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Breathing mechanics at different exercise intensity domains

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To my sister,
my strength and the best part of me.

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Abstract

Understanding how the changes in breathing mechanics with exercise are reflected in biophysical responses is essential to interpret sports performance. It was implemented an intermittent incremental protocol until exhaustion according to the exercise mode: (i) in swimming, 200 m steps with $0.05 \text{ m}\cdot\text{s}^{-1}$ velocity increments were performed on an indoor 25 m swimming pool, (ii) in running, 800 m steps with $1 \text{ km}\cdot\text{h}^{-1}$ velocity increments were performed on an outdoor track field and (iii) in cycling, 3 min steps with 25 W increments were performed on a smart trainer system. All the steps were separated by 30 s passive rest intervals. In some studies, a 100 m, 400 m and 1 min maximal bouts were added at the end of the protocol (respectively). Results pointed out the repeatability of the most frequently assessed ventilatory, metabolic and biomechanical variables during the incremental protocol, well characterizing the swimmers physiological condition at the evaluation moment. When using a respiratory snorkel, especially at higher intensities, swimmers seem to adopt a different breathing pattern, losing the imposed synchronization with stroke rate (due to face immersion and restricted ventilation). Given the advantages of assessing oxygen uptake through backward extrapolation and the analysis of its off-transient kinetics, the development and validation of VO₂FITTING (an open-source software to characterize oxygen uptake kinetics during continuous exercise) for post-exercise data was conducted. Regarding the bioenergetic profiles, the aerobic and anaerobic metabolisms have different behaviours and relative contributions along the velocities in the three exercise modes. When considering low to extreme velocities, exercise economy is better described by an exponential model. With the increase in intensity and energy demand, there is a constant adaptation of physiological responses and technical patterns, reflecting the close interaction between these mechanisms. In the three exercise modes, the ventilation rise along the intensities seems to be mainly caused by increases in respiratory frequency, while tidal volume was maintained. This analysis allowed a deeper understanding of performance depending on the intensity, improving training evaluation and prescription.

Keywords: ventilatory responses, oxygen uptake, blood lactate, bioenergetics, cyclic exercise modes

Resumo

Perceber de que forma as alterações nos mecanismos respiratórios com o exercício são refletidas nas respostas biofísicas é essencial para a interpretação da performance desportiva. As avaliações basearam-se num protocolo incremental intermitente até à exaustão, adaptado a cada modalidade, realizando-se (i) na natação, patamares de 200 m e incrementos de velocidade de $0.05 \text{ m}\cdot\text{s}^{-1}$, numa piscina de 25 m, (ii) na corrida, patamares de 800 m e incrementos de velocidade de $1 \text{ km}\cdot\text{h}^{-1}$ na pista e (iii) no ciclismo, patamares de 1 min e incrementos de potência de 25 W num rolo de treino. O intervalo entre patamares foi de 30 s. Para alguns estudos, uma repetição máxima de 100 m, 400 m e 1 min foi incluída no final do protocolo (respetivamente). Os resultados evidenciaram a repetibilidade das variáveis ventilatórias, metabólicas e biomecânicas mais avaliadas, caracterizando corretamente os sujeitos no momento da avaliação. Quando o *snorkel* respiratório é usado, os nadadores adotam um padrão respiratório diferente, perdendo a sincronização imposta com a frequência gestual (devido à posição horizontal e ventilação restrita), sobretudo a intensidades mais altas. Dado as vantagens de avaliar o consumo de oxigénio através da retroextrapolação e análise da cinética da curva de recuperação, o VO₂FITTING (software gratuito para a caracterização da cinética da curva de consumo de oxigénio durante exercício contínuo) foi desenvolvido e validado para dados pós-exercício. Quanto aos perfis bioenergéticos, os metabolismos aeróbio e anaeróbio têm diferentes comportamentos e contribuições relativas ao longo do espectro de velocidades estudadas, nas três modalidades. Quando se consideram velocidades baixas a máximas, a economia de exercício é melhor descrita por um modelo exponencial. Com o aumento da intensidade e demanda energética, há uma adaptação constante das respostas fisiológicas e padrões técnicos, refletindo a interação entre estes mecanismos. Nas três modalidades, o aumento da ventilação parece ser causado pelo aumento da frequência respiratória, enquanto o volume corrente é mantido. Esta análise permitiu um conhecimento mais aprofundado da performance dependendo da intensidade melhorando a avaliação e prescrição do treino.

Palavras-chave: respostas ventilatórias, consumo de oxigénio, lactato sanguíneo, bioenergética, modalidades cíclicas

List of abbreviations and symbols

ANOVA	Analysis of variance
AnT	Anaerobic threshold
A_{off}	Amplitude of the onset of the fast oxygen uptake component during exercise
A_{on}	Amplitude of the onset of the fast oxygen uptake component during recovery
A_p	Amplitude of the fast oxygen uptake component
A_{sc}	Amplitude of the slow oxygen uptake component
C	Energy cost of locomotion
CI	Confidence intervals
CO ₂	Carbon dioxide
CO _{2exp}	Carbon dioxide expired
CV	Coefficient of variation
$\dot{E}$	Energy expenditure
ES	Effect size
EE $\dot{V}O_2$	End-exercise oxygen uptake value
e.g.	As example
FeCO ₂	Fraction of expired carbon dioxide
FeO ₂	Fraction of expired oxygen
FetCO ₂	Fraction of end tidal carbon dioxide
FetO ₂	Fraction of end tidal oxygen
f_R	Respiratory frequency
HR	Heart rate
ICC	Intraclass correlation coefficient
[La ⁻]	Blood lactate concentration
O ₂	Oxygen
O _{2exp}	Oxygen expired
R	Respiratory exchange ratio
r	Pearson's correlation coefficient
SD	Standard deviation

SL	Stroke length
SR	Stroke rate
T_{on}	Time constant of the onset of the fast oxygen uptake component during exercise
T_{off}	Time constant of the onset of the fast oxygen uptake component during recovery
T_p	Time constant of the fast oxygen uptake component
T_{sc}	Time constant of the slow oxygen uptake component
TD_{on}	Time delay of the onset of the fast oxygen uptake component during exercise
TD_p	Time delay of the fast oxygen uptake component
TD_{sc}	Time delay of the slow oxygen uptake component
v	Velocity
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E$	Minute ventilation
$\dot{V}E/\dot{V}CO_2$	Ventilatory equivalents for carbon dioxide
$\dot{V}E/\dot{V}O_2$	Ventilatory equivalents for oxygen
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
V_T	Tidal volume
T_e	Duration of expiration
T_i	Duration of inspiration
T_{tot}	Duration of total breathing cycle
η_p^2	Partial eta-squared

Chapter 1

General introduction

Breathing and pulmonary ventilation are fundamental physiological processes that support life by enabling gas exchange. This exchange involves the oxygen uptake ($\dot{V}O_2$) and the carbon dioxide elimination, which are crucial for cellular metabolism and homeostasis (Jones & Poole, 2005; Wasserman, 1978), as displayed in Figure 1. Breathing mechanics, i.e., how the air is drawn into and expelled from the lungs, is controlled by a complex interaction of respiratory muscles, lung compliance, airway resistance and neural feedback mechanisms (Dominelli & Sheel, 2012). The pressure differences between the alveoli and the environment are the driving forces for the gases exchange that occur during ventilation.

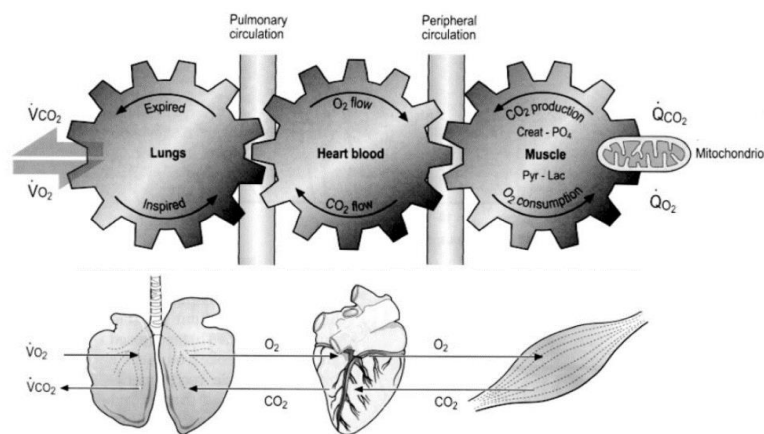


Figure 1. Schematic illustration of the relationship between muscle oxygen consumption and its pulmonary uptake. Oxygen is inspired from the atmosphere and delivered by the cardiovascular system to the working muscles, where it is consumed in the mitochondria to produce ATP. The deoxygenated blood is then transported back to the pulmonary circulation enabling muscle $\dot{V}O_2$ to be estimated from pulmonary oxygen exchange (adapted from Jones & Poole, 2005).

At rest, breathing follows a rhythmic cycle involving the diaphragm and intercostal muscles. Their contraction lowers the diaphragm, raises and expands the chest and lungs, creating a negative pressure in the thoracic cavity that allows air to enter (Dominelli & Sheel, 2012). During expiration, the diaphragm relaxes, reducing the volume of the chest and increasing the pressure in the lungs, forcing the air out. Since this action occurs primarily due to intrinsic elastic recoil of the

lungs, expiration is described as passive at rest (Dominelli & Sheel, 2012; West, 2012). This process maintains oxygen consumption and carbon dioxide elimination without a significant conscious effort, primarily controlled by the brainstem respiratory centres (Forster et al., 2012).

While breathing at rest is relatively well-described and understood, the respiratory system faces unique challenges during exercise, particularly as intensity rises. The increased demand for oxygen and the need to eliminate the excess of carbon dioxide require the respiratory system to dynamically adapt to meet the needs of working muscles (Jones & Poole, 2005). The utilization of physiological indicators and the interpretation of the $\dot{V}O_2$ kinetics curve enables a deeper comprehension of the individual responses to exercise (Capelli, 1999; Burnley and Jones, 2007). Consequently, understanding breathing mechanics across different exercise intensity domains is essential to know how the body maintains an efficient gas exchange and meets the metabolic demands under stress.

Tests like spirometry, plethysmography and maximal voluntary ventilation are commonly used to analyse breathing mechanics, measuring lung volumes, airway resistance and respiratory muscle function (Dominelli & Sheel, 2012). However, these tests are often impractical during dynamic exercise due to their intrusive nature and the need for controlled environments. This challenge highlights the importance of using portable systems like gas analysers which provide continuous, non-invasive monitoring of ventilatory variables as $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$), respiratory frequency (f_R) and tidal volume (V_T) in real-time during exercise, allowing for effective assessment of respiratory efficiency in practical, performance-oriented contexts.

The relationships between these ventilatory and metabolic variables during incremental exercise protocols provide valuable insights into breathing mechanics, even when direct mechanical assessments are not possible. As exercise intensity increases, the proportional rise in $\dot{V}E$ to $\dot{V}O_2$ and $\dot{V}CO_2$ reflects how the respiratory system adapts to meet the metabolic demands (Glaad & Taube, 2022). A linear increase in $\dot{V}E$ relative to $\dot{V}O_2$ indicates efficient oxygen delivery, while a steeper rise in $\dot{V}E$ relative to $\dot{V}CO_2$ signals the ventilatory threshold, where breathing compensates for accumulating carbon dioxide and

metabolic acidosis. Modifications in f_R and V_T describe the breathing pattern, where a balanced increase suggests efficient mechanics, while a rapid and shallow breathing indicates mechanical limitations or respiratory fatigue. By analysing these variables through incremental protocols, it is possible to infer key aspects of breathing efficiency and potential mechanical constraints, offering a more comprehensive view of breathing mechanics under exercise stress.

The delimitation of different exercise intensity domains has been evolving over time. The single utilization of a percentage (%) of maximal oxygen uptake ($\dot{V}O_{2max}$) to describe an exertion was frequently observed. However, it is known that a specific % $\dot{V}O_{2max}$ may correspond to different intensities within a sports practitioner's group (Coyle et al., 1988; Fernandes, 2013) due to differences regarding training adaptations and metabolic profiles, leading to an inadequate characterization of the exercise domains. Thus, the inclusion of blood acid-basis profile, particularly through the assessment of blood lactate concentrations ($[La^-]$), is essential to a precise identification of the different intensities and a correct and individualized prescription of training workloads (Heck et al., 1985; Fernandes, 2013).

Considering the anaerobic threshold (AnT, the boundary after which lactate production exceeds its transformation, signalling a shift from predominantly aerobic energy production to an increase in reliance of anaerobic metabolism) (Carvalho et al., 2020; Pelarigo et al., 2016) and $\dot{V}O_{2max}$ as the primary physiological indicators used for exercise intensity domains delimitation, it is possible to establish five different domains: low, moderate, heavy, severe and extreme. Each one is characterized by unique respiratory demands and challenges that elicit distinct physiological responses (Fernandes et al., 2024; Wassermann et al., 1973). First, the low intensity domain corresponds to the exercise velocities or power outputs below the AnT, characterized by $[La^-]$ values closer to baseline (Lopes et al., 2023), where $\dot{V}O_2$ achieves a steady-state, after a rapid increase, that can be sustained for extended periods (Pelarigo et al., 2016; Sousa et al., 2014a).

The AnT, that is related to the maximum velocity or power output at which the body can maintain its homeostasis, is considered one of the most consistent

predictors of performance in cyclic and individual exercise modes (Carvalho et al., 2020; Wassermann et al., 1973), being its velocity or power output corresponding to the moderate domain (Carter et al., 2002; de Jesus et al., 2015). In practical terms, it is given by the break point on the $[La^-]$ vs. workload curve during incremental exercise (Fernandes et al., 2011; Cardoso et al., 2022). At these low and moderate domains, the aerobic energy system supports the vast majority of the energy requirements (Fernandes, 2013; Gustin, 2001). Above this point, the oxygen supply is unable to meet the demands of the working muscles, leading to an increase in the contribution of the anaerobic pathways. Consequently, the accumulation of lactic acid in the bloodstream is notable, indicated by increasingly high $[La^-]$ values (Barstow & Molé, 1991; Bell et al., 2017; Carvalho et al., 2020).

The heavy intensity domain is characterized by velocities or power outputs above the AnT and below the $\dot{V}O_{2max}$, existing a substantial accumulation of $[La^-]$ over time, together with an elevated $\dot{V}O_2$ response (de Jesus et al., 2015; Sousa et al., 2014b). Often, a $\dot{V}O_2$ stabilization does not occur, being observed a second increase until a delayed steady-state or exhaustion are attained (Billat, 2000; Reis et al., 2012; Whipp, 1994). This phenomenon is particularly evidenced when square-wave exercises at this exertion are performed, and seems to be related to the progressive recruitment and activation of fast twitch fibres and $[La^-]$ increase (Barstow & Molé, 1991; Poole et al., 1988). In each subject, different velocities or power outputs may be included in this domain, rendering its characterization more challenging.

The severe intensity domain is determined by the identification of the $\dot{V}O_{2max}$, or the minimum velocity or power output that elicits the $\dot{V}O_{2max}$, based on specific physiological criteria, namely the occurrence of a plateau in $\dot{V}O_2$ ($< 2.1 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) despite an increase in workload, high levels of $[La^-]$ ($\geq 8 \text{ mmol}\cdot\text{L}^{-1}$) and elevated respiratory exchange ratio (≥ 1.15) (Howley et al., 1995). At this intensity domain, neither $\dot{V}O_2$ nor $[La^-]$ can be stabilized (de Jesus et al., 2015; Gaesser & Poole, 1996), showing a pronounced magnitude compared with heavy exertions (Faina et al., 1997; Pringle et al., 2003), and a $\dot{V}O_2$ slow component is commonly reported (Fernandes et al., 2008; Özyener et al., 2001).

Finally, the extreme intensity domain accounts for short duration and maximal intensity efforts at velocities or power outputs where exhaustion occurs before $\dot{V}O_2$ max is attained (Hill et al., 2002; Ribeiro et al., 2017). It includes a significant range of exercise durations, from explosive movements (as the starting phase of a specific event) to maximal exertions until 2 min (Alexander et al., 2019; Almeida et al., 2020). Due to the high involvement of the anaerobic pathways (relying on its availability on the muscles and the speed at which they can use the available energy), $[La^-]$ is much more expressive than in the previous domains (Hellard et al., 2018; Santos et al., 2021).

Thus, the performance of an incremental protocol until exhaustion combining (i) sufficiently long steps to allow the attainment of a $\dot{V}O_2$ steady-state, (ii) workload increments that facilitate a progressive intensity increase without premature exhaustion and (iii) short-time intervals for the capillary blood sample collection, enables the individual determination of the exercise velocities or power outputs corresponding to the distinct exercise intensity domain (Bentley et al., 2007; McNaughton et al., 2006; Sousa et al., 2015). Consequently, it allows for a comprehensive characterization of the associated ventilatory responses. Depending on the exercise mode, the incremental protocol may be adjusted, according to their specificity, to fulfil its purpose. In general, steps lasting ~3 min are used, given the time needed to a $\dot{V}O_2$ stabilization or for the emergence of a $\dot{V}O_2$ slow component, depending on the velocity or power output and the corresponding intensity domain (Bentley et al., 2007; Billat, 2000).

The current thesis aims to conduct a biophysical analysis along the spectrum of exercise intensities to better understand the ventilatory responses and to comprehensively characterize cyclic sports performance. Beyond the current chapter, where a general introduction about the research topic is conducted, this thesis comprises five experimental and a perspective related studies (**Chapters 2 to 7**). Afterwards, a general discussion is presented, where the results obtained in the experimental studies are related between them and compared with the literature (**Chapter 8**), followed by the conclusions (**Chapter 9**), the recommendations for future studies (**Chapter 10**) and the bibliographic references supporting each chapter of this work (**Chapter 11**).

A significant number of studies conducted both pulmonary gas exchange and ventilation assessment and biomechanical analysis in laboratory conditions under controlled environments, using treadmill running or cycle ergometer protocols (Lopes et al., 2023; Sousa et al., 2014a; Swinnen et al., 2018). The development of portable breath-by-breath gas analyser systems allowed a more ecological evaluation, without the need of using ergometers to mimic the sports specific movements. From the three exercise modes studied in the current Thesis, swimming was the most challenging to explore given the constraints of the aquatic environment. Thus, some purposes of this work were conducted based exclusively on swimmers' evaluation.

In **Chapter 2**, the accuracy of a specific protocol test was assessed. In fact, it is expected that the output test did not vary in short-term repeated measurements on the same subject, independently of the exercise type performed, otherwise it would not be helpful for its characterization and further training prescription (Nevill & Atkinson, 1997; Smith et al., 2002). Thus, our first purpose was to determine the repeatability of different ventilatory, metabolic and biomechanical variables assessed during a front crawl intermittent incremental protocol until exhaustion frequently used for a biophysical monitoring (Zacca et al., 2019b). It was hypothesized that, from low to severe exercise intensities, a strong agreement, combined with a small range of variation, would be observed for all the selected variables.

The aquatic environment restrictions imposed to the human body lead to an augmented work of the inspiratory muscles, restricted ventilation due to the horizontal position and the need to synchronize the respiratory phases with upper and lower limbs actions (Lomax & McConnel, 2003; Lehay et al., 2019). So, a continuous ventilatory assessment is only possible when swimmers use a breathing snorkel that connects them to the portable gas analyser (Ribeiro et al., 2015), allowing to describe the relationship between f_R and stroke rate. In **Chapter 3**, we aimed to assess the acute ventilatory responses and to characterize the adopted breathing patterns when swimming front crawl from low to extreme intensity domains. It was hypothesized that, despite the respiratory constraints, gas exchange variables would increase concomitantly with the swimming velocity rise. In addition, we suggest that swimmers would keep the

breathing patterns used during free swimming when breathing into a snorkel due to the breathing synchronization with stroke rate.

As mentioned, to continuously characterize swimmers' physiological responses to exercise, it is mandatory the use of the breathing snorkel. However, this apparatus may cause additional restrictions to swimmers' evaluation, particularly due to the impossibility of include block starts, tumbling turns and underwater gliding phases (Barbosa et al., 2010; Figueiredo et al., 2013). Despite there is no modification in swimming velocity (no increase in active drag while swimming with the snorkel was showed), the total time is affected by the use of open turns and the absence of underwater gliding (Ribeiro et al., 2016). To better characterize swimmers' intensity domains, the backward extrapolation method may be conducted, allowing swimmers to free perform and, consequently, to achieve competitive velocities (Chaverri et al., 2016; Laffite et al., 2004; Montpetit et al., 1981).

By using data from the recovery periods, it is also possible to study the $\dot{V}O_2$ off-transient kinetics, allowing to better understand the responsiveness to training stimuli, particularly regarding the physiological significance of the fast and slow components of the dynamic $\dot{V}O_2$ response (Monteiro et al., 2020; Sousa et al., 2011). In **Chapter 4**, we aimed to present an updated version of VO_2 FITTING, a validated, free and open-source software (previously developed to characterize $\dot{V}O_2$ kinetics during continuous exercise; Zacca et al., 2019a) that allows to dynamically analyse and model $\dot{V}O_2$ post-exercise data. In addition, we aimed to contribute to the existent knowledge about the $\dot{V}O_2$ on/off symmetry by directly comparing the exercise and its subsequent recovery at different intensity domains. It was hypothesized that an on/off symmetry would be observed at low and moderate intensities, while at heavy and severe domains different exponential models for the exercise and recovery phases would be evidenced.

Cyclic sports performance depends on a complex interaction of factors: (i) combination of high cardiac output and high rate of oxygen delivery to working muscles, leading to a large capacity for adenosine triphosphate regeneration, i.e., high $\dot{V}O_{2max}$, (ii) the balance between lactate production and clearance, given by the AnT and (iii) the exercise economy (Fernandes et al., 2005; Saunders et

al., 2004; Swinnen et al., 2018). Exercise economy is typically calculated as the steady-state $\dot{V}O_2$ for a given velocity. This is a complex and multifactorial concept that represents the sum of various metabolic, cardiorespiratory, biomechanical and neuromuscular characteristics (Barnes and Kilding, 2015). Two determinant aspects of exercise economy calculation refer to the lack of consideration of the anaerobic energy system and velocities or power outputs above the AnT (Fernandes & Vilas-Boas, 2020).

In **Chapter 5**, a perspective is presented about the methods used to calculate exercise economy. Considering that merely relating a $\dot{V}O_2$ value to an exercise velocity or power output when characterizing exercise economy is reductive, since it does not consider possible differences in metabolism which may exist between sport practitioners or under different conditions (Daniels, 1985), we seek to deeper understand the bioenergetic responses on a large spectrum of workloads, particularly from low to extreme. In **Chapter 6**, we aimed to evidence the participation of the anaerobic metabolism and its influence in energy expenditure (and respective relative contributions) and energy cost estimation and to assess the intensity effect on exercise economy profile, given by the slope of the regression line between energy expenditure and velocity. It was hypothesized that, even at lower velocities, energy cost would be higher when considering the anaerobic metabolism and that athletes profile would be underestimated when considering only submaximal velocities and the aerobic metabolism.

After a comprehensive description of the ventilatory and metabolic responses to different exercise modes and intensities, we deemed it would be interesting to also characterize the biomechanical responses through a large spectrum of intensity domains. In fact, a wide number of studies evaluating running (both physiologically and biomechanically) are performed on a treadmill in laboratory conditions, under a controlled environment. However, the high association and applicability between field data, training and competing contexts are undeniable. Considering that changes in running velocity will alter the adopted biomechanical pattern, especially in overground running, that, consequently, will modify the pulmonary and ventilatory response (Cavanagh & Williams, 1982; Williams, 2000), in **Chapter 7** we aimed to characterize the biomechanical and

physiological adaptations while running overground at low, moderate, heavy and severe exercise intensities and to assess the consistency of the running gait pattern within each intensity domain. It was hypothesized that there would be a decrease in temporal and an increase in frequency- and distance-related variables, concurrently with a rise in the physiological variables. A maintenance of linear and angular kinematic variables from the beginning to the end of each exertion was also expected.

Repeatability of ventilatory, metabolic and biomechanical responses to an intermittent incremental swimming protocol

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Abstract

Objective. This study aimed to determine the repeatability of ventilatory, metabolic and biomechanical variables assessed at a large spectrum of front crawl swimming intensities. We hypothesized a strong agreement (combined with a small range of variation) between a typical step protocol performed in two experimental moments. **Approach.** Forty competitive swimmers performed a 7 x 200 m front crawl intermittent incremental protocol ($0.05 \text{ m}\cdot\text{s}^{-1}$ velocity rises and 30 s intervals) on two different occasions (48-72 h apart). Pulmonary gas exchange and ventilation were continuously measured breath-by-breath, metabolic variables were assessed during the intervals and biomechanical analysis was done at every protocol step. **Main results.** Concomitantly with the velocity increment, oxygen uptake, carbon dioxide production, ventilation, respiratory frequency, respiratory exchange ratio, averaged expiratory concentrations, end tidal oxygen and ventilatory equivalents for oxygen and carbon dioxide and blood lactate concentrations rose ($p < 0.001$), averaged expiratory concentrations and end tidal carbon dioxide and duration of inspiration, expiration and total breathing cycle decreased ($p < 0.001$), while tidal volume and volumes of oxygen and carbon dioxide expired maintained constant. Stroke frequency and stroke length increased and decreased (respectively) with the swimming velocity raise. No differences between experimental moments were observed in most of the assessed variables ($p > 0.05$), with a low dispersion (0.49%-9.94%) except for lactate concentrations and inspiration and expiration durations (11.00%-17.16%). Moderate-nearly perfect direct relationships and a good-excellent degree of reliability between moments were verified for all the assessed variables ($r = 0.50-1.00$, $\text{ICC} = 0.76-1.00$, $p < 0.001$), except for respiratory exchange ratio. **Significance.** The reliability analysis confirmed the repeatability of the assessed ventilatory, metabolic and biomechanical variables, with the obtained data well representing swimmers physiological condition when monitoring performance through a commonly used step protocol.

Keywords: repeatability, ventilation, metabolism, biomechanics, incremental protocol, swimming.

Introduction

In swimming, the assessment of aerobic fitness related variables should be part of the overall monitoring process and subsequent training prescription since oxidative energy provision plays a critical role in swimmers performance independently of the effort duration (de Jesus et al., 2015; Ribeiro et al., 2017). Oxygen uptake ($\dot{V}O_2$) evaluation allows better understanding variables such as: (i) maximal oxygen consumption ($\dot{V}O_{2max}$), the highest rate at which O_2 can be spent during severe exercise, a widely used indicator of cardiorespiratory fitness (Bassett and Howley, 2000; Sousa et al., 2014) with notable implications for swimming performance (Fernandes et al., 2005; Matos et al., 2022); (ii) energy expenditure, an accurate method for assessing swimming economy (Vilas-Boas and Santos, 1994; Barbosa et al., 2006; Figueiredo et al., 2012) and (iii) $\dot{V}O_2$ kinetics, allowing to better understand the mechanistic bases of the $\dot{V}O_2$ response to exercise at different intensity domains (Fernandes et al., 2013; Sousa et al., 2014; de Jesus et al., 2015).

However, assessing $\dot{V}O_2$ using automated gas analysis systems is performed at the mouth and may not reflect gas exchanges at the cellular level (Lamarra et al., 1987; Capelli et al., 2011). Since the concurrent assessment of pulmonary and ventilatory responses to exercise appears to partially overcome that issue (Wasserman et al., 1973; Whipp et al., 1982; Ribeiro et al., 2017), multiple ventilatory variables should be considered concomitantly with $\dot{V}O_2$ analysis when evaluating swimmers. Particularly, it should be included ventilation ($\dot{V}E$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (R), respiratory frequency (f_R), tidal volume (V_T) and other outputs that, in close combination, add valuable information about swimmers physiological status (Wasserman et al., 1973; Sun et al., 2002; Zacca et al., 2019a).

Independently of the used swimming monitoring protocol, it is important to notice a number of factors affecting swimmers day-to-day variability that can be more or less controlled, such as sleeping quality, physical activity, hydration, nutrition and motivation (Kline et al., 2007; Zacca et al., 2019b). Thus, keeping a constant routine in the previous and testing days is extremely important to ensure the minimum effect of these biological constraints (Barbosa et al., 2015; Zacca et al.,

2019a). Moreover, the number of swimming physiological and performance related testing protocols has increased significantly in the last decades, being fundamental to carry out a previous rigorous evaluation particularly considering its validity and reliability (Atkinson and Nevill, 1998). In fact, to assure the accuracy of a specific test is of utmost importance since it will be used to characterize swimmers performance determinants and ultimately assure proper training prescription (Stuart et al., 2019).

The determination of the repeatability and agreement of a specific test is of great importance in the sport context (Nevill and Atkinson 1997) and particularly in swimming (Smith et al., 2002; Reis et al., 2012). If the test outputs considerably vary in repeated measurements on the same subject, it would not be helpful in practical terms. We have already observed that square-wave exercises performed at the minimum velocity that elicits $\dot{V}O_{2max}$ (previously determined by an incremental protocol) presented a good agreement (e.g., Zacca et al., 2019a), but the repeatability of ventilatory responses to different swimming intensities is still scarce. The purpose of the current study is to determine the short-term repeatability of ventilatory, metabolic and biomechanical variables obtained during an intermittent incremental swimming protocol from low to severe intensities performed in two moments 48-72 h apart. It was hypothesized that a strong agreement, combined with a small range of variation, would be observed for all selected variables.

Methods

Ethical approval

The current study was approved by the Institutional Review Board committee of the University of Porto (CEFADE 25 2020) and conducted in accordance with the latest revision of the Declaration of Helsinki. All volunteers were informed about the experimental procedures, associated risks and benefits of participation, and gave their written informed consent before the commencement of the study.

Subjects

Forty trained swimmers (28 males and 12 females) of 32.0 ± 14.2 versus 17.4 ± 2.1 years of age, 72.9 ± 11.1 versus 58.7 ± 6.0 kg of body mass and 177.1 ± 7.5

versus 164.4 ± 4.5 cm of height (respectively) were recruited to participate. They were engaged in a swimming training frequency > three times per week (2 h per training unit) for at least one year before the start of the study and presented 229-639 Fédération Internationale de Natation (FINA) points in their best competitive performance at 400 m front crawl event. No subject was smoker or used any dietary supplementation or medication that could have affected his/her somatic and cognitive functions. All reported to be in good health and none presented medical contraindications for participation in the current study.

Study procedures

All swimmers were instructed to arrive at the evaluation centre in a rested and fully hydrated state, and refrain from alcohol and caffeine consumption for the previous 24 h. They were also asked to avoid vigorous exercise for at least 24 h before each testing moment. Throughout the study period, subjects maintained their normal daily activities, as well as food and fluid intake. All experiments were performed at the same time of the day (to avoid circadian variance) in a 25 m indoor pool, with the same water and air temperatures. Each swimmer was tested on two moments separated by 48-72 h (M1 and M2, respectively). Following a 600 m low-to-moderate intensity warm-up (Fernandes et al., 2010; de Jesus et al., 2014), swimmers performed a 7 x 200 m front crawl intermittent incremental protocol with $0.05 \text{ m} \cdot \text{s}^{-1}$ step increases and 30 s rest intervals (Fernandes et al., 2005, Barbosa et al., 2015). Initial swimming velocity was defined according to the swimmers performance on the 400 m front crawl at the moment of the experiments (by consulting swimmers and coaches) minus six increments of velocity (Figure 1; de Jesus et al., 2015; Carvalho et al., 2020). Swimming velocity was controlled using a visual pacer with flashing lights (Pacer2Swim, KulzerTEC, Aveiro, Portugal) placed on the bottom of the pool and in-water starts and open turns (without underwater gliding) were used (Ribeiro et al., 2017; Monteiro et al., 2020).

