historic methods but also evidencing and detailing studies that conducted VO₂ measurements in ecologic conditions. Once the historical concepts and methods in swimming research history are well developed that in Chapter 2, we will focus in this discussion on the studies that were conducted in specific competition conditions.

Considering the online computer searches on PubMed and Scopus databases, and on the books of the International Symposiums on Biomechanics and Medicine in Swimming, it was found a total of 113 studies conducted without direct VO₂ assessment in specific ergometers or in free swimming conditions, the first study occurring in 1920 and the last in 2010. Moreover, it was also found that 30 studies were conducted with direct VO₂ measurement in free swimming conditions, the pioneer study occurring in 1994 (Vilas-Boas & Santos, 1994). From those studies, the subjects in the sample, the competitive level of the subjects, the type of effort conducted, the snorkel and valve system used, the oxymeter and the VO₂ sampling interval used were distinct. To improve the understanding of the previous data, Figure 1 shows the VO₂ assessment studies conducted in free swimming with direct VO₂ measurements, evidencing the different methodologies used.

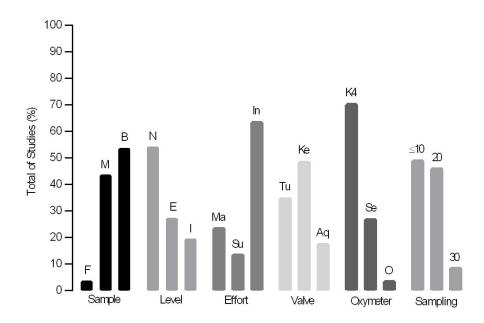


Figure 1. Schematic representation of VO₂ assessment studies conducted in free swimming with direct VO₂ measurements. F=female, M=male, B=both, N=national, E=elite, I=international, Ma=maximal or supra-maximal, Su=sub-maximal, In=incremental, Tu=Toussaint's valve, Ke=Keskinen's valve, Aq=Aquatrainer valve, K4=K4b², Se=sensormedics 2900, O=other, ≤10=inferior or equal to 10 s average, 20=20 s average, 30=30 s average.

As shown in Figure 1, most studies were conducted with male or both genders, being the female swimmers less attractive for VO₂ swimming research, as often occurs in Sport Sciences. The subjects used in the studies are largely from national level, being the elite swimmers less studied, since these are fewer. The incremental protocols are frequently conducted, being the maximal/ supramaximal and sub-maximal efforts less evaluated. Having at our disposal some valve systems to collect expired gases, the most frequently used is from (Keskinen et al., 2003), being the Aquatrainer (Cosmed, Rome, Italy) less used, probably due to the fact that is more recent. The K4b² (Cosmed, Rome, Italy), by allowing the collection of expired gas in a breath-by-breath basis, is the oxymeter mostly used. Therefore, the sampling interval most privileged are the most frequent, namely the 5 and 10 s, although some studies (especially those conducted at sub-maximal intensities) have used the 20 s interval.

Over time, VO₂ measurement has progressed to the point where it has become more effortless, practical and relevant to conduct in specific swimming conditions. Nevertheless, there have been few studies attempting to assess gas exchange parameters in specific swimming pool conditions and throughout direct measurements of VO₂ breath-by-breath technology. In fact, essential to the utilization and interpretation of these data is the consideration of substantial inter-breath fluctuations of gas exchange during exercise periods (Midgley et al., 2007). The comparison between different time-averaging intervals used to remove breath-by-breath fluctuations has remained neglected in sport, in general, and swimming, in particular. Thus, it was conducted a study -Appendix I - with the purpose of investigate the influence that different time averaging intervals had in aerobic power related parameters, namely VO_{2peak} and VO_{2max}. This study (n=10) concluded that the intensity at which the 200m front crawl was performed (supra-maximal intensity - VO_{2peak} and maximal intensity – VO_{2max}) had a significant effect (71%) on VO_{2peak} and VO_{2max} values obtained for each averaging intervals studied.

Gathering information from the two pioneering and unique studies conducted in time averaging swimming research (Sousa et al., 2010 and Fernandes et al., 2012), the results of the above referred study corroborated the literature conducted in other cyclic sports, namely treadmill running and cycle ergometry exercise, which state that less frequent sampling frequencies underestimate the VO₂ values (Astorino & Robergs, 2001; Astorino, 2009; Myers et al., 1990). This fact seems to be explained by the greater temporal resolution that breath-by-breath sampling offers, allowing a better examination of small changes in high VO₂ values. Moreover, it was concluded that at supra-maximal intensities the mean VO_{2peak} value was higher in the shortest sampling interval (breath-by-breath) compared with the 5, 10, 15 and 20 s sampling averages. Contrarily, at maximal intensities, differences were found only between the breath-by-breath and time sampling interval of 10, 15 and 20 s, and between 5 and 20 s. Such fact was clearly stated in the higher mean value variation in VO_{2peak} (61.1 - 77.7 to ml.kg⁻¹.min⁻¹).

From this study it was possible to infer that for VO_{2peak} and VO_{2max} assessment it should be taken into account the intensity at which the effort occurs since this may lead to distinct averaging intervals strategies. At the time of this study, it was still unanswered which time-averaging interval (breath-by-breath, 5, 10, 15 or 20 s) was the most appropriate sampling strategy to be used in swimming exercise, and therefore, we selected the 5 s mean value as a standardized criterion (as a way to remove the possibility of selecting an artefact) to be used in the data analysis of the studies presented on Chapters 3 to 8 and Appendix II. However, a recent study conducted in swimming exercise and within the moderate, heavy and severe intensity exercise domains (along an incremental discontinuous protocol – 7*200m) have recommended the use of 10 and 15 s time averaging intervals to determine relevant respiratory gas exchange parameters (de Jesus et al., 2014). Notwithstanding, the most appropriate sampling strategy to be use within other cyclic sports is still a matter of debate, hampering VO_2 analysis.

Moreover, the intensity at which an exercise is performed does not only influences the selection of the appropriate sampling interval, but also has a major role in the magnitude and nature of the VO_2 adjustment at the beginning of exercise (Burnley & Jones, 2007). In this sense, it was conducted a study - **Appendix II** - in which the VO_2 kinetics at different swimming intensities was compared. Ten male swimmers of international level performed a progressive and intermittent front crawl protocol of 7x200m to determine the anaerobic threshold and corresponding step (moderate intensity - Lan_{ind}), and 200m at maximal velocity (extreme intensity). Although both intensities were well described by mono-exponential fittings, differences were found in amplitude and time constant on the VO_2 kinetics in-between swimming intensities.

This study has shown that at moderate intensity, the VO₂ response to exercise is characterized by the existence of three distinct phases: cardio-dynamic, fast component and steady state, corroborating previous findings (Xu & Rhodes, 1999). Thus, this response was better characterized by a mono-exponential

fitting (Özyener et al., 2001). In fact, by using the F-Test (p=0.91) for comparison of the mono-exponential and bi-exponential fitting of the VO₂ curves, it was concluded that the double-exponential fitting was unnecessary to fit the VO₂ at moderate intensity. Similar to this latter, the extreme intensity was also better described by a mono-exponential fitting, corroborating previous studies conducted in this exercise intensity domain (Sousa et al., 2011).

Regarding the VO₂ kinetics parameters, this study showed that higher VO₂ amplitudes were obtained in the 200m performed at maximal velocity (38 vs. 26 ml.kg⁻¹.min⁻¹), contrarily to the time constant whose mean values was higher at the moderate intensity (19 vs. 13 s). This trend for higher values in amplitude was observed previously in cycle ergometry (Carter et al., 2002; Cleuziou et al., 2004; Pringle et al., 2003b) and at the high intensity domain (Scheuermann & Barstow, 2003). These differences were attributed to the higher values of VO₂ reached in the extreme domain (higher oxygen demand), since as the effort intensity increases, the amplitude gain is higher. The differences observed in time constant (lower at maximal velocity) clashed with some studies conducted in cycle ergometry which refer the constancy of this parameter along the different intensities (Carter et al., 2000; Cleuziou et al., 2004; Pringle et al., 2003a). Representing the adaptation profile of the cardiovascular and muscular systems at the corresponding intensity (Markovitz et al., 2004), the difference in time constant was attributed to the extreme intensity at which the 200m were performed.

This study has also shown that swimmers whose VO_2 fast component started earlier (shorter time delay) were those who also needed more time (longer time constant) until they reached stabilization in the VO_2 (r=-0.74, $p \le 0.05$) which was never reported before. From this pilot study it was possible to conclude that both moderate and extreme intensities analysed were well described by monoexponential approaches, although differences were found between them regarding the VO_2 kinetics parameters studied.

The finding from Appendix II study allowed us to conclude that under the Lan $_{ind}$ and above VO $_{2max}$ intensities, similarly to what was previously described for other exercise modes, swimming VO $_{2}$ kinetics retained to a first order profile. Having this is mind, in Chapters 6 and 7, and only for the 105% VO $_{2max}$ intensity, mono-exponential and double-exponential approaches were simultaneously tested to isolate the VO $_{2}$ fast and possible slow components response, respectively. For that, an F-Test (p>0.05) was used to evaluate whether the mono-exponential or double-exponential models provided the best fit to each data set. We have concluded that for the 105% VO $_{2max}$ swimming intensity (and although the intensity performed was only 5% above the VO $_{2max}$ intensity), the VO $_{2}$ kinetics still retain a two-component form, similar to what was proposed for intensities under VO $_{2max}$. Thus, we have used the double-exponential approach as the more suitable since it allowed a significant reduction in the sum of squared residuals as the criterion measure for the goodness of fit of the regression model.