Data collection

Pulmonary gas exchange and ventilation were measured breath-by-breath using a portable gas analysis system (Cosmed K4b², Cosmed, Rome, Italy; $\pm 0.02\%$ accuracy) suspended on a steel cable 2 m above the water surface and connected to the swimmer via a specific respiratory snorkel and valve system

(Aquatrainer®, Cosmed, Rome, Italy). This is a low hydrodynamic resistance device (Fernandes et al., 2013; Ribeiro et al., 2016) that allows swimming front crawl closer to training and competitions conditions, without changes in kinematics and swimming efficiency (Barbosa et al., 2010; Sousa et al., 2014). Before each test, the gas analysis system was calibrated using ambient air, in accordance with the manufacturers guidelines, against known concentrations of cylinder gases (16% O₂ and 5% CO₂) and a precision 3 L calibration syringe (Hans Rudolph, Kansas City, United States). Capillary blood samples for lactate concentration ([La⁻]) analysis were collected from the fingertip at rest, immediately after each step and at 1, 3, 5 and 7 min after the protocol end, using a portable analyser (Lactate Pro2, Arkay, Inc., Kyoto, Japan; Carvalho et al., 2020). Stroke frequency was assessed as the number of cycles per min (using a Finis stopwatch with a three upper limb cycles frequency meter function) and stroke length was calculated by dividing the mean velocity by stroke frequency.

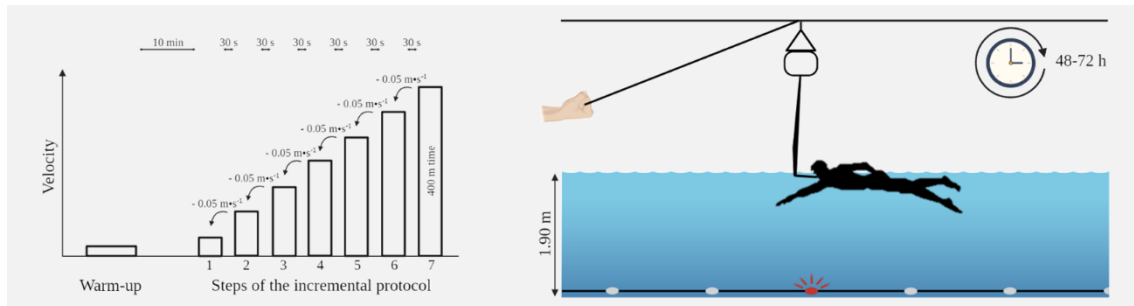


Figure 1. 7 x 200 m front crawl intermittent incremental swimming protocol (left panel) and data collection set-up (right panel).

Data processing

The pulmonary gas exchange and ventilation data from each experimental moment were examined to exclude occasional errant breaths caused by swallowing, coughing or signal interruptions. Only $\dot{V}O_2$ values between ± 3 SDs were included for analysis and subsequently smoothed using a 3-breath moving average and 10 s time average (Lamarra et al., 1987; Fernandes et al., 2012; Monteiro et al., 2020). $\dot{V}O_{2max}$ was defined according to conventional physiological criteria (Howley et al., 1995; Zacca et al., 2019a), with the occurrence of a plateau in $\dot{V}O_2$ (≤ 2.1 mL·kg⁻¹·min⁻¹) despite an increase in swimming velocity, levels of maximal [La⁻] ≥ 8 mmol·L⁻¹ or end-test respiratory exchange ratio ≥ 1.15 . $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, f_R , R , V_T , $\dot{V}O_2$ and $\dot{V}CO_2$ during the current

breath ($\text{O}_{2\text{exp}}$ and $\text{CO}_{2\text{exp}}$, respectively), fractions of expired O_2 and CO_2 (FeO_2 and FeCO_2 , respectively), fractions of end tidal O_2 and CO_2 (FetO_2 and FetCO_2 , respectively), ventilatory equivalents for O_2 and CO_2 ($\dot{V}\text{E}/\dot{V}\text{O}_2$ and $\dot{V}\text{E}/\dot{V}\text{CO}_2$, respectively) and duration of inspiration, expiration and total breathing cycle (T_i , T_e and T_{tot} , respectively) measures were obtained using the mean values of each step last 30 s.

Statistical analysis

Based on a statistical power (β) of 0.80, a moderate effect size (0.40) and an overall level of significance of 0.05, a sample size of 40 participants seemed adequate for carrying out the current study (G*Power 3.1.9.7, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Data are reported as mean $\pm$ SD and normal data distribution was confirmed using Shapiro-Wilk test. One-way repeated measures ANOVA was used to determine the incremental behaviour of the physiological variables across the intermittent front crawl swimming protocol (using partial eta-squared (η^2) for effect size calculation: 0.05-0.25 – small, 0.25-0.64 – moderate and ≥ 0.64 – strong: Ferguson, 2009) and paired t-tests were applied to identify differences between test and retest data per step. Intraclass correlation coefficients (ICC) and Pearson's correlation coefficients (r) were used to assess the degree of reliability (< 0.5 – poor, 0.5-0.75 – moderate, 0.75-0.9 – good and > 0.9 – excellent; Koo and Li, 2016) and association (< 0.1 – trivial, 0.1-0.3 – small, 0.3-0.5 – moderate, 0.5-0.7 – large, 0.7-0.9 – very large and > 0.9 – nearly perfect: Hopkins et al., 2009) between experimental moments (respectively), while coefficients of variation (CV) were calculated to express typical error. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, United States).

Results

The mean ventilatory, metabolic and biomechanical responses when swimming front crawl at increasing intensities are shown in Figure 2 and Table 1. In both experimental moments, the velocity increase along the protocol ($F_{6,234} = 507.3$, $\eta^2 = 0.93$ in M1 and $F_{6,234} = 585.3$, $\eta^2 = 0.94$ in M2) implied a raise in stroke frequency, $[\text{La}^-]$, $\dot{V}\text{O}_2$, $\dot{V}\text{CO}_2$, $\dot{V}\text{E}$, f_R , R , FeO_2 , FetO_2 , $\dot{V}\text{E}/\dot{V}\text{O}_2$ and $\dot{V}\text{E}/\dot{V}\text{CO}_2$ ($\eta^2 = 0.40$ -0.91) and a decrease in stroke length, FeCO_2 , FetCO_2 , T_i , T_e and T_{tot} ($\eta^2 =$

0.05-0.80), all for $p < 0.001$. In contrast, V_T , O_{2exp} and CO_{2exp} remained stable in both testing moments despite the velocity increment.

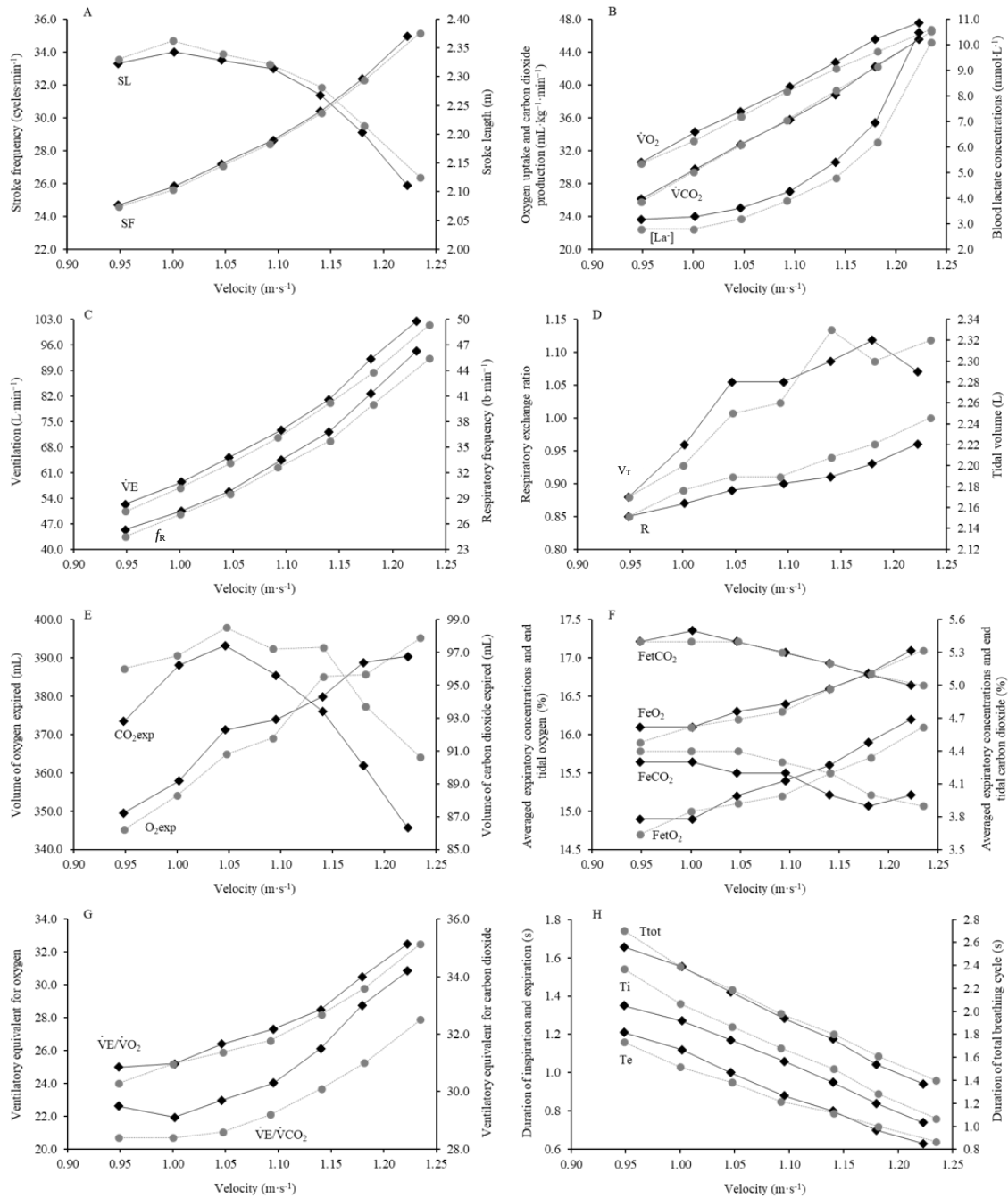


Figure 2. Mean physiological responses along the 7 x 200 m front crawl intermittent incremental protocol in moments one and two (black and grey, respectively).

Similar ventilatory, metabolic and biomechanical values were found between testing moments during the entire protocol excepting for the higher M2 versus M1 scores for velocity in the seventh step, $FeCO_2$ in the third and sixth steps, Ti in the first and second steps, and T_{tot} in the first and sixth steps, as well as the

lower M2 versus M1 scores for $[La^-]$ from the first to the sixth steps, $\dot{V}O_2$ in the second step, $\dot{V}E$ in the first, fourth and sixth steps, FeO_2 in the first step, $FetO_2$ in the first and fourth steps, $\dot{V}E/\dot{V}O_2$ in the first step, $\dot{V}E/\dot{V}CO_2$ in the first and from the third to the seventh steps, and Te in the second step ($p < 0.01$). The CV values for each protocol step are presented in Table 2, with most of the assessed variables presenting a low variation (1.29%-11.63%) since swimmers performance was very similar in both experimental moments (CV = 0.49%-1.35%), with exception of the large variation evidenced by $[La^-]$ (14.37%-17.1%).

In addition, it was observed very large to nearly perfect relations between M1 and M2 for most of the assessed variables, particularly for velocity, stroke frequency, stroke length, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, f_R , V_T , O_{2exp} , CO_{2exp} , $FetO_2$, $FetCO_2$, $\dot{V}E/\dot{V}CO_2$ and T_{tot} ($r = 0.71$ - 0.997 ; $p < 0.001$). Relevant very large associations between moments were also found for $[La^-]$ in the fourth and fifth steps, FeO_2 , $FeCO_2$ and Te from the first to the sixth steps, and FeO_2 and $\dot{V}E/\dot{V}O_2$ from the first to the fifth steps and $\dot{V}E/\dot{V}O_2$ from the first to the fifth steps ($r = 0.683$ - 0.879 , $p < 0.001$), moderate to large relations were noted in the remaining steps ($r = 0.498$ - 0.690 , $p < 0.001$) and R was the only variable that did not present an association between moments ($p > 0.05$). The reliability degree between moments was excellent for velocity, stroke frequency, stroke length, $\dot{V}O_2$, $\dot{V}E$, V_T , O_{2exp} and CO_{2exp} in each protocol step, for $FetCO_2$ from the first to the fourth and in the sixth steps, for $\dot{V}CO_2$ from the first to the fourth steps, for T_{tot} from the second to the fourth and in the sixth steps, for $FeCO_2$ in the first, second and fourth steps, for f_R , FeO_2 and $\dot{V}E/\dot{V}CO_2$ in the first step and for $FetO_2$ in the third step (ICC = 0.90-1.00, 95% CI = 0.81-1.00, $p < 0.001$). All the remain variables at other protocol steps presented a good reliability (ICC = 0.76-0.90, 95% CI = 0.55-0.95, $p < 0.001$) except for $[La^-]$ in the first, second and sixth steps, f_R and $FeCO_2$ in the last step and Ti in the first and fifth steps that evidenced a moderate reliability (ICC = 0.49-0.74, 95% CI = 0.04-0.85, $p < 0.001$). R presented a moderate reliability only in the first step (ICC = 0.49, 95% CI = 0.03-0.73, $p < 0.05$).

Table 1. Mean $\pm$ SD values of the selected ventilatory, metabolic and biomechanical responses along the front crawl intermittent incremental protocol.

		Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
v (m·s ⁻¹)	M1	0.95 $\pm$ 0.10 ⁺	1.00 $\pm$ 0.12 [†]	1.05 $\pm$ 0.12 [†]	1.10 $\pm$ 0.12 [†]	1.14 $\pm$ 0.12 [†]	1.18 $\pm$ 0.11 [†]	1.22 $\pm$ 0.11 ^{*†}
	M2	0.95 $\pm$ 0.11 ⁺	1.00 $\pm$ 0.12 [†]	1.05 $\pm$ 0.13 [†]	1.09 $\pm$ 0.12 [†]	1.14 $\pm$ 0.12 [†]	1.18 $\pm$ 0.12 [†]	1.23 $\pm$ 0.11 [†]
SF (cycles·min ⁻¹)	M1	24.7 $\pm$ 2.7 ⁺	25.8 $\pm$ 3.3 [†]	27.2 $\pm$ 3.3 [†]	28.6 $\pm$ 3.5 [†]	30.4 $\pm$ 3.5 [†]	32.4 $\pm$ 3.5 [†]	35.0 $\pm$ 3.4 [†]
	M2	24.6 $\pm$ 2.7 ⁺	25.6 $\pm$ 3.2 [†]	27.1 $\pm$ 3.3 [†]	28.4 $\pm$ 3.3 [†]	30.3 $\pm$ 3.8 [†]	32.3 $\pm$ 3.8 [†]	35.2 $\pm$ 4.0 [†]
SL (m·min ⁻¹)	M1	2.32 $\pm$ 0.26 ⁺	2.34 $\pm$ 0.30	2.33 $\pm$ 0.29	2.31 $\pm$ 0.27	2.27 $\pm$ 0.26 [†]	2.20 $\pm$ 0.25 [†]	2.11 $\pm$ 0.21 [†]
	M2	2.33 $\pm$ 0.25 ⁺	2.36 $\pm$ 0.31	2.34 $\pm$ 0.30	2.32 $\pm$ 0.27	2.28 $\pm$ 0.27 [†]	2.21 $\pm$ 0.25 [†]	2.12 $\pm$ 0.23 [†]
[La ⁻] (mmol·L ⁻¹)	M1	3.2 $\pm$ 0.8 ^{**}	3.3 $\pm$ 0.9 [*]	3.6 $\pm$ 1.2 ^{*†}	4.3 $\pm$ 1.4 ^{*†}	5.4 $\pm$ 1.9 ^{*†}	7.0 $\pm$ 2.3 ^{*†}	10.5 $\pm$ 3.3 [†]
	M2	2.8 $\pm$ 0.7 ⁺	2.8 $\pm$ 0.9	3.2 $\pm$ 1.1 [†]	3.9 $\pm$ 1.5 [†]	4.8 $\pm$ 1.7 [†]	6.2 $\pm$ 2.5 [†]	10.1 $\pm$ 3.9 [†]
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	M1	30.6 $\pm$ 5.1 ⁺	34.3 $\pm$ 7.3 ^{*†}	36.8 $\pm$ 8.4 [†]	39.8 $\pm$ 8.8 [†]	42.8 $\pm$ 9.1 [†]	45.6 $\pm$ 9.2 [†]	47.6 $\pm$ 10.0 [†]
	M2	30.5 $\pm$ 4.9 ⁺	33.2 $\pm$ 6.1 [†]	36.2 $\pm$ 6.7 [†]	39.2 $\pm$ 7.2 [†]	42.0 $\pm$ 7.5 [†]	44.1 $\pm$ 7.9 [†]	46.8 $\pm$ 8.5 [†]
$\dot{V}CO_2$ (mL·kg ⁻¹ ·min ⁻¹)	M1	26.2 $\pm$ 4.6 ⁺	29.8 $\pm$ 6.1 [†]	32.8 $\pm$ 7.2 [†]	35.8 $\pm$ 7.5 [†]	38.9 $\pm$ 8.4 [†]	42.3 $\pm$ 9.0 [†]	45.6 $\pm$ 10.1 [†]
	M2	25.8 $\pm$ 4.3 ⁺	29.5 $\pm$ 5.5 [†]	32.7 $\pm$ 6.1 [†]	35.7 $\pm$ 6.7 [†]	39.3 $\pm$ 7.1 [†]	42.3 $\pm$ 7.8 [†]	46.5 $\pm$ 8.2 [†]
$\dot{V}E$ (L·min ⁻¹)	M1	52.3 $\pm$ 11.0 ^{*†}	58.4 $\pm$ 13.8 [†]	65.2 $\pm$ 15.0 [†]	72.7 $\pm$ 16.4 ^{*†}	81.0 $\pm$ 16.5 [†]	92.2 $\pm$ 18.0 ^{*†}	102.5 $\pm$ 19.2 [†]
	M2	50.4 $\pm$ 11.7 ⁺	56.8 $\pm$ 14.2 [†]	63.6 $\pm$ 15.5 [†]	70.6 $\pm$ 16.1 [†]	80.1 $\pm$ 18.0 [†]	88.4 $\pm$ 19.0 [†]	101.5 $\pm$ 19.2 [†]
f_R (b·min ⁻¹)	M1	25 $\pm$ 6 ⁺	28 $\pm$ 6 [†]	30 $\pm$ 7 [†]	34 $\pm$ 7 [†]	37 $\pm$ 8 [†]	41 $\pm$ 7 [†]	46 $\pm$ 8 [†]
	M2	25 $\pm$ 6 ⁺	27 $\pm$ 5 [†]	30 $\pm$ 6 [†]	33 $\pm$ 6 [†]	36 $\pm$ 8 [†]	40 $\pm$ 8 [†]	45 $\pm$ 9 [†]
R	M1	0.85 $\pm$ 0.07 ⁺	0.87 $\pm$ 0.08	0.89 $\pm$ 0.09 [†]	0.90 $\pm$ 0.08	0.91 $\pm$ 0.11	0.93 $\pm$ 0.11	0.96 $\pm$ 0.11 [†]
	M2	0.85 $\pm$ 0.05 ⁺	0.89 $\pm$ 0.05 [†]	0.91 $\pm$ 0.05 [†]	0.91 $\pm$ 0.05	0.94 $\pm$ 0.06 [†]	0.96 $\pm$ 0.07 [†]	1.00 $\pm$ 0.09 [†]
V_T (L)	M1	2.17 $\pm$ 0.54	2.22 $\pm$ 0.57	2.28 $\pm$ 0.58	2.28 $\pm$ 0.57	2.30 $\pm$ 0.57	2.32 $\pm$ 0.54	2.29 $\pm$ 0.50
	M2	2.17 $\pm$ 0.54	2.20 $\pm$ 0.59	2.25 $\pm$ 0.60	2.26 $\pm$ 0.59	2.33 $\pm$ 0.58	2.30 $\pm$ 0.57	2.32 $\pm$ 0.54
O ₂ exp (mL)	M1	349.5 $\pm$ 86.4	357.8 $\pm$ 89.0	371.2 $\pm$ 91.3	373.9 $\pm$ 91.9	379.8 $\pm$ 91.3	388.7 $\pm$ 89.4	390.3 $\pm$ 84.7
	M2	345.1 $\pm$ 86.3	354.1 $\pm$ 97.5	364.8 $\pm$ 99.1	369.0 $\pm$ 97.8	385.1 $\pm$ 97.6	385.6 $\pm$ 97.2	395.2 $\pm$ 92.9
CO ₂ exp (mL)	M1	92.8 $\pm$ 24.9	96.2 $\pm$ 27.6	97.4 $\pm$ 31.0	95.6 $\pm$ 29.4	93.4 $\pm$ 30.8	90.1 $\pm$ 28.4	86.3 $\pm$ 26.0
	M2	96.0 $\pm$ 27.3	96.8 $\pm$ 29.4	98.5 $\pm$ 29.9	97.2 $\pm$ 29.5	97.3 $\pm$ 28.6	93.7 $\pm$ 28.1	90.6 $\pm$ 28.0
FeO ₂ (%)	M1	16.1 $\pm$ 0.6 ^{**}	16.1 $\pm$ 0.6	16.3 $\pm$ 0.6 [†]	16.4 $\pm$ 0.7	16.6 $\pm$ 0.8 [†]	16.8 $\pm$ 0.8 [†]	17.1 $\pm$ 0.7 [†]
	M2	15.9 $\pm$ 0.6 ⁺	16.1 $\pm$ 0.6 [†]	16.2 $\pm$ 0.6	16.3 $\pm$ 0.6	16.6 $\pm$ 0.6 [†]	16.8 $\pm$ 0.7 [†]	17.1 $\pm$ 0.7 [†]
FeCO ₂ (%)	M1	4.3 $\pm$ 0.5 ⁺	4.3 $\pm$ 0.5	4.2 $\pm$ 0.6 [*]	4.2 $\pm$ 0.6	4.0 $\pm$ 0.7 [†]	3.9 $\pm$ 0.7 ^{*†}	4.0 $\pm$ 1.9 [†]
	M2	4.4 $\pm$ 0.5 ⁺	4.4 $\pm$ 0.5	4.4 $\pm$ 0.5	4.3 $\pm$ 0.5	4.2 $\pm$ 0.6 [†]	4.0 $\pm$ 0.5 [†]	3.9 $\pm$ 0.6 [†]
FetO ₂ (%)	M1	14.9 $\pm$ 0.7 ^{**}	14.9 $\pm$ 0.7	15.2 $\pm$ 0.8 [†]	15.4 $\pm$ 0.8 ^{*†}	15.6 $\pm$ 0.8 [†]	15.9 $\pm$ 0.9 [†]	16.2 $\pm$ 0.8 [†]
	M2	14.7 $\pm$ 0.7 ⁺	15.0 $\pm$ 0.7	15.1 $\pm$ 0.7	15.2 $\pm$ 0.7	15.5 $\pm$ 0.7 [†]	15.7 $\pm$ 0.8 [†]	16.1 $\pm$ 0.8 [†]
FetCO ₂ (%)	M1	5.4 $\pm$ 0.6 ⁺	5.5 $\pm$ 0.6	5.4 $\pm$ 0.6	5.3 $\pm$ 0.6 [†]	5.2 $\pm$ 0.6	5.1 $\pm$ 0.6	5.0 $\pm$ 0.6
	M2	5.4 $\pm$ 0.6 ⁺	5.4 $\pm$ 0.6	5.4 $\pm$ 0.6	5.3 $\pm$ 0.6	5.2 $\pm$ 0.6 [†]	5.1 $\pm$ 0.7	5.0 $\pm$ 0.7 [†]
$\dot{V}E/\dot{V}O_2$	M1	25.0 $\pm$ 3.0 ^{**}	25.2 $\pm$ 3.2	26.4 $\pm$ 3.8 [†]	27.3 $\pm$ 4.2	28.5 $\pm$ 5.3 [†]	30.5 $\pm$ 6.1 [†]	32.5 $\pm$ 6.1 [†]
	M2	24.0 $\pm$ 2.7 ⁺	25.2 $\pm$ 3.0 [†]	25.9 $\pm$ 3.3	26.6 $\pm$ 3.6	28.2 $\pm$ 4.2 [†]	29.8 $\pm$ 4.7 [†]	32.5 $\pm$ 5.8 [†]
$\dot{V}E/\dot{V}CO_2$	M1	29.5 $\pm$ 4.1 ^{**}	29.1 $\pm$ 4.1	29.7 $\pm$ 4.7 [*]	30.3 $\pm$ 5.0 [*]	31.5 $\pm$ 5.7 ^{*†}	33.0 $\pm$ 6.7 ^{*†}	34.2 $\pm$ 7.6 ^{*†}
	M2	28.4 $\pm$ 3.5 ⁺	28.4 $\pm$ 3.3	28.6 $\pm$ 3.7	29.2 $\pm$ 3.9	30.1 $\pm$ 4.1 [†]	31.0 $\pm$ 4.2 [†]	32.5 $\pm$ 5.1 [†]
Ti (s)	M1	1.35 $\pm$ 0.26 ^{**}	1.27 $\pm$ 0.29 [*]	1.17 $\pm$ 0.29 [†]	1.06 $\pm$ 0.25 [†]	0.95 $\pm$ 0.21 [†]	0.84 $\pm$ 0.17 [†]	0.74 $\pm$ 0.13 [†]
	M2	1.54 $\pm$ 0.46 ⁺	1.36 $\pm$ 0.33 [†]	1.24 $\pm$ 0.33 [†]	1.13 $\pm$ 0.26 [†]	1.02 $\pm$ 0.23 [†]	0.89 $\pm$ 0.19 [†]	0.76 $\pm$ 0.15 [†]
Te (s)	M1	1.21 $\pm$ 0.35 ⁺	1.12 $\pm$ 0.42 [*]	1.00 $\pm$ 0.29 [†]	0.88 $\pm$ 0.26 [†]	0.80 $\pm$ 0.22 [†]	0.70 $\pm$ 0.17 [†]	0.63 $\pm$ 0.13 [†]
	M2	1.16 $\pm$ 0.27 ⁺	1.03 $\pm$ 0.22 [†]	0.95 $\pm$ 0.23 [†]	0.85 $\pm$ 0.18 [†]	0.79 $\pm$ 0.19 [†]	0.72 $\pm$ 0.19 [†]	0.64 $\pm$ 0.16 [†]
Ttot (s)	M1	2.56 $\pm$ 0.49 ^{**}	2.39 $\pm$ 0.61	2.17 $\pm$ 0.51 [†]	1.94 $\pm$ 0.42 [†]	1.76 $\pm$ 0.36 [†]	1.54 $\pm$ 0.28 ^{*†}	1.37 $\pm$ 0.21 [†]
	M2	2.70 $\pm$ 0.65 ⁺	2.39 $\pm$ 0.49 [†]	2.19 $\pm$ 0.48 [†]	1.98 $\pm$ 0.38 [†]	1.80 $\pm$ 0.36 [†]	1.61 $\pm$ 0.33 [†]	1.40 $\pm$ 0.28 [†]

Velocity (v), stroke frequency (SF), stroke length (SL), blood lactate concentrations ([La⁻]), oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), ventilation ($\dot{V}E$), respiratory frequency (f_R), respiratory exchange ratio (R), tidal volume (V_T), volumes of oxygen and carbon dioxide expired during each breath (O₂exp and CO₂exp), fractions of expired oxygen and carbon dioxide (FeO₂ and FeCO₂), fractions of end tidal oxygen and carbon dioxide (FetO₂ and FetCO₂), ventilatory equivalents for oxygen and carbon dioxide ($\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$), duration of inspiration, expiration and total breathing cycle (Ti, Te and Ttot), moment one and two (M1 and M2). *, †, + differences from M2, previous step and last step, respectively ($p < 0.05$).

Table 2. Test-retest coefficient of variation (CV) and 95% CI of the selected cardiorespiratory, metabolic and biomechanical variables.

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
v (m·s ⁻¹)	1.15 (0.53-1.76)	0.63 (0.39-0.88)	0.64 (0.42-0.86)	0.49 (0.32-0.65)	0.51 (0.35-0.68)	0.86 (0.60-1.12)	1.35 (0.99-1.73)
SF (cycles·min ⁻¹)	2.25 (1.55-2.69)	2.88 (2.29-3.47)	2.63 (2.09-3.17)	1.95 (1.47-2.44)	2.14 (1.65-2.63)	2.10 (1.57-2.63)	2.49 (1.75-3.23)
SL (m·min ⁻¹)	2.06 (1.35-2.78)	2.88 (2.16-3.61)	2.47 (1.99-2.95)	1.91 (1.46-2.35)	2.03 (1.49-2.57)	2.18 (1.70-2.65)	2.39 (1.79-2.98)
[La ⁻] (mmol·L ⁻¹)	14.70 (11.48-17.92)	15.12 (11.21-19.03)	14.71 (10.95-18.47)	14.63 (11.36-17.91)	14.37 (11.59-17.15)	17.16 (12.16-22.15)	15.70 (11.73-19.68)
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	4.10 (2.75-5.45)	4.86 (3.75-5.97)	4.85 (3.54-6.17)	4.93 (3.55-6.30)	4.47 (3.28-5.66)	5.44 (3.97-6.91)	5.71 (3.94-7.48)
$\dot{V}CO_2$ (mL·kg ⁻¹ ·min ⁻¹)	5.39 (3.95-6.82)	6.16 (4.71-7.61)	6.74 (5.08-8.40)	5.83 (4.51-7.15)	7.72 (5.63-9.80)	7.62 (5.85-9.40)	8.55 (6.32-10.78)
$\dot{V}E$ (L·min ⁻¹)	5.59 (3.88-7.31)	5.27 (3.87-6.67)	5.63 (3.97-7.29)	5.41 (4.27-6.54)	6.21 (4.84-7.58)	5.63 (3.85-7.41)	5.52 (3.39-7.64)
f_R (b·min ⁻¹)	6.26 (4.10-8.43)	6.77 (4.91-8.62)	7.23 (5.11-9.35)	6.42 (4.18-8.66)	7.72 (5.44-10.01)	6.57 (4.59-8.55)	7.56 (4.57-10.55)
R	4.52 (3.20-5.84)	5.80 (4.43-7.16)	5.92 (4.58-7.27)	5.27 (3.91-6.63)	7.62 (5.15-10.09)	8.25 (6.05-10.46)	9.20 (6.88-11.52)
V_T (L)	5.90 (3.82-7.98)	5.33 (3.78-6.87)	5.70 (4.03-7.37)	4.91 (3.84-5.97)	4.59 (3.32-5.86)	4.75 (3.64-5.87)	4.01 (2.48-5.54)
O ₂ exp (mL)	6.44 (4.22-8.66)	5.46 (3.75-7.18)	5.79 (4.03-7.55)	5.04 (3.87-6.22)	4.96 (3.68-6.23)	5.46 (4.21-6.71)	4.38 (2.84-5.91)
CO ₂ exp (mL)	6.55 (4.53-8.58)	6.71 (4.85-8.56)	7.82 (5.77-9.87)	6.93 (5.16-8.69)	8.81 (6.65-10.97)	9.49 (7.16-11.81)	8.69 (6.26-11.13)
FeO ₂ (%)	1.29 (0.98-1.60)	1.35 (0.99-1.71)	1.33 (0.98-1.67)	1.40 (1.05-1.74)	1.68 (1.23-2.13)	1.88 (1.39-2.36)	1.49 (0.93-2.05)
FeCO ₂ (%)	3.28 (2.21-4.36)	3.98 (2.99-4.96)	5.09 (3.94-6.24)	5.13 (3.95-6.31)	6.03 (4.58-7.48)	6.73 (4.78-8.68)	8.37 (4.75-11.99)
FetO ₂ (%)	1.84 (1.44-2.24)	1.95 (1.39-2.52)	1.46 (1.00-1.93)	1.68 (1.20-2.17)	1.99 (1.40-2.57)	2.24 (1.69-2.79)	1.72 (1.02-2.42)
FetCO ₂ (%)	2.27 (1.49-3.05)	3.09 (2.32-3.86)	3.28 (2.45-4.11)	3.15 (2.28-4.02)	3.98 (3.00-4.97)	3.83 (2.72-4.93)	3.76 (2.65-4.87)
$\dot{V}E/\dot{V}O_2$	4.75 (3.65-5.85)	5.17 (3.92-6.41)	5.06 (3.79-6.32)	5.70 (4.36-7.04)	7.05 (5.15-8.94)	8.52 (6.34-10.69)	7.48 (4.97-9.98)
$\dot{V}E/\dot{V}CO_2$	3.78 (2.64-4.92)	4.39 (3.28-5.50)	5.45 (4.19-6.71)	5.58 (4.25-6.92)	6.48 (4.90-8.06)	7.19 (5.10-9.27)	7.48 (5.66-9.31)
Ti (s)	11.00 (7.73-14.28)	9.65 (7.15-12.15)	11.23 (7.76-14.70)	9.80 (7.45-12.16)	11.63 (8.55-14.71)	8.73 (6.21-11.25)	8.00 (5.51-10.48)
Te (s)	9.94 (7.30-12.59)	10.56 (7.97-13.15)	10.26 (7.72-12.80)	8.32 (5.99-10.64)	8.59 (6.00-11.18)	7.93 (5.72-10.14)	8.55 (5.89-11.22)
Ttot (s)	6.22 (4.11-8.32)	5.74 (4.02-7.47)	6.74 (4.72-8.76)	5.36 (3.70-7.03)	7.77 (5.96-9.58)	6.40 (4.62-8.19)	6.65 (4.44-8.86)

Velocity (v), stroke frequency (SF), stroke length (SL), blood lactate concentrations ([La⁻]), oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), ventilation ($\dot{V}E$), respiratory frequency (f_R), respiratory exchange ratio (R), tidal volume (V_T), volumes of oxygen and carbon dioxide expired during each breath (O₂exp and CO₂exp), fractions of expired oxygen and carbon dioxide (FeO₂ and FeCO₂), fractions of end tidal oxygen and carbon dioxide (FetO₂ and FetCO₂), ventilatory equivalents for oxygen and carbon dioxide ($\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$), duration of inspiration, expiration and total breathing cycle (Ti, Te and Ttot), moment one and two (M1and M2).

Discussion

The main purpose of the current study was to determine the short-term repeatability (through the best and up to date existent methods for its assessment) of ventilatory, metabolic and biomechanical variables during a front crawl intermittent incremental protocol that is frequently used for swimmers biophysical monitoring. The initial hypothesis of a strong agreement between two experimental moments separated by 48-72 h, in tandem with a small range of variation, was partially confirmed. In fact, swimming velocity (the gold standard measure of swimmers performance) presented similar values to the literature in moderately trained swimmers (Fernandes et al., 2010; Fernandes et al., 2011; Barbosa et al., 2015) and did not differ between M1 and M2. The only exception occurred at the seventh step of the protocol, where it was asked a maximal exertion, evidencing only a very low typical error (less than a half of a velocity step increment, i.e., $0.02 \text{ m}\cdot\text{s}^{-1}$).

Since the assessed variables behaviour depends on the swimming intensity domain, the velocity similarity between M1 and M2 ensured the incremental protocol reliability. Of note, both stroke frequency and stroke length kinetics along the protocol agrees with the literature (Barbosa et al., 2006; Zacca et al., 2020) and was similar between M1 and M2. This similitude is particularly important and corroborates these variables strong relation with swimming performance. It also reinforces why these variables continue to be the best and easiest strategy for characterizing biomechanical changes on swimmers technique due to their stability and high reliability in repeated tests with different intensities (Zacca et al., 2020).

$\dot{V}O_2$ and $[La^-]$ are the most used physiological indicators to describe metabolic contributions during exercise and are expected as highly reliable variables. $\dot{V}O_2$ values in both experimental moments corroborate our hypothesis, evidencing a high $\dot{V}O_2$ reliability and stability along the incremental protocol. The higher $\dot{V}O_2$ observed in M1 versus M2 in the second step reflects a residual variation ($1.64 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), that is lower than the criteria used for $\dot{V}O_{2\text{max}}$ identification (Howley et al., 1995; de Jesus et al., 2014), presenting a negligible practical relevance. Conversely, $[La^-]$ values were only reliable in the last and more intense

step, presenting lower scores in M2 than M1 in the previous steps. This behaviour was associated with an increased typical error along the protocol (0.44, 0.46, 0.50, 0.60, 0.73 and 1.13 mmol·L⁻¹, respectively), probably due to an eventual fitness change (Pyne et al., 2001) even if the experimental moments were conducted at the same velocities. The [La⁻] analyser associated error of measurement (0.05 mmol·L⁻¹) should also be considered, meaning that a 0.01 mmol·L⁻¹ real difference can be presented as 0.1 mmol·L⁻¹ (e.g., 7.04 = 7.0 and 7.05 = 7.1 mmol·L⁻¹ for M1 and M2), being the real and difference 10 times inferior than the assessed one.

The $\dot{V}CO_2$ incremental behaviour along the protocol and its similitude between M1 and M2 demonstrates a high reliability and stability. This corroborates the importance of its assessment as a metabolic rate variable, as was stated before when evaluating competitive swimmers (Pelarigo et al., 2018). $\dot{V}CO_2$ is highly related to $\dot{V}E$ (Nicolò et al., 2018) since this latter variable allows the CO₂ removal, preventing or constraining the perturbation in arterial blood gas and acid-base status (like the elimination of the increased heat generate by exercise; Forster et al., 2012; Poole and Jones, 2012). As expected, $\dot{V}E$ increased along the studied incremental protocol, in line with what is reported in the literature (Ribeiro et al., 2015). However, the lower $\dot{V}E$ values founded in the M2 first, fourth and sixth steps were not anticipated given the observed similarity of f_R and V_T in M1 and M2. These protocol steps are representative of different training zones, particularly the low, moderate and severe intensities that can present subtle differences when associating a protocol step to the specific moment when they occur (e.g., when the anaerobic threshold – the gold standard for moderate intensity exercise – is identified between two steps).

The R value well characterizes the metabolic process by indicating how much CO₂ needs to be released and O₂ brought in per respiration, and is highly related to $\dot{V}E$ (Howley et al., 1995). The R similar values observed in both experimental moments seem to indicate that swimmers had the same metabolic response along the M1 and M2 incremental protocol (Zacca et al., 2019a). Although the observed similitude between moments, the lack of association and reliability (represented by the different incremental behaviour in M1 and M2) demonstrates

that this variable should be used carefully. Considering the alveolar expired air composition (16% O₂ and 5% CO₂), the observed O_{2exp}, CO_{2exp}, FeO₂, FeCO₂, FetO₂ and FetCO₂ values evidenced that these variables maintained in the same despite the velocity increase along the protocol. The differences founded between M1 and M2 in FeO₂, FeCO₂ and FetO₂ also corresponded to a residual variation ([0.21%-0.26%]), indicating a similarity between experimental moments.

Due to what was previously verified in $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$, the $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ ratios tended to increase with the swimming intensity raise both in M1 and M2, particularly from the fourth step, i.e., at exertions higher than the moderate domain. In fact, concurrently with the $\dot{V}O_2$ and $\dot{V}CO_2$ increase throughout the protocol, the $\dot{V}E$ exponential raise above the anaerobic threshold intensity (Ribeiro et al., 2015) contributes to this behaviour. $\dot{V}E/\dot{V}O_2$ provides an estimation of the $\dot{V}E$ efficiency with respect to $\dot{V}O_2$, while $\dot{V}E/\dot{V}CO_2$ (an indicator of ventilatory efficiency) is commonly used to determine the point at which $\dot{V}E$ increases out of proportion to $\dot{V}CO_2$, i.e., the respiratory compensation point (Bongers et al., 2016). However, the $\dot{V}E/\dot{V}O_2$ different behaviour along the swimming velocity raise and the lower $\dot{V}E/\dot{V}CO_2$ values in M2 indicate that both variables need to be carefully considered in swimmers physiological characterization. Lastly, the T_i , T_e and T_{tot} rise with swimming velocity along the M1 and M2 step protocol is a common response (Kay et al., 1975; Scheuermann and Kowalchuk 1999), with the higher T_i and T_{tot} (and lower T_e) values in M2 being related to a residual difference ([0.10-0.16 s]) with a negligible practical relevance.

Conclusions

When conducting swimming monitoring, independently of the experimental protocol used, similar criteria of validity must be ensured to provide useful and associative performance related information (Smith et al., 2002; Fernandes et al., 2012; de Jesus et al., 2014). Despite our initial hypothesis was not confirmed for all the selected variables, the observed similar variables behaviour in the experimental moments (their increase, decrease or maintenance along with the intensity rise in M1 were verified in M2), and the large similarity between each M1

and M2 steps, showed a high sensibility to the swimming intensity increment and their repeatability and validity as performance indicators. The most used ventilatory and biomechanical variables really characterize the swimmers at the moment of the evaluation. The $[La^-]$ is highly sensitive to the swimmer effort, remaining an important variable for the aerobic and anaerobic characterisation. The R different response to the swimming intensity raise highlighted that this specific variable needs to be used with caution in future swimmers physiological characterization.

Acute ventilatory responses to swimming at increasing intensities

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Abstract

Background: Physical exercise is a source of stress to the human body, triggering different ventilatory responses through different regulatory mechanisms and the aquatic environment imposes several restrictions to the swimmer, particularly regarding the restricted ventilation. Thus, we aimed to assess the acute ventilatory responses and to characterize the adopted breathing patterns when swimming front crawl at increasing intensity domains. **Methods:** Eighteen well-trained swimmers performed 7 x 200 m front crawl (0.05 m·s⁻¹ velocity increments) and a maximal 100 m (30 s rest intervals). Pulmonary gas exchange and ventilation were continuously measured (breath-by-breath) and capillary blood samples for lactate concentration ([La⁻]) analysis were collected at rest, during intervals and at the end of the protocol, allowing the identification of the low, moderate, heavy, severe and extreme intensity domains. **Results:** With the swimming velocity rise, respiratory frequency (f_R), [La⁻] and stroke rate (SR) increased ([29.1-49.7] breaths·min⁻¹, [2.7-11.4] mmol·L⁻¹, [26.23-40.85] cycles; respectively) and stroke length (SL) decreased ([2.43-2.04] m·min⁻¹; respectively). Oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), carbon dioxide production ($\dot{V}CO_2$) and heart rate (HR) increased until severe ([37.5-53.5] mL·kg⁻¹·min⁻¹, [55.8-96.3] L·min⁻¹, [32.2-51.5] mL·kg⁻¹·min⁻¹ and [152-182] bpm; respectively) and stabilized from severe to extreme (53.1 ± 8.4, mL·kg⁻¹·min⁻¹, 99.5 ± 19.1 L·min⁻¹, 49.7 ± 8.3 mL·kg⁻¹·min⁻¹ and 186 ± 11 bpm; respectively) while tidal volume (V_T) was similar from low to severe ([2.02-2.18] L) and decreased at extreme intensities (2.08 ± 0.56 L). Lastly, the f_R /SR ratio increased from low to heavy and decreased from severe to the extreme intensity domains (1.12 ± 0.24, 1.19 ± 0.25, 1.26 ± 0.26, 1.32 ± 0.26 and 1.23 ± 0.26). **Conclusions:** Our findings confirm a different ventilatory response pattern at extreme intensities when compared to the usually evaluated exertions. This novel insight helps to understand and characterize the maximal efforts in swimming and reinforces the importance to include extreme efforts in future swimming evaluations.

Keywords: ventilation, respiratory frequency, tidal volume, breathing patterns, swimming.

Introduction

Breathing is a natural and fundamental human behaviour that allows the exchange of respiratory gases between the lungs and the atmosphere. When we are under stress, as physical exercise, minute ventilation ($\dot{V}_E$) increases (Pelarigo et al., 2016; Tipton et al., 2017) due to the respiratory frequency (f_R) and tidal volume (V_T) rise. During an incremental exercise, f_R increases non-linearly and V_T tends to present a plateau, with the $\dot{V}_E$ rise at lower intensities depending on both f_R and V_T increases. The further growth in $\dot{V}_E$ at higher exercise intensities seems to be explained by the increase in f_R , a phenomenon known as the tachypnoeic breathing pattern (Sheel & Romer, 2012; Nicolò et al., 2020). Despite the well-established knowledge on the f_R and V_T contributions for $\dot{V}_E$ increase during an incremental exercise, further research focusing on the different regulatory mechanisms that drive these contributions is welcome (Figueiredo et al., 2013; Tipton et al., 2017).

Central command, muscle afferent feedback and metabolic inputs are the major $\dot{V}_E$ behaviour determinants despite acting with different timings when exercise intensity changes (Forster et al., 2012; Duffin, 2014; Tipton et al., 2017). The regulation of f_R and V_T is less studied but it was previously suggested that the inputs driving $\dot{V}_E$ act separately on these variables, with central command and muscle afferent feedback preferentially regulating f_R (Amann et al., 2010; Nicolò et al., 2017), while metabolic responses are responsible for the V_T regulation (Nicolò et al., 2017). Considering the great importance of the central command on f_R control and the close association between breathing patterns, exercise modes and limbs movement (Sheel & Romer, 2012; Forster et al., 2012), it is of great importance to understand how the ventilatory response adapts to different exercise related constraints.

In swimming, the aquatic environment imposes significant restrictions on the human body, such as the increase of the hydrostatic pressure around the chest, resulting in an augmented work of the inspiratory muscles (Lomax & McConnell, 2003; Leahy et al., 2019). In addition, the swimming typical horizontal position leads to face immersion and, consequently, to restricted ventilation (Holmér et al., 1974; McCabe et al., 2015). These restrictions oblige swimmers to

synchronize active inspiratory and expiratory phases with movements of upper and lower limbs, resulting in specific swimming breathing patterns (Leahy et al., 2019). Front crawl is the most common (in training and competition conditions) from the four swimming conventional techniques, with swimmers more generally inspiring on every two or three upper limbs actions, i.e., using unilateral and bilateral breathing patterns (Seifert et al., 2005; Figueiredo et al., 2013). Despite the existing variability of the $\dot{V}_E$ responses along the different intensity domains, particularly when using the front crawl technique (Ribeiro et al., 2015; Monteiro et al., 2022), the f_R and V_T behaviours are still scarcely studied when swimming at increasing paces.

Since further research on the f_R and V_T responses is necessary to improve the overall understanding of breathing physiology and ventilatory control, we have aimed to assess the acute ventilatory responses when swimming from low to extreme intensity domains. For achieving that purpose, swimmers were required to wear a breathing snorkel attached to a gas analyser along a standard incremental front crawl protocol. Complementarily, we aimed to characterize the swimmers breathing patterns along the exercise intensity rise to understand if the synchronization with the upper and lower limbs motion is maintained even when using the respiratory snorkel, i.e., without constraining the inspiratory and expiratory phases. We have hypothesized that: (i) despite the respiratory constraints, gas exchange variables increase concomitantly with the swimming velocity rise, with f_R and V_T presenting a nonlinear increase and a stabilization (respectively); and (ii) swimmers keep the breathing patterns used in free swimming when breathing into a snorkel (due to the breathing synchronization with stroke rate).

Materials and Methods

Participants

Eighteen well-trained swimmers (nine male) volunteered to participate in the current study. Their main anthropometric, training background and competitive characteristics were (for males and females, respectively): 20.1 ± 8.0 vs. 16.8 ± 1.8 years of age, 176.6 ± 7.6 vs. 163.4 ± 4.7 cm of body height, 67.5 ± 12.1 vs. 57.3 ± 6.5 kg of body mass, 21.5 ± 2.7 vs. 21.4 ± 1.6 kg·m⁻² of body mass index,

8.3 ± 3.8 vs. 7.3 ± 3.4 years of swimming practice and 489 ± 66 vs. 478 ± 83 Fédération Internationale de Natation points of their best competitive performance event. Participants were recruited via personal contact and based on the following eligibility criteria: (i) without a history of cardiorespiratory and physical diseases or injuries within the previous 6 months; (ii) having ≥ 2 years of swimming training background and (iii) being engaged at ≥ 5 training sessions per week. All the experiments were approved by the Faculty of Sport of University of Porto ethics committee (CEFADE 25 2020) and participants were informed about the purpose, benefits and any associated risks (providing their written individual consent for participation in accordance with the Helsinki Declaration).

Experimental protocol

Subjects were asked to be rested and fully hydrated, and refrained from alcohol and caffeine consumption (and from vigorous exercise) for, at least, 24 h prior the evaluation. Test sessions were conducted in a 25 m indoor pool, with 27° C and 26.5° C of water and air temperatures (respectively) and 75% of humidity. After a 600 m low intensity in-water warm-up, each swimmer performed a front crawl discontinuous incremental protocol, consisting of 7 x 200 m (with 0.05 m·s⁻¹ velocity increments), followed by a maximal 100 m, with 30 s rest intervals in-between (adapted from Fernandes et al., 2005; Carvalho et al., 2020; Monteiro et al., 2022). The paces for each swimmer 7th step were established based on the individual 400 m front crawl performance on the evaluation day, then six velocity increments were subtracted. Swimming velocities were controlled using flashing lights on the bottom of the pool (Pacer2Swim; KulzerTEC, Aveiro, Portugal), with in-water starts and open turns (without underwater gliding) being used due to the impossibility of performing flip turns with deep water gliding when using a respiratory snorkel.

A portable gas analysis system (K4b²; Cosmed, Rome, Italy) was transported on a steel cable above the water surface allowing to measure breath-by-breath pulmonary gas exchange and ventilation by connecting the swimmer through a low hydrodynamic resistance respiratory snorkel and valve system (Aquatrainer®, Cosmed, Rome, Italy; Ribeiro et al., 2015). This gas analysis system was calibrated before each experimental session using ambient air against known concentrations (16% O₂ and 5% CO₂) and a 3 L calibration

syringe. Heart rate (HR) was continuously recorded at the baseline and during the incremental protocol using a Polar Vantage NV (Polar Electro Oy, Kemple, Finland) that telemetrically emitted to the portable gas analyser unit (de Jesus et al., 2015). Lactate concentration ($[La^-]$) values were obtained using fingertip capillary blood samples collected at rest, immediately after the end of each step and at 1, 3, 5 and/or 7 min post-protocol (until obtaining maximal values) using a portable analyser (Lactate Pro2; Arkay Inc., Kyoto, Japan; Carvalho et al., 2020). Stroke rate (SR) was assessed through the number of upper limbs cycles per minute in the last 50 m of each step (using a Finis stopwatch with a frequency meter function) and stroke length (SL) was calculated by dividing the mean velocity by SR (Fernandes et al., 2005; Monteiro et al., 2022).

Data analysis

The pulmonary gas exchange and ventilation data were examined to exclude occasional errant breaths (eventually caused by swallowing, coughing or signal interruptions). For the analysis, only the values of oxygen uptake ($\dot{V}O_2$) between ± 3 SD were considered (Monteiro et al., 2020), which were then smoothed using a three-breaths moving average and 10 s time average (Fernandes et al., 2012). The mean values from the last 30 s of exercise per step were selected and conventional physiological criteria were applied to establish the maximal oxygen uptake $\dot{V}O_{2max}$; (Howley et al. 1995; Zacca et al., 2020). The lactate-velocity curve modelling method, through the determination of the interception point of the best fit of a combined linear and exponential pair of regressions, was used to determine the individual anaerobic threshold (Carvalho et al., 2020; Monteiro et al., 2022). Using the $\dot{V}O_{2max}$ and the anaerobic threshold as physiological indicators, the following intensity domains were identified (Figure 1): (i) the low and moderate domains, corresponding to two steps below and the step at the anaerobic threshold; (ii) the heavy and severe domains, matching the step below and the step where $\dot{V}O_{2max}$ was elicited; and (iii) the extreme domain, allocated to the maximum 100 m at the end of the incremental protocol (Fernandes et al., 2012; de Jesus et al., 2015; Ribeiro et al., 2017). Swimmers breathing patterns were determined by calculating the ratio between f_R and SR.

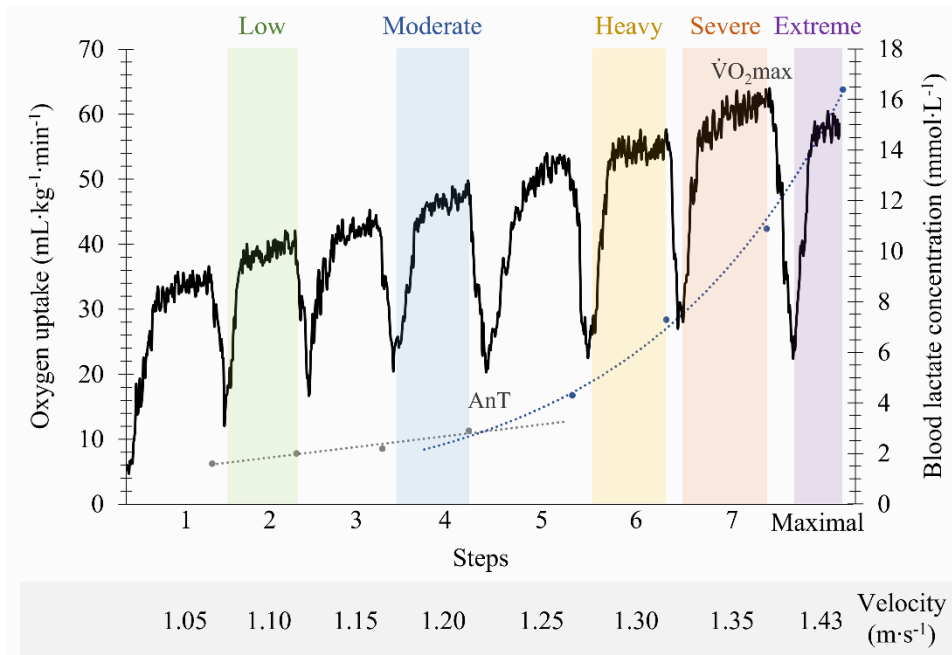


Figure 1. Identification of the front crawl swimming intensity domains on a representative participant based on anaerobic threshold (AnT) and maximal oxygen uptake ($\dot{V}O_{2max}$) indicators.

Statistical analysis

A sample size of 18 subjects was required for a paired sample design to detect a moderately large effect size (0.83) with a 5% significance level and 95% power (G*Power 3.1.9.7; Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany). Statistical procedures were conducted using SPSS (version 27.0.1.0; IBM Corporation, Armonk, NY, USA) and the normal data distribution was checked for all variables using the Shapiro-Wilk test. Mean and standard deviation values were computed for descriptive analysis of all variables and one-way repeated measures analysis of variance with Bonferroni adjustment was used to compare the assessed physiological and performance variables along the intensity domains spectrum ($p \leq 0.05$ level). Partial eta-squared (η_p^2) for effect size calculation was computed to compare the magnitude of changes between swimming intensity domains.

Results

Depending on the swimmer, the establishment of the low and moderate intensity domains corresponded to the swimming velocity between the first-third and third-

fifth steps (respectively), while the heavy, severe and extreme intensity domains corresponded to the sixth, seventh and maximal last protocol steps (in this order). The low, moderate, heavy, severe and extreme efforts were performed at 1.04 ± 0.11 , 1.13 ± 0.11 , 1.22 ± 0.10 , 1.26 ± 0.10 and 1.39 ± 0.11 $\text{m}\cdot\text{s}^{-1}$ (respectively) and all the physiological and performance variables are presented in Figure 2. With the swimming intensity rise ($Z_{4.68} = 305.79$, $\eta_p^2 = 0.95$, $p < 0.001$), f_R , $[\text{La}^-]$ and SR increased ($\eta_p^2 = 0.81$, $\eta_p^2 = 0.95$ and $\eta_p^2 = 0.88$, respectively; $p < 0.001$) and SL decreased ($\eta_p^2 = 0.59$, $p < 0.001$). $\dot{V}\text{O}_2$, $\dot{V}\text{E}$, $\dot{V}\text{CO}_2$ and HR increased from low to severe intensities ($\eta_p^2 = 0.91$, $\eta_p^2 = 0.88$, $\eta_p^2 = 0.89$ and $\eta_p^2 = 0.83$, respectively; $p < 0.001$), but all stabilized at extreme exertion and V_T presented similar values from low to severe exertions and decreased at the extreme intensity ($p = 0.006$). The f_R/SR ratio increased from low to moderate ($p = 0.01$) and from moderate to heavy domains ($p = 0.02$) and lower values were observed at extreme compared to severe intensity ($p = 0.02$; Figure 3).

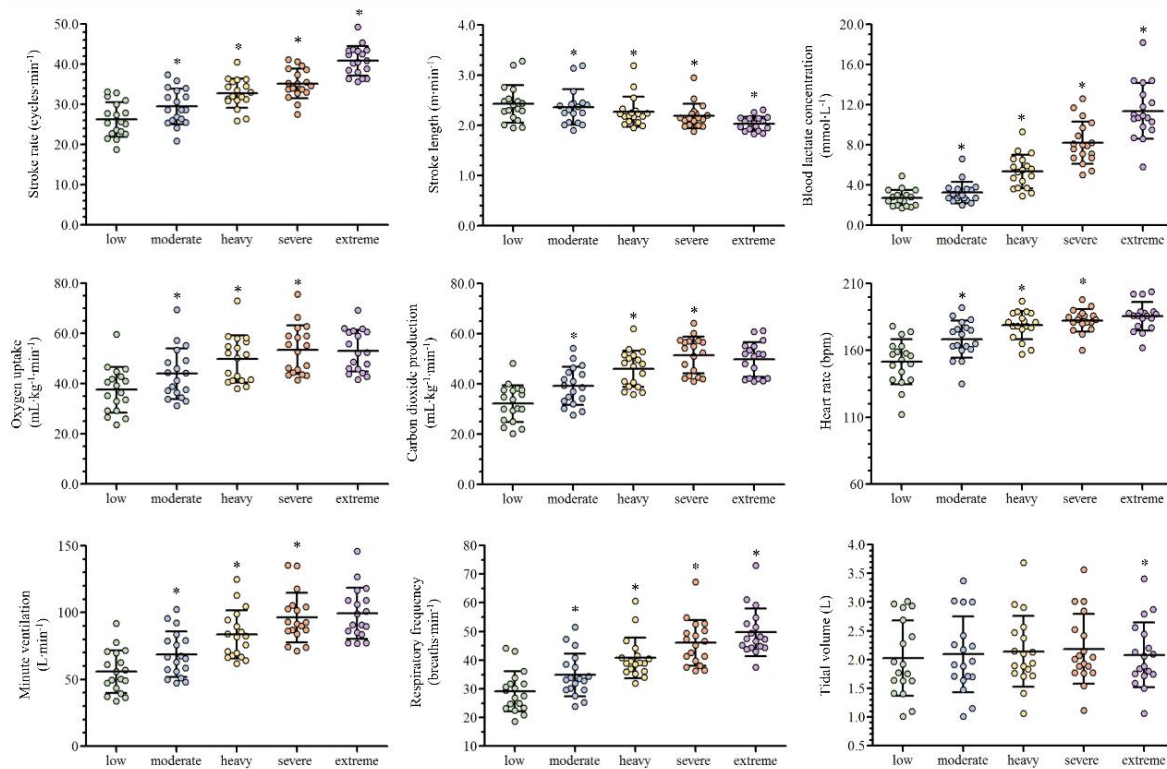


Figure 2. Scatter plots with mean and standard deviation of the ventilatory and performance variables responses along with the swimming intensity rise. An asterisk (*) indicates difference from the previous intensity domain ($p < 0.05$).

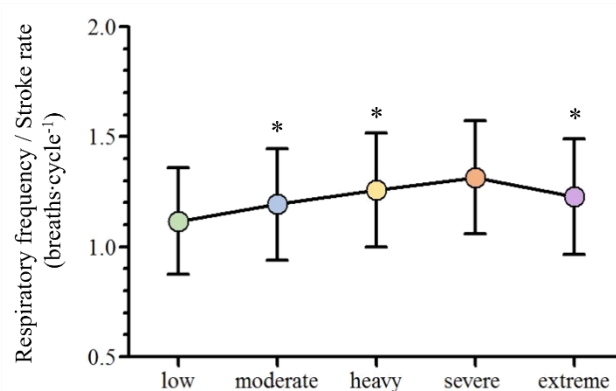


Figure 3. Mean and standard deviation of the adopted breathing patterns along with the swimming intensity rise. An asterisk (*) indicates difference from the previous intensity domain ($p < 0.05$).

Discussion

The main purpose of the current study was to assess swimmers acute ventilatory responses when performing front crawl from low to extreme intensities. As hypothesized, the values of the selected gas exchange variables increased along with the swimming intensity rise (until the severe intensity domain) and f_R and V_T presented a nonlinear increase and a plateau (respectively) in the incremental protocol. Concurrently, we aimed to analyse swimmers breathing pattern behaviour as swimming pace was rising, being observed an f_R/SR ratio increase until reaching the severe intensity domain and a decrease at extreme exertions. This does not confirm our initial hypothesis that the front crawl breathing pattern was going to remain stable when swimming with a respiratory snorkel.

It is well established that the 7 x 200 m front crawl intermittent incremental protocol allows collecting capillary blood for $[La^-]$ analysis (Fernandes et al., 2005; Pelarigo et al., 2016; Carvalho et al., 2020) and, together with the gas exchange assessment, ensures a complete physiological characterization of the low, moderate, heavy and severe intensity domains (de Jesus et al., 2015; Zacca et al., 2019; Monteiro et al., 2022). The current results are similar to those previously presented for the low-severe swimming intensity domains when using the same methodological approach, particularly regarding $\dot{V}O_2$ (Fernandes et al., 2012; de Jesus et al., 2015), $\dot{V}E$ and f_R (Pelarigo et al., 2016; Monteiro et al., 2022), and

[La⁻] values (Štrumbelj et al., 2007; Sousa, Vilas-Boas & Fernandes, 2014; Monteiro et al., 2022).

However, most official swimming events (such as the 50, 100 and 200 m distances) occur at the extreme intensity domain, and is why a complete swimming ventilatory characterization should also include extreme efforts. This is a fundamental training zone for excelling competitive swimmers performances where the exertions are so intense that fatigue occurs and exercise ends before $\dot{V}O_{2max}$ can be reached (Hill et al., 2002; Ribeiro et al., 2017). Studies focusing on the anaerobic capacity development are very scarce, with swimmers acute ventilatory responses remaining almost unexplored, justifying the inclusion of a 100 m maximal bout at the end of the front crawl incremental protocol. This maximal intensity short duration effort, when swimming up at the standard 7 x 200 m step protocol, allows swimmers to have their physiological profile fully characterized.

The current ventilatory results at the maximal 100 m front crawl evidenced lower $\dot{V}O_2$ and $\dot{V}E$ values compared to those reported for the same intensity domain (Sousa et al., 2014), probably due to the sample characteristics (higher level and male swimmers only) and the higher swimming velocity achieved (the current 100 m bout was part of an incremental protocol instead of an isolated rectangular test). Additionally, higher f_R values were observed at the current extreme intensity domain compared to a maximal 200 m front crawl bout performed at a lower swimming velocity (Štrumbelj et al., 2007). This demonstrates that the extreme exertions (only inferiorly delimited by the $\dot{V}O_{2max}$) can include a wider range of swimming velocities, being important to consider them when comparing the results obtained at this intensity domain.