The bioenergetic characterization, as an important goal of this Thesis, implied the assessment of the controversial Ana_{alac}. In this sense, in **Chapter 3** was conducted a study to estimate the Ana_{alac} in a 200m front crawl swimming trial by means of two different methods — Ana_{pcr} and Ana_{recovery}. The study concluded that, despite the existence of some caveats regarding both estimations of the Ana_{alac}, both methods yield similar results and allowed to estimate this contribution in supra-maximal swimming trials. In fact, and although each method was already studied before (Capelli et al., 1998; Figueiredo et al., 2011; Guidetti et al., 2007; Guidetti et al., 2008; Zamparo et al., 2000), this study was the first attempt to compare both methods, supporting the necessity of Ana_{alac} assessment, at least for swimming exercise.

Never used before in swimming related literature, the Ana_{recovery} method (~33kJ) presented lower values than those reported for treadmill running (Roberts & Morton, 1978) and cycling exercise (Beneke et al., 2002), but higher than those reported in ballet exercise (Guidetti et al., 2007; Guidetti et al., 2008). These

differences were attributed to different exercise time durations, exercise intensities and muscle masses activation patterns. Already used before in swimming, the Ana_{alac} contribution assessed through the Ana_{pcr} method (~32kJ) was similar to those reported for swimming events of the same duration and intensity (Capelli et al., 1998; Figueiredo et al., 2011; Zamparo et al., 2000).

The early explanation of the dynamics of recovery VO₂ after exercise was termed "alactic" (without lactate build-up) and "alactic" oxygen debt (Margaria et al., 1933). The authors based these two explanations after observing that the initial phase of recovery VO₂ ended before blood lactate begun to decline. However, and as stated in Chapter 3 study, the fact that the VO₂ fast component during recovery is independent of lactic acid removal from blood has been questioned. In fact, the term "oxygen debt" received some critical appraisal and other contemporary concept was proposed – EPOC (excess post-exercise VO₂) which included the much more prolonged increase in aerobic power that is observed for hours after exercise (Gaesser & Brooks, 1983). Despite the existence of some caveats regarding this method, Chapter 3 study based on the "alactic debt" concept.

Notwithstanding the comparison between methods evidenced similar values, suggesting that both could be utilized to estimate Ana_{alac} contributions at supramaximal competitive swimming speeds, this study draw attention for the inclusion of this energy pathway in order to adequately assess E_{tot} and consequently C. In fact, despite the existence of some caveats regarding both methods, and despite the absence of other non-invasive approaches, not considering the Ana_{alac} contribution lead to an underestimation of E_{tot} . Therefore, and although this study did not showed differences between the $Ana_{recovery}$ and Ana_{pcr} , we selected the Ana_{pcr} method as a standardized criterion to assess the contribution of anaerobic alactic system in Chapters 4 and 6.

The studies described previously were useful to gather the necessary knowledge to analyse the VO₂ kinetics and E_{tot} at different exercise modes at

100% of VO_{2max} intensity. In fact, the bioenergetics of cyclic sports have been studied since the 1920s (Fernandes & Vilas Boas, 2012), with a major scientific interest on the energetics of locomotion and its contribution to athletic performance, although comparison in-between different exercise modes is almost inexistent. In this sense, the purpose of **Chapter 4** study was to assess Tlim-100% VO_{2max} , for the first time, in swimming, rowing, running and cycling and to determine the corresponding VO_2 kinetics response and E_{tot} .

Have been used 40 subjects (10 swimmers, 10 rowers, 10 runners and 10 cyclists), the study concluded that no differences were found in Tlim-100%VO_{2max} in-between exercise modes, contrarily to VO₂ kinetics profile which showed a slower response in swimming compared to the other three modes of exercise and in running compared with cycling. Also, E_{tot} was lower in swimming comparing to rowing and running and the Ana_{alac} was larger in swimming compared with the other sports, with no differences in the Aer and Ana_{lac}.

For assessing Tlim-100%VO $_{2max}$ at each exercise mode, it was necessary, in first place, to determine the vVO $_{2max}$ (in swimming and running) and wVO $_{2max}$ (in rowing and cycling), which were assessed through an exercise mode specific incremental protocol. It was observed that VO $_{2max}$ values were higher in runners compared with cyclists and swimmers, and similar values were found inbetween the other exercise modes (~61, 67, 71, 65 ml.kh⁻¹.min⁻¹, for swimmers, rowers, runners and cyclists, respectively). As extensively reported that runners present higher VO $_{2max}$ in comparison with other exercise modes (e.g. cycling), this difference is traditionally attributed to the larger muscular mass used in running exercise (Gleser et al., 1974; Secher et al., 1974). In addition, in the current study, the movement of the upper limbs and trunk may have demanded a significant O $_{2}$ requirement in running compared with cycling, where the upper limbs and trunk may have had a smaller contribution to the total exercise VO $_{2}$ (Hill et al., 2003).

Furthermore, during the incremental protocols, cyclists evidenced a greater rise of energy expenditure per step, followed by rowers/ runners, and swimmers, as showed by $E_{tot-inc}$ - ~6, 4 and 3 ml.kg⁻¹.min⁻¹ (respectively). One notable difference between these exercise modes is the cost of exercise, which suggests that this measure depended not only on the aerobic and anaerobic contributions, but also on its interval range during incremental intensities. Contrarily, no differences were found in Tlim-100%VO_{2max} between exercise modes, a fact that was consistent with previous reports for other forms of locomotion (Billat et al., 1996; Faina et al., 1997). However, considering the variability that Tlim-100%VO_{2max} evidenced in-between exercise modes, it is suggested a total exercise duration of ~200 (for swimming and rowing) and ~250 s (for running and cycling) whenever VO_{2max} training intensity is to be enhanced.

Regarding E_{tot-tlim}, the absolute Aer was similar between exercise modes and the Ana_{lac} and Ana_{alac} were lower in swimming and running (respectively) comparing with the other exercise modes due to the lower net accumulation of lactate after exercise (Ana_{lac}) and to the lower runners' body mass (Ana_{alac}). This is justified since no differences were found in Tlim-100%VO_{2max} (the exercise time) and the same % of the overall body mass was assumed to correspond to the maximally working muscle mass (30%) in all exercise modes. The relative contribution (%) evidenced no substantial differences between exercise modes, with the exception of Ana_{alac} that was higher in swimming compared with the other exercise modes. This difference suggested a greater recruitment of type II muscle fibres in swimming, as reported during arm-crank exercise compared with cycling (Koppo et al., 2002), knowing that the proportion of type I muscle fibres is substantially lower in the muscles of the upper body vs. the lower body (Johnson et al., 1973).

The VO_2 kinetics analysis showed that τ_1 was longer in swimming compared with other sports, even though the swimmers were younger. In fact, and contrarily to Tlim-100% VO_{2max} , the mechanical differences between exercise

modes were sufficient to promote differences, at least for swimming. A key postural difference between swimming and the other exercise modes is that swimmers are in an horizontal position, where an increased venous blood return occurs but a reduced blood hydrostatic pressure in the legs is evident (Libicz et al., 2005). Also, the muscle perfusion pressure is lower, combining in a longer (Koga et al., 1999). Moreover, the inability to produce maximal muscle contractions (due to environment constraints) could have limited a faster increase in VO_2 kinetics in swimming exercise. Considering the previous findings, it is suggested that swimmers, compared with athletes from the other exercise modes, would benefit more from a longer duration (~90 s) of exercise or training intervals whenever VO_{2max} training intensity is to be enhanced.

This study still evidenced a faster VO₂ kinetics in runners compared with cyclists, a fact already reported during an exercise intensity that leaded to exhaustion in ~5 min (Hill et al., 2003), being attributed to differences in the type of muscle actions involved in both exercise modes. In contrast to running, cycling involves high levels of muscular tension, which could lead to occlusion of vessels, and, consequently, impede blood flow and oxygen delivery, delaying the VO₂ response. Running, on the other hand, has periods of low force production (e.g. when the body is airborne), which should facilitate muscle blood flow and oxygen delivery, and consequently, speed the VO₂ response (Clarys et al., 1988). This outcome suggest that runners would benefit from a shorter duration of training intervals (~50 s), compared with cyclists (~70 s), whenever VO_{2max} intensity is the training goal.

No differences were found in the VO_2 slow component kinetic response. This phenomenon was first evidenced in 1923 after Hill and Lupton proposed the concept of VO_{2max} in the year before. In fact, these authors found a substantial VO_2 increase for one subject running at constant speed on the treadmill but attributed this phenomenon to a "painful blister" (Hill & Lupton, 1923). Together with other pioneer researchers (e.g. Astrand and Saltin) begun to described the so called VO_2 slow component as an accepted standard for the ensuing

decades (Poole & Jones, 2012). This slow component is not trivial and could represent \geq 25% of the total increase in VO₂ in the severe intensity exercise domain, being distinct from the "O2 drift" (<200 ml) that may attend moderate exercise of a prolonged duration (\geq 60 min) (Jones et al., 2011). Therefore, and although this topic is still a matter of debate in the specialized literature, we considered the 200 ml value as a cut-off value of physiological meaningful in the VO₂ slow component phenomenon throughout this Thesis.