Swimming faster implied a $\dot{V}O_2$, $\dot{V}E$, f_R , $\dot{V}CO_2$, [La⁻] and SR increase and a SL decrease from low to severe exertions, as previously described (Figueiredo et al., 2013; de Jesus et al., 2015; Monteiro et al., 2022), while $\dot{V}O_2$, $\dot{V}E$, $\dot{V}CO_2$ and HR stabilized from severe to extreme intensities (Sousa et al., 2014). The attainment of $\dot{V}O_{2max}$ at severe intensity paces, and the fact that these abovementioned variables are highly related, explain the maintenance of similar values despite the swimming intensity rise (Sousa et al., 2014; Nicolò et al., 2018; Monteiro et al.,

2022). HR at $\dot{V}O_{2\max}$ corresponded to $90.4\% \pm 3.1\%$ of its maximum, in accordance with the secondary criteria used to confirm $\dot{V}O_{2\max}$ (Howley et al., 1995; Zacca et al., 2020). Maximal $[La^-]$ values were observed at extreme exertions, where the energy production is highly dependent on anaerobic metabolism, with a higher production of lactate and, consequently, its progressive accumulation in the bloodstream (Hargreaves & Spriet, 2020). Contrarily to what was described (Sousa et al., 2014), our $[La^-]$ values increase from severe to extreme intensity domains is explained by a bigger velocity rise ($\sim 10\%$ instead of 5%), corroborating the existence of a wide range of swimming velocities at this domain.

The observed $\dot{V}E$, f_R and V_T behaviours along the low to extreme swimming intensity domains spectrum corroborates what is described in the literature, independently of the exercise modality performed (Amann et al., 2010; Nicolò et al., 2017). This seems to indicate that, regardless of the swimming movements and the different constraints imposed by the aquatic environment, the central command, muscle afferent feedback and metabolic inputs have the same influence on their regulation along the intensity domains spectrum (Štrumbelj et al., 2007; Sheel & Romer, 2012; Forster et al., 2012). However, the swimming intensity rise resulted in the selection of different breathing patterns at each intensity domain. Diversely to what was initially expected, the f_R/SR ratio tended to increase until the heavy intensity domain, indicating that swimmers took advantage of free breathing while using the respiratory snorkel (Štrumbelj et al., 2007). The f_R/SR ratio decrease from severe to extreme intensity domains can be justified by both the maximal intensity and the short time duration effort of the 100 m exertion, where SR increased more than f_R (16% vs. 8%, respectively). In addition, the lower increase in f_R compared to SR seems to indicate that this extreme effort is characterized by moments of apnoea.

Conclusions

The f_R and, consequently, the f_R/SR ratio values were influenced by the use of the respiratory snorkel and its interpretation may be different compared to free swimming. However, this is the only methodology that provides a real time and breath-by-breath assessment of the swimmers ventilatory responses. In

conclusion, by proposing the addition of a maximal effort at the end of the front crawl intermittent incremental swimming protocol, the current study provides a novel framework of the acute ventilatory responses to the large spectrum of swimming intensity domains, particularly at the extreme exertion, used both in training and competition contexts. $\dot{V}O_2$, $\dot{V}E$ and $\dot{V}CO_2$ stabilized, and V_T decreased, from severe to extreme intensity domains, differently to what happened from low to severe exertions, while f_R and SR increased along the swimming intensities spectrum. The breathing pattern varied along the incremental protocol and its synchronization with stroke rate was not verified when using the respiratory snorkel.

VO₂FITTING software: new insights and practical applications

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Abstract

This study aimed to present an updated version of VO₂FITTING software, where it is possible to dynamically edit, process, filter and model $\dot{V}O_2$ post-exercise data and to characterize the $\dot{V}O_2$ on/off symmetry along different exercise intensity domains. Validation datasets were developed and applied to four widely used models for describing low, moderate, heavy and severe intensity transitions. Perfect fits were observed and parameter estimates perfectly matched the known inputted values for all available models (standard error = 0; $p < 0.001$). In addition, an experiment with 10 trained swimmers performing a 5 x 200 m front crawl protocol (with 0.05 m·s⁻¹ velocity step increments and 3 min passive rest intervals) was conducted. The on- and off-transient phases were symmetrical in their shape since they were both adequately fitted by a mono-exponential regression model and no slow component was observed independently of the intensity domain. Furthermore, the mono-exponential model without time delay best fitted the $\dot{V}O_2$ off-transient data. The mean time constant of the on-transient period value was lower than the respective off-transient for all the intensities (15.8 ± 11.4 vs. 30.8 ± 10.4, 11.3 ± 2.3 vs. 29.7 ± 8.4, 13.9 ± 7.0 vs. 28.7 ± 10.8 and 10.3 ± 4.6 vs. 37.0 ± 9.2 s; $p < 0.05$). VO₂FITTING is valid, free and open-source software for characterizing $\dot{V}O_2$ kinetics during both the exercise and recovery periods, helping researchers to give rapid feedback also about the off-transient kinetic parameters.

Keywords: software, oxygen uptake, off-transient kinetics modelling, symmetry, swimming.

Introduction

The assessment of oxygen uptake ($\dot{V}O_2$) kinetics and the interpretation of its parameters allow the quantification of the physiological mechanisms responsible for the dynamic $\dot{V}O_2$ response to exercise (on-transient kinetics) and its subsequent recovery (off-transient kinetics) (Jones & Poole, 2013; Özyener et al., 2001). It enables a non-invasive assessment of the control mechanisms of muscle energetics and oxidative metabolism (Jones & Poole, 2013; Rossiter et al., 2005) and, consequently, the effectiveness of a training program, providing relevant information about the exercise tolerance determinants (Poole and Jones, 2012; Zacca et al., 2019). Despite the importance of maximal $\dot{V}O_2$ ($\dot{V}O_{2\max}$) for training control and prescription (Duquette & Adam, 2024), the interpretation of $\dot{V}O_2$ kinetic parameters help researchers and coaches to better understand the responsiveness to training stimuli, particularly regarding the physiological significance of the fast and slow components of the dynamic $\dot{V}O_2$ response (Burnley & Jones, 2007; Fernandes et al., 2024).

Following the onset of a specific exercise intensity, $\dot{V}O_2$ on- and off-transient kinetics may have different profiles. Below and at the anaerobic threshold (AnT), i.e., at low and moderate intensities, the $\dot{V}O_2$ profile, after an initial rise that usually lasts ~15–20 s (phase I or cardiodynamic phase), is described by a mono-exponential function, where an exponential increase is visible (phase II or fast component), followed by a steady-state (phase III) (Monteiro et al., 2020; Özyener et al., 2001; Poole & Jones, 2012). At the recovery period after these exercise intensity domains, $\dot{V}O_2$ presents a similar behaviour, i.e., a rapid decrease until reaching the baseline values. Above the AnT, at the heavy intensity domain, $\dot{V}O_2$ response profile starts to differ from less stressful intensities, being usually described by a bi-exponential function (Özyener et al., 2001; Reis et al., 2012). Here, a second $\dot{V}O_2$ elevation is observed after phase II (after ~90–120 s), known as a $\dot{V}O_2$ slow component (Billat, 2000; Burnley & Jones, 2007), until a delayed steady-state or exhaustion are attained. Different behaviours of the off-transient kinetics have been described, since it is identified by both mono- and bi-exponential functions (Monteiro et al., 2020; Özyener et al., 2001; Pelarigo et al., 2017). At the severe intensity domain, a bi-exponential

function is typically observed both during on- and off-transient kinetics, with a $\dot{V}O_2$ slow component with significant amplitude (Jones & Poole, 2013; Sousa et al., 2015; Zacca et al., 2019).

Usually, $\dot{V}O_2$ kinetics has been analysed through mathematical modelling (both for on- and off-transient kinetics) using complex programs that requires some mastery beyond the knowledge of respiratory physiology (de Jesus et al., 2015; Özyener et al., 2001; Sousa et al., 2011). Considering the importance of giving rapid feedback from experimental testing, it became relevant to create a tool for effectively and straightforward analysis of the $\dot{V}O_2$ response during exercise. Therefore, VO_2 FITTING, a validated, free and open-source software, was developed to characterize $\dot{V}O_2$ kinetics during continuous exercise (e.g., running, cycling or swimming), allowing to dynamically edit, process, filter and model the typical $\dot{V}O_2$ responses (Zacca et al., 2019). However, it did not include the possibility of analysing the exercise $\dot{V}O_2$ off-transient kinetics that can provide additional information on gas exchange dynamics, aiding to better interpret the physiological events supporting the $\dot{V}O_2$ on-transient response (Jones & Poole, 2013).

The $\dot{V}O_2$ off-transient kinetics also becomes very useful when the backward extrapolation method is applied, e.g., allowing swimmers to perform without a breathing mask and perform flip turns, achieving competitive velocities, since the testing environment is more ecological (Ribeiro et al., 2016). The aim of the current study was to present an updated version of VO_2 FITTING software that allows to dynamically process $\dot{V}O_2$ post-exercise data of a large spectrum of exercise intensities, demonstrating the possibility to equally analyse and model $\dot{V}O_2$ recovery data. In addition, we aimed to contribute to the existent knowledge about the $\dot{V}O_2$ on/off symmetry by directly comparing the exercise and its subsequent response at low, moderate, heavy and severe intensity domains. We hypothesized that an on/off symmetry would be observed at low and moderate intensities, while above the AnT, i.e., at heavy and severe domains, different exponential models for the exercise and recovery phases would be evidenced.

Methods

Development and validation of VO₂FITTING software for post-exercise data analysis

The already available VO₂FITTING software (Zacca et al., 2019) was extended for $\dot{V}O_2$ off-transient kinetics analysis and a swimming experiment was conducted to illustrate how it can be used to edit, process, filter and model the $\dot{V}O_2$ post-exercise data. The respective installation instructions and other documentation are available at https://shiny.cespu.pt/vo2_news/, and the corresponding author can be reached for further follow-up. Validation $\dot{V}O_2$ datasets were developed for post-exercise data involving two mono- and two bi- exponential widely used mathematical models for describing different intensity transitions (Özyener et al., 2001; Pelarigo et al., 2017; Sousa et al., 2015). $\dot{V}O_2$ was used in raw form as input, without any filtering or processing, and the models were applied without any parameter constraints.

Participants

Ten trained swimmers (five male) voluntarily participated in the current study, all being engaged in $\geq$ five swimming training sessions per week. Their main physical characteristics were 16.1 ± 1.7 vs. 15.3 ± 1.2 years of age, 64.0 ± 6.6 vs. 56.5 ± 6.8 kg of body mass and 174.8 ± 4.6 vs. 163.5 ± 6.0 cm of body height for male and female swimmers, respectively, and 495 ± 80 World Aquatics swimming points of best actual competitive performance at the 400 m freestyle event. Swimmers were informed about the purpose of the evaluations and individual written informed consent was provided before data collection, which was approved by the Institutional Ethics Committee of the Faculty of Sport of the University of Porto (protocol code: CEFAD 25 2020; approval date: 11 November 2020) and performed in accordance with the Declaration of Helsinki.

Design and procedures

In a 25-m indoor swimming pool (with 27°C water temperature), and after a 600-m low-to-moderate intensity warm-up, swimmers performed a 5 x 200 m front crawl incremental protocol with $0.05 \text{ m}\cdot\text{s}^{-1}$ velocity increments and 3 min passive rest intervals between steps (Carvalho et al., 2020; Fernandes et al., 2011; Monteiro et al., 2020). The velocity of the last step was calculated according to

each swimmer's 400 m front crawl time, with a 0.83 s per turn adjustment (due to the use of the respiratory snorkel) (Ribeiro et al., 2016). Subsequently, four successive velocity increments were subtracted to define the subsequent step paces. Velocity was controlled by a visual pacer with flashing lights in the bottom of the pool (Pacer2Swim, KulzerTEC, Aveiro, Portugal) and measured with a manual stopwatch (Seiko, Tokyo, Japan). In-water starts and open turns without underwater gliding were used as previously described (Monteiro et al., 2020).

Pulmonary gas exchange and ventilation were continuously measured breath-by-breath using a portable gas analyser (K4b², Cosmed, Rome, Italy) suspended on a steel cable above the water surface and connected to the swimmer by a low hydrodynamic resistance respiratory snorkel and valve system (Aquatrainer®, Cosmed, Rome, Italy). The respiratory variables were continuously monitored for 3 min during the recovery period (Ribeiro et al., 2016). The gas analysis system and the turbine volume transducer were calibrated before the experiments (following the manufacturer instructions) with gases of known concentrations (16% O₂ and 5% CO₂) and a 3 L syringe (respectively). Lactate concentration ([La⁻]) values were obtained using capillary blood samples from the swimmers' fingertip at rest, immediately after each step and at the 1st, 3rd, 5th and/or 7th min post-protocol until reaching the maximal individual value (Lactate Pro2; Arkay Inc., Kyoto, Japan) (Carvalho et al., 2020; Monteiro et al., 2023).

$\dot{V}O_2$ data were analysed for each incremental protocol step and categorized as low, moderate, heavy and severe intensity domains according to the intensities corresponding to the AnT and $\dot{V}O_{2max}$ (Fernandes et al., 2024). To this end, the lactate-velocity curve modelling method was used to determine the interception point of a combined linear and exponential pair of regressions (Carvalho et al., 2020), while conventional physiological criteria were applied to establish $\dot{V}O_{2max}$ (de Jesus et al., 2015; Howley et al., 1995). Therefore, the low and moderate exercise domains were identified as corresponding to the step below and the step at the AnT (respectively), and the heavy and severe domains as corresponding to the step below and the step where $\dot{V}O_{2max}$ was elicited (respectively) (de Jesus et al., 2015; Pelarigo et al., 2017).

$\dot{V}O_2$ off-transient kinetic parameters were estimated by bootstrapping and the goodness of fit of each model was analysed with raw data by only excluding errant breaths (Lamarra et al., 1987; Sousa et al., 2015). The off-transient for each intensity domain was estimated with two mono- and two bi-exponential models (Equations 1–4, respectively), and the one that best fitted the data by presenting lower standard error of regression was selected:

1. $\dot{V}O_2(t) = EE\dot{V}O_2 - A_p(1 - e^{-(t - TD_p) / \tau_p}), TD_p = 0$
2. $\dot{V}O_2(t) = EE\dot{V}O_2 - H(t - TD_p)A_p(1 - e^{-(t - TD_p) / \tau_p})$
3. $\dot{V}O_2(t) = EE\dot{V}O_2 - H(t - TD_p)A_p(1 - e^{-(t - TD_p) / \tau_p}) - H(t - TD_{sc})A_{sc}(1 - e^{-(t - TD_{sc}) / \tau_{sc}})$
4. $\dot{V}O_2(t) = EE\dot{V}O_2 - H(t - TD)A_p(1 - e^{-(t - TD) / \tau_p}) - H(t - TD)A_{sc}(1 - e^{-(t - TD) / \tau_{sc}})$

where $\dot{V}O_2(t)$ ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) is $\dot{V}O_2$ normalized to the body mass at the time t , $EE\dot{V}O_2$ is the end-exercise $\dot{V}O_2$ value, A_p and A_{sc} ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), TD_p and TD_{sc} (s), and τ_p and τ_{sc} (s) are the amplitudes, time delays and time constants of the fast and slow $\dot{V}O_2$ components, respectively (Özyener et al., 2001; Sousa et al., 2015), and H represented the Heaviside step function (Ma et al., 2010). To characterize the on/off symmetry response at the different swimming intensity domains, $\dot{V}O_2$ on-transient kinetic parameters were also estimated using $VO_2\text{FITTING}$ by choosing the model that best fitted to the different intensity swimming efforts from those described in the literature (de Jesus et al., 2015; Özyener et al., 2001; Zacca et al., 2019).

Statistical analysis

Noisy (gaussian) and non-noisy validation datasets were developed for the four above-referred models to describe the different intensity transitions. Subsequently, $\dot{V}O_2$ data as a function of time were uploaded into the software, verifying whether the fitted parameters perfectly matched the known input values. For the experimental study, a post-hoc power calculation indicated that a sample size of 10 subjects with a large effect size would result in a statistical power of 75% ($\alpha = 0.05$; G*Power 3.1.9.7; Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany). Bootstrapping with 1000 samples was employed to estimate the parameters of mono- and bi-exponential fitting models (a feature available in $VO_2\text{FITTING}$). When multiple models were applied to $\dot{V}O_2$ data, an ANOVA F-test was conducted to verify the goodness-of-fit. The mean, standard

deviation and the coefficient of variation were calculated for each parameter estimated. A Student's paired *t*-test was conducted to test for differences between the on- and off-transient kinetic parameters within each intensity domain and between consecutive intensities, using Cohen's *d* standardized effect sizes (ES) and 95% confidence intervals (CI). For the three comparisons between consecutive intensities, a Hochberg (step-up) correction was used to reduce the type I errors (Menhyart et al., 2021). Furthermore, linear regression and Pearson's correlation (*r*) and determination (*r*²) coefficients were also used to assess the relationships between the considered variables. These statistical analyses were conducted using SPSS (version 29.0.0.0; IBM Corporation, Armonk, NY, USA) with a significance level of 5%.

Results

$\dot{V}O_2$ data as a function of time, obtained from the validation datasets, generated perfect fits, with the parameter estimates perfectly matching the known inputted values for all the four available models (standard error = 0; *p* < 0.001). An example of the different smoothing filters available in VO₂FITTING and applied to the $\dot{V}O_2$ post-exercise curve is presented in Figure 1. The mono-exponential models resulted in best fits during swimming and the recovery period for all swimmers, independently of the exercise intensity (Figure 2) since they were the only ones that fitted or because they presented a smaller standard error of regression and a residual sum of squares. Particularly for $\dot{V}O_2$ off-transient kinetics data modelling, the mono-exponential function without TD had the best fit. Mean parameter estimates for all swimmers (individually fitted), standard deviations, and mean coefficients of variation are presented in Table 1. The coefficients of variation of the estimated *A*_{on}, *A*_{off}, *T*_{on} and *T*_{off} ranged between 1.4–2.7, 1.8–5.4, 18.1–26.0 and 5.6–15.1%, respectively, and 4.5–11.5% for *TD*_{on}.

The mean swimming velocities increased with the exercise intensity, corresponding to 1.18 ± 0.06 , 1.24 ± 0.06 , 1.31 ± 0.06 and 1.40 ± 0.06 m·s⁻¹ for the low, moderate, heavy and severe domains (*p* < 0.001; ES [95% CI]: -2.6 [-4.0 to -1.3], -2.5 [-3.8 to -1.2] and -3.6 [-5.3 to -1.8], respectively). An increase in $\dot{V}O_{2peak}$ with the intensity rise was observed, while both *A*_{on} and *A*_{off} increased from moderate to heavy and from heavy to severe intensities (*p* < 0.05).

When analysing the on/off symmetry, the τ_{on} was lower than τ_{off} at all swimming intensity domains ($p < 0.05$). Significant correlations between on- and off-transient kinetic parameters were observed (Figure 3), with direct associations between A_{on} and A_{off} at severe and between $\dot{V}O_{2peak}$ and $EE\dot{V}O_2$ at moderate and severe intensity domains ($p < 0.01$).

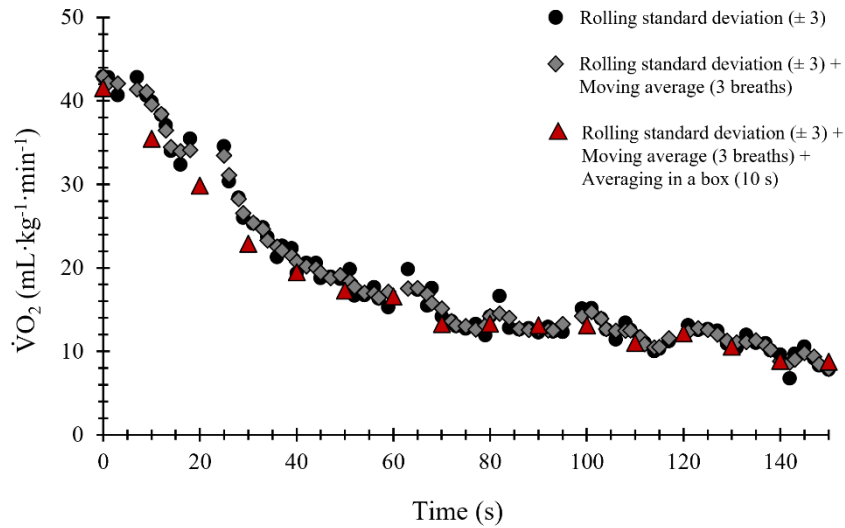


Figure 1. Example of oxygen uptake ($\dot{V}O_2$) recovery curve smoothing using different available filters in VO₂FITTING.

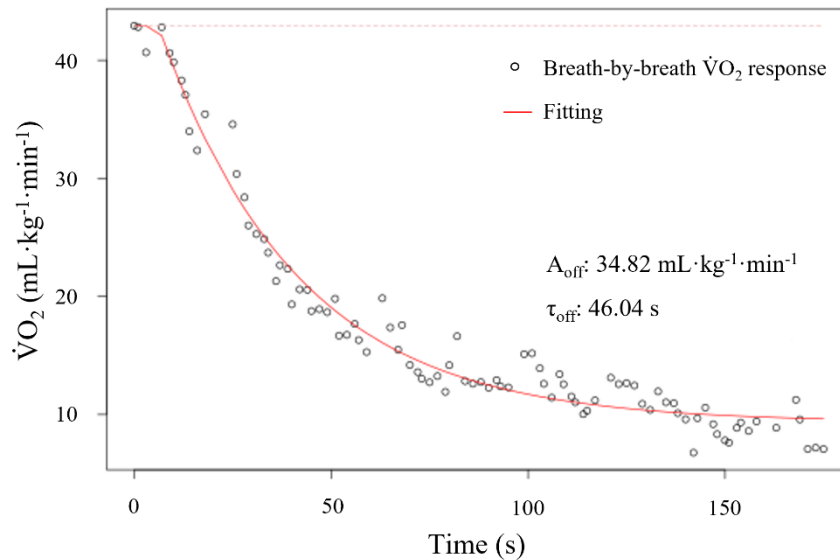


Figure 2. Representation of a recovery oxygen uptake ($\dot{V}O_2$) to time curve response using VO₂FITTING with the respective amplitude (A_{off}) and time constant (τ_{off}) identified, using the mono-exponential model without time delay.

Table 1. Estimated $\dot{V}O_2$ on- and off-transient kinetic parameters at the low, moderate, heavy and severe swimming intensity domains.

	Low	Moderate	Heavy	Severe	Low vs. Moderate		Moderate vs. Heavy		Heavy vs. Severe	
					<i>p</i>	ES (95% CI)	<i>p</i>	ES (95% CI)	<i>p</i>	ES (95% CI)
$\dot{V}O_{2peak}$ (mL·kg ⁻¹ ·min ⁻¹)	39.0 ± 6.1	42.2 ± 4.4	48.0 ± 3.4	51.4 ± 1.3	0.03	-0.8 (-1.5 to 0.1)	< 0.001	-2.2 (-3.4 to -1.0)	0.001	-1.5 (-2.4 to -0.6)
EE $\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	38.8 ± 4.9	38.9 ± 8.1	46.3 ± 6.2	51.0 ± 8.4	0.97	-0.01 (-0.6 to 0.6)	0.006	-1.1 (-1.9 to -0.3)	0.07	-0.7 (-1.3 to 0.1)
<i>p</i>	0.91	0.06	0.45	0.81	-	-	-	-	-	-
ES (95% CI)	0.04 (-0.6 to 0.7)	0.7 (-0.02 to 1.4)	0.3 (-0.4 to 0.9)	0.1 (-0.6 to 0.7)	-	-	-	-	-	-
A_{on} (mL·kg ⁻¹ ·min ⁻¹)	31.6 ± 3.9	33.2 ± 5.9	38.4 ± 5.6	40.3 ± 6.1	0.32	-0.3 (-1.0 to 0.3)	< 0.001	-2.0 (-3.1 to -0.9)	0.01	-1.0 (-1.8 to -0.2)
A_{off} (mL·kg ⁻¹ ·min ⁻¹)	30.1 ± 5.8	29.9 ± 7.4	36.0 ± 5.7	40.3 ± 7.0	0.96	0.02 (-0.6 to 0.6)	0.03	-0.8 (-1.6 to -0.1)	0.05	-0.7 (-1.4 to 0.01)
<i>p</i>	0.49	0.13	0.28	0.99	-	-	-	-	-	-
ES (95% CI)	0.2 (-0.4 to 0.9)	0.5 (-0.2 to 1.2)	0.4 (-0.3 to 1.0)	-0.004 (-0.6 to 0.6)	-	-	-	-	-	-
T_{on} (s)	15.8 ± 11.4	11.3 ± 2.3	13.9 ± 7.0	10.3 ± 4.6	0.18	0.5 (-0.2 to 1.1)	0.17	-0.5 (-1.1 to 0.2)	0.04	0.5 (-0.2 to 1.1)
T_{off} (s)	30.8 ± 10.4	29.7 ± 8.4	28.7 ± 10.8	37.0 ± 9.2	0.82	0.1 (-0.6 to 0.7)	0.85	0.06 (-0.6 to 0.7)	0.08	-0.6 (-1.3 to 0.07)
<i>p</i>	0.03	< 0.001	0.002	< 0.001	-	-	-	-	-	-
ES (95% CI)	-0.8 (-1.5 to -0.1)	-2.5 (-3.8 to -1.2)	-1.3 (-2.2 to -0.4)	-2.7 (-4.1 to -1.3)	-	-	-	-	-	-
TD_{on} (s)	22.4 ± 6.6	19.8 ± 4.4	20.5 ± 3.4	19.6 ± 1.3	0.30	0.4 (-0.3 to 1.0)	0.69	-0.1 (-0.7 to 0.5)	0.44	0.3 (-0.4 to 0.8)

Abbreviations: $\dot{V}O_{2peak}$ - peak oxygen uptake; EE $\dot{V}O_2$ - end-exercise $\dot{V}O_2$ value estimated using the off-transient kinetics model; A_{on} and A_{off} , T_{on} and T_{off} and TD_{on} - amplitude, time constant and time delay of the onset of the fast $\dot{V}O_2$ component during swimming and recovery periods; ES - effect size; CI - confidence intervals.

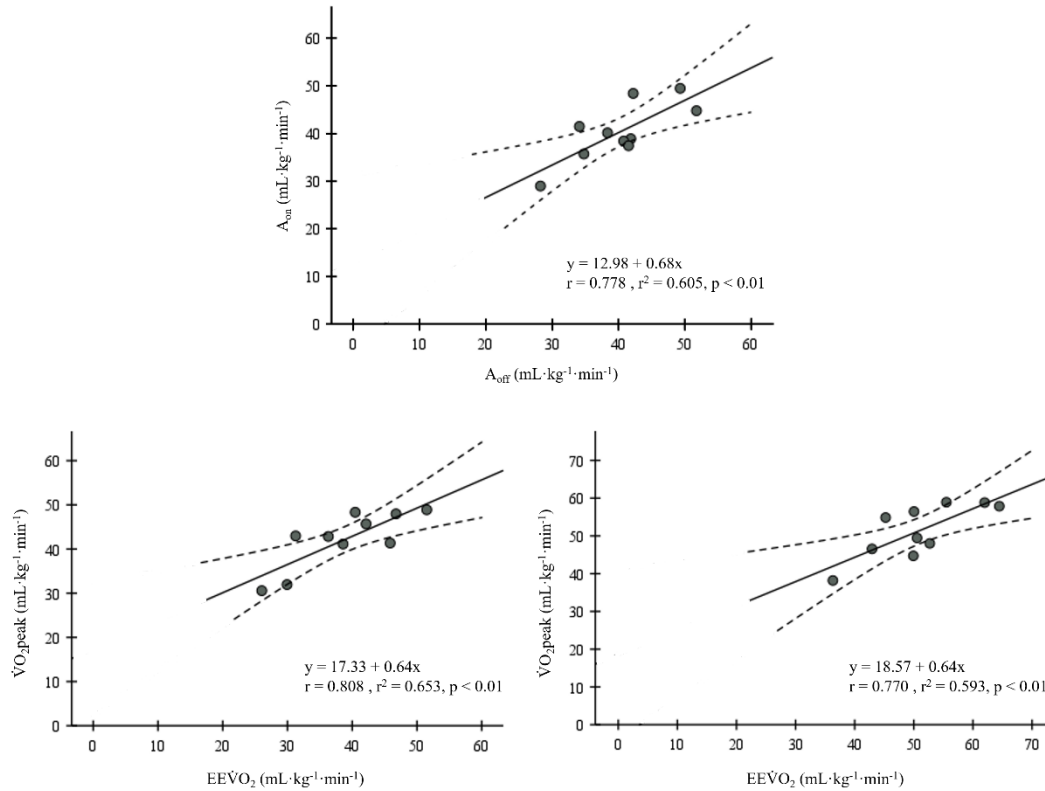


Figure 3. Relationships between on- and off-transient amplitude (A_{on} and A_{off}) at severe, between peak $\dot{V}O_2$ determined by the last 30 s of swimming ($\dot{V}O_{2peak}$) and the end-exercise $\dot{V}O_2$ determined by the fitting models and ($EE\dot{V}O_2$) at moderate and severe intensity domains (upper and left and right lower panels, respectively).

Discussion

Despite the importance of $\dot{V}O_{2max}$ assessment (Duquette & Adam, 2024), $\dot{V}O_2$ kinetic parameters' interpretation is crucial to better understand the physiological response to a given effort (Jones & Poole, 2013). Although different commercial software is available to analyse $\dot{V}O_2$ kinetic data, $VO_2FITTING$ is free and open-source, enabling an easier analysis and rapid feedback from continuous exercise, being a useful tool to monitor performance (Zacca et al., 2019). Our results obtained with the validation datasets demonstrated that $VO_2FITTING$ allowed to dynamically edit, process, filter and model also $\dot{V}O_2$ post-exercise data with the available features commonly used in $\dot{V}O_2$ kinetic modelling. The addition of this feature permits many advantages like allowing to compare $\dot{V}O_2$ off-transient data

with the respective previous exercise phase, with different assessments over time or with different exercise modes.

Breath-by-breath measurements have inherent non-uniformities in the breathing pattern, resulting in some variability around the mean $\dot{V}O_2$ response, known as noise, that can produce some uncertainty in the estimation of the kinetic parameters (Keir et al., 2014; Lamarra et al., 1987). To improve this signal-to-noise ratio and have a clearer and more representative $\dot{V}O_2$ profile, each participant usually performs several exercise transitions that are then time-aligned by interpolating to 1 s time intervals (Lamarra et al., 1987). This tool is available in VO_2 FITTING, both for on- and off-transient period analysis (Zacca et al., 2019). However, in the current study the incremental protocol was performed only once by each swimmer due to the complexity of the measurements in the aquatic environment. Thus, the estimation of different $\dot{V}O_2$ kinetic parameters from a single transition was carried out using the bootstrapping method that provides reliable information about the estimated parameters (Curran-Everett, 2009; Millet & Borrani, 2009).

Increasing $\dot{V}O_{2peak}$ mean values were observed along the swimming intensity domains spectrum (Fernandes et al., 2006; Monteiro et al., 2023), with the higher amplitude mean values (A_{on} and A_{off}) occurring at heavy and severe compared to moderate and heavy exertions (respectively) being directly linked with the greater $\dot{V}O_{2peak}$ mean values in these latter efforts. Regarding τ , it is a major focus of interest in the $\dot{V}O_2$ kinetic related literature since it indicates the time needed to attain a $\dot{V}O_2$ steady-state, having great importance from a practical perspective and being its accurate estimation highly relevant in the understanding of $\dot{V}O_2$ kinetic on-response control (Carter et al., 2002). An invariant τ_{on} is related to a non-limiting oxygen delivery, at least until the heavy intensity domain, and control of muscle kinetics by intracellular processes (Carter et al., 2002; Grassi, 2000). The observed longer τ_{off} seems to be related to a slower rate of response towards reaching the $\dot{V}O_2$ steady-state (Xu & Rhodes, 1999) that can be attributable to the external load on the thorax and increased airway resistance caused by the hydrostatic pressure from water immersion (Leahy et al., 2019), as to a lower muscle oxidative capacity related to the different body position adopted during

swimming and recovery periods (Sousa et al., 2015). In addition, it seems that this kinetic parameter tends to remain constant along different intensities (Cleuziou et al., 2003), as it was evidenced in the current study.

Actually, the observed estimated coefficients of variation for A_{on} , TD_{on} , A_{off} and τ_{off} at the four different intensity domains were suitable, as previously reported (Zacca et al., 2019). τ_{on} presented slightly higher mean coefficient of variation values, which may be related to the natural variability of $\dot{V}O_2$ response (Cooper & Garfinkel, 2022) and not to the constraints in the breathing pattern while swimming caused by the body position and the aquatic environment, as it was previously thought. In fact, when swimmers used the respiratory snorkel, a loss of synchronization was observed between the breathing pattern and the swimming movement, particularly at higher intensities, indicating that swimmers took advantage from the snorkel to breathe whenever they needed to (and not only when it was possible, as it occurs in free swimming) (Monteiro et al., 2023), approaching what takes place in other exercise modes (e.g., running or cycling).

There is a general consensus in the literature on a mono-exponential response for the low and moderate intensity domains (Cleuziou et al., 2003; Özyener et al., 2001; Poole & Jones, 2012), being consistent with the ideas that O_2 debt matches the O_2 deficit and of a linear control dynamic (Rossiter et al., 2005). Actually, the AnT is described as the point up to which there is no change or a small increase in $[La^-]$ and the $\dot{V}O_2$ steady state is attained following the fast $\dot{V}O_2$ response (Burnley & Jones, 2007; Pelarigo et al., 2017). Above the AnT there is a loss of body homeostasis, with higher participation of the anaerobic metabolism (Carvalho et al., 2020; Pelarigo et al., 2017). Thus, in the $\dot{V}O_2$ kinetic related literature, heavy and severe intensity domains are commonly described by bi-exponential models (Cleuziou et al., 2003; Özyener et al., 2001; Sousa et al., 2015), where a delayed increase in $\dot{V}O_2$ kinetics, known as a slow component, appears after approximately 2–3 min of exercise (Jones & Poole, 2013) as a sign of decreased efficiency of muscle contractions and of the recruitment of fibres with inherently slower $\dot{V}O_2$ kinetics (Grassi et al., 2015; Zoladz et al., 2008).

At these higher intensities (above the AnT), an on/off symmetry is not always verified, contrary to what happens at low and moderate efforts (Özyener et al.,

2001; Paterson & Whipp, 1991). However, the results of our experimental study did not evidence any second and delayed exponential increase during exercise nor recovery periods at the different swimming intensity domains. As a consequence of the swimming velocity rise along the incremental protocol, there was an evident decrease in the 200 m step duration (mean $\pm$ SD of 169.2 ± 8.0 , 161.4 ± 8.1 , 152.9 ± 7.0 and 142.8 ± 5.7 s for the low, moderate, heavy and severe intensities, respectively). On the one hand, and despite the 200 m step length validity and practical application during training sessions (Fernandes et al., 2011), the duration of the protocol steps may not have been sufficient to allow the development of the $\dot{V}O_2$ slow component (Jones & Poole, 2013), suggesting that its emergence is closer to the third minute of exercise (Billat, 2000). On the other hand, the predominance of the aerobic component in the swimmers' training sessions (Santos et al., 2024) may have decreased the $\dot{V}O_2$ slow component due to an increased distribution of type I fibres and an increase in mitochondrial and capillary density (Billat, 2000; Holloszy & Coyle, 1984). In addition, the lack of isometric contractions in swimming and the effort distribution on all four limbs may suggest that no slow component should be expected (Demarie et al., 2001).

Despite the on/off symmetry observed in the present study, since the mono-exponential model presented the best fit, differences between both phases were found, particularly regarding TD. At the transition from rest to exercise, there must be a coordinated pulmonary, cardiovascular and muscular system response aiming to rapidly increase the O_2 flux from the atmosphere to muscle mitochondria (Poole & Jones, 2012). Our results are in agreement with a commonly described delay of about 10–20 s, representing the time needed to O_2 be unloaded in the muscle and the arrival of the same blood in the pulmonary vasculature for gas exchange (Barstow et al., 1996; Burnley & Jones, 2007). When the exercise ceases, a TD is often described but little interpreted and discussed, being possible to find a high range of mean values along the different exercise intensity domains (Billat et al., 2002; Cleuziou et al., 2003; Sousa et al., 2015).

However, the current results did not evidence a TD at the transition from exercise to recovery, at any of the studied intensity domains. Using VO_2 FITTING with breath-by-breath data without constraints and smoothing processes, it was

possible to choose between different functions the one with the best fit and lower error. In this sense, raw $\dot{V}O_2$ off-transient data with their inherent variability (Keir et al., 2014) demonstrated to be better characterized by a mono-exponential model without TD, indicating that the recovery period started immediately when swimmers stopped after each effort (Sousa et al., 2011) as it has been demonstrated at a muscle level (Behnke et al., 2009). The transition from the horizontal to the vertical position during the intervals and at the end of the protocol seems to facilitate the beginning of the recovery process (Leahy et al., 2019; Sousa et al., 2015), helping to explain the non-delay in our results.

Studies related to $\dot{V}O_2$ kinetics at different exercise intensities and particularly focusing on the $\dot{V}O_2$ slow component and off-transient phase have been carried out mainly in treadmill running and/or with a cycle ergometer (Cleuziou et al., 2003; Jones & Poole, 2013; Özyener et al., 2001), being this topic less studied in swimming. Despite the individual and cyclic characteristics of swimming (like running and cycling), the different environment conditions and the body position seem to affect the $\dot{V}O_2$ kinetic response (Demarie et al., 2001; Leahy et al., 2019). With the use of VO₂FITTING, and considering its availability to also analyse the $\dot{V}O_2$ post-exercise data dynamically, we intend to contribute to future challenges, particularly to expand the knowledge about the on/off symmetry and $\dot{V}O_2$ kinetics in such a peculiar exercise mode as swimming. In addition, future works including the comparison of the current methodological approach with only the $\dot{V}O_2$ post-exercise data assessment, also allowing the inclusion of the undulatory underwater phases (Ruiz-Navarro et al., 2024), would add valuable information to this topic.

Conclusions

VO₂FITTING proved to be valid for characterizing $\dot{V}O_2$ kinetics not only during continuous exercise, but also during the subsequent recovery period. With this free and open-source software applied for research and performance, it is possible to have rapid feedback about the $\dot{V}O_2$ kinetics parameters without resorting to the use of complex mathematical programming. When characterizing $\dot{V}O_2$ kinetics and the on/off symmetry at different swimming efforts, it seems that

protocol steps of 200 m may not be sufficient to observe the development of the $\dot{V}O_2$ slow component at higher intensity domains, particularly in high aerobically trained swimmers. In addition, due to the use of dynamic analysis software it was possible to observe that, after each effort, the recovery started immediately, a fact that could be more evident in swimming due to the body position and environment characteristics. We hope to encourage further research about these less studied topics, particularly in swimming.

Revisiting the concept of exercise economy

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To be submitted

Abstract

Exercise economy is a critical determinant of endurance performance, expressing the efficiency of the chemical energy conversion into mechanical work. It is traditionally measured by determining the energy cost of exercise, i.e., the ratio between the energy expenditure (usually using the oxygen uptake values) per the distance covered in a submaximal incremental protocol. Although energy cost monitoring traditionally focused on evaluating the aerobic metabolism, it may overlook other relevant physiological determinants, particularly the anaerobic energy contribution. With the development of new technological devices and by using step tests until voluntary exhaustion, a more comprehensive assessment of the energy systems contribution is available, allowing the incorporation of the anaerobic components derived from lactate production and phosphocreatine splitting. This approach permits a detailed examination of the interplay between energy systems across a broader range of velocities/power outputs, providing new insights into the adaptations occurring during exercise. By extending the exercise economy concept to encompass both aerobic and anaerobic pathways, using a wider spectrum of intensities, it is possible to gain a deeper understanding of the actual training effects. This might allow for more effective strategies aiming at optimising endurance performance in a variety of contexts.

The evaluation of exercise economy, particularly regarding endurance-related sports, has been a topic of interest for a long time. In fact, concurrently with the assessment of maximal oxygen uptake and blood lactate concentrations (commonly accepted indicators of oxidative and glycolytic metabolisms), and its specific percentages that can be sustained over time, exercise economy is an important performance influencing factor (Joyner et al., 2020; Saunders et al., 2004; Swinnen et al., 2018). Even if the first attempts to describe the energetics of human locomotion on land have been dedicated to walking, over the last decades the economy of cyclic exercise modes (particularly running, cycling and swimming) has been widely analysed (di Prampero, 1986; Kjendlie et al., 2004; Saunders et al., 2004; Swinnen et al., 2018). These studies were centred on thermodynamic principles and attended to how efficiently the chemical energy derived from substrates (e.g., carbohydrates and fats) is converted into mechanical work (Figueiredo et al., 2013; Sparrow and Newell, 1998). However, the methodology of assessing the energy demand for a given submaximal velocity by measuring the steady-state oxygen uptake and the recent technological advances lead us to question whether the traditional exercise economy assessment follows the best practices and is still accurate.

The most common method to assess exercise economy on land employs a submaximal intensity incremental protocol on a treadmill, increasing the velocity or grade step by step until the moment where the blood lactate concentrations exponentially increase (Mayhew, 1977; Morgan et al., 1989; Shaw et al., 2014). The exercise economy value is calculated by the inverse of the energy cost of locomotion, usually expressed in $\text{kJ}\cdot\text{km}^{-1}$ or $\text{J}\cdot\text{m}^{-1}$, representing the amount of energy expended transporting the body at a given distance. The ratio between energy expenditure and velocity allows calculating energy cost (di Prampero et al., 1976), and overall indicates that a larger value of this variable corresponds to a less economical runner (di Prampero, 1986). Consequently, it evidences that velocity is dependent on the ratio between energy expenditure and energy cost. Considering that energy cost is influenced by the athlete technical skills (due to the influence of work and efficiency in both on land and aquatic exercises) (Figueiredo et al., 2013; Folland et al., 2017; Zamparo et al., 2013), performance

improvements can be achieved through physiological and/or technical enhancement(s).

In general terms, the optimal strategy for a practitioner to achieve the best exercise economy levels is to minimize his/her energy expenditure while maintaining a consistent velocity or power output. This is true regardless of performing long distance at low/moderate intensity efforts (like marathons, open water or cycling road racing events) or engaging in short and fast exertions (like 100-400 m track run, 50-100 m swim or sprint track cycling trials). We understand that the relative contribution of the different energy systems depends on the exercise duration and intensity, i.e., low intensity physical activities predominantly (but not exclusively) are dependent on aerobic based energy, while high intensity exertions use a larger amount of energy provided from the anaerobic pathways (Cahill et al., 1997; Ribeiro et al., 2017). Nevertheless, it is our conviction that a comprehensive evaluation of exercise economy requires the assessment of all energy systems along a protocol that includes a larger spectrum of power outputs (Cardoso et al., 2025; Fernandes & Vilas-Boas, 2020).

When traditionally assessing exercise economy (using constant velocity steps in a submaximal protocol), the basis for energy expenditure estimation is the aerobic energy metabolism (di Prampero, 1986; Morgan et al., 1989; Swinnen et al., 2018) through the measurement of oxygen uptake (Zamparo et al., 2013). In this case there is the assumption that the energy required for the resynthesis of the adenosine triphosphate (ATP) is derived entirely from the oxidation of carbohydrate and fat substrates (Saunders et al., 2004). From our perspective, when this method is employed, a more specific nomenclature should be used, like oxygen cost or aerobic energy expenditure (Margaria et al., 1963; Mayhew, 1977), given that only this energy system is taken in consideration in the calculations and a narrow range of exercise velocities is considered. Furthermore, when exclusively relating oxygen uptake to a specific velocity or power output to describe exercise economy, this overlooks other contributing factors since it does not consider different (aerobic and anaerobic) metabolic efficiencies and/or biomechanical determinants (Cardoso et al., 2025; Daniels, 1985).

To conduct a more comprehensive assessment of the exercise economy of a specific sport activity, it is important to consider a broader range of velocities that are representative of its various competitive events. The aerobic energy system is capable of producing a substantial amount of ATP that would endure exercise through time, but its production rate is constrained by the limits of oxidative phosphorylation and of the respiratory and cardiovascular systems that regulate the muscular oxygen delivery (Gastin, 2001). Furthermore, both aerobic and anaerobic energy systems are active at every exercise intensity, with the onset of every effort being a predominantly anaerobic process. Since this energy yielding system is also trainable (Cahill et al., 1997; Gastin, 2001), its end products should also be integrated in the overall energy cost calculation by estimating energy expenditure through the sum of both aerobic and anaerobic (lactic and alactic) pathways (Cardoso et al., 2022; Fernandes et al., 2006; Sousa et al., 2015).

The relevance of the anaerobic metabolism contribution during running, cycling and swimming have been gradually addressed (di Prampero, 1981; Faude et al., 2009; Fernandes et al., 2005), with the first attempts to quantify it using invasive biochemical and histological methods. This was done by collecting venous blood samples (Cahill et al., 1997; Wasserman et al., 1973) and by determining the oxygen debt as a reflection of lactate accumulation originated from exercise (Åstrand, 1981; di Prampero, 1981). Afterwards, portable analysers were developed, aiming to a less invasive, easier and faster assessment, simplifying the lactate concentrations evaluation in both research and training contexts. Nowadays, high-invasive methods are harsh to be used in daily training sessions since the extensive use blood of lactate analysis has a high degree of consistency across a range of intensities, in different populations and in various exercise modes (even if estimated from capillary rather than muscle) (Lopes et al., 2023; Monteiro et al., 2022; Wasserman et al., 1973). In addition, the identification of a 2.0-5.0 mmol·L⁻¹ range of blood lactate concentrations until the power outputs corresponding to the anaerobic threshold evidences that not all athletes have negligible lactate accumulation at low intensities (Capelli et al., 1998; Carvalho et al., 2020; Monteiro et al., 2023). Depending on the individual characteristics and

on the training process, the anaerobic energy component may be visible even at moderate exercise intensities.

When the obtained capillary blood lactate concentration values are used to estimate the anaerobic contribution that supports exercise, some energy equivalents should be considered (Cardoso et al., 2025; di Prampero, 1981). The lactic component (the amount of energy derived from full exploitation of the anaerobic glycolytic pathway) is calculated as the product of body mass, net lactate accumulation and a constant for its oxygen equivalent (that varies according to the type of exercise). The pioneer studies reported that 1 mM increase in blood lactate concentration obtained through venous samples is equivalent to the energy release by the oxygen consumption of 3.1-3.3 and 2.7 mL·kg⁻¹·mM⁻¹ in running (Margaria et al., 1963) and in swimming (di Prampero et al., 1978), respectively. More recently, similar values have been widely used for on-land activities (Bertuzzi et al., 2013; Sousa et al., 2015; Zamparo et al., 2013) and swimming (Fernandes et al., 2005; Thevelein et al., 1984) to estimate energy expenditure through capillary blood samples.

The alactic component of the overall energy input (the one derived from phosphocreatine splitting at the onset of exercise) is calculated based on the body mass, exercise duration, time constant of the phosphocreatine splitting at exercise onset (23.4 s) (Mebdo and Tabata, 1984) and phosphocreatine concentration at rest - 0.418 kJ·kg⁻¹ (assuming that it decreases by 18.5 mM·kg⁻¹ in a maximally active muscle mass in the transition from rest to exhaustion, corresponding to ~30% of body mass, and a phosphate/oxygen ratio of 6.25) (Capelli et al., 1998; Zamparo et al., 2011). Moreover, it should be highlighted that the 0.418 kJ·kg⁻¹ value, obtained during maximal exertions, is applicable only to individuals aged between 21 and 30 years, and that it has been adjusted to 0.393 and 0.407 kJ·kg⁻¹ for the 18-19 and 20 years old age groups, respectively (Capelli et al., 1998).

It is also assumed that, for each litre of oxygen utilised in the respiratory chain, there is a production of ~20.9 kJ of energy that is used for conversion into energy units (di Prampero, 1981; Sousa et al., 2015). This illustrates that, despite the less invasive nature of the above-referred methods and their ease of use, the process of assessing oxygen uptake and blood lactate concentrations for energy

expenditure estimation remains an indirect measurement. Here, the assumptions about what happens at the muscle are made based on the values obtained from a peripheral pathway. We recognize that these estimations may comprise some errors, particularly regarding the phosphocreatine splitting on the method of calculating anaerobic alactic contribution, since it was proposed for maximal exertions (Capelli et al., 1998). However, this is the only way available to assess it at different intensities and exercise modes, meaning that further research is encouraged to enhance the precision of these calculations in diverse contexts.

Considering the existing technology and knowledge pertaining to this topic, we consider that it is of paramount importance to extend the concept of exercise economy to encompass a diverse range of velocities or power outputs and considering the interplay between the aerobic and anaerobic energy systems. In practical terms, this approach would allow for a more comprehensive understanding of the adaptations that result from the training sessions. Such an expanded framework permits a more profound examination on how training intensities and durations affect energy utilization, providing invaluable insights into the training process optimization.

Effects of velocity and energy contributions on exercise economy assessment

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Abstract

Purpose: This study aimed to bioenergetically characterize traditional cyclic exercise modes from low to extreme intensities, including exertions above anaerobic threshold (AnT) and maximal oxygen uptake ($\dot{V}O_{2\max}$). **Methods:** Thirty male trained subjects (10 runners, 10 swimmers and 10 cyclists) performed an incremental protocol until exhaustion, tailored to each exercise mode, to assess ventilatory and bioenergetical responses, particularly oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), respiratory frequency (f_R), tidal volume (V_T), heart rate (HR), blood lactate concentrations ($[La^-]$), energy expenditure ($\dot{E}$, calculated as the sum of aerobic and anaerobic lactic and alactic contributions) and energy cost (C). The slope of the regression lines between $\dot{E}$ and velocities (C_{slope}) were also determined. **Results:** $\dot{V}O_2$, $\dot{V}E$, f_R , HR, $[La^-]$ and $\dot{E}$ progressively increased with exercise intensity ($p < 0.001$), while V_T remained relatively stable (particularly in swimming). Running exhibited a velocity-independent C ($[0.29-0.35 \text{ kJ}\cdot\text{m}^{-1}]$, with no variation between consecutive step increments, except between maximal step $[0.46\pm0.06 \text{ kJ}\cdot\text{m}^{-1}]$). Swimming and cycling showed velocity-dependent increases ($[0.86-1.44 \text{ kJ}\cdot\text{m}^{-1}]$ and $[0.10-0.23 \text{ kJ}\cdot\text{m}^{-1}]$). The inclusion of velocities above AnT in the incremental protocol constantly changed C_{slope} ($[5.71-10.18]$, $[30.65-42.76]$ and $[3.32-7.21] \text{ kJ}\cdot\text{m}^{-1}$ for running, swimming and cycling), highlighting the limitations of assessing exercise economy based solely on submaximal velocities. The inclusion of extreme intensities (above $\dot{V}O_{2\max}$) revealed a shift towards glycolytic metabolism, emphasizing the importance of evaluating a broader spectrum of velocities to more accurately characterize exercise economy. **Conclusions:** These findings emphasize the limitations of traditional submaximal assessments and underline the importance of including a wider range of velocities to a better training prescription by identifying performance limitations and improve training interventions.

Keywords: energy expenditure, energy cost, running, swimming, cycling.

Introduction

Cyclic individual exercise modes performance depends on a complex interaction of different factors, including maximal oxygen uptake - $\dot{V}O_{2\max}$ (representing the maximal rate at which an individual can consume oxygen to produce energy), anaerobic threshold - AnT (reflecting the balance between lactate production and clearance, marking the intensity at which the anaerobic metabolism contribution starts to be more pronounced) and exercise economy (Fernandes et al., 2005; Foster & Lucía, 2007; Morgan et al., 1989). The assessment of this latter represents a topic of interest for a long time, centred on understanding how efficiently the human body converts chemical energy derived from substrates into biomechanical work (Sparrow & Newell, 1998). Through the calculation of energy cost (C), obtained by the ratio between energy expenditure and velocity or power output, it is possible to ascertain the amount of energy used in transporting the body over a given distance (di Prampero, 1986; Zamparo et al., 2013).

The assessment of exercise economy is traditionally performed through submaximal incremental protocols by measuring the steady-state oxygen uptake (Saunders et al., 2004), assuming that the energy required for the resynthesis of the adenosine triphosphate is entirely derived from the oxidation of carbohydrate and fat substrates (di Prampero, 1986; Morgan et al., 1989; Zamparo et al., 2013). However, besides the fact that the rate of aerobic energy production is limited to the oxidative phosphorylation and respiratory and cardiovascular systems capacity (Gastin, 2001), it is known that aerobic and anaerobic energy systems are simultaneously active at every exercise intensity and that every movement initiation is a predominantly anaerobic process (Cahill et al., 1997; Gastin, 2001). In addition, it has been demonstrated that below the AnT, blood lactate concentrations $[La^-]$ are not always neglectable (Lopes et al., 2023; Monteiro et al., 2023), meaning that the anaerobic energy system may have a higher contribution even at lower intensities, depending on individual characteristics and training. Thus, merely relating a $\dot{V}O_2$ value to an exercise velocity/power when characterizing exercise economy seems to be reductive, since it does not consider possible differences in metabolism that may exist between practitioners or experimental conditions (Daniels, 1985; Fernandes et al., 2005).

Besides the calculation of C for a specific velocity, the slope of the regression line obtained from the relationship between energy expenditure ($\dot{E}$) and velocity along an incremental protocol (C_{slope}) is presented as an indicator of exercise economy profile (Fernandes et al., 2005; Kjendlie et al., 2004). In this case, a lower slope indicates that less energy is being required to achieve a given increase in velocity, reflecting a better exercise economy, while a steeper slope will reflect a higher energy demand for the same increment, reflecting a poorer exercise economy. Thus, the exclusive use of submaximal velocities to describe this relationship does not fully characterize the exercise economy profile of an athlete (Morgan et al., 1989). To conduct a more comprehensive evaluation of exercise economy of a specific sport activity all energy systems should be assessed and considered of broader range of velocities representative of competitive events (Capelli et al., 1998; Fernandes et al., 2005; Sousa et al., 2015).

Running, swimming and cycling are individual and cyclic exercise modes influenced by common factors (Swinen et al., 2018; Zamparo et al., 2011). However, these sports differ regarding the environment where they are performed, the body posture and the muscles groups predominantly engaged. Running and cycling are on-land activities primarily involving the contraction of lower limb muscles, yet they exhibit distinct mechanical and physiological responses (Millet et al., 2009). Running is characterized by alternating phases of lower limbs support, with a vertical displacement of the body (Folland et al., 2017), whereas cycling involves minimal impact forces due to the continuous support provided by the saddle and cyclic pedalling motion with alternated power and recovery phases (Turpin & Waiter, 2020). Swimming takes place in an aquatic environment, requiring a horizontal body position, dealing with the aquatic drag and facing a restricted ventilation (Leahy et al., 2019), resulting in higher $\dot{E}$ compared to running and cycling (Sousa et al., 2015).

The scarce inclusion of a wider range of velocities and, consequently, the exclusive use of aerobic metabolism when describing the energy demand, seems to indicate that there is an incomplete characterization of exercise economy along the above-referred cyclic exercise modes. The main purpose of the current study was to bioenergetically characterize running, swimming and cycling from low to extreme exertions, aiming: (i) to evidence the participation of the anaerobic

metabolism and its influence in energy expenditure (and respective relative contributions) and C estimation; and (ii) to assess the intensity effect on the exercise economy profile. It was hypothesized that the bioenergetic responses would increase with the velocity rise but differently between exercise modes and that exercise economy would change depending on the inclusion of intensities above the AnT.

Methods

Subjects

Thirty male trained subjects (engaged in, at least, five training sessions per week and regularly involved in competitive events) participated in the current study. The sample consisted of 10 runners, 10 swimmers and 10 cyclists with 26.4 ± 5.4 , 19.0 ± 7.4 and 32.5 ± 8.8 years of age, 177.7 ± 7.0 , 174.9 ± 8.5 and 174.4 ± 8.0 cm of height, and 67.8 ± 8.4 , 65.0 ± 11.9 and 72.6 ± 7.0 kg of body mass. The anthropometric characteristics were similar between subjects, but swimmers were younger than cyclists ($p < 0.001$). All participants provided informed written consent (or parents, when minors) before data collection, avoided strenuous exercise in the 24 h before each testing session and were well hydrated and abstained from caffeine in the three hours before testing. The institutional ethics committee approved the study design (CEFADE 25 2020) performed in accordance with the Declaration of Helsinki.

Design

All subjects completed an intermittent incremental protocol until exhaustion that varied according to each exercise mode specificity (Sousa et al., 2015). Swimmers performed 200 m front crawl steps with increments of $0.05 \text{ m}\cdot\text{s}^{-1}$, followed by a maximal 100 m, with 30 s passive intervals in-between, on a 25 m indoor swimming pool (Fernandes et al., 2011; Monteiro et al., 2023). The initial velocity was established according to each swimmer 400 m front crawl time, less six velocity increments, controlled by a visual pacer with flashing lights on the bottom of the pool (Pacer2Swim; KulzerTEC, Aveiro, Portugal). Runners performed 800 m steps with increments of $1 \text{ km}\cdot\text{h}^{-1}$, followed by a maximal 400 m, with 30 s passive intervals in-between, on a 400 m outdoor track field. The initial velocity was defined according to the individual performance on the 3000

m less six velocity increments, controlled by audio feedback (whistle) at every 100 m. (Cardoso et al., 2024). Cyclists performed 3 min steps with increments of 25 W and an average cadence between 70-90 rpm, followed by a maximal 1 min, with 30 s passive intervals in-between, on their own bicycle positioned on a Tacx Neo 2T smart trainer (Garmin, Olathe, KS, USA). The initial power was established according to each performance on a recent aerobic power test and controlled by visual feedback. (Lucía et al., 1999; Morais et al., 2024).

Methodology

Pulmonary gas exchange and ventilation were measured using a telemetric portable gas analyser (K5, Cosmed, Rome, Italy) calibrated before each testing session in accordance with the manufacturer instructions. In swimming, this equipment was transported on a steel cable 2 m above the water surface and connected to the swimmer through a low hydrodynamic resistance respiratory snorkel (Aquatrainer®, Cosmed, Rome, Italy). In-water starts and open turns without underwater gliding were used (Ribeiro et al., 2016). In running and cycling tests, the subjects breathed through a traditional face mask (K5, Cosmed, Rome, Italy) and the gas analyser was placed near the body centre of mass. Heart rate (HR) was continuously recorded at baseline and during the protocol using a Polar Vantage NV (Polar Electro Oy, Kemple, Finland) that telemetrically emitted to the portable gas analyser unit. Capillary blood samples for blood lactate concentrations $[La^-]$ analysis were collected from the fingertip at rest, during the intervals and 1, 3, 5 and/or 7 min post-exercise (until obtaining the maximal value) using a portable analyser (LactatePro2, Arkay Inc., Kyoto, Japan) (Sousa et al., 2015).

Errant $\dot{V}O_2$ breaths (caused by swallowing, coughing or signal interruptions) were omitted from the analysis by excluding those deviating more than three standard deviations (Lamarra et al., 1987). Afterwards, individual breath-by-breath $\dot{V}O_2$ values were smoothed using a three-breaths moving average and 10 s time interval (Fernandes et al., 2012), and the mean values from the last 30 s of exercise per step were selected, also for minute ventilation ($\dot{V}E$), respiratory frequency (f_R), tidal volume (V_T) and heart rate (HR) (Monteiro et al., 2022). $\dot{E}$ was estimated as the sum of aerobic (Aer), anaerobic lactic (AnL) and anaerobic alactic (AnAl) energy contributions. Aer was obtained by from the time integral of

the net $\dot{V}O_2$ (difference between the last 30 s mean values and the baseline values) vs. time relationship. AnL and AnAI were estimated using the following equations:

$$1. \text{AnL} = [\text{La}^-]_{\text{net}} \cdot \beta \cdot M$$

$$2. \text{AnAI} = \text{PCr} \cdot (1 - e^{-t/\tau}) \cdot M$$

where $[\text{La}^-]_{\text{net}}$ is the difference between each step $[\text{La}^-]$ and the baseline values, β is the constant for oxygen equivalents of $[\text{La}^-]_{\text{net}}$ (3.0 for running and cycling and $2.7 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{mM}^{-1}$ for swimming) (di Prampero, 1987; Zamparo et al., 2010), M is the body mass, PCr is the phosphocreatine at rest, t is the exercise time and τ is the time constant of the PCr splitting at exercise onset (23.4 s) (Mebdo and Tabata, 1984).

The energy contributions were expressed in kJ by assuming an energetic equivalent of $20.9 \text{ kJ} \cdot \text{L}^{-1}$ for Aer and AnL, and 0.418, 0.407 or $0.393 \text{ kJ} \cdot \text{kg}^{-1}$ (depending on subjects' age) for AnAI sources (Capelli et al., 1998). C was estimated as the ratio between $\dot{E}$ and velocity or power for each protocol step. C at AnT (C_{AnT}) and at $\dot{V}O_{2\text{max}}$ ($C_{\dot{V}O_{2\text{max}}}$) were calculated for comparison between exercise modes and C_{slope} was obtained based on the slope of the regression line between energy expenditure and velocity. Also, linear and exponential regression models were applied to this relationship based on the steps until the anaerobic threshold (considering Aer and $\dot{E}$), until the maximal oxygen uptake and with all the protocol steps.

Statistical Analysis

Assuming an effect size of 0.5, a power of 0.80 and an α error probability of 5%, a sample size of nine subjects for each exercise mode was required (G*Power, version 3.1.9.7; Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany). Statistical procedures were conducted using SPSS (version 29.0.0.0; IBM Corporation, Armonk, NY, USA) with a significance level of 0.05. The normal data distribution was checked for all variables with the Shapiro-Wilk test and data is presented as mean $\pm$ standard deviation. A one-way ANOVA for repeated measures was conducted to identify differences along the incremental protocol within each exercise mode. Partial eta-squared (η_p^2) for effect size calculations

was determined, with values of 0.04, 0.25 and above 0.64 considered as minimum, moderate and strong, respectively (Ferguson, 2009).

A two-way ANOVA for repeated measures [steps*exercise modes] was used to assess the exercise mode effect on $\dot{V}E$, f_R and C along the velocities, with sphericity (homogeneity of variance and covariance) verified by means of the Mauchley test. If the assumption of sphericity was not met, the significance of the F -ratios was adjusted according to the Greenhouse-Geisser procedure. When a significant F -value was achieved, Bonferroni post-hoc analyses were performed to locate the pairwise differences between the means. The association between $\dot{V}E$ and f_R with C were analyzed through Pearson correlation coefficient (r : < 0.1 - trivial, 0.1-0.3 - small, 0.3-0.5 - moderate, 0.5-0.7 - large, 0.7-0.9 - very large and > 0.9 - nearly perfect: Hopkins et al., 2009). A paired sample Student's t -test was performed to compare the coefficients of determination (r^2) between the linear and exponential regression models. When similar r^2 values were identified, the simpler regression model (linear) was selected.

Results

The mean and standard deviation values of the incremental protocol responses in running, swimming and cycling are reported in Table 1. In running, the velocity rise led to consecutive step increments in $\dot{V}O_2$ from 1-6 ($p = [< 0.001-0.004]$), except from 3-4 ($p = 0.057$), in $\dot{V}E$ until 7 ($p = [< 0.001-0.003]$), except from 2-3 ($p = 0.106$), in f_R and $[La^-]$ only from 6-7 ($p = 0.042$ and 0.006), in HR from 1-7 ($p = [< 0.001-0.016]$), in $\dot{E}$ ($p = [< 0.001 - 0.006]$) and in C only from 7 to maximal ($p = 0.004$). In swimming, the velocity rise led to consecutive step increments in $\dot{V}O_2$ from 2-3 and 6-7 ($p = 0.014$ and < 0.001), in $\dot{V}E$ from 1-3 and from 4-5 ($p = [0.006-0.011]$), in f_R only from 1-2 ($p = 0.043$), in HR from 1-3 ($p = [0.004-0.022]$), in $[La^-]$ from 3-5 and 6 to maximal ($p = [0.020-0.037]$), in $\dot{E}$ ($p < 0.001$), except from 1-2 ($p = 0.059$) and in C from 2-3 and 6 to maximal ($p = [< 0.001-0.005]$).