After observing the variability induced in VO₂ on-kinetics, it was our aim to test if the mechanical differences between exercise modes had also a potential effect on the VO₂ off-kinetics. In this sense, the purpose of **Chapter 5** was to compare the VO₂ off-transient kinetics response between swimmers, rowers, runners and cyclists during their specific mode of exercise at 100% of VO_{2max} intensity, examining also the on/off symmetry of the VO₂ response. With an n=36 (8 swimmers, 8 rowers, 8 runners and 8 cyclists), we have observed in all exercise modes that both transient periods were symmetrical in shape once they were both adequately fitted by a double exponential mathematical model. However, the amplitude of the fast-component VO₂ off-response was higher in running compared with cycling and the time constant of the same phase was higher in swimming compared with rowing and cycling.

In the specialized literature, there is still no consensus of which mathematical model should be used in the VO_2 off-kinetics phase (Cleuziou et al., 2004). In fact, some studies modelled the off-response by using a double exponential approach with independent time delays for the fast and slow components (Dupont et al., 2010) whereas others used a common time delay for both (Özyener et al., 2001). It was reported that when analyzing recovery kinetics any slow component term is assigned the same time delay as the primary exponential term (Jones & Poole, 2005). The rationale behind it is that the mechanisms responsible for the emergence of the slow component during exercise would be expected to be present early in the recovery phase after that exercise. Having this is mind, in our study, the off-transient phase was better

described by a double exponential model with a common fast and slow components time delay, with which a significant increase in the sum of squared residuals occurred. Notwithstanding, more studies are needed to clarify the physiological explanation for the presence of a common time delay in the recovery period.

Similar to what was observed in Chapter 4, differences were found in the off-transient kinetic parameters between exercise modes. In fact, $A_{1\text{off}}$ was larger in running compared with cycling (~24% on average), perhaps simply reflecting the ~16% higher $VO_{2\text{peak}}$ values of the group of runners. Despite this study did not focus on this phenomenon on the O_2 debt assessment, it was suggested that running exercise induced a greater accumulation of high-energy phosphate, thus explaining higher $A_{1\text{off}}$ values. Moreover, the possible explanations presented previously for the higher $VO_{2\text{max}}$ values found for runners compared with cyclists (Chapter 4) should be taken into account.

Moreover, in the above referred study, it was found a longer τ_{1off} in swimming compared with rowing (~16% on average) and cycling (~13%). Since τ_{1off} reflects the rate at which the VO₂ response achieves the VO₂ steady state, swimmers evidenced a slower rate of response towards reaching that balance. Once again, the key postural difference between this exercise and others could have underpinned this slower response. Therefore, and similar to what was proposed for the VO₂ on-kinetics, we suggest that swimmers, contrarily to rowers and cyclists, would benefit more of a longer duration of training intervals after each set of exercise in VO_{2max} training intensity enhancement. Notwithstanding, no differences were found in the A_{2off} values between exercise modes, similar to Chapter 4 findings in this phase.

From this study it was possible to conclude that the exercise modes related differences contributed to distinct off-transient VO₂ kinetics pattern at this particular exercise domain, and both on and off-phases were well described by a double exponential model. Normally to overcome the limitations inherent to

the VO₂ assessment during exercise, many studies have preferentially collected consecutive samples of expired air at the end of the exercise – backward extrapolation method. The 20 s recovery gas sample was first used in speed skating (Di Prampero et al., 1976) and later was to be valid and reproducible in treadmill cycling, treadmill testing, and indoor track running (Léger et al., 1980). Surprisingly, and although the VO₂ off-kinetics can aid in the interpretation of the physiological events underpinning the VO₂ on-response, few studies were conducted in the recovery phase to provide additional information on gas exchange dynamics.

Although the 100% of VO_{2max} intensity expresses the subjects' maximal metabolic aerobic performance, the intensities around the VO_{2max} intensity are assumed as being also important for training purposes since they are very close to the ones adopted in competitive events. Thus, it was the purpose of **Chapter** 6 to compare, for the first time, the VO_2 kinetic responses and E_{tot} whilst swimming until exhaustion at different velocities (95, 100 and 105%) around the VO_{2max} intensity. It was concluded that no differences were observed in the fast component response but the absolute slow-component amplitude was higher in 95 and 100% comparing to 105% intensity (n=12).

For assessing the time sustained at the above referred intensities, it was previously necessary to determine the velocity associated with VO_{2max} intensity. For that purpose it was used an incremental discontinuous protocol to it (Fernandes et al., 2003; Fernandes et al., 2008), and similar values were observed in the ventilatory and metabolic parameters between the incremental protocol and the square wave exercises, in agreement with what was reported before for 100% vVO_{2max} (Billat et al., 1996; Fernandes et al., 2008; Renoux, 2001). In the square wave exercises, the time sustained at 95% (~344s) and 100% (~194s) of VO_{2max} intensities was similar to the values found for 96% (Demarie et al., 2001) and for 100% (Fernandes et al., 2006a; Fernandes et al., 2008; Fernandes et al., 2006b) of VO_{2max} intensities in previous related studies. Comparison with the literature regarding the 105% of VO_{2max} intensity was not

possible due to the inexistence of studies conducted at this intensity in swimming.

In this study it was also observed that the VO₂ fast-component kinetics response was similar in-between swimming intensities (~36, 34 and 37 ml.kg⁻¹ ¹.min⁻¹ and ~15, 18 and 16 s, for amplitude and time constant in 95, 100 and 105% of vVO_{2max} intensity, respectively). Several studies, although carried out in cycling and treadmill ergometer, showed that the fast-component time constant remains constant as exercise intensity increases from moderate to heavy and to severe intensity domains, despite the increasing acidosis (Barstow et al., 1996; Pringle et al., 2003a; Scheuermann & Barstow, 2003), corroborating the lack of differences found in the current study. Thus, similar fast-component time constant values observed suggest that an O2 delivery is not a distinguishing factor in a 5% external arousal in swimming at vVO_{2max}. Contrarily, the similar fast-component amplitudes across conditions did not corroborate previous findings referring that the increase in amplitude was described to be linearly related to the increase in exercise intensity (Barstow et al., 1996; Pringle et al., 2003a; Scheuermann & Barstow, 2003). This fact suggested that a 5% difference in the magnitude of velocity across our experimental was not sufficient to promote changes in fast-component amplitude.

The slow component phase showed physiological meaning (≥200 ml.min⁻¹) only at 95 and 100% of vVO_{2max}. In fact, in constant exercise performed at the severe intensity domain – 95 and 100% of the vVO_{2max}), the attainment of a VO₂ steady state was delayed due to the emergence of a supplementary slowly developing component of the VO₂ response (Jones et al., 2011). However, at intensities higher than VO_{2max}, the exercise duration is so short (≤2 min) that a VO₂ slow component is not readily observed (Jones & Burnley, 2009), which could have happened at the extreme intensity domain – 105% of the vVO_{2max}. No differences were found in absolute and relative slow-component amplitudes in-between 95 and 100% of vVO_{2max} intensities, a fact attributed at the similar

intensity exercise domain in which both efforts took place. Contrarily to previous results in cycling ergometry, it was not observed a direct relationship between time sustained and VO_2 slow component. Regarding E_{tot} , Aer was higher at 95% compared with 100 and 105% of vVO_{2max} intensities and the Ana_{lac} and Ana_{alac} showed the opposite trend. From this study it was possible to infer that 5% velocity variability across conditions was not sufficient to promote changes in the kinetics of the VO_2 fast component, but resulted in differences between all intensities for the kinetics of the VO_2 slow component and the corresponding metabolic profiles.

After observing the variability induced in VO₂ kinetics behavior, it was our aim to check if 5% variability in swimming velocity would retain for general biomechanical parameters. In this sense, the purpose of the study presented in **Chapter 7** was to compare the VO₂ kinetics and biomechanical responses in three square wave swimming transitions exercises (95, 100 and 105% of vVO_{2max} intensity, n=5). We concluded that the exercise intensities performed were not sufficient to promote significant changes in both fast and slow components, with the exception of slow component amplitude, but were sufficient to promote an increase in SF and SI (without changing in SL).

In fact, the 5% external arousal was not sufficient to promote changes in fast-component amplitude and time constant, but the slow component amplitude was higher in the 100% comparing to 105% vVO_{2max} condition, although no differences between 95 and 105% intensities were seen (4.3, 7.4 and 2.6 ml.kg⁻¹.min⁻¹, for 95, 100 and 105% of vVO_{2max} intensity, respectively). At 105% swimming intensity the slow component phenomenon did not evidenced physiological meaning (<200ml.min⁻¹), and, as expected, at the severe domain (95 and 100% of vVO_{2max} intensity) the VO₂ slow component was much more developed, with the magnitude dependent on the duration and type of exercise.

Complementarily, the biomechanical behavior evidenced a progressive increase of the SF (higher in 100 and 105% compared to 95% of vVO_{2max} intensity) and

SI (higher in 105% compared to 95 and 100% of vVO_{2max} intensity) as intensity increased (0.59, 0.63 and 0.66 Hz, and, 3.08, 3.16 and 3.34 m².s⁻¹, for SF and SI at 95, 100 and 105% of vVO_{2max} intensity, respectively). A tendency for an opposite trend was observed for SL (2.29, 2.24 and 2.25 m.cycle⁻¹ for 95, 100 and 105% of vVO_{2max} intensity, respectively). The increase in SF compensated the reduction in SL, in line with previous observations (Alberty et al., 2008; Alberty et al., 2009). In fact, the swimmers showed a mechanic adaptation at higher velocities by increasing SF, with a concomitant tendency to decrease the SL, which contributed to higher SI values in 105% vVO_{2max} compared to the 100% intensity condition. Notwithstanding, at 95% swimming condition, inverse correlations were found between fast component time constant and SL, and SI. This fact suggested that swimmers with a higher SF and SI experienced more difficulties in achieving VO₂ steady state phase at 95% intensity. Hence, the capacity to maintain high rates of SL and SI at 95% vVO_{2max} indicated an improvement in the VO₂ kinetic response.