Table 1. Mean $\pm$ standard deviation values of performance, ventilatory and bioenergetical responses in running, swimming and cycling.

	Incremental protocol steps								η_p^2	p
	1	2	3	4	5	6	7	Max		
Running										
v (km·h ⁻¹)	13.6 $\pm$ 1.9	14.7 $\pm$ 2.0	15.6 $\pm$ 1.9	16.5 $\pm$ 1.9	17.5 $\pm$ 1.9	18.5 $\pm$ 1.9	19.3 $\pm$ 1.9	21.3 $\pm$ 2.0	0.988	<0.001
v (m·s ⁻¹)	3.79 $\pm$ 0.53 ^{2-M}	4.08 $\pm$ 0.56 ^{3-M}	4.32 $\pm$ 0.51 ^{4-M}	4.57 $\pm$ 0.52 ^{5-M}	4.86 $\pm$ 0.53 ^{6-M}	5.13 $\pm$ 0.52 ^{7-M}	5.36 $\pm$ 0.53 ^M	5.92 $\pm$ 0.56	0.988	<0.001
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	46.3 $\pm$ 6.2 ^{2-M}	50.3 $\pm$ 5.9 ^{3-M}	53.5 $\pm$ 6.1 ^{4-M}	56.2 $\pm$ 6.8 ^{5-M}	60.0 $\pm$ 7.1 ⁶⁻⁷	62.2 $\pm$ 7.4	65.3 $\pm$ 8.6	62.5 $\pm$ 6.1	0.920	<0.001
$\dot{V}E$ (L·min ⁻¹)	83.0 $\pm$ 11.6 ^{2-M}	96.3 $\pm$ 13.0 ^{4-M}	105.3 $\pm$ 13.2 ^{4-M}	116.2 $\pm$ 14.2 ^{5-M}	132.7 $\pm$ 15.8 ^{6-M}	143.6 $\pm$ 17.3 ^{7-M}	159.9 $\pm$ 14.2	165.2 $\pm$ 15.0	0.963	<0.001
f_R (breaths·min ⁻¹)	40 $\pm$ 8 ^{5-M}	49 $\pm$ 14 ^M	50 $\pm$ 12 ^M	51 $\pm$ 12 ^M	53 $\pm$ 8 ^M	56 $\pm$ 8 ^M	64 $\pm$ 7 ^M	71 $\pm$ 8	0.750	<0.001
V_T (L)	2.14 $\pm$ 0.52 ⁶⁻⁷	2.18 $\pm$ 0.41 ^{5-M}	2.32 $\pm$ 0.40 ⁶⁻⁷	2.44 $\pm$ 0.48 ⁶	2.59 $\pm$ 0.49	2.67 $\pm$ 0.47	2.60 $\pm$ 0.40	2.43 $\pm$ 0.39	0.664	<0.001
HR (bpm)	148 $\pm$ 12 ^{2-M}	158 $\pm$ 14 ^{3-M}	164 $\pm$ 13 ^{4-M}	170 $\pm$ 13 ^{5-M}	177 $\pm$ 12 ⁶⁻⁷	181 $\pm$ 12 ⁷	185 $\pm$ 12	184 $\pm$ 11	0.949	<0.001
[La] (mmol·L ⁻¹)	1.9 $\pm$ 1.0 ^{6-M}	2.9 $\pm$ 1.8 ^{5-M}	3.1 $\pm$ 1.7 ^{6-M}	4.2 $\pm$ 2.2 ^{6-M}	5.6 $\pm$ 3.2 ^{7-M}	7.8 $\pm$ 3.7 ^{7-M}	12.2 $\pm$ 4.9	17.4 $\pm$ 4.8	0.874	<0.001
$\dot{E}$ (kJ·kg ⁻¹ ·min ⁻¹)	0.96 $\pm$ 0.13 ^{2-M}	1.07 $\pm$ 0.13 ^{3-M}	1.15 $\pm$ 0.13 ^{4-M}	1.24 $\pm$ 0.16 ^{5-M}	1.37 $\pm$ 0.18 ^{6-M}	1.48 $\pm$ 0.20 ^{7-M}	1.67 $\pm$ 0.23 ^M	2.40 $\pm$ 0.32	0.956	<0.001
C (kJ·m ⁻¹)	0.29 $\pm$ 0.05 ^{7-M}	0.30 $\pm$ 0.04 ^{7-M}	0.30 $\pm$ 0.04 ^{7-M}	0.31 $\pm$ 0.04 ^{7-M}	0.32 $\pm$ 0.03 ^{7-M}	0.32 $\pm$ 0.04 ^M	0.35 $\pm$ 0.04 ^M	0.46 $\pm$ 0.06	0.880	<0.001
Swimming										
v (m·s ⁻¹)	1.02 $\pm$ 0.10 ^{2-M}	1.07 $\pm$ 0.09 ^{3-M}	1.12 $\pm$ 0.09 ^{4-M}	1.17 $\pm$ 0.09 ^{5-M}	1.22 $\pm$ 0.09 ^{6-M}	1.26 $\pm$ 0.09 ^{7-M}	1.31 $\pm$ 0.08 ^M	1.45 $\pm$ 0.08	0.971	<0.001
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	38.5 $\pm$ 5.6 ^{3-M}	42.1 $\pm$ 4.9 ^{3-M}	46.1 $\pm$ 5.8 ^{5-M}	49.7 $\pm$ 7.4 ^{6-M}	53.6 $\pm$ 7.4 ^{7-M}	57.3 $\pm$ 6.8 ⁷	60.7 $\pm$ 6.6	59.2 $\pm$ 5.2	0.913	<0.001
$\dot{V}E$ (L·min ⁻¹)	55.0 $\pm$ 13.1 ^{2-M}	63.7 $\pm$ 12.6 ^{3-M}	70.6 $\pm$ 14.6 ^{5-M}	76.4 $\pm$ 16.4 ^{5-M}	86.7 $\pm$ 17.3 ^{7-M}	94.4 $\pm$ 20.1 ^M	105. $\pm$ 18.3	109.7 $\pm$ 18.3	0.902	<0.001
f_R (breaths·min ⁻¹)	24 $\pm$ 5 ^{2-M}	28 $\pm$ 5 ^{4-M}	31 $\pm$ 6 ^{7-M}	33 $\pm$ 4 ^{7-M}	36 $\pm$ 5 ^{7-M}	38 $\pm$ 2 ^M	43 $\pm$ 6	48 $\pm$ 5	0.839	<0.001
V_T (L)	2.36 $\pm$ 0.55	2.38 $\pm$ 0.57	2.38 $\pm$ 0.57	2.44 $\pm$ 0.62	2.48 $\pm$ 0.62	2.52 $\pm$ 0.63	2.50 $\pm$ 0.60	2.37 $\pm$ 0.55	0.143	0.251
HR (bpm)	141 $\pm$ 19 ^{2-M}	149 $\pm$ 17 ^{3, 5-M}	159 $\pm$ 16 ^{6-M}	163 $\pm$ 15 ^{7-M}	170 $\pm$ 14 ^M	175 $\pm$ 11 ^M	179 $\pm$ 9	183 $\pm$ 11	0.801	<0.001
[La] (mmol·L ⁻¹)	2.7 $\pm$ 0.7 ^{4-M}	3.0 $\pm$ 0.9 ^{4-M}	3.4 $\pm$ 1.1 ^{4-M}	4.0 $\pm$ 1.4 ^{5-M}	4.9 $\pm$ 1.8 ^{7-M}	6.1 $\pm$ 2.4 ^{7-M}	8.8 $\pm$ 2.2 ^M	12.0 $\pm$ 3.0	0.882	<0.001
$\dot{E}$ (kJ·kg ⁻¹ ·min ⁻¹)	0.81 $\pm$ 0.14 ^{3-M}	0.90 $\pm$ 0.13 ^{3-M}	1.00 $\pm$ 0.15 ^{4-M}	1.09 $\pm$ 0.19 ^{5-M}	1.20 $\pm$ 0.20 ^{6-M}	1.31 $\pm$ 0.19 ^{7-M}	1.45 $\pm$ 0.19 ^M	1.93 $\pm$ 0.22	0.956	<0.001
C (kJ·m ⁻¹)	0.86 $\pm$ 0.17 ^{3-M}	0.90 $\pm$ 0.15 ^{3-M}	0.96 $\pm$ 0.18 ^{5-M}	1.00 $\pm$ 0.21 ^{6-M}	1.06 $\pm$ 0.24 ^{7-M}	1.12 $\pm$ 0.25 ^{7-M}	1.19 $\pm$ 0.24 ^M	1.44 $\pm$ 0.30	0.891	<0.001
Cycling										
P (W)	169 $\pm$ 60	194 $\pm$ 60	219 $\pm$ 60	244 $\pm$ 60	269 $\pm$ 60	294 $\pm$ 60	319 $\pm$ 60	392 $\pm$ 78	0.982	<0.001
v (m·s ⁻¹)	9.36 $\pm$ 1.54 ^{2-M}	9.95 $\pm$ 1.35 ^{3-M}	10.43 $\pm$ 1.20 ^{4-M}	10.89 $\pm$ 1.09 ^{5-M}	11.32 $\pm$ 1.01 ^{6-M}	11.71 $\pm$ 0.93 ^{7-M}	12.07 $\pm$ 0.87 ^M	12.94 $\pm$ 1.04	0.949	<0.001
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	36.5 $\pm$ 9.1 ^{2-M}	41.2 $\pm$ 9.2 ^{3-M}	44.8 $\pm$ 8.6 ^{4-M}	48.4 $\pm$ 8.8 ^{5-M}	53.2 $\pm$ 9.5 ^{6-M}	58.3 $\pm$ 9.8	61.6 $\pm$ 8.5	60.0 $\pm$ 8.1	0.912	<0.001
$\dot{V}E$ (L·min ⁻¹)	58.8 $\pm$ 16.4 ^{2-M}	67.7 $\pm$ 15.8 ^{3-M}	77.8 $\pm$ 18.0 ^{4-M}	91.6 $\pm$ 19.2 ^{5-M}	109.1 $\pm$ 22.1 ^{6-M}	131.1 $\pm$ 24.1 ^M	157.8 $\pm$ 18.5	159.9 $\pm$ 20.3	0.949	<0.001

f_R (breaths·min ⁻¹)	26 ± 5 ^{4-M}	29 ± 5 ^{4-M}	33 ± 6 ^{5-M}	36 ± 7 ^{7-M}	40 ± 8 ^{6-M}	45 ± 10 ^M	56 ± 8 ^M	63 ± 9	0.879	<0.001
V_T (L)	2.31 ± 0.47 ⁵⁻⁷	2.42 ± 0.48 ⁶	2.42 ± 0.41	2.59 ± 0.39	2.79 ± 0.39 ⁶	2.97 ± 0.41 ^M	2.87 ± 0.38 ^M	2.57 ± 0.36	0.565	<0.001
HR (bpm)	124 ± 10 ^{2-M}	137 ± 14 ^{3-M}	149 ± 14 ^{4-M}	159 ± 15 ^{5-M}	168 ± 13 ^{6-M}	175 ± 13	181 ± 12	178 ± 13	0.961	<0.001
[La ⁻] (mmol·L ⁻¹)	1.8 ± 0.5 ^{4-M}	2.0 ± 0.5 ^{5-M}	2.4 ± 0.7 ^{4-M}	3.5 ± 1.4 ^{5-M}	5.5 ± 2.2 ^{6-M}	9.1 ± 3.3 ^{7-M}	12.6 ± 4.3	17.1 ± 3.0	0.911	<0.001
$\dot{E}$ (kJ·kg ⁻¹ ·min ⁻¹)	0.79 ± 0.18 ^{2-M}	0.89 ± 0.19 ^{3-M}	0.97 ± 0.18 ^{4-M}	1.07 ± 0.19 ^{5-M}	1.21 ± 0.21 ^{6-M}	1.40 ± 0.24 ^{7-M}	1.54 ± 0.23 ^M	2.49 ± 0.22	0.963	<0.001
C (kJ·m ⁻¹)	0.10 ± 0.02 ^{5-M}	0.11 ± 0.01 ^{5-M}	0.11 ± 0.01 ^{4-M}	0.12 ± 0.01 ^{5-M}	0.13 ± 0.01 ^{6-M}	0.14 ± 0.02 ^M	0.15 ± 0.02 ^M	0.23 ± 0.04	0.911	<0.001

Velocity (v), power (P), oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), respiratory frequency (f_R), tidal volume (V_T), heart rate (HR), blood lactate concentrations ([La⁻]), total energy expenditure ($\dot{E}$), energy cost (C), partial eta-squared (η_p^2). Superscript numbers represent differences from noted steps ($p < 0.05$).

In cycling, the intensity rise led to consecutive increments in $\dot{V}O_2$ and $\dot{V}E$ until step 6 ($p = [0.001 - 0.011]$ and $[< 0.001-0.015]$), in f_R and V_T only from 5-6 and 7 to maximal ($p < 0.001$ and $= 0.042$, and $p = 0.038$ and 0.015), in HR until step 6 ($p = [< 0.001-0.008]$), in $\dot{E}$ ($p = [< 0.001-0.004]$) and in C from 3-6 and 7 to maximal ($p = [< 0.001-0.031]$). C_{AnT} and $C\dot{V}O_{2max}$ were 0.31 ± 0.04 and 0.35 ± 0.03 for running, 1.02 ± 0.21 and 1.19 ± 0.24 for swimming, and 0.11 ± 0.01 and $0.15 \pm 0.01 \text{ kJ}\cdot\text{m}^{-1}$ for cycling (respectively). Running and swimming presented higher $C\dot{V}O_{2max}$ ($p = 0.008$ and < 0.001), while cycling showed similar values ($p = 0.204$). Also, running revealed higher C_{AnT} and $C\dot{V}O_{2max}$ than cycling and swimming C_{AnT} and $C\dot{V}O_{2max}$ were higher than running and cycling (all for $p < 0.001$).

Distinct profiles were observed throughout the increasing velocities, as a [steps*exercise mode] interaction effect was verified for $\dot{V}E$ ($p < 0.001$, $\eta_p^2 = 0.562$), f_R ($p = 0.002$, $\eta_p^2 = 0.212$), V_T ($p = 0.018$, $\eta_p^2 = 0.171$) and C ($p < 0.001$, $\eta_p^2 = 0.733$). Differences in $\dot{V}E$ values were observed between exercise modes ($p < 0.001$, $\eta_p^2 = 0.589$), with running eliciting higher $\dot{V}E$ compared to both swimming ($p < 0.001$) and cycling ($p = 0.035$). In addition, $\dot{V}E$ was higher during cycling than swimming ($p = 0.005$). The observed differences in f_R between exercise modes ($p < 0.001$, $\eta_p^2 = 0.654$) reflected higher f_R also in running compared to both swimming and cycling ($p < 0.001$). Regarding the differences in C values between exercise modes ($p < 0.001$, $\eta_p^2 = 0.920$), running presented higher C compared to cycling ($p = 0.005$) and swimming had higher C than both running and cycling ($p < 0.001$). Differently, V_T values were similar between exercise modes ($p = 0.557$, $\eta_p^2 = 0.042$). Large to nearly perfect positive relationships were found between C and $\dot{V}E$ ($r = [0.785-0.999]$, $p = [< 0.001-0.021]$) and C and f_R ($r = [0.920-0.996]$, $p = [< 0.001-0.009]$) in the three exercise modes, both when considering the velocities until or above $\dot{V}O_{2max}$ (Figure 1).

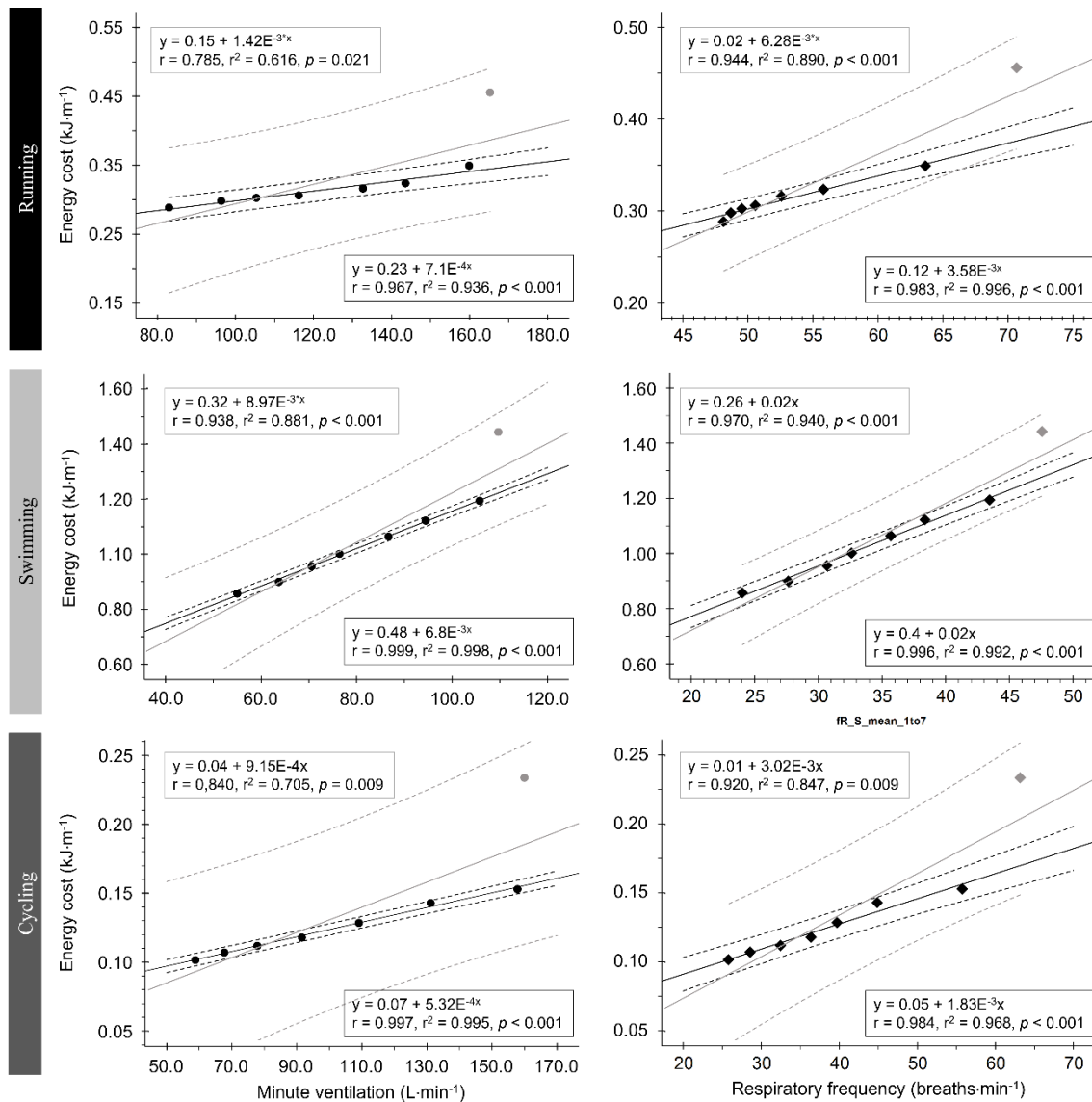


Figure 1. Relationships between energy cost (C) and minute ventilation ($\dot{V}E$) (left panels) and respiratory frequency (f_R) (right panels) in running, swimming and cycling (upper, middle and lower panels) including the velocities until and above $\dot{V}O_2\max$ (black and grey). The regression equations, coefficients of determination (r^2) and significance level (p) values are also identified.

The absolute Aer, AnL and AnAI energy expenditures and relative energy contributions of the three assessed exercise modes are presented in Figure 2. In running, the changes in absolute Aer ($p < 0.001$, $\eta_p^2 = 0.945$) evidenced a decrease only at maximal step ($p < 0.001$), with similar values between first, AnT and $\dot{V}O_2\max$ corresponding steps ($p = [0.714-1.000]$). Absolute AnL changes ($p < 0.001$, $\eta_p^2 = 0.875$) reflected an increase from steps 6-7 ($p = 0.008$), with an

increase between the first and the AnT corresponding steps and from this latter to $\dot{V}O_{2\max}$ ($p = 0.017$ and < 0.001). Absolute AnAI variation ($p < 0.001$, $\eta_p^2 = 0.928$) demonstrated a decrease from step 4 until maximal ($p = [< 0.001-0.022]$) with also a decrease between the first, AnT and $\dot{V}O_{2\max}$ corresponding steps ($p = 0.016$ and < 0.001). Similarly, relative Aer contribution changes ($p < 0.001$, $\eta_p^2 = 0.965$) reflected a decrease from steps 6-7 and 7 to maximal ($p = 0.003$ and < 0.001), while the relative AnL modifications ($p < 0.001$, $\eta_p^2 = 0.943$) were an increase from step 6 ($p = [< 0.001-0.039]$) and in relative AnAI ($p < 0.001$, $\eta_p^2 = 0.765$) reflected a decrease from steps 6-7 and an increase from 7 to maximal ($p = 0.032$ and < 0.001).

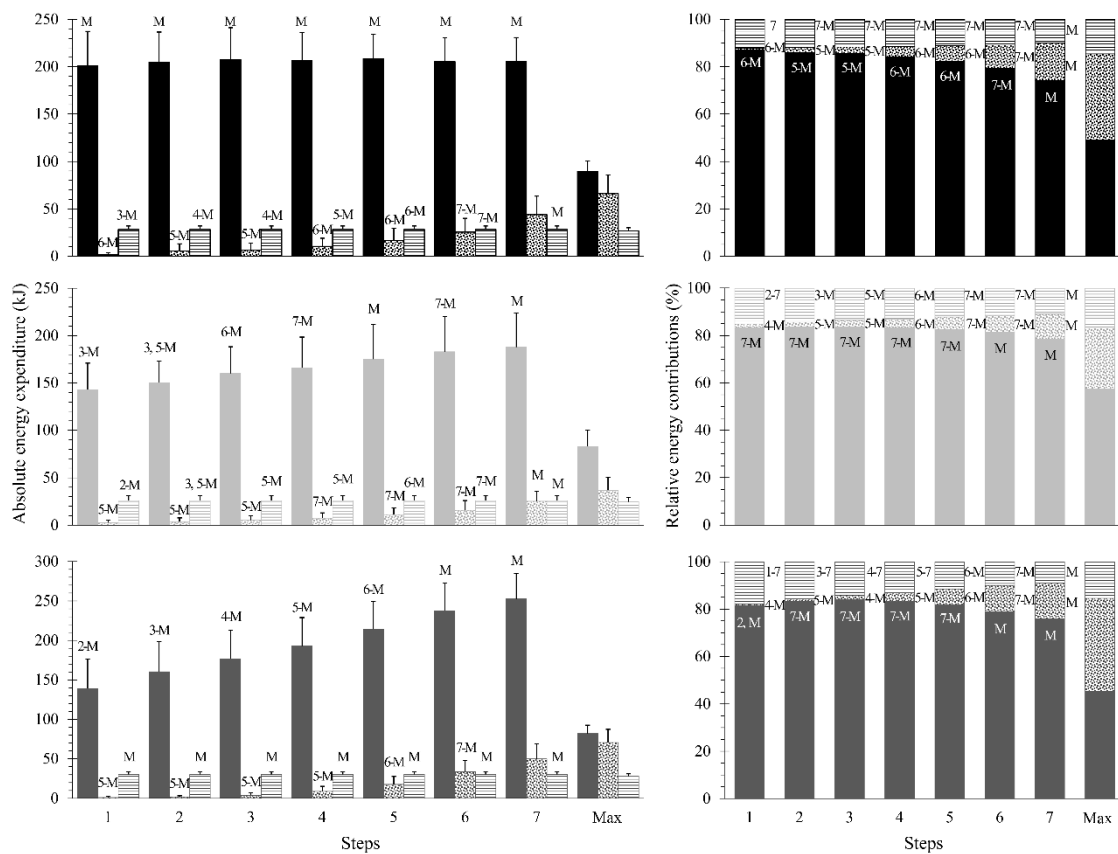


Figure 2. Absolute and relative (left and right panels) aerobic, anaerobic lactic and anaerobic alactic energy contributions (filled, dotted and stripped bars) along the steps of the incremental protocol in running, swimming and cycling (black, light and dark grey graphs). Superscript numbers represent differences from noted steps ($p < 0.05$).

In swimming, absolute Aer changes ($p < 0.001$, $\eta_p^2 = 0.916$) reflected an increase from steps 2-3 and 6-7 ($p = 0.017$ and 0.043) and a decrease from steps 7 to

maximal ($p < 0.001$), in absolute AnL ($p < 0.001$, $\eta_p^2 = 0.846$) was an increase from steps 6-7 and 7 to maximal ($p = 0.008$ and 0.040) and in absolute AnAI ($p < 0.001$, $\eta_p^2 = 0.931$) was a decrease along the incremental protocol steps ($p = [< 0.001-0.018]$, except from steps 3-4 ($p = 0.162$). An increase between the first, AnT and $\dot{V}O_{2\max}$ corresponding steps was observed for Aer ($p = 0.002$ and < 0.001), while a decrease between these steps for AnL ($p = 0.007$ and < 0.001) and AnAI ($p = 0.003$ and < 0.001) was verified. Regarding the relative contributions, changes in Aer ($p < 0.001$, $\eta_p^2 = 0.949$) reflected also a decrease from steps 7 to maximal ($p < 0.001$) while in AnL ($p < 0.001$, $\eta_p^2 = 0.929$) evidenced an increase from steps 6-7 and 7 to maximal ($p = 0.031$ and < 0.001) and in AnAI ($p < 0.001$, $\eta_p^2 = 0.887$) reflected a decrease from steps 2-3, 6-7 and 7 to maximal ($p = [< 0.001-0.003]$).

In cycling, absolute Aer changes ($p < 0.001$, $\eta_p^2 = 0.940$) reflected an increase until step 6 ($p = [<0.001-0.020]$) and a decrease from steps 7 to maximal ($p < 0.001$) and in absolute AnL ($p < 0.001$, $\eta_p^2 = 0.904$) reflected an increase from steps 4-5, 5-6 and 6-7 ($p = [0.002-0.007]$), both presenting an increase between the first, AnT and $\dot{V}O_{2\max}$ corresponding steps ($p = [< 0.001-0.002]$). Given the dependence of AnAI on exercise duration, only a decrease from steps 7 to maximal was observed ($p < 0.001$). Relative Aer contribution changes ($p < 0.001$, $\eta_p^2 = 0.969$) were evidenced by an increase from steps 1-2 and a decrease from 7 to maximal ($p = 0.43$ and <0.001), in relative AnL ($p < 0.001$, $\eta_p^2 = 0.961$) reflected an increase from the steps 4 until maximal ($p = [< 0.001-0.039]$) and in relative AnAI ($p < 0.001$, $\eta_p^2 = 0.842$) there was a constant decrease with the power output increase ($p = [< 0.001-0.015]$).

The individual relationships between $\dot{E}$ and running, swimming and cycling workloads along the incremental protocol are described in Figure 3. The maximal step was performed without imposing a pace or cadence, above the velocity or power that elicits $\dot{V}O_{2\max}$. Regarding C_{slope} , it was verified that comparing $\dot{E}_{\text{tot}}$ vs. Aer contribution using the velocities until the AnT led to higher values for running, swimming and cycling (5.71 ± 1.61 vs. 4.36 ± 1.06 , 30.65 ± 9.79 vs.

25.33 ± 8.86 and 3.32 ± 1.54 vs. 3.08 ± 1.58 , respectively, all for $p < 0.001$). Also, higher C_{slope} was observed when higher velocities were considered, particularly until and above $\dot{V}O_{2max}$ (7.06 ± 1.75 and 10.18 ± 2.20 ; $p < 0.001$, $\eta_p^2 = 0.867$ for running, 36.21 ± 7.74 and 42.76 ± 6.91 ; $p < 0.001$, $\eta_p^2 = 0.875$ for swimming, and 4.66 ± 1.22 and 7.21 ± 1.48 ; $p < 0.001$, $\eta_p^2 = 0.864$ for cycling). The adjustment of linear and exponential regression models between $\dot{E}$ and the different running, swimming and cycling velocities are described in Figure 4. Regardless of the velocities and type of regression used, all the fittings presented $r^2 > 0.80$. Both in running and swimming, this relationship is better described with a linear regression when considering velocities until AnT and $\dot{V}O_{2max}$, and with an exponential regression when considering velocities above $\dot{V}O_{2max}$. In cycling, also a linear regression had the best fit for velocities until AnT , but the exponential regression better described the $\dot{E}_{tot}/velocity$ relationship when considering velocities until and above $\dot{V}O_{2max}$.

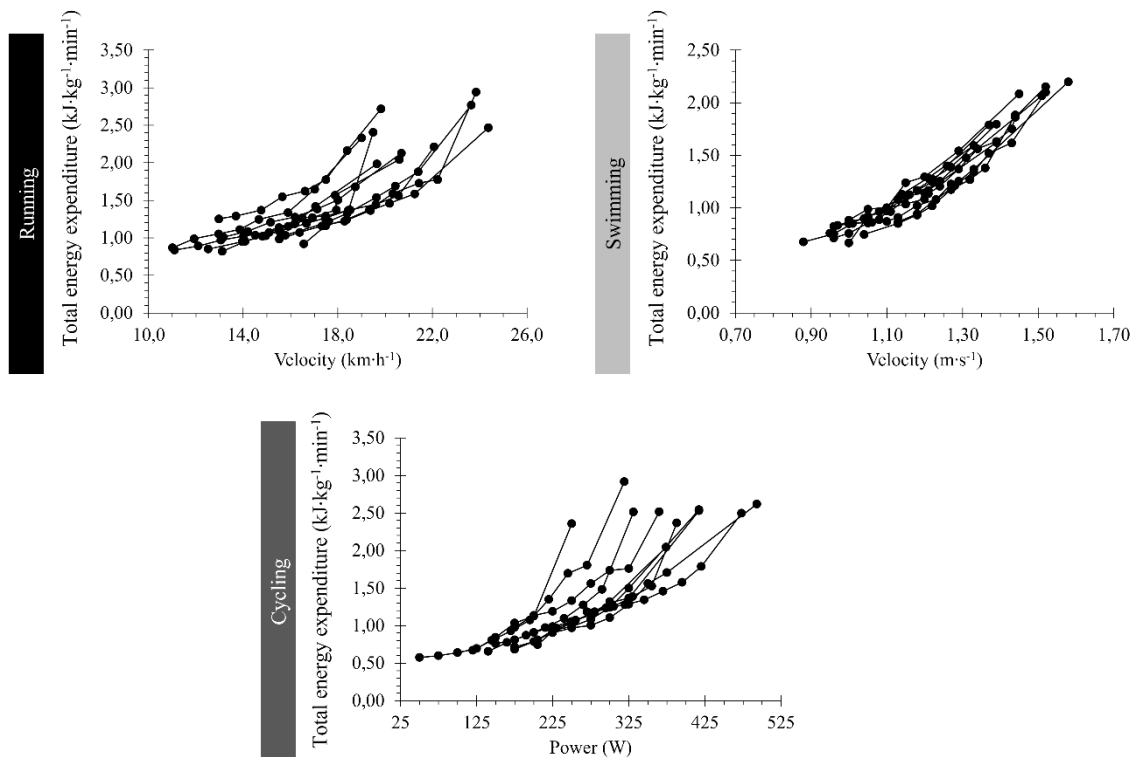


Figure 3. Individual relationships between total energy expenditure and running, swimming and cycling velocities and power output along the steps of the incremental protocol.