From this study it was possible to conclude that the exercise intensities performed were not sufficient to promote significant changes in both fast and slow components, with the exception of slow component amplitude. However, the different intensities were sufficient to promote an increase in SF and SI, without changing in SL, which reflected the mechanic adaptation of the swimmers at higher velocities. The studies conducted in Chapters 6 and 7 had the purpose to verify whether a 5% change in swimming velocity was sufficient to promote changes in VO₂ kinetics and biomechanical behaviour. In fact, a biophysical analysis at intensities near the 400 m competitive distance (in which the maximal aerobic power evidences its full potential) was inexistent. Therefore, these studies allowed knowing more detailed the physiological and biomechanical alterations induced with a 5% change under and above the 100% of VO_{2max} swimming intensity, which are traditionally used whenever VO_{2max} training zone is enhanced.

Prior exercise has been used extensively as an intervention to investigate the limitations of VO₂ following the onset of a subsequent exercise bout, such as those performed in Chapters 4, 5, 6 and 7. Trying to evaluate the influence of prior exercise, the purpose of **Chapter 8** study was to examine the influence of prior moderate and heavy intensity exercise on VO₂ kinetics and subsequent rowing performance at 100% of VO_{2max} intensity (n=6). We concluded that the performance of prior heavy exercise, although useful in accelerating the VO₂ kinetics response, resulted in a shorter time sustained at 100% of VO_{2max} intensity, compared to the other two conditions – no prior and prior moderate exercise. Moreover, prior moderate exercise resulted in a faster VO₂ kinetics response, but contrarily to prior heavy exercise, lead to an improvement in subsequent rowing performance.

This study was the first attempt to measure VO₂ kinetics under the influence of prior different exercise. In fact, only one study was conducted comparing the VO₂ kinetic responses to step transitions to moderate and heavy intensity exercises during upright cycle and rowing ergometer exercises (Roberts et al., 2005). Although this latter study has concluded that the VO₂ kinetic responses were similar between both types of exercise, this was not an expected outcome since rowing exercise engaged a higher percentage of active muscular mass (Secher, 1993), and therefore, a slower VO₂ kinetics would be expected in rowing comparing with cycling. However, there were differences in Tlim-100%VO_{2max} between the three studied conditions, with higher values when prior moderate exercise was performed. In this latter, Tlim-100%VO_{2max} was increased in 10.9% and 34.9% compared with the without prior and prior heavy exercise conditions, respectively. Specialized literature reported that prior exercise may predispose subjects to increase exercise tolerance in the subsequent bout of exercise, due to the sparing of anaerobic energy as a result of the increase in muscle aerobic energy turnover (Gerbino et al., 1996; Krustrup et al., 2001).

However, the unexpected shorter Tlim-100%VO_{2max} was shorter in the prior heavy exercise condition compared with the other two conditions was attributed to the significant higher [La-] values that were observed before the exhaustive enhanced. In fact, the exaggerated accumulation of metabolites in the vascular beds in the exercised muscles consequent decrease in blood pH, may have contributed to decreased performance (Bailey et al., 2009; Jones et al., 2008). Moreover, the VO₂ baseline and HR mean values in the prior heavy exercise were significantly elevated in the baseline period preceding the exhaustive exercise bout, suggesting that the recovery period may have had not long enough to allow sufficient time for the necessary restoration. In fact, and contrarily to the 6 min recovery between the end on prior exercise bout and the subsequent exhaustive exercise, it was reported that prior high intensity exercise enhanced the tolerance to subsequent high intensity exercise if it was coupled with adequate recovery duration (≥9 min) in-between bouts (Bailey et al., 2009). A recent review on warm-up exercise evidenced that this topic is still a matter of debate, and as a result of that, athletes and coaches design their warm-up routines based on their individual experiences, considering the volume, intensity, recovery interval and techniques of it (Neiva et al., 2014).

Regarding VO₂ kinetics, there were significant differences in fast-component time constant between all studied conditions, with the values being 16.2% and 42.6% longer when no prior exercise was performed, compared to the conditions with prior moderate and heavy exercise conditions, respectively. Moreover, when prior moderate exercise was performed, subjects needed more time to achieve VO₂ steady state, compared to the condition when prior heavy exercise was performed (~13 vs. 9 s, respectively). These results for rowing were not consistent with previous studies conducted in cycling exercise (Bailey et al., 2009; Burnley et al., 2000; Burnley et al., 2001; Burnley et al., 2002; DiMenna et al., 2010), suggesting that VO₂ steady-state was achieved faster than in cycling exercise.

From the Chapter 8 study it was possible to conclude that performance of prior moderate exercise resulted in faster VO_2 kinetics and an improved exercise time at 100% of VO_{2max} intensity. Prior heavy exercise, although effective in accelerating VO_2 kinetics in a subsequent exhaustive exercise, it resulted in a shorter exercise time compared to no prior exercise and prior moderate exercise conditions. Thus, the intensity used (50% of VO_{2max} – clearly under the lactate threshold intensity) in the warm-up exercise performed previously to each square wave transition exercise (Chapters 4, 5, 6 and 7), plus the rest period (300 s) used, gives us certainty that the prior exercise used did not influenced the time sustained as well as the VO_2 kinetics response to exercise.

Chapter 10. Conclusions

After the findings obtained in the collection of studies presented in this Thesis, it seems reasonable to stress out the following conclusions:

- (i) Research in human exercise physiology emerges in the 1920s and VO₂ uptake research in swimming is very scarce during the first half of the 20th century;
- (ii) There are few studies attempting to assess VO_{2max} in elite swimmers in ecological swimming-pool conditions (not in treadmill running or cycling ergometer) and through direct measurements of VO₂;
- (iii) The intensity at which a 200 m front crawl is performed (maximal VO_{2max} and supra-maximal intensities VO_{2peak}) has a significant effect on VO_{2max} and VO_{2peak} values obtained for each averaging intervals studied.
- (iv) The breath-by-breath sampling interval gas exchange acquisition present greater VO₂ values than sampling intervals of 10, 15 and 20 s at the 200 m front crawl performed at maximal and supra-maximal intensities;
- (v) The breath-by-breath sampling interval gas exchange acquisition can induce a significant variability of the VO₂ values acquired at maximal and supra-maximal intensities;
- (vi) The VO₂ kinetics at 200 m front crawl performed at the moderate (corresponding to Lan_{ind}) and extreme (above the VO_{2max}) intensities are well described by mono exponential fittings;
- (vii) Higher values of amplitude and time constant are obtained in 200 m crawl performed above the VO_{2max}, contrarily to the time delay whose mean is higher at the intensity corresponding to Lan_{ind};
- (viii) No significant differences in Ana_{alac} are observed between the Ana_{recovery} and Ana_{pcr} methods, suggesting that both approaches can be utilized to estimate Ana_{alac} at extreme swimming intensities;

- (ix) The inclusion of the Ana_{alac} is necessary to adequately assess C being this underestimate in about 10% when the Ana_{alac} is not taken into account at extreme swimming intensities;
- (x) Swimmers exhibit lower pulmonary and metabolic values compared with rowers, runners and cyclists at both sub-maximal (under the VO_{2max} intensity) and maximal intensities (100% of VO_{2max} intensity);
- (xi) The Tlim-100%VO_{2max} is similar in-between exercise modes;
- (xii) Swimmers evidence a slower response in VO₂ kinetics and a lower amplitude of the fast component compared with rowers, runners and cyclists at 100% of VO_{2max} intensity;
- (xiii) Runners show a faster VO₂ kinetics response compared with cyclists at 100% of VO_{2max} intensity;
- (xiv) Ana_{lac} contribution is smaller in swimming compared with rowing, running and cycling at 100% of VO_{2max} intensity;
- (xv) The VO₂ off-kinetics response is better described by a double exponential equation with a common time delay for both the fast and slow components (compared with a double exponential equation with independent time delays for the fast and slow VO₂ components) after an exercise performed at 100% of VO_{2max} intensity in swimming, rowing, running and cycling;
- (xvi) The VO₂ off-kinetics analysis evidence a higher amplitude of the fast component in running compared with cycling after an exercise performed at 100% of VO_{2max} intensity;
- (xvii) The time constant of the VO₂ off-kinetics fast-component response is longer in swimming compared with rowing and cycling after an exercise performed at 100% of VO_{2max} intensity;
- (xviii)The 5% external arousal in velocity do not induce differences in the fast component of the VO₂ in-between 95, 100 and 105% of VO_{2max} swimming intensities;
- (xix) The amplitude of the slow component is higher in 95 and 100% compared with 105% swimming intensity and the Aer increases with the time sustained;

- (xx) SF is lower in 95% compared with 100 and 105% and SI is higher in 105% swimming intensity compared with 95 and 100%;
- (xxi) Performance of prior moderate exercise results in faster VO₂ pulmonary kinetics and also improves the Tlim-100%VO_{2max} rowing exercise;
- (xxii) Prior heavy exercise, although effective in accelerating VO₂ kinetics in a subsequent rowing exhaustive exercise, results in a shorter Tlim-100%VO_{2max} compared with no prior exercise and prior moderate exercise conditions.