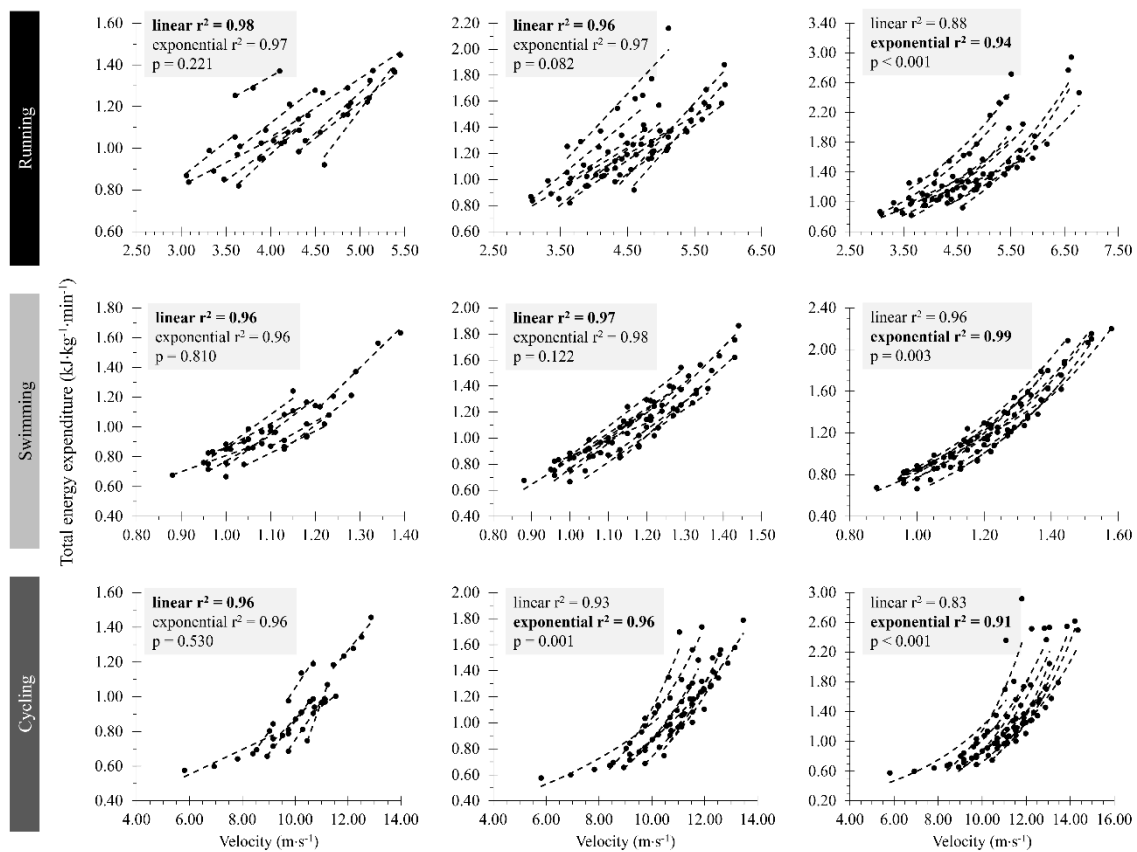


Figure 4. Adjustment of linear and exponential relationships between total energy expenditure and velocity in running, swimming and cycling considering the steps until the anaerobic threshold (left panels), until the maximal oxygen uptake (central panels) and all the velocities obtained in the incremental protocol (right panels). The best fit is presented in the graphs (indicated by bold text).

Discussion

The main purposes of the current study were to characterize running, swimming and cycling bioenergetic responses from low to extreme velocities and to assess the effect of different intensities and energy pathways effect on exercise economy estimation. The velocity at the AnT was obtained in steps 3-6 in running, 3-5 in swimming and 2-5 in cycling, while the $\dot{V}O_{2\max}$ was achieved in steps 6 and 7 in running and cycling, and always in step 7 in swimming. The main findings evidenced that, in general, $\dot{V}O_2$, $\dot{V}E$, f_R and HR increased with the velocity rise, whereas modifications in V_T were less evident (particularly in swimming). Concurrently with $[La^-]$, a progressive increase in $\dot{E}$ was observed, while C increases were verified in the last steps in running and swimming, and from step

4 in cycling. Different behaviours were found for absolute Aer, AnL and AnAI depending on the exercise mode, while relative energy contributions had similar behaviours in running and swimming (except for the anaerobic components in cycling that started to change their contribution in earlier steps). It was also evidenced that velocities above AnT and the anaerobic metabolism are crucial for a complete characterization of the exercise economy profile, in the three exercise modes.

Conducting an incremental protocol until exhaustion allows a complete physiological and biomechanical evaluation, involving velocities/power outputs performed in training context and competitive events and, consequently, to improve training control and prescription. This protocol is implemented to assess $\dot{V}O_2\text{max}$ and its corresponding velocity for further prescription of this specific intensity (Bentley et al., 2007; Cardoso et al., 2024; Fernandes et al., 2008). Since the current study aimed to bioenergetically characterize a larger spectrum of exertions, a velocity above $\dot{V}O_2\text{max}$ was included aiming for a more extended and completed characterization (Fernandes et al., 2024; Monteiro et al., 2023).

According to each exercise mode, the incremental protocol was adapted regarding steps length/duration and velocity/power increments (Bentley et al., 2007; Sousa et al., 2015). In cycling, despite the laboratory assessment, practitioners used their own bikes instead of a stationary cycle ergometer, with an efficient and accurate system without physical transmissions and no loss of power (<https://www.garmin.com/en-US/garmin-technology/tacx/neo-principles/>) (accessed 18 September 2024). Consequently, and contrary to running and swimming, the protocol steps in cycling were based on time, with similar mean duration of the remaining exercise modes steps (Billat, 2000; Fernandes et al., 2011). To avoid larger rises of $\dot{E}$ per power increment (Sousa et al., 2015) and seeking for similar and gradual increments between exercise modes, the 25 W power increments were selected for the cycling test (Bell et al., 2017; Lucía et al., 2001).

The performance and metabolic response along the incremental protocol observed in the current study is in accordance with data previously reported for running (Cardoso et al., 2024; Monteiro et al., 2024), swimming (Barbosa et al.,

2006; Monteiro et al., 2023) and cycling (Lucía et al., 1999; Power et al., 2012). Due to the velocity rise, the metabolic demand will imply modifications in ventilatory responses, particularly the increase in $\dot{V}O_2$, HR and $[La^-]$ and, consequently the increase in $\dot{E}$ (Sousa et al., 2015). The constant V_T values between consecutive steps, and their similarity in swimming, indicates that the increases in $\dot{V}E$ are mainly explained by the increases in f_R (Tipton et al., 2017).

Notwithstanding the similar behaviours along the velocities rise, differences between exercise modes were observed, particularly higher $\dot{V}E$ and f_R in running compared to swimming and cycling and lower $\dot{V}E$ in swimming. Despite it has been verified that, when using a respiratory snorkel, swimmers took advantage of it by breathing more often, losing the coordination between respiration and upper limbs movements (Monteiro et al., 2023), f_R values were lower compared to running at the different exertions. In fact, the horizontal body position (that is not verified in the remaining exercise modes) and the hydrostatic pressures while swimming increases flow resistance (Leahy et al., 2019), resulting in smaller airways and a decrease in lung compliance. Given the close interaction between f_R and $\dot{V}E$ (Tipton et al., 2017), the lower f_R values in swimming reflected the also lower $\dot{V}E$.

The correlations between C and $\dot{V}E$ as well as f_R provide valuable insights into the influence of respiratory muscles activity on exercise economy. The observed nearly perfect positive relationships across velocities until $\dot{V}O_{2max}$ suggest that increased ventilatory demand is closely linked to higher energy expenditure. This relationship likely reflects the metabolic cost associated with the activation of respiratory muscles (Tipton et al., 2017), which become progressively more significant as exercise intensity approaches $\dot{V}O_{2max}$, without contributing directly to forward locomotion. Notably, when velocities above $\dot{V}O_{2max}$ are included in the analysis, the regression line demonstrates a steeper slope and broader 95% confidence intervals, suggesting a shift in the underlying metabolic demands. At intensities exceeding $\dot{V}O_{2max}$, the energy expenditure relies more heavily on glycolytic pathways (with a reduced contribution from oxidative metabolism, as evidenced in Figure 2) (Bar-Even et al., 2012), which are characterized as less

efficient due to the lower production of energy per glucose molecule and the consequent accumulation of $[La^-]$, justifying the C exponential increase.

Although $\dot{E}$ demonstrates progressive changes along the velocities, the same trend is not observed for C . This discrepancy arises from the distinct nature of these measures: $\dot{E}$ represents the energy expended per unit of time, whereas C reflects the energy used per unit of distance. The specific mechanical demands of the three exercise modes appear to account for the differing C patterns observed. In running, C remains relatively constant, indicating its independence from velocity (Capelli et al., 1998; di Prampero et al., 1993). In contrast, C during cycling changes at lower relative velocities, highlighting its velocity dependence (Capelli et al., 1998). Similarly, in swimming, C increases more consistently with the velocity rise. These patterns can likely be attributed to the unique mechanical and physiological requirements of each mode. In cycling, the need for synergistic muscle actions, particularly involving the trunk and upper limbs, facilitated greater force production as velocity increases. For swimming, the rise in C may be explained by compensatory movements for stabilization, driven by the constant displacement of the water caused by segmental body motions.

The inclusion of higher velocities when characterizing the individual exercise economy profile highlights the limitations of traditional assessment conducted exclusively at submaximal velocities. Such assessments fail to fully represent the athletes' profile and are not accurate for extrapolating to higher velocities, as demonstrated by the increasing values of C_{slope} when higher velocities are included. Expanding the velocity range allows for the identification of weaker velocities or training areas and provides a deeper understanding of the effects of specific training interventions (such as technical, strength or power-focused programs). Moreover, given the sensitivity of C_{slope} to the inclusion of additional velocities, incorporating shorter and more intense exertions above $\dot{V}O_{2max}$ (lasting less than 1 min) may provide further insights into the impact of these high-intensity exertions on exercise economy profile. This broader approach offers enhanced applicability across athletes specializing in short-, middle- or longer distances disciplines.

We acknowledge some limitations in the current study that should be addressed. First, the use of different subjects for the running, swimming and cycling tests may introduce some variability, potentially limiting the accuracy of direct comparisons across the assessed variables. Second, the estimation $\dot{V}O_2$ contribution, based solely on exercise duration without accounting for intensity, may have reduced its relevance, particularly in cycling, where intensity-dependent factors are more pronounced. Addressing these limitations in future research could improve the robustness of findings and enhance the generalizability of the results.

Conclusion

This study provides a comprehensive bioenergetic characterization of running, swimming and cycling across a broad spectrum of velocities, including those exceeding $\dot{V}O_{2max}$, revealing distinct mechanical and physiological responses unique to each exercise mode. While $\dot{E}$ demonstrated progressive increases with velocity, $\dot{V}O_2$ exhibited specific behaviours influenced by mechanical and physiological demands. Running showed a consistent $\dot{V}O_2$ across velocities, whereas cycling and swimming revealed velocity-dependent increases, driven by synergistic muscle actions and compensatory stabilization movements, respectively. The inclusion of higher velocities allowed for a more complete assessment of exercise economy, highlighting the metabolic costs of ventilatory demands and anaerobic contributions at high intensities. These findings emphasize the limitations of traditional submaximal assessments and underline the importance of including a wider range of velocities to a better training prescription and improve performance strategies across different specialties. Future research addressing inter-subject variability and refining anaerobic contribution estimation will further enhance our understanding of exercise economy and its application to sports performance.

Kinematical and physiological responses of overground running gait pattern at different intensities

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Abstract

Runners achieve forward locomotion through diverse techniques. However, understanding the behavior of the involved kinematic variables remains incomplete, particularly when running overground and along an intensity spectrum. We aimed to characterize the biomechanical and physiological adaptations while running at low, moderate, heavy and severe intensities. Ten middle- and long-distance runners completed an incremental intermittent protocol of 800 m steps until exhaustion ($1 \text{ km} \cdot \text{h}^{-1}$ velocity increments and 30 s intervals) on an outdoor track field. Biomechanical data were captured using two high-resolution video cameras, and linear and angular kinematic variables were analyzed. With the intensity rise, a decrease in stride, step and contact times ($[0.70\text{--}0.65]$, $[0.35\text{--}0.33]$ and $[0.42\text{--}0.37]$ s) and an increase in stride length and frequency and flight time ($[3.13\text{--}3.52]$ m, $[1.43\text{--}1.52]$ Hz and $[0.28\text{--}0.29]$ s; $p < 0.05$) were observed, together with an increase in oxygen uptake and blood lactate concentrations ($[54.7\text{--}67.6]$ $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and $[3.1\text{--}10.2]$ $\text{mmol} \cdot \text{L}^{-1}$). A more flexed hip at initial contact and toe-off ($152.02\text{--}149.36$ and $165.70\text{--}163.64$) and knee at initial contact ($[162.64\text{--}159.57]$; $p < 0.05$) were also observed. A consistent gait pattern along each protocol step was exhibited, with minor changes without practical significance. Runners are constantly adapting their gait pattern, reflected in both biomechanical and physiological responses, both of which should be considered for better characterization.

Keywords: gait analysis, linear kinematics, angular kinematics, intensity domains, overground running

Introduction

Running is a popular physical activity characterized by continuous, cyclic and fairly unconstrained movements. A specific combination of stride length and frequency is adopted by runners (mainly subconsciously) for each velocity, resulting in a large interindividual variation in stride patterns and lower-limb kinematics (Cavanagh and Kram, 1989; Folland et al., 2017). The changes in running patterns with the velocity rise seek to optimize movement at slower paces and maximize power output at sprinting velocities. These kinematical modifications of the segmental movements seek to improve several aspects, including metabolic energy expenditure, tissue stress, muscle power and fatigue (Williams, 2000).

The quantitative analysis of running kinematics is a powerful tool that has been evolving over the last decades, aiming to better understand the influence of technique in performance, identify injury risk factors and/or facilitate recovery through more accurate and automated systems (Coyler et al., 218). Despite the current feasibility of motion analysis using automatic, non-invasive and markerless methodologies (Needham et al., 2022), their utilization remains predominantly confined to laboratory conditions under controlled environments. Conversely, the indirect techniques of gait analysis based on video recording and planar analysis with manual digitation have also progressed, allowing athlete monitoring in ecological conditions, such as overground running on a track field instead of running on a treadmill (Cardoso et al., 2022; Coyler et al., 218; Madden et al., 2016).

Notwithstanding the controversial and inconsistent biomechanical and physiological responses when comparing overground vs. treadmill running (Miller et al., 2019; Van Hooren et al., 2020), the high association and applicability between field data and training and competing contexts are undeniable. In fact, during overground running, the body moves over the supporting lower limbs while when exercising on a treadmill, the running belt moves the supporting lower limbs at a constant velocity, leading to changes in propulsive forces between conditions (Riley et al., 2008). Also, differences in surface stiffness, air resistance, comfort and altered velocity perception should be taken into account (Van Hooren et al., 2020).

It is well known that running involves a complex interaction between both mechanical and physiological mechanisms, since there is a constant processing of different types of information coming from external and internal sources that are related to the movements involved and the corresponding consequences (Williams, 2000). Changes in running velocity will alter the adopted biomechanical pattern that, consequently, will modify the pulmonary and ventilatory response (Cavanagh and Williams, 1982; Williams, 2000). The current study aimed to comprehensively characterize the biomechanical and physiological adaptations while running overground at low, moderate, heavy and severe exercise intensities. It was hypothesized that there would be a decrease in temporal and an increase in frequency- and distance-related variables, concurrently with a rise in the physiological variables, along the intensity spectrum. In addition, we sought to assess the consistency of the running gait pattern within each intensity domain, hypothesizing that the linear and angular kinematic responses would be maintained from the beginning to the end of each exertion.

Materials and Methods

Participants

Ten middle- and long-distance male runners volunteered to participate (age: 26.8 ± 5.7 years, body mass: 68.2 ± 8.2 kg and body height: 180.0 ± 6.5 cm). They were involved in running training practice for 9.9 ± 3.9 years and in 12.9 ± 2.2 h of weekly running training. Participants were recruited through direct contact and selected if there was no history of cardiorespiratory and physical diseases or injuries within the previous six months and if they had more than two years of running training background. The current research was approved by the local ethics committee and the participants were informed about the purpose, benefits and any associated risks (providing their written individual consent for participation in accordance with the Helsinki Declaration).

Experimental Protocol

After an individualized warm-up of ~20 min at low intensity, participants performed an incremental intermittent running protocol of 800 m steps until exhaustion (with $1 \text{ km} \cdot \text{h}^{-1}$ increments and 30 s rest intervals in-between) on a 400

m outdoor track field (Cardoso et al., 2022; Sousa et al., 2015). The velocities for each runner's last step were established based on their best individual 3000 m performance at the time of the data collection or according to their own experience in previous tests. Afterward, six velocity increments were subtracted to define the subsequent step paces, with velocities being controlled for each step by audio feedback emitted at every 100 m where fluorescent cones were placed (Cardoso et al., 2024; Sousa et al., 2015).

Before the evaluation moment, the acromion, iliac crest, greater trochanter, lateral femoral condyle, lateral malleolus and second metatarsal head of the runners' left body side were manually marked (Cardoso et al., 2024) using black skin landmarks. The lower-limb kinematical data were recorded from the runners' left sagittal plane using two high-resolution video cameras previously calibrated (1920×1080 pixels; GoPro HERO6 Black, CA, USA) at a sampling rate of 120 Hz, fixed on tripods and positioned 3 m from the middle of the 100–200 running track section and 4.5 m from the first lane.

During the protocol, pulmonary gas exchange and ventilation were continuously measured breath-by-breath by a telemetric portable gas analyzer (K5, Cosmed, Rome, Italy) previously calibrated according to manufacturer instructions (using ambient air against known concentrations [16% O₂ and 5% CO₂] and a 3 L syringe) and placed on runners' backs, near the body center of mass. Heart rate was also continuously recorded using a Polar Vantage NV (Polar Electro Oy, Kemple, Finland) that telemetrically emitted to the portable gas analyzer unit (Lopes et al., 2023). Capillary blood samples for lactate concentration analysis were collected from the fingertip at rest, during the 30 s intervals and at 1, 3, 5 and/or 7 min at the end of the protocol (until obtaining maximal values) using a portable analyzer (Lactate Pro2; Arkay Inc., Kyoto, Japan) (Sousa et al., 2018).

Data Analysis

For each running protocol step, a total of twelve strides were analyzed frame-by-frame using two-dimensional motion analysis software (Kinovea, version 0.8.27, Boston, MA, USA) to determine the stride time (time between the left foot touchdown and the next left foot touchdown), step time (time between the left foot touchdown and the right foot touchdown), stride length (mean velocity/stride frequency) and frequency (1/stride time) and contact and flight times (times from

initial contact to toe-off and from toe-off to the initial contact of the same foot, respectively) as linear kinematic variables (Folland et al., 2017). The flight and contact times normalized to the stride duration were also included (flight time/stride time and contact time/stride time). Based on the marked anatomic points described above, the hip, knee and ankle joint angles (both at initial contact and toe-off moments) were calculated as angular kinematic variables (Figure 1) (Cardoso et al., 2024). Body marks were carefully detected, and the mean of three measurements was used. A 0.98 intraclass correlation coefficient was verified.

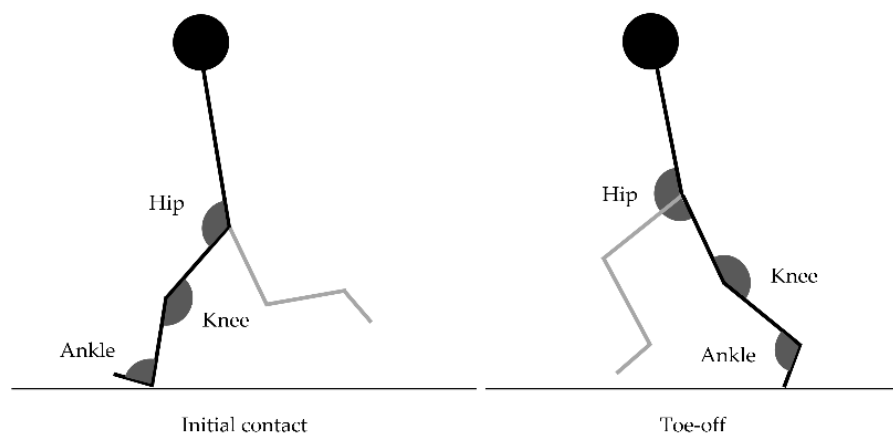


Figure 1. Hip, knee and ankle joint angle determination at initial contact and toe-off moments.

The lactate-velocity curve modeling method, through the determination of the interception point of the best fit of a combined linear and exponential pair of regressions, was used to determine the individual anaerobic threshold (Cardoso et al., 2022; Carvalho et al., 2020). The mean oxygen uptake values from the last 30 s of each protocol step were selected, and conventional physiological criteria were applied to establish the maximal oxygen uptake, particularly the occurrence of a plateau in oxygen uptake despite a velocity increase ($< 2.1 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and level of maximal blood lactate higher than $8 \text{ mmol} \cdot \text{L}^{-1}$ (Lopes et al., 2023). Using these two physiological indicators, the low, moderate, heavy (the steps below, at and above the anaerobic threshold, respectively) and severe (the step where maximal oxygen uptake was elicited) exercise intensity domains were identified.

Statistical Analysis

Statistical procedures were conducted using SPSS (version 29.0.0.0; IBM Corporation, Armonk, NY, USA) with a 5% significance level. The normal data distribution was checked for all variables with the Shapiro-Wilk test. The Wilcoxon signed-rank test was used to compare stride length and frequency, flight time, normalized flight time and respiratory frequency along the running intensity domains and between laps, with the respective median and interquartile range presented here. Their effect size estimation was conducted using eta-square based on the Friedman test results and interpreted as small (0.01), medium (0.06) and large (0.14). For the remaining kinematical and physiological variables, presented as mean $\pm$ standard deviation, a repeated-measures ANOVA was performed with Bonferroni post hoc analysis to identify the pairwise differences, and eta-square was calculated, with values of 0.04, 0.25 and above 0.64 considered as minimum, moderate and strong, respectively (Ferguson, 2009).

A repeated-measures Student's T-test with effect size estimation (Cohen's d : 0.20, 0.50 and 0.80 considered as small, medium and large, respectively [Cohen, 2013]) was used to compare the kinematical responses between the first and second laps of the protocol steps corresponding to each intensity domain. The coefficient of variation (CV) with the respective 95% confidence interval (CI) was also determined. A sample size of 10 participants was deemed sufficient, assuming a statistical power of 80%, an effect size of 0.85 and an α error probability of 5% (G*Power, version 3.1.9.7; Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany).

Results

The biomechanical and physiological responses when running at low, moderate, heavy and severe intensities are described in Table 1. With the increase in velocity, a decrease in stride time, step time, contact time and normalized contact time and an increase in stride frequency and normalized flight time were observed. Stride length increased from low to moderate ($p = 0.005$) and from low, moderate and heavy to severe ($p = 0.001$), whereas flight time was smaller at low ($p < 0.003$) and similar between moderate, heavy and severe domains. Hip and knee joint angles at initial contact were similar at low and moderate and

decreased at heavy ($p = 0.008$ and 0.002) and severe ($p = 0.023$ and 0.004), hip joint angle at toe-off only decreased at severe ($p = 0.004$), and knee joint angle at toe-off and ankle joint angle both at initial contact and toe-off remained unchanged along the running intensities. From low to severe, a progressive increase in oxygen uptake, minute ventilation, heart rate and blood lactate concentrations was also verified, while respiratory frequency only increased at heavy and severe intensity domains ($p < 0.05$).

Table 1. Kinematical and physiological responses at low, moderate, heavy and severe running intensity domains.

	Low	Moderate	Heavy	Severe	Eta-square	<i>p</i>
Velocity ($\text{m}\cdot\text{s}^{-1}$)	$4.44 \pm 0.55^{\text{m,h,s}}$	$4.74 \pm 0.53^{\text{h,s}}$	$5.05 \pm 0.5^{\text{s}}$	5.34 ± 0.54	0.925	<0.001
Stride time (s)	$0.70 \pm 0.03^{\text{m,h,s}}$	$0.68 \pm 0.03^{\text{h,s}}$	$0.67 \pm 0.03^{\text{s}}$	0.65 ± 0.03	0.905	<0.001
Step time (s)	$0.35 \pm 0.01^{\text{m,h,s}}$	$0.34 \pm 0.01^{\text{h,s}}$	$0.34 \pm 0.01^{\text{s}}$	0.33 ± 0.02	0.905	<0.001
Stride length (m)	3.13 (2.79–3.39) ^{m,s}	$3.23 \pm$ (2.95–3.52) ^s	$3.28 \pm$ (2.98–3.56) ^s	3.52 (3.22–3.71)	0.239	<0.001
Stride frequency (Hz)	1.43 (1.40–1.49) ^{m,h,s}	1.46 (1.41–1.52) ^{h,s}	1.51 (1.43–1.58)	1.52 (1.46–1.60)	0.248	<0.001
Flight time (s)	0.28 (0.27–0.28) ^{m,h,s}	0.29 (0.28–0.29)	0.29 (0.28–0.30)	0.29 (0.28–0.31)	0.168	0.017
Normalized flight time (%)	40.0 (36.8–42.3) ^{m,h,s}	41.4 (39.9–43.6) ^{h,s}	43.2 (40.6–45.3) ^s	44.6 (42.9–46.9)	0.246	<0.001
Contact time (s)	$0.42 \pm 0.03^{\text{m,h,s}}$	$0.40 \pm 0.03^{\text{h,s}}$	$0.39 \pm 0.03^{\text{s}}$	0.37 ± 0.02	0.911	<0.001
Normalized contact time (%)	$60.8 \pm 3.7^{\text{m,h,s}}$	$58.8 \pm 3.2^{\text{h,s}}$	57.4 ± 3.5	56.1 ± 2.7	0.784	<0.001
Hip joint angle at initial contact (°)	$152.02 \pm 2.85^{\text{h,s}}$	151.20 ± 2.31	150.45 ± 2.68	149.36 ± 2.94	0.530	<0.001
Hip joint angle at toe-off (°)	$165.70 \pm 5.62^{\text{s}}$	$165.16 \pm 5.84^{\text{s}}$	164.70 ± 6.49	163.64 ± 6.39	0.580	<0.001
Knee joint angle at initial contact (°)	$162.64 \pm 5.30^{\text{h,s}}$	$161.94 \pm 4.91^{\text{h,s}}$	$161.19 \pm 4.93^{\text{s}}$	159.57 ± 4.49	0.698	<0.001
Knee joint angle at toe-off (°)	168.38 ± 5.47	168.67 ± 5.64	168.60 ± 5.92	168.77 ± 5.55	0.053	0.683
Ankle joint angle at initial contact (°)	100.10 ± 6.20	100.05 ± 6.08	100.43 ± 5.62	101.37 ± 6.21	0.164	0.212
Ankle joint angle at toe-off (°)	129.95 ± 5.97	129.73 ± 5.72	129.65 ± 6.43	129.55 ± 6.84	0.009	0.916
Oxygen uptake ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	$54.7 \pm 5.2^{\text{m,h,s}}$	$58.7 \pm 5.4^{\text{h,s}}$	$62.5 \pm 6.5^{\text{s}}$	67.6 ± 9.4	0.787	<0.001
Minute ventilation ($\text{L}\cdot\text{min}^{-1}$)	$112.2 \pm 17.5^{\text{m,h,s}}$	$124.2 \pm 20.4^{\text{h,s}}$	$138.7 \pm 19.5^{\text{s}}$	157.7 ± 16.9	0.911	<0.001
Respiratory frequency ($\text{breaths}\cdot\text{min}^{-1}$)	40.0 (41.0–54.9) ^s	51.8 (41.5–57.9) ^{h,s}	56.1 (47.8–59.2) ^s	62.1 (56.1–68.6)	0.220	<0.001
Heart rate (bpm)	$163 \pm 13^{\text{m,h,s}}$	$170 \pm 13^{\text{h,s}}$	$176 \pm 12^{\text{s}}$	181 ± 13	0.906	<0.001
Blood lactate concentration ($\text{mmol}\cdot\text{L}^{-1}$)	$3.1 \pm 1.2^{\text{m,h,s}}$	$3.5 \pm 1.4^{\text{h,s}}$	$5.6 \pm 2.4^{\text{s}}$	10.2 ± 3.9	0.761	<0.001

^{m,h,s} Different from moderate, heavy and severe intensity domains, respectively ($p < 0.05$).

The variation between laps within each intensity domain of the assessed linear and angular kinematic variables are illustrated in Figures 2 and 3, respectively. From the first to the second laps, the mean velocity was maintained (4.45 ± 0.57 and 4.38 ± 0.51 , 4.75 ± 0.52 and 4.67 ± 0.48 , 5.05 ± 0.53 and 4.96 ± 0.50 and 5.29 ± 0.50 and $5.32 \pm 0.57 \text{ m}\cdot\text{s}^{-1}$, $d = 0.14\text{--}0.61$, for low, moderate, heavy and severe intensities, respectively; $p > 0.05$). The assessed kinematic variables were similar along each protocol step at the different intensities, except for flight time, normalized flight time and normalized contact time at low ($d = 0.84$, 0.74 and 0.74 ; CV [95% CI]: 1.70 [$0.96\text{--}2.45$], 1.82 [$1.02\text{--}2.61$] and 1.21 [$0.68\text{--}1.74$], respectively), hip joint angle at toe-off at moderate ($d = 0.95$; CV [95% CI]: 0.42 [$0.24\text{--}0.60$]) and stride time, step time, stride frequency, contact time and knee joint angle at toe-off at heavy intensity domains ($d = 0.98$, 0.97 , 0.97 , 0.97 and 0.82 ; CV [95% CI]: 1.18 [$0.66\text{--}1.69$], 1.17 [$0.66\text{--}1.68$], 1.17 [$0.66\text{--}1.68$], 2.04 [$1.15\text{--}2.94$] and 0.44 [$0.25\text{--}0.63$], respectively).

Discussion

The main purpose of this study was to analyze the effect of exercise intensity on overground running gait patterns, with a particular focus on linear and angular kinematical variables, through video recording and two-dimensional analysis. Concurrently, it was intended to ascertain if the self-selected patterns remained consistent within each exertion. As expected, temporal variables tended to decrease, and frequency and distance variables tended to increase along the intensity domain spectrum. In addition, an increase in the assessed physiological variables was observed, but all these changes were not always verified between consecutive intensities. It was also observed that hip joint angle at initial contact and toe-off and knee joint angle at initial contact had greater sensitivity to variations in running intensity. Differently, knee joint angle at toe-off and ankle joint angle at initial contact and toe-off demonstrated a consistent behavior with the intensity rise. In addition, the gait pattern was not always maintained, particularly at low, moderate and heavy intensities, since differences between laps were identified despite the maintenance of a constant velocity.

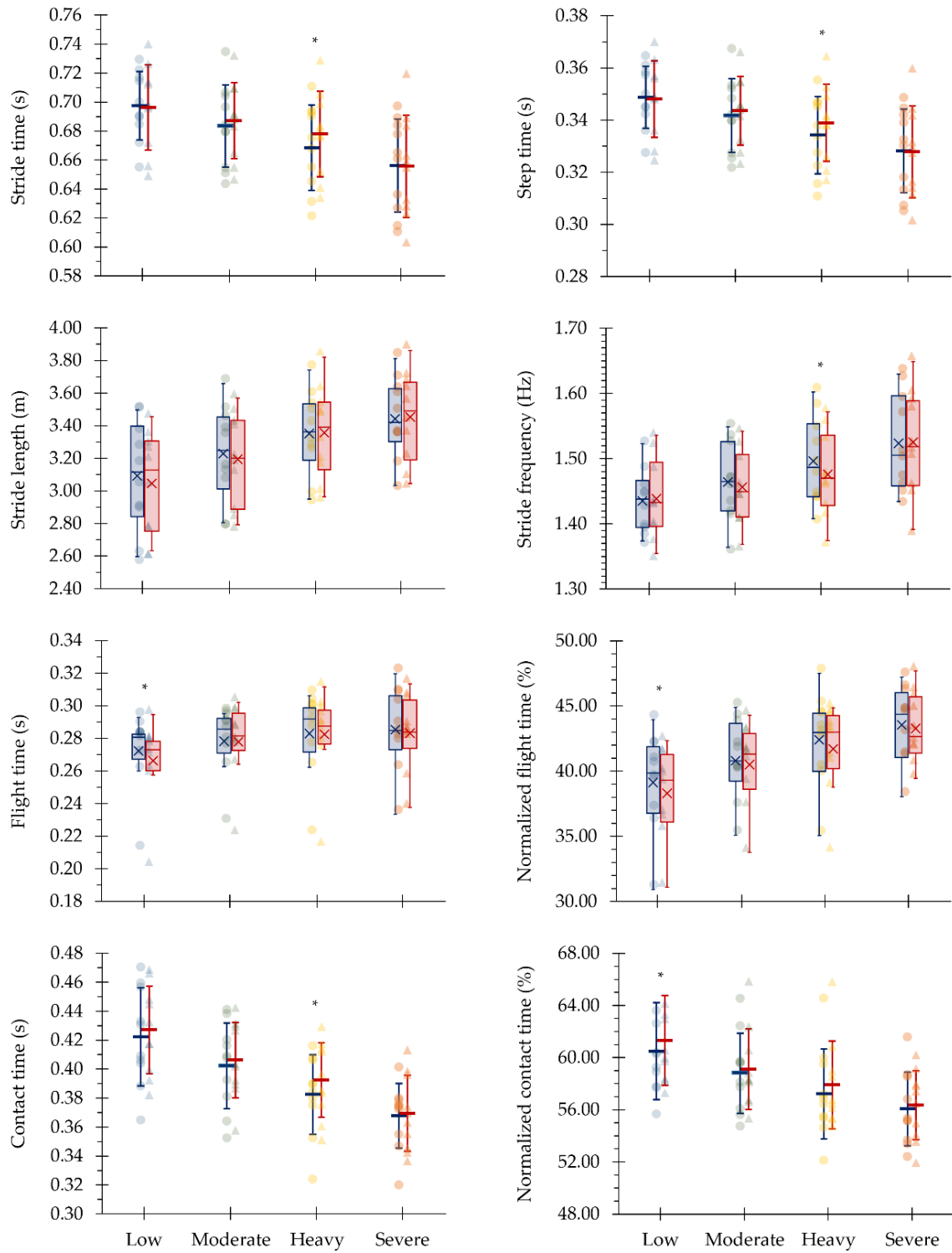


Figure 2. Individual (light circles and triangles), mean/median and standard deviation/interquartile range of the linear kinematical variables from the first (dark blue) to the second (dark red) laps of each protocol step corresponding to low, moderate, heavy and severe intensity domains. * Indicates differences between laps ($p < 0.05$).

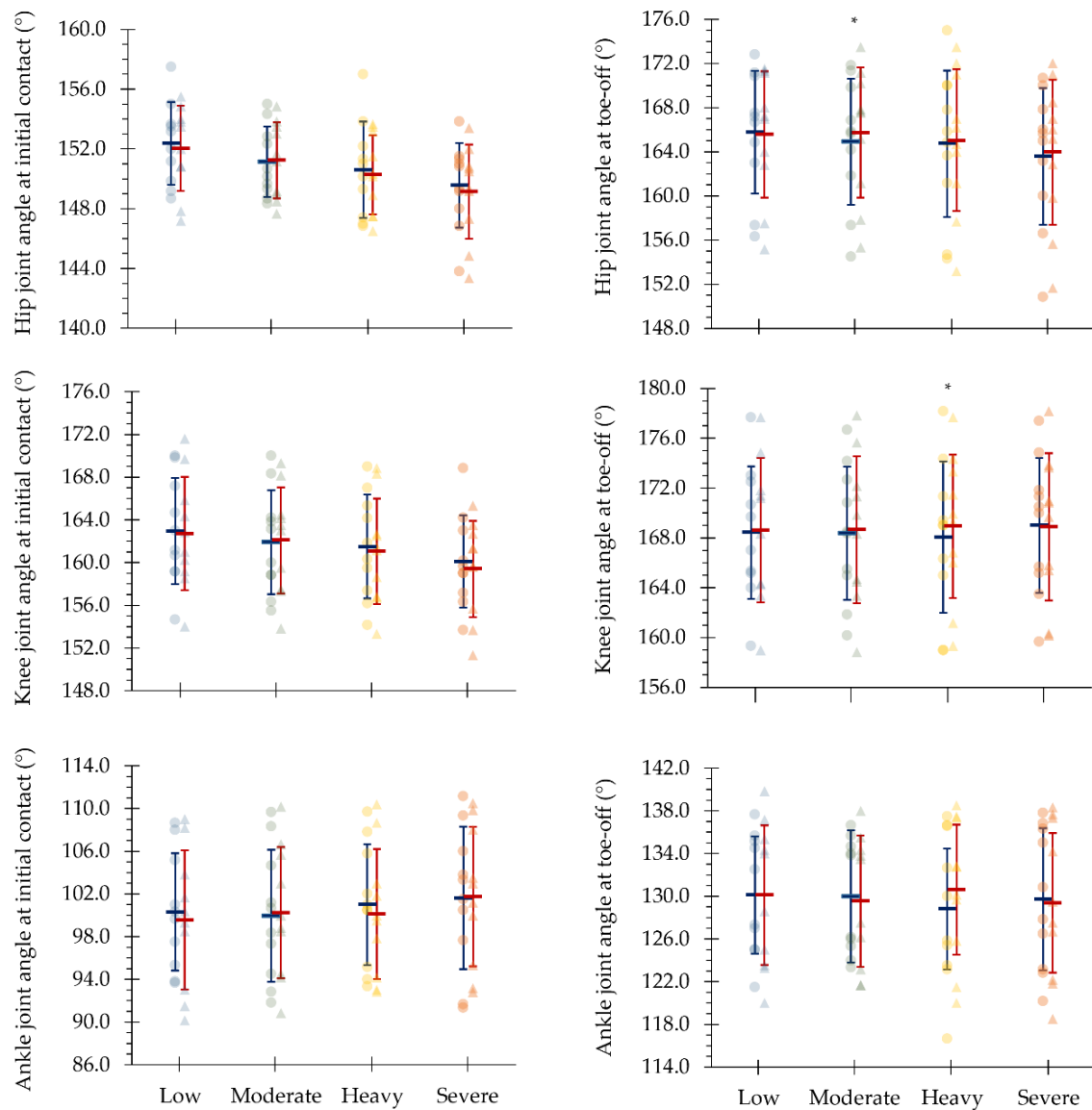


Figure 3. Individual (light circles and triangles), mean and standard deviation of the angular kinematic variables from the first (dark blue) to the second (dark red) laps of each protocol step corresponding to low, moderate, heavy and severe intensity domains.

* Indicates differences between laps ($p < 0.05$).

The implemented intermittent incremental protocol is widely used for sport practitioner evaluation and training control and prescription, particularly for individual and cyclic exercise modes (Monteiro et al., 2022; Patoz et al., 2023; Sousa et al., 2015). It allows an individual characterization through the delimitation of the exercise intensity domains and the prescription of appropriate training velocities. To this end, a range of different biomechanical and physiological variables are assessed, including stride length and frequency,

oxygen uptake, heart rate and blood lactate concentrations (Folland et al., 2017; Miller et al., 2019). The former two variables are often reported, also in physiological-related studies, due to their easy assessment and implementation into training sessions or competitive events and their relationship with performance (Cardoso et al., 2022; Cavanagh and Williams, 1982; De Ruiter et al., 2014). However, the application of the step protocol combining kinematical analysis with physiological data on overground running on a track field is scarce.

It has been reported that runners tend to select (usually subconsciously) a combination of stride frequency and length that is in close proximity to their optimal condition, minimizing their metabolic cost (Cavanagh and Kram, 1989; Cavanagh and Williams, 1982). Accordingly, the current study did not impose a specific stride frequency, enabling runners to fulfill the pre-defined velocities of the incremental protocol at a self-selected gait pattern, with similar values verified for comparable velocities in the literature (Dorn et al., 2012). It was also observed that, with the increase in intensity from heavy to severe exertions, runners achieved the selected velocity by maintaining stride frequency (with a trivial increase of 0.7%) and increasing stride length by 7.3% (Cavanagh and Kram, 1989; Dorn et al., 2012; Patoz et al., 2023). As a consequence, flight time increased and contact time decreased along the intensity spectrum when normalized to the stride time, reflecting a more aerial gait pattern, leading to a higher level of generated power within each stride (Lussiana et al., 2019; Nummela et al., 2007; Patoz et al., 2020).

With the intensity rise and so the stride frequency, an increase in hip and knee flexion was observed (Schubert et al., 2014). In the current study, from low to moderate intensities, the lower-limb joint angles remained consistent while above the anaerobic threshold, where the higher anaerobic contribution to energy production induces metabolic acidosis (Carvalho et al., 2020), notable variations were observed. In comparison to moderate-intensity running, a reduction in hip joint angle at initial contact at heavy and severe exertions (0.5 and 1.2%, respectively) and a reduction in hip joint angle at toe-off at severe intensity (0.9%) were detected, reflecting a greater hip flexion (Winter et al., 2016). Also, a reduction in knee joint angle at initial contact was observed at heavy and severe domains (0.5 and 1.5%, respectively), indicating a greater knee flexion

(Heiderscheit et al., 2011) without an increase in its extension at toe-off. The lack of change in ankle joint angle with the velocity rise has already been reported (Williams, 2000) and may be attributed to the constant stride frequency, particularly at higher intensities, as well as the submaximal velocity attained (since muscular changes have been described at faster running) (Dorn et al., 2012).

The adaptations of spatiotemporal biomechanical variables (e.g., stride frequency and flight and contact times) have been described during running at a self-selected pace (Dierks et al., 2010; Riley et al., 2008) or at different intensities during constant velocity exhaustive runs (Derrick et al., 2002; Madden et al., 2016; Patoz et al., 2023). Despite the duration of the incremental protocol (~24 min), no evidence of a fatigue effect was observed during each of the 800 m steps. If a fatigue effect had been present, differences between laps would be expected when running at severe intensity, since this exertion is characterized by the highest velocities achieved at the final steps of the incremental protocol. Complementarily, the variation of the kinematical variables observed from the first to the second laps at low, moderate and heavy intensities corresponded to a mean decrease of 0.02 Hz in stride frequency, 0.01 s in flight time and 0.83% in normalized flight time and a mean increase of 0.01 s in stride time, step time and contact time and 0.81 and 0.87° in hip and knee joint angles at toe-off, respectively. These minor discrepancies fall within the calculated CV, indicating trivial differences between the first to the second step corresponding to each intensity domain, displaying a negligible practical significance.

The findings of the current study provide valuable insights about how kinematical and physiological variables adjust with varying intensities during overground running. The observed alterations reflect the neuromuscular adaptations that are requisite to accommodate higher running velocities and optimize propulsion in conjunction with the physiological adaptations necessary to achieve each exertion. The use of these indicators will help to improve runners' technical ability in each exercise domain. In addition, monitoring their technical stability and body positioning at a constant running velocity is crucial to mitigate the risk of injury due to fatigue and thus enhance running performance.

We acknowledge that this research has some limitations that should be considered, particularly regarding weather conditions (wind and air temperature) and the limitations of the two-dimensional kinematic analysis. Upcoming research on overground running gait patterns should include advanced motion analysis techniques (such as three-dimensional and markerless approaches), aiming to reduce sources of error such as digitation and data smoothing. Complementarily, the addition of dynamometry (e.g., by using a force plate during the protocol) to analyze the behavior of force production with the intensity increase, as well as its relationship with spatiotemporal and kinematical data, would provide important information. Also, the inclusion of extreme exertions (above the maximal oxygen uptake boundary) to better describe and understand the modifications in running gait patterns along the intensity domain spectrum would be valuable. Our results enhance the understanding of progressive changes in overground gait patterns with the running intensity increment, being closer to runners' training and competitive contexts.

Conclusions

A modification in overground running gait patterns was observed with the intensity rise, particularly a decrease in temporal variables and an increase in frequency, distance and physiological values. In addition, more flexed hip (at initial contact and toe-off) and knee (at initial contact) joint angles were verified. Runners exhibited a consistent gait pattern throughout the 800 m steps corresponding to each intensity domain, with the minor adaptations observed during the second lap not representing a practical significance. The complex and close interaction between biomechanical and physiological mechanisms enhances the importance of a biophysical approach in runner monitoring, especially to improve the individual prescription of adequate training velocities and decrease the risk of injury.

General discussion

During exercise, the respiratory system adapts through faster and deeper breathing, activation of additional muscles and precise neural and chemical control to meet the body metabolic demands (Nicolò et al., 2018; Dominelli & Sheel, 2012). These adaptations ensure adequate oxygen delivery and carbon dioxide removal to sustain physical performance (Jones & Poole, 2005). The changes in breathing mechanics are reflected in increased f_R , which facilitates the delivery of more oxygen to the bloodstream and the efficient expulsion of carbon dioxide. Simultaneously, the V_T increase from rest to exercise allows deeper breaths to maximize $\dot{V}O_2$ at the alveolar level (Tipton et al., 2017). Higher activation of respiratory muscles, particularly the diaphragm and intercostal muscles, enables the system to handle the increased ventilatory workload (Dominelli & Sheel, 2012). Additionally, accessory muscles (as sternocleidomastoid and scalene muscles) assist in ribcage elevation for deeper inhalations, while the abdominal and internal intercostal muscles contribute to forceful exhalations. Consequently, $\dot{V}E$, as the product of f_R and V_T , increases significantly during exercise, aiming to maintain arterial blood gases at appropriate levels (Dominelli & Sheel, 2012). This is accompanied by improved ventilation-perfusion ratio, increased $\dot{V}O_2$ and $\dot{V}CO_2$, supporting the metabolic demand of working muscles.

Plethysmograph and spirometer are some of the primary devices used to measure volumes to characterize breathing mechanics under different conditions. They are also critical for diagnosing obstructive or restrictive lung diseases. The use of a turbine flowmeter also allows the measurement of flow with vanes that spin in relation to the gas velocity, detected by an opto-electric system that is temporarily broken when a vane passes through it (Dominelli & Sheel, 2012). In addition, it can be used while moving, as during exercise in different conditions and intensities (Baldari et al., 2013; Macfarlane, 2017), while connected to a portable gas analyser. Following the purposes of the current Thesis, ventilatory variables as $\dot{V}E$, f_R , V_T , $\dot{V}O_2$ and $\dot{V}CO_2$ offer a robust foundation for characterizing breathing mechanics and ventilatory responses

during exercise (Sheel & Romer, 2011). These variables are practical and informative, allowing for the evaluation of overall respiratory performance under physical stress. Consequently, they provide valuable insights into how the respiratory system dynamically adapts to meet the physiological demands of exercise.

From the various individual and cyclic exercise modes, swimming presents unique challenges, not only due to the need for adapted equipment to function in proximity to the swimmer, but also due to the distinct physiological and biomechanical demands imposed by the aquatic environment (Fernandes, 2013; Sousa et al., 2014). The synchronization of breathing with stroke cycles induces specific respiratory constraints, significantly limiting opportunities for inhalation, with a tendentially lower f_R (but with very rapid inhalation) and higher V_T compared to spontaneously breathing activities (Rodríguez, 2000). The horizontal body position and hydrostatic pressure exerted by the water compress the thoracic and abdominal cavities, reducing lung volumes and increasing the workload of the diaphragm and other respiratory muscles (Holmér et al., 1974; Leahy et al., 2019; Lomax et al., 2003). Additionally, swimmers are required to exhale forcefully against water resistance, resulting in heightened activation of expiratory muscle, while inhalation should be rapid and coordinated to avoid water aspiration (Wells et al., 2005). Nevertheless, regular swimming induces beneficial adaptations, including enhanced respiratory muscles strength, improved neuromuscular coordination and more efficient use of lung volumes, allowing swimmers to overcome these challenges and maintain effective ventilation under the unique demands of aquatic environment (Holmér et al., 1974; Mickleborough et al., 2008).

The relevance of these ventilatory variables extends to the assessment of the short-term repeatability of a specific test. The assessment of a protocol short-term repeatability ensures that the observed variations in the measured variables reflect true physiological responses or adaptations rather than measurement errors or inconsistencies in protocol execution. The well-known incremental protocol performed until voluntary exhaustion (Pyne et al., 2001) allows the characterization of a large spectrum of intensity domains that will further improve training prescription, being largely used in swimming evaluations (Capelli et al.,

1998; Carvalho et al., 2020; Fernandes et al., 2008). By integrating ventilatory, metabolic and biomechanical variables, the short-term repeatability of the incremental protocol can be further strengthened. This dual approach ensures that both respiratory and musculoskeletal responses are captured consistently, providing a comprehensive and reproducible assessment of the physiological demands of exercise (Zacca et al., 2020). Such consistency is crucial for accurately interpreting individual performance, tracking adaptations, and establishing reliable benchmarks in research and practice.

Among the various ventilatory variables assessed (**Chapter 2**), some alterations were observed between the first and second evaluation moments, likely reflecting a combination of physiological and psychological adaptations. These adaptations seem to indicate improved ventilatory efficiency (given the reduced $\dot{V}_E$ in some steps), enhanced respiratory muscle performance, increased familiarity with the protocol and potentially improved swimming economy. The ability to maintain a similar R across moments, despite the differing patterns of $\dot{V}_E/\dot{V}O_2$ and a reduced $\dot{V}_E/\dot{V}CO_2$, highlights a more refined and efficient ventilatory response during the second evaluation. This suggests an improved integration between respiratory and metabolic systems, likely due to better coordination and optimization of ventilatory patterns. These occasional differences do not compromise the validity or repeatability of the protocol but, instead, indicate that certain variables may be more sensitive to external and internal factors. Despite the efforts to standardize conditions (as similar training, nutrition and recovery before both evaluation moments), some factors as physical well-being, psychological stress and sleep quality are inherently difficult to control.

The observed progressive increase in $\dot{V}_E$ and f_R with the velocity rise, along with the constant V_T , reflected a constant tachypnoeic breathing pattern since the rise in $\dot{V}_E$ is mainly caused by increases in f_R (Martin & Weil, 1979; Sheel & Romer, 2012). These different behaviours may be attributed to different determinants on their regulation, particularly central command (by activating motoneurons for muscle contraction and respiratory centres) and muscle afferent feedback (with sensory feedback from exercising muscles) controlling f_R , and metabolic responses (given the increased carbon dioxide production, decreased pH and the

need for greater oxygen) regulating V_T (Amann et al., 2010; Nicolò et al., 2017). Part of these ventilatory response may arise due to direct stimulation at the spinal level of α -motoneurons innervating the intercostal muscles and diaphragm (Forster et al., 2012; Tipton et al., 2017).

Another critical consideration when assessing ventilatory responses in swimming is the use of breath-by-breath analysis. Similar to other exercise modes, as running or cycling, swimmers must be directly connected to the portable gas analyser to enable precise characterization of ventilatory responses, which are essential for understanding the respiratory demands and specific adaptations. In the aquatic environment, this approach introduces logistical and technical challenges, necessitating the use of a specialized respiratory snorkel that connects the swimmer to the portable gas analyser (Fernandes, 2013). While its design to minimize hydrodynamic resistance (with inspiration and expiration tubes aligned in the swimmer's forward direction and positioned vertically over his head to avoid drag) it imposes some limitations. These include the inability to perform block starts, flip turns and underwater gliding, which are integral components of competitive swimming performance (Ribeiro et al., 2016).

During free swimming, particularly in front crawl, inspiration typically occurs every two or three upper limbs actions ($1/2$ or $1/3$), following unilateral or bilateral breathing patterns (Figueiredo et al., 2013; Seifert et al., 2005). This results in a theoretical f_R/SR ratio of 0.5 or 0.3, respectively. However, when using a respiratory snorkel along a range of exercise intensities, higher f_R/SR ratios were observed, with a noticeable increase with the intensity rise (**Chapter 3**). These findings suggest that the respiratory snorkel allows swimmers to breathe more freely, enabling them to inhale as often as desired or needed, particularly at higher intensities. Consequently, when the respiratory snorkel is used, there is a loss of the tightly regulated breathing patterns characteristic of free swimming. In addition, with the inclusion of the extreme intensity domain, similar $\dot{V}_E$ (as well as $\dot{V}O_2$ and $\dot{V}CO_2$), increased f_R and reduced V_T were evidenced compared to the severe domain (**Chapter 3**). It was already showed a decrease in breathing times (reduced T_i , T_e and T_{tot}) with the velocity rise (Scheuermann & Kowalchuk, 1999) (**Chapter 2**), which will limit the amount of air that can be exchanged in

each breath (Nicolò & Sacchetti, 2023). Given the greater f_R values, V_T is not able to increase and, consequently, $\dot{V}E$ maintains stable.

Beyond the inability to perform the specific actions previously mentioned, the use of the respiratory snorkel seems to alter the natural coordination between breathing and SR, potentially affecting the typical ventilatory and biomechanical patterns observed during unrestricted swimming (Štrumbelj et al., 2007). These limitations underscore the importance of interpreting data obtained with the snorkel with some caution, as it may not fully represent the physiological and biomechanical demands of free swimming. A direct comparison of two incremental protocols (performed with and without the respiratory snorkel), would provide valuable insights into the extent of these alterations and their impact on swimming performance and ventilatory responses. Although this comparison was beyond the scope of the current Thesis, it represents an important recommendation for future research to better understand the physiological and biomechanical implications of using the respiratory snorkel in swimming assessments.

One way to address these challenges is through the backward extrapolation method (Lavoie, et al., 1983; Montpetit et al., 1981), which allow swimmers to perform without the respiratory snorkel, including flip turns, and achieve competitive velocities, thereby creating a more ecologically valid test environment (Ribeiro et al., 2016). Upon completing the test, the breathing mask is immediately connected to the swimmer to assess the recovery $\dot{V}O_2$ values, which are then extrapolate them to time zero to determine the $\dot{V}O_2$ peak or maximal values (Laffite et al., 2004). This approach provides the natural biomechanics of swimming (by maintaining the natural longitudinal body rotations for inhalation moments), while still providing accurate measures of $\dot{V}O_2$. Furthermore, analysing the exercise recovery curve (i.e., the $\dot{V}O_2$ off-transient kinetics), offers additional insights into gas exchange dynamics, enhancing the interpretation of the physiological mechanisms underlying the $\dot{V}O_2$ on-transient response (Cleuziou et al., 2003; Özyener et al., 2001). The development of advanced tools such as the updated version of VO_2 FITTING (**Chapter 4**) further facilitates this

process, enabling researchers to dynamically edit, process, filter and model the $\dot{V}O_2$ post-exercise data, without depending on complex mathematical programs.

The discussed challenges and the variation of breathing mechanics will also have significant implications for another performance influencing factor, the exercise economy (Foster & Lucía, 2007; Morgan et al., 1989). Estimating exercise economy will allow to understand how efficiently the human body converts chemical energy derived from substrates into biomechanical work (Sparrow & Newell, 1998). In endurance sports, optimal performance is highly dependent on the efficient utilization of the available energy. This emphasizes the importance of a high capacity to supply energy to the working muscles and the ability to sustain high intensity exertions without negatively affecting the rate of utilization of total energy sources (Daniels, 1985). Exercise economy reflects the intricate relationship between energy expenditure and velocity, offering a comprehensive perspective on the physiological and biomechanical profile of an athlete. Understanding and optimizing exercise economy is thus essential for enhancing performance and prescribing targeted interventions (**Chapter 5**).

Exercise economy is typically quantified as C, the ratio between energy expenditure and velocity, reflecting the amount of energy required to transport the body over a given distance (di Prampero, 1986). It serves as a critical indicator, with lower values reflecting a more economical use of energy for a given velocity. Over the decades, exercise economy has been primarily assessed through the measurement of $\dot{V}O_2$ at steady-state during submaximal exercise, while C has also been calculated for higher velocities or power outputs, without neglecting the anaerobic contribution (Capelli, 1999; Ribeiro et al., 2017; Zamparo et al., 2011). However, given the highly inter-individual variation of $[La^-]$ values, even below the AnT (Carvalho et al., 2020) also observed in the studies of the current Thesis (**Chapters 3, 6 and 7**), we believe that considering the anaerobic pathways when calculating C will provide more accurate values to better understand the exercise responses.

It is also valuable to characterize the individual exercise economy profile through the slope of the regression line derived from the relationship of energy expenditure and increasing velocities (Fernandes et al., 2005; Kjendlie et al.,

2004). This approach provides a detailed information on how energy expenditure increases with the exercise intensity, contributing to a better understanding of the individual metabolic response to physical effort across varying workloads. The modification in C_{slope} values when velocities above the AnT are included indicates that the submaximal estimation of C does not allow to extrapolate to higher velocities, independently of the cyclic exercise mode performed (**Chapter 6**).

The positive correlations between C, $\dot{V}E$ and f_R using the velocities until $\dot{V}O_{2\text{max}}$ suggest that increased ventilatory demand is closely linked to higher energy expenditure. This relationship likely reflects the metabolic cost associated with the activation of respiratory muscles (Tipton et al., 2017), which become progressively more significant as exercise intensity approaches $\dot{V}O_{2\text{max}}$, without contributing directly to forward locomotion. When higher velocities are included in the analysis, the regression lines suggest a shift in the underlying metabolic demands, given the notable contribution of the glycolytic pathways (Bar-Even et al., 2012). Expanding the velocity range allows for the identification of weaker velocities or training zones and provides a deeper understanding of the effects of specific training interventions.

The inclusion of running, swimming and cycling ventilatory responses analysis along the current Thesis evidenced that exercise mode influences the breathing pattern (Sheel & Romer, 2012), given the interaction effect observed (**Chapter 6**). While in swimming $\dot{V}E$ progressively increases with velocity along with f_R and a constant V_T (**Chapters 2, 3 and 6**), running and cycling evidenced $\dot{V}E$ increases also due to rises in V_T , particularly at higher exertions (**Chapter 6**). Modifications in V_T are expected during on-land exercises (Sheer & Romer, 2012). Its non-variation in swimming seems to be related to the water hydrostatic pressure exerted on the chest and abdomen, restricting lung expansion, and to the increased airway resistance caused by water immersion, making deep breaths more difficult. Also, excessive lung inflation and deflation may alter body position and, consequently, alter buoyancy and hydrodynamic resistive forces.

The observed variations between consecutive steps in $\dot{V}E$, f_R , V_T and C were different in the three exercise modes (e.g., f_R in running increased from steps 7 to maximal, in swimming increased immediately from the first to the second steps

and in cycling increased from steps 5 to 6 and 7 to maximal), evidencing the different breathing patterns. These phenomena may be explained by the exercise modes internal characteristics (in swimming, due to the water movements, performing slower may be challenging than swimming faster, due to the difficulty to produce propulsive forces) and training characteristics (running and swimming have similarly long competitive events, whereas road cycling is characterized by longer events).

The specific mechanical demands of the three exercise modes also account for different C patterns. In running, C remains relatively constant, indicating its independence from velocity (Capelli et al., 1998; di Prampero et al., 1993). In contrast, C during cycling changes at lower relative velocities, highlighting its velocity dependence (Capelli et al., 1998). Similarly, in swimming C increases more consistently with the velocity rise. These patterns can likely be attributed to the unique mechanical and physiological requirements of each mode. In cycling, the need for synergistic muscle actions, particularly involving the trunk and upper limbs, facilitated greater force production as velocity increases. For swimming, the rise in C may be explained by compensatory movements for stabilization, driven by the constant displacement of the water caused by segmental body motions.

Similarly to how the relationship between f_R and SR in swimming was investigated (**Chapter 3**), we find it relevant to analyse the modifications in movement patterns during running (**Chapter 7**). From low to severe intensities, a progressive increase in $\dot{V}E$ was also verified, while f_R only increased at heavy and severe exertions. Simultaneously, the velocity increase from heavy to severe domains was achieved by a maintenance of stride frequency and an increase in stride length (Cavanagh and Kram, 1989; Dorn et al., 2012; Patoz et al., 2023). Therefore, flight time increased and contact time decreased along the intensity spectrum when normalized to the stride time, reflecting a more aerial gait pattern, leading to a higher level of generated power within each stride (Lussiana et al., 2019; Nummela et al., 2007; Patoz et al., 2020).

In swimming, the velocity increase was achieved by an increase in SR and a decrease in SL (**Chapter 3**), from heavy to severe intensity domains, contrarily

to what was observed in running. Once again, the water environment has significant implications in the adopted mechanical patterns. From a certain velocity, its increase is given by solely increases in SR (Craig & Pendergast, 1979), particularly due to the volume of water displaced in each upper limb movement, that limit the capability of producing propulsive forces (Toussaint & Truijens, 2005). We are aware that this type of analysis would provide important insight also into cycling. However, the adopted methodology, particularly the stationary test, with controlled cadence and mandatory seated position) and the use of their own bikes limited this characterization. This latter factor, despite fundamental for the physiological characterization, limited the mechanical analysis due to the different bike length segments (saddle to pedal and saddle to handle) (Krämer et al., 2009; Too & Landwer, 2000) and different chainrings used (round and oval) (Sinclair et al., 2018).

Conclusions

In summary, the current Thesis demonstrated that the ventilatory responses vary depending on the exercise mode, from low to extreme intensities, influenced by the distinct characteristics and demands between running, swimming and cycling. Specifically, the increased ventilatory response (reflected by the higher $\dot{V}_E$) in swimming was primarily driven by a rise in f_R , whereas in running and cycling both f_R and V_T contributed, particularly at higher exertions. Based on the findings obtained in the experimental moments, it is pertinent to emphasize the following conclusions:

- I. The most frequently used ventilatory and biomechanical variables during an intermittent incremental protocol until exhaustion truly characterize the swimmers at the moment of the evaluation.
- II. When the respiratory snorkel is used, it might be observed a loss of the tightly regulated breathing patterns (f_R/SR ratio) characteristic of free swimming.
- III. The inclusion of the extreme intensity domain in swimming led to similar $\dot{V}_E$, increased f_R and reduced V_T compared to the severe domain.
- IV. $VO_2FITTING$ is a valid, free and open-source software for characterizing $\dot{V}O_2$ kinetics also during the recovery periods.
- V. A $\dot{V}O_2$ kinetics on/off symmetry was observed at low, moderate, heavy and severe intensity domains, characterized by a mono-exponential model.
- VI. The duration of the protocol steps, particularly above the AnT, may not be sufficient to allow the development of the $\dot{V}O_2$ slow component.
- VII. It was not observed a TD at the transition from exercise to recovery at any intensity domain, indicating that the recovery period started immediately when swimmers stopped after each effort.
- VIII. The modification in C_{slope} values at velocities above AnT suggests that submaximal C estimation cannot be extrapolated to higher velocities, regardless of the cyclic exercise mode performed.
- IX. There is a nearly perfect correlation between C, $\dot{V}_E$ and f_R until $\dot{V}O_{2max}$, reflected the increased C given by respiratory muscles activity.

- X. It was confirmed the velocity independence of C in running, contrastingly with the responses in swimming and cycling, highlighting the specific environmental and technical characteristics of the exercise modes.
- XI. In running, from heavy to severe domains, the velocity is achieved by maintaining stride frequency and increasing stride length, while in swimming this transition is represented by increased