Chapter 11. Suggestions for Future Research

The importance of VO_2 kinetics study in sports is well accepted by coaches and scientist but, although our main purpose was the study of cyclic individual sports, there is yet a lack of research focusing on this thematic in swimming. This seems to be due to the water environments that implies several constraints to VO_2 testing. Thus, it is our purpose to continue studying deeply the parameters and conditions that could influence the VO_2 kinetics in swimming, namely:

- (i) Prior Continuous Exercise;
- (ii) Pacing Strategy;
- (iii) Cadence;
- (iv) High Interval Intensity Training;
- (v) Inspiratory Muscle Training;
- (vi) Hypoxia/ Hyperoxia Conditions.

Notwithstanding the previously suggested, a more invasive approach would be necessary in swimming related research. In fact, the VO₂ kinetics response is measured most conveniently breath-by-breath using rapidly responding gas analyzers. Although there is a tacit presumption that pulmonary VO₂ kinetics faithfully reflects muscle VO₂ kinetics, the main challenge would be studying in swimming some intra-muscle parameters (e.g. oxyhaemoglobin, PCr and pH), as has already been done in other cyclic modes, particularly in cycling and running. This analysis would be crucial to convey valuable information on understanding the physiological mechanisms that underpin the performance of one of the most challenging exercise mode there is. Therefore, this crucial information would subsequent allow an enhancement of a more objective and specific prescription of training planning.

Chapter 12. References

Chapter 1

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Appendix I

Comparison between aerobic power parameters at different timeaveraging intervals in swimming: an update

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Published on The Open Sports Science Journal (2013), 6, 22-25.

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Abstract

Sousa et al. (Open Sports Sci J, 3: 22 - 24, 2010) showed that different time averaging intervals lead to distinct VO₂ values in a maximal 200m front crawl effort, evidencing higher VO₂ values for breath-by-breath sampling, and differences between this latter data acquisition and all the other less frequent time intervals studied (5, 10, 15 and 20 s). These are interesting outputs in the field of exercise physiology applied to swimming once: (1) VO₂ assessment is conducted in a swimming pool with a portable gas analyser which allowed breath-by-breath measurements, and not in a swimming flume with a Douglas bag technique or mixing chamber analyser, as traditionally occurs, and (2) the comparison between different time-averaging intervals used to remove breathby-breath fluctuations during exercise periods has remained neglected, in sport in general and swimming in particular. Therefore, in the present study, we investigate the influence that different time averaging intervals have in aerobic power related parameters (VO_{2peak} and VO_{2max}). Ten subjects performed 200 m front crawl effort at supra-maximal intensities (all-out test) and other ten subjects performed 200 m front crawl effort at maximal aerobic intensities (100% of VO_{2max}). The intensity at which the 200 m front crawl was performed (supra-maximal and maximal intensities) had a significant effect on VO_{2peak} and VO_{2max} values obtained for each averaging intervals studied.

Introduction

The goal of competitive swimming is to obtain the fastest speed of locomotion during a race, being success determined by several influencing factors, particularly the energetic and biomechanical ones. This is possible to infer from the swimming performance equation: $v = E^*$ (ept / D), where v is the swimming velocity, E represents the energy expenditure, ept is the propulsive mechanic efficiency and D represents the hydrodynamic drag (Pendergast et al., 1977). Among the evaluation of the energetic factors, the assessment of maximal oxygen uptake (VO_{2max}) is a key point of contemporary research in sport science in general and in "swimming science" in particular (Fernandes & Vilas Boas, 2012). Considered to express the maximal metabolic aerobic performance capability of a subject, the VO_{2max} assessment is crucial for a better understanding of human energetics, and therefore, is related to one of the primary areas of interest in swimming training and performance diagnoses (Fernandes & Vilas Boas, 2012; Sousa et al., 2011b).

Acknowledging that the evaluation of aerobic performance is very relevant for swimming training purposes, it is important to study the specific VO₂ kinetics at different swimming intensities. In fact, the physiology of a maximal performance encompasses distinct neuromuscular processes, intramuscular energy turnover, cardiovascular and respiratory elements, which interconnect differently across different swimming intensities (Aspenes & Karlsen, 2012). Furthermore, when studying the VO₂ response to a specific effort it is essential to analyze the variability on the VO₂ data imposed by the used sampling intervals (Dwyer, 2004). In fact, the selection of optimal sampling intervals strategy is fundamental to the validation of the research findings, as well as to the correct training diagnosis and posterior prescription of the intensity of the training series (Fernandes et al., 2012). (Myers et al., 1990) reported 20% of variability on the VO₂ values due to different chosen data sampling intervals, and that the greatest VO_{2max} values were systematically higher as fewer breaths were included in an average. (Midgley et al., 2007) evidenced that short time-average

intervals appear to be inadequate in reducing the noise in pulmonary VO_2 , resulting in artificially high VO_{2max} values. Moreover, (Hill et al., 2003) showed higher peak VO_2 (VO_{2peak}) values at different intensities when based on smaller sampling intervals. These last referred studies (Hill et al., 2003; Midgley et al., 2007; Myers et al., 1990) were conducted in laboratory conditions, not in real swimming situation.

Regarding swimming, only our group (Fernandes et al., 2012; Sousa et al., 2010) analyzed the VO_2 variability when considering distinct time averaging intervals, but different swimming intensities were never compared. In this sense, the purpose of this study is to compare the variability of the VO_2 values obtained in a 200 m front crawl effort performed at maximal and supra-maximal aerobic intensities, using five different time averaging intervals: breath-by-breath and average of 5, 10, 15, and 20 s, respectively. We hypothesized that the different intensity performed in the 200m front crawl would lead to significant effect on VO_{2peak} and VO_{2max} values obtained for each averaging intervals.

Methods

Participants

Ten male well trained swimmers $(20.5 \pm 2.3 \text{ years old}, 185.2 \pm 2.3 \text{cm}, 77.4 \pm 5.3 \text{kg}$ and $10.1 \pm 1.8\%$ of fat mass) and ten trained male swimmers $(20.7 \pm 2.8 \text{ years old}, 182.0 \pm 0.1 \text{cm}, 75.2 \pm 4.1 \text{kg}$ and $11.1 \pm 1.6\%$ of fat mass) volunteered to participate in (Sousa et al., 2010) and (Fernandes et al., 2012) studies, respectively. All subjects were informed of the protocol before the beginning the measurement procedures, and were usually involved in physiological evaluation and training control procedures.

Procedures

Both studies were conducted in a 25 m indoor swimming pool, 1.90 m deep, water temperature of 27.5°C and humidity of 55%. In (Sousa et al., 2010) each

swimmer performed an all-out 200 m front crawl (with an individual freely chosen pace). VO_{2peak} was accepted as the highest single value on breath-by-breath, 5, 10, 15 and 20 s sampling obtained. In (Fernandes et al., 2012), each swimmer performed a 7x200 m front crawl intermittent incremental protocol until exhaustion, with 30 s rest intervals and with velocity increments of 0.05 m.s⁻¹ between each step. The velocity of the last step was determined through the 400 m front crawl best time that swimmers were able to accomplish at that moment (using in-water starts and open turns); then, 6 successive 0.05 m/s were subtracted from the swimming velocity corresponding to the last step, allowing the determination of the mean target velocity for each step. This was controlled by underwater pacemaker lights (GBK-Pacer, GBK Electronics, Aveiro, Portugal), placed on the bottom of the pool. VO₂ data analysis was centred in the step where VO_{2max} occurred, being this considered as the average values of the breath-by-breath, 5, 10, 15 and 20 s sampling obtained.

As swimmers were attached to a respiratory valve (cf. Figure 1), allowing measuring the VO_2 kinetics in real time, open turns without underwater gliding and in-water starts were used. For a detailed description of the breathing snorkels used in the supra-maximal and maximal intensities cf. (Keskinen et al., 2003) and (Fernandes & Vilas Boas, 2012), respectively. These respiratory snorkels and valve systems were previously considered to produce low hydrodynamic resistance and, therefore, not significantly affect the swimmers performance. VO_2 kinetics was measured breath-by-breath by a portable metabolic cart (K4b², Cosmed, Italy) that was fixed over the water (at a 2 m height) in a steel cable, allowing following the swimmer along the pool and minimizing disturbances of the swimming movements during the test.

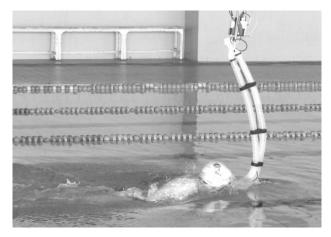


Figure 1. Specific snorkel and valve system for breath-by-breath VO₂ kinetics assessment in swimming.

Statistical Analysis

Mean \pm SD computations for descriptive analysis were obtained for the studied variable using SPSS package (version 14.0 for Windows). In addition, ANOVA of repeated measures was used to test: (i) the differences between the five different sampling intervals considered in the maximal and supra-maximal intensity, and (ii) the interaction effect of intensity in the VO₂ values in the five different sampling intervals studied. When a significant F value was achieved, Bonferroni post hoc procedures were performed to locate the pairwise differences between the averages. A significance level of 5% was accepted. Since a limited sample was used, effect size was computed with Cohen's f. It was considered (1) small effect size if $0 \le |f| \le 0.10$; (2) medium effect size if $0.10 < |f| \le 0.25$; and (3) large effect size if |f| > 0.25 (Cohen, 1988a).

Results

The VO₂ values (expressed in ml.kg⁻¹.min⁻¹) obtained in the breath-by-breath, 5, 10, 15 and 20 s time averaging intervals studied in the 200 m front crawl effort performed at supra-maximal (Sousa et al., 2010) and maximal aerobic intensities (Fernandes et al., 2012) are presented in Fig.2.

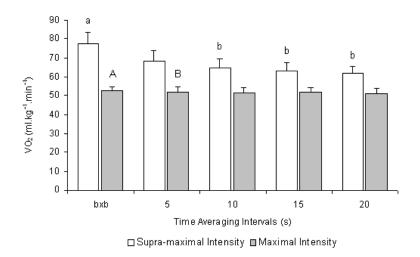


Figure 2. VO_2 values (expressed in ml.kg⁻¹.min⁻¹) obtained in the breath-by-breath, 5, 10, 15 and 20 s time averaging intervals studied in the 200 m front crawl effort performed at supramaximal (Sousa et al., 2010) and maximal aerobic intensities (Fernandes et al., 2012). Bars indicate standard deviations. ^a Significantly different from time averaging interval of 5, 10, 15 and 20 s, ^b Significantly different from time averaging interval of 5 s, ^A Significantly different from time averaging interval of 10, 15 and 20 s, respectively, ^B Significantly different from time averaging interval of 20 s, p<0.05.

In (Sousa et al., 2010), VO_{2peak} ranged from 61.1 to 77.7 to ml.kg⁻¹.min⁻¹ (F_(1.82; 16.38)=59.55, p<0.001, f=0.86). Higher VO_{2peak} values were reported for breath-by-breath interval, being observed differences between the 5 s averaging interval and the other less frequent data acquisitions considered (10, 15 and 20 s). In (Fernandes et al., 2012), VO_{2max} ranged from 51.1 to 53.2 ml.kg⁻¹.min⁻¹ (F_(2.18; 19.63)=4.12, p<0.05, f=0.31). The breath-by-breath time interval was only significantly different from the three less frequent averaging intervals studied (10, 15 and 20 s), being also reported differences between the 5 and 20 s intervals methods. The intensity at which the 200 m front crawl was performed (supra-maximal and maximal intensities) had a significant effect on VO_{2peak} and VO_{2max} values obtained for each averaging intervals studied (F_(1.87; 33.75)=44.15, p<0.001, f=0.71).

Discussion

It is well accepted that for modern diagnostics of swimming performance, new more precise and accurate analytical techniques for VO₂ kinetics assessment are needed. In fact, after the Douglas bags procedures, VO2 became to be directly assessed using mixing chamber's devices, and only afterwards an upgrade enabled real time breath-by-breath data collection with portable gas measurement systems (Fernandes et al., 2013). Furthermore, this improvement also allowed testing in normal swimming pool conditions, overlapping the standard laboratory conditions that do not perfectly reflect the real-world performances (Fernandes et al., 2008a; Fernandes & Vilas Boas, 2012; Sousa et al., 2011b). The VO_{2peak} mean value obtained in (Sousa et al., 2010) study was similar to those described in the literature for experienced male competitive swimmers (Fernandes et al., 2008a; Rodríguez & Mader, 2003), but higher than the VO_{2max} mean value reported by (Fernandes et al., 2012). This may be due to the different intensity domain in which both efforts occurred. In fact, the sudden and exponential increase in VO₂ that occurs close to the beginning of the effort at intensities above VO_{2max} triggers the attainment of high VO₂ values (Sousa et al., 2011b). Moreover, the intensity at which the 200 m front crawl was performed (supra-maximal and maximal intensities) had a significant effect (71%) on VO_{2peak} and VO_{2max} values obtained for each sampling intervals studied.

Regarding the primary aim of the current study, both (Fernandes et al., 2012; Sousa et al., 2010) studies corroborate the specialized literature conducted in other cyclic sports (namely treadmill running and cycle ergometer), which state that less frequent sampling frequencies underestimate the VO₂ values (Astorino & Robergs, 2001; Astorino, 2009; Myers et al., 1990). Regarding the swimming specialized literature, both studies are unique and both reported that the breath-by-breath acquisition presented greater values than sampling intervals of 10, 15 and 20 s. This fact seems to be explained by the greater temporal resolution that breath-by-breath sampling offers, allowing a better examination of small

changes in high VO_2 values. However, it should be taken into account that the breath-by-breath gas acquisition could induce a significant variability of the VO_2 values acquired. Moreover, while (Sousa et al., 2010) evidenced significant differences between the two shortest sampling intervals (breath by breath and 5s), (Fernandes et al., 2012) only reported significant differences between the breath by breath and time sampling interval of 10, 15 and 20 s, and between time sampling interval of 5 and 20 s. These apparently incongruent results may be due to the distinct swimming intensities at which both efforts occurred.

In conclusion, we have shown that the intensity at which the 200 m front crawl was performed (supra-maximal and maximal intensities) had a significant effect on VO_{2peak} and VO_{2max} values obtained for each averaging intervals studied, still being unanswered which of the models tested is the most appropriate sampling interval to be used. In this sense, in VO_{2peak} and VO_{2max} assessment it must be taken into account the intensity at which the effort occurred because this may lead to distinct averaging intervals strategies. At supra-maximal intensity, and considering the higher ventilation, respiratory frequency and VO₂, the possibility of selecting an artifact with lower averaging intervals (e.g. breath-by-breath), is higher. Such fact is clearly stated in the significant difference between VO_{2peak} values obtained (ranging from 61.1 to 77.7 to ml.kg-1.min-1). At maximal intensities, being this range lower (51.1 to 53.2 to ml.kg⁻¹.min⁻¹), the associated error is less obvious. A limitation to our study is the fact that the swimmers who performed the 200 m front crawl at supra-maximal intensity were not the ones that held the 200 m at maximal intensity. Such lack of uniformity could lead to inter individual differences possible to interfere in the VO_{2peak} and VO_{2max} values obtained. Future research about this topic, also conducted in ecologic swimming conditions, i.e., in swimming-pool (not in laboratory based ergometers and swimming flumes) is needed. Although VO2 is difficult to measure due to technical limitations imposed by the swimming pool and the aquatic environment, its assessment in non-ecological conditions could influence the VO_{2max}, compromise the assessment of the corresponding velocity at VO_{2max} (vVO_{2max}) and the time to exhaustion at vVO_{2max} . These two latter

problems could induce errors in training intensities prescriptions. In this sense, the most advanced (valid, accurate and reliable) monitoring methods that could be used during actual swimming must be used in order to assess VO₂ in ecological swimming conditions, allowing more reliable, accurate and valid results.

The selection of optimal sampling strategies is fundamental to the validation and comparison of research findings, as well as to the correct training diagnosis and training intensities prescription. Literature results should be taken with caution when comparing VO_{2peak} and VO_{2max} values assessed with different sampling intervals and in different intensity domains. In addition, a standardized criterion should be found to accurate set the VO_{2peak} and VO_{2max} that removes the possibility of selecting an artifact.

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Appendix II

Oxygen uptake kinetics at moderate and extreme swimming intensities

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Published on Brazilian Journal of Sporting Medicine (2013), 19 (3), 186-190.

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Abstract

Traditionally, studies regarding oxygen consumption kinetics are conducted at lower intensities, very different from those in which the sports performance occurs. Knowing that the magnitude of this physiological parameter depends on the intensity in which the effort occurs, it was intended with this study compare the oxygen consumption kinetics in the 200 m front crawl at two different intensities: moderate and extreme. Ten international male level swimmers two separate tests by 24 h: (i) progressive and intermittent protocol of 7 x 200 m. with 30 s intervals and with increments of 0.05 m.s⁻¹, to determine the anaerobic threshold correspondent step; and, (ii) 200 m at maximal velocity: in both expiratory gases were continuously collected breath-by-breath. Significant differences were obtained between amplitude and time constant determine in the 200 m at extreme and moderate intensities, respectively (38,53 ± 5,30 ml. $kg^{-1}.min^{-1}$ versus 26,32 ± 9,73 ml. $kg^{-1}.min^{-1}$ e 13,21 ± 5,86 s versus 18,89 ± 6,53 s (p≤0,05). No differences were found in time delay (9,47 ± 6,42 s versus 12,36 \pm 6,62 s, at extreme and moderate intensity, respectively ($p \le 0.05$). A negative correlation between time delay and time constant at the moderate intensity was reported (r=-0.74, $p\leq0,05$).

Key words: Swimming, VO₂ kinetics, moderate intensity, extreme intensity

Introduction

The magnitude and nature of the adjustment of the oxygen consumption (VO₂) at the beginning of any physical exercise strongly depends on the intensity at which the effort is performed (Jones & Burnley, 2009). In fact, at moderate intensities, where exercise is performed below the anaerobic threshold, the VO₂ reaches a quick balance state after a single growth phase, which is named fast component (Burnley & Jones, 2007). At high intensity though, for example, above the anaerobic threshold, the VO₂ kinetics reveals a new phase – the slow component -, which, when appearing after the fast component, delays the onset of the balance state of VO₂ (Barstow & Mole, 1991). At severe intensities, where exercise is performed significantly above the anaerobic threshold, the VO₂ and blood lactate values ([La⁻]) are not able to stabilize, and therefore, the VO₂ kinetics exposes two components (fast and slow), finishing the exercise before it is possible to obtain a balance state (Gaesser & Poole, 1996). Although it has been described very recently, the extreme intensity domain, being performed at intensity above maximal oxygen consumption (VO_{2max}) , reflects the intensity at which the majority of the competitive efforts occur (Burnley & Jones, 2007). However, few studies have been conducted in this domain, being almost unexplored in swimming, especially at higher intensities. The aim of the present work is to analyze and compare the VO₂ kinetics at two distinct swimming intensities, in conditions as close as possible to the ones obtained during competition: (i) moderate intensity, analyzing 200 m crawl at intensity corresponding to the individual anaerobic threshold - lanind); and (ii) extreme intensity, evaluating 200 m *crawl* swam at maximum intensity.

Methods

10 male swimmers of international level participated in this study. The individual and mean (± SD) values of their main physical characteristics and of

competitive swimming practice are presented in table 1. The body weight and fat mass values were determined through bioelectrical impedance (Tanita TBF 305, Tokyo, Japan). All subjects were previously informed about the details of the experimental protocol before the data collection, having offered their written consent for the participation. The protocol was approved by the ethics committee of the local Institution.

Table 1. Individual and mean \pm SD values of the main physical characteristics and sports performance of the swimmers.

Swimmer	Age (yrs)	Height (m)	Weight (kg)	Fat Mass (%)	Points Len 200m	Years of Training (yrs)	% World Record 200m
#1	17	1.77	68.1	12.5	1707.0	8	80.6
#2	24	1.82	73.4	9.2	1376.2	17	88.8
#3	24	1.92	81.5	9.1	1480.3	17	86.1
#4	19	1.78	73.7	12.8	1752.7	8	79.6
#5	22	1.84	75.2	9.7	1511.5	15	85.3
#6	21	1.89	74.6	10.1	1794.7	13	78.6
#7	22	1.72	74.2	13.6	1906.7	13	76.2
#8	16	1.87	81.0	11.2	1734.8	7	79.9
#9	21	1.82	72.3	12.3	1688.6	12	81.0
#10	21	1.83	78.4	11.2	1622.5	15	82.4
Mean±SD	20.71±2.82	1.82±0.06	75.24±4.07	11.17±1.60	1657.5±160.7	12.50±3.71	81.9±3.9

Instruments and Procedures

All experimental sessions occurred in an indoors 25 m acclimatized swimming pool (27°C), with relative humidity of 45%. Each subject performed two distinct protocols in the *crawl* style, and an interval of 24 hr between them was respected. A progressive and interval protocol of 7 x 200 m, with 30 s of interval with increments of 0.05 m.s⁻¹ between each step (Fernandes et al., 2003; Fernandes et al., 2008a). The velocity of the last step was determined according to the performance hypothetically reached at that time at 400 m crawl, subtracting later to six intensity thresholds; swimming velocity was controlled with a light pacer (TAR 1.1, GBK – electronics, Aveiro, Portugal), placed in the bottom of the pool. This test was used to determine the 200 m which was closer (or coinciding) with the velocity corresponding to the lan_{ind}, 24 hr after that, the 200 m crawl at maximum velocity was performed (Sousa et al., 2011b). In both protocols, the starts were performed from the water, and the

swimmers were told to perform open laps, always to the same side and without gliding. The VO₂ was measured through continuous expired gas collection breath-by-breath through a portable gas analyzer (K4b², Cosmed, Italy), which was connected to the swimmer through a respiratory tube and valve considered suitable for ventilatory gas parameters collection in swimming situations (Baldari et al., 2011). All that experimental equipment was lifted 2 m above the water surface on a steel cable, which made it possible to follow the swimmer along the pool, minimizing discomfort to the swimmer's movements (figure 1).

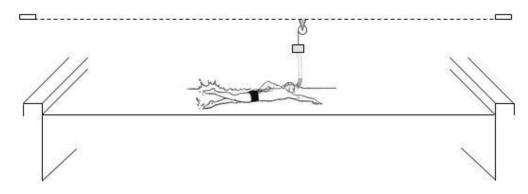


Figure 1. Experimental instrument used for collection of ventilatory gas.

In order to minimize the noise resulting from the gas collection breath-by-breath, data were then edited to exclude faulty breathing (e.g. coughing), which do not realistically represent the subjacent kinetics, being only considered the values comprised between the mean ± four SD (Özyener et al., 2001). Subsequently, the data obtained breath-by-breath were softened through a movable mean of three breaths (Guidetti et al., 2008) and recorded in mean periods of 5 s (Sousa et al., 2010), increasing the validity of the estimated parameter. Capillary blood was collected from the earlobe and used to determine the [La-] using a portable analyzer (Lactate Pro analyzer, Arcay, Inc). The collections occurred before each protocol, during the recovery periods (incremental protocol) and at the end of them (at minutes 1, 3, 5 and 7 of recovery). The [La-] enabled the determination of lan_{ind}, in the incremental protocol through the [La-] curve modelling *versus* velocity, assuming it was the interception point of the best adjustment of linear and exponential regressions used for determination of the exact point of the beginning of exponential increase of [La-] (Fernandes et al.,

2012; Machado et al., 2006). In all swimmers from the sample, the inflexion point of the [La] occurred at the 4th step of the incremental protocol. Heart rate values were continuously monitored (at each 5 s) through a monitor system (Polar Vantage NV, Polar Electro Oy, Kempele, Finland).

In order to analyze the VO₂ kinetics, the curves considered (from the 200 m corresponding to the lan_{ind} and from 200 m at maximal velocity) were modelled considering a mono-exponential fitting (equation 1):

$$VO_{2}(t) = V_{b} + A * (1 - e^{-(t-TD/\tau)})$$
 (1)

Where t is the time (s), V_b is the VO_2 value at the beginning of the exercise (ml.kg⁻¹.min⁻¹), A is the amplitude of the fast component (ml.kg⁻¹.min⁻¹), TD is time of beginning of the fast component (s) and t is the time constant of the fast component (s), i.e., the time needed to reach 63% of the plateau of this phase. Additionally, the VO_2 curves corresponding to the Ian_{ind} were also modelled considering two exponential phases (equation 2 – bi-exponential):

$$VO_{2}(t) = V_{b} + A_{1} * (1 - e^{-(t - TD_{1}/\tau_{1})}) + A_{2} * (1 - e^{-(t - TD_{2}/\tau_{2})})$$
 (2)

Where t is the time (s), V_b is the VO_2 value at the beginning of the exercise (ml.kg⁻¹.min⁻¹), A_1 and A_2 are the amplitude of the fast and slow components (ml.kg⁻¹.min⁻¹), TD_1 and TD_2 are the times of the beginning of the fast and slow components (s) and τ_1 and τ_2 are the time constants of the fast and slow components (s), respectively. The linear method of the minimum squares was implemented in the Matlab program for the adjustment of this function to the VO_2 data.

Statistical Analysis

The mean values (± standard deviation) for the descriptive analysis were obtained for all the variables of the study, for the total sample and each subject, and normality of its distribution was verified through the Shapiro-Wilk test. The SPSS program (linear regression, and the T-Test of repeated measures) was used for the inferential statistical analysis, and significance level was established at 0.05. The F-Test was used for comparison of the

monoexponential and bi-exponential fitting of the VO₂ curves corresponding to the lan_{ind} swimming intensity.

Results

The F-Test (p=0.91) presented the homogeneity of the variance of the monoexponential and bi-exponential models used to analyze the 200 m *crawl* performed at the intensity corresponding to the lan_{ind}, which was confirmed by the equality of mean values through the T-Test (p=0.97). Thus, in the present study the VO₂ kinetics at moderate and extreme intensities seem to be well-described by a monoexponential function, not being positive to use a bi-exponential function. Figure 2 presents two illustration curves of the VO₂ kinetics of one swimmer, in the 200 m corresponding to the lan_{ind}, and in the 200 m performed at maximum intensity.

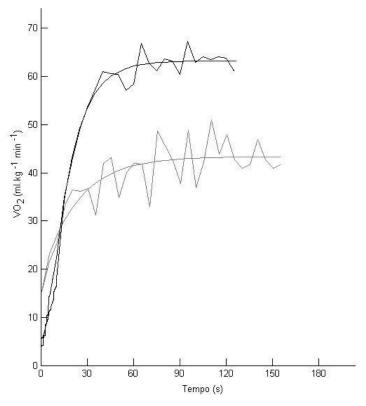


Figure 2. Example of two curve of the oxygen consumption kinetics corresponding to two distinct intensities – to the individual anaerobic threshold (gray color) and to the maximum velocity of 200 m *crawl* (black color).

The mean values (\pm SD) of A_{lan}, A₂₀₀, τ_{lan} , τ_{200} , TD_{lan} and TD₂₀₀, at moderate and extreme intensities, are presented in table 2. Statistically significant differences were obtained in two kinetic parameters (amplitude and time constant) between the 200 m performed at the lan_{ind} and maximal velocity intensities. Additionally, negative correlations were found between TD_{lan} and τ_{lan} (r=-0.74, p=0.01, figure 3). Nonetheless, further significant relations were not found in the remaining studied parameters.

Table 2. Individual and mean \pm SD values of A_{lan}, A₂₀₀, τ _{lan}, τ ₂₀₀, TD_{lan} and TD₂₀₀ corresponding to the threshold where lan_{ind} occurred in the incremental protocol and at 200 m performed at maximum velocity.

Swimmer	A _{lan} (ml.kg ⁻¹ .min ⁻¹)	A ₂₀₀ (ml.kg ⁻¹ .min ⁻¹)	$ au_{lan}$ (s)	τ ₂₀₀ (s)	TD _{lan} (s)	TD ₂₀₀ (s)
#1	38.52	44.83	23.60	18.16	4.90	19.51
#2	31.05	32.03	19.17	22.32	9.99	15.00
#3	22.86	32.54	23.75	8,82	9.51	2.36
#4	26.73	33.57	8.85	9.33	4.99	9.99
#5	37.32	36.81	20.63	14.56	7.90	9.00
#6	18.57	45.18	23.49	22.41	17.37	4.32
#7	28.92	45.63	12.93	7.05	9.99	5.15
#8	34.52	40.72	7.79	11.01	25.0	4.98
#9	31.45	36.02	9.91	7.39	20.0	19.59
#10	22.94	36.97	20.24	11.14	13.99	4.81
Mean±SD	26.32±9.73	38.43±5.30*	18.89±6.53	13.21±5.86*	12.36±6.62	9.47 ±6.4

 A_{lan} , A_{200} = amplitude of the 200 m at the intensity corresponding to lan_{ind} and maximum velocity, respectively; TD_{lan} , TD_{200} = time delay of the 200 m at intensity corresponding to lan_{ind} and maximum velocity, respectively; τ_{lan} , τ_{200} = time constant of the 200 m at intensity corresponding to lan_{ind} and maximum velocity, respectively. *Significantly different from the respective kinetic parameter corresponding to the intensity individual anaerobic threshold.

Discussion

The aim of the present study was to assess and compare the VO_2 kinetics in 200 m *crawl* performed at two distinct swimming intensities: moderate (corresponding to the lan_{ind}) and extreme (at maximal intensity).

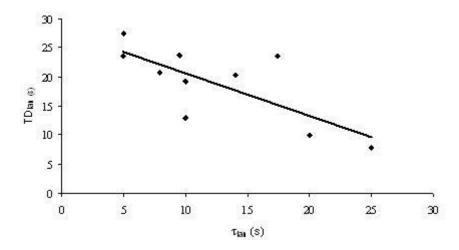


Figure 3. Ratio obtained between time of the beginning of the fast component to the intensity corresponding to the individual anaerobic threshold (TD_{lan}) and the time constant of the fast component to the intensity corresponding to the individual anaerobic threshold (τ_{lan}) (y=26.62 – 0.75x, n = 10, r=-0.74, p<0.05).

Since these two intensities are considered very important in the swimming training, as they are used for the development of the aerobic and anaerobic capacities, respectively, it seems crucial to provide better understanding on the VO₂ kinetic parameters. The literature has highlighted the study of low and moderate effort intensities, while studies concerning higher intensities are scarcer, which are representative of the swimming rhythm used during competition. Moreover, the existing studies occurred at unspecific and/or laboratory evaluation conditions (e.g. cycling ergometer and treadmills), compromising hence the validity and applicability of their results. Concerning swimming, only (Rodríguez et al., 2003; Rodríguez et al., 2008; Sousa et al., 2011b) carried out studies at high intensities and at conditions as close as possible to the real swimming conditions, and there are no comparative studies between intensity domains.

Exercise intensity below the lan_{ind} is characterized by the presence of three distinct phases: cardiodynamic, fast and the VO₂ stabilization which occurs three minutes after the beginning of the exercise (Xu & Rhodes, 1999). The intensity immediately above the lan_{ind} presents an additional phase (slow component), which delays the onset in the VO₂ stabilization, appearing

approximately 10 min after the beginning of the effort (Burnley & Jones, 2007). However, being the upper boundary of the moderate intensity and, consecutively, the lower one in the high intensity domain, the lan_{ind} is an intensity little studied concerning the VO₂ kinetics. However, (Özyener et al., 2001) refer that moderate intensities are well-described by monoexponential fittings, instead of the high intensities (high and sever intensity domains) which are better characterized by bi-exponential fittings.

In the present study, and considering the F-Test values, it was verified that the intensity corresponding to the lan_{ind}, the VO₂ kinetics will be possibly described considering the existence of a single phase (fast component) and, consequently, the use of a bi-exponential fitting becomes unnecessary. Although no study has been carried out at this specific intensity, other ones conducted at the moderate intensity domain presented monoexponential fitting in the VO₂ kinetics (Carter et al., 2000; Carter et al., 2002; Cleuziou et al., 2004; Fawkner et al., 2002; Fawkner & Armstrong, 2003; Pringle et al., 2003a). Concerning extreme intensity, monoexponential fittings were previously defined as being more positive for this intensity domain (Sousa et al., 2011b).

Concerning the kinetic parameters, we verified that they are significantly different between the two exercise intensities studied, especially regarding amplitude and time constant. Thus, higher values of these two parameters were obtained in the 200 m *crawl* performed at maximal velocity, contrary to the time delay whose mean values were higher at the intensity corresponding to the lan_{ind}. The amplitude values corroborate the ones presented in the literature, either for the moderate (Barstow & Mole, 1991; Carter et al., 2002; Cleuziou et al., 2004; Pringle et al., 2003a) or for the extreme domain (Sousa et al., 2011b), where only the later was carried out with swimming. The tendency for higher values of amplitude in the extreme domain supports the literature carried out in cycle ergometer (Carter et al., 2002; Cleuziou et al., 2004; Pringle et al., 2003a) and in domains of high intensity (Scheuermann & Barstow, 2003). These differences are due to the higher values of VO₂ reached in the extreme domain

(higher oxygen demand), since as the effort intensity increases, the amplitude gain is higher. This fact is well explained in figure 2, where the higher VO₂ values reached at the end of the exercise can be observed.

Despite this, higher VO₂ values are also observed at the beginning of the moderate effort, comparatively to the effort performed at extreme intensity. Such fact is due to the previous performance of the 200 m crawl steps included in the protocol used (cf. instrument and procedures section) and that, despite being performed at low intensity, induced an increase in the VO₂ baseline values at the beginning of the following step. However, studies conducted refer that only previous exercise of high intensity conditions and influences the following efforts, namely slow component VO2 (Koppo & Bouckaert, 2000a; Koppo & Bouckaert, 2000b) kinetics. Thus, it seems that the existence of low intensity plateaus preceding the effort corresponding to the laning did not influence the respective VO₂ kinetics to lan_{ind}. Significant differences went to the time constant, being higher at the intensity corresponding to the laning, clashing hence with some studies which refer the constancy of this parameter along the different intensities (Carter et al., 2000; Cleuziou et al., 2004; Pringle et al., 2003a). However, it should be mentioned that the later ones were performed in cycle ergometer and comparing moderate to high intensity and/or severe domains.

In spite of this information, the values of the time constant observed for the 200 m *crawl* performed at maximal velocity are lower than the ones reported in the literature (Rodríguez et al., 2003; Rodríguez et al., 2008), especially for the 100 and 400 m distances, but similar to the ones by Sousa et al. (2011) for the same distance. Regarding the intensity corresponding to the lan_{ind}, the values presented corroborate the ones reported in the literature for efforts performed in cycle ergometer (Carter et al., 2000; Carter et al., 2002; Cleuziou et al., 2004; Fawkner et al., 2002; Pringle et al., 2003a). In the present study, the fact the time constant is not similar between the two intensities seems to be due to the extreme intensity at which the 200 m *crawl* were performed. Therefore, and

since the value of the time constant describes the adaptation profile of the cardiovascular and muscular systems at the intensity of the performed effort (Markovitz et al., 2004), the sudden and exponential need of VO₂ to higher intensities (figure 2) will be able to explain the lower values of this parameter.

The time delay was the only kinetic parameter where significant differences have not been verified between the two studied intensities, corroborating the studies which compare the moderate and high exercise domains (Carter et al., 2002) and moderate and severe domains (Cleuziou et al., 2004). However, Pringle et al. (2003) showed that this parameter ranges between the moderate, high and severe domains. Although the mean values found in our study are lower than the ones found in the literature for the moderate domain (Carter et al., 2000; Cleuziou et al., 2004; Pringle et al., 2003a), the values corresponding to the extreme domain agree with the only study conducted in the swimming environment for the 200 m distance (Sousa et al., 2011b). In the moderate domain, the differences found may be due to the fact the studies mentioned have been conducted in different sports modalities.

The negative correlation observed between the delay and time constant in the 200 m *crawl* performed at lan_{ind} intensities has not been previously reported in the literature; nevertheless, in the present sample the swimmers, whose fast component of VO₂ started earlier (shorter time delay), were those who also needed more time (longer time constant) until they reached stabilization in the VO₂ consumption. Thus, the sports performance level of our sample (high level) as well as its specialty (sprinters) seem to be two factors which explain the correlations reported here.

Conclusion

Both were well described by mono exponential fittings and significant differences have been verified between them concerning amplitude and time constant. Thus, higher values of these two kinetic parameters have been obtained in 200 m *crawl* performed at maximum velocity, contrary to the timed delay whose mean was higher at the intensity corresponding to the lan_{ind}. Additionally, negative correlations have been obtained between TD_{lan} and τ_{lan} .

Acknowledgments

Science and Technology Foundation (PTDC/DES/101224/2008; SFRH/BD/72610/2010). Ana Sousa acknowledges the Portuguese Science and Technology Foundation (FCT) for the PhD grant (SFRH/BD/72610/2010).

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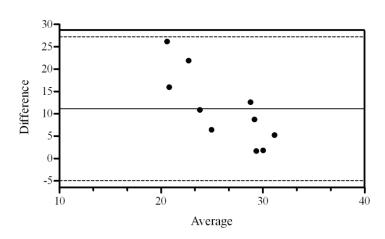
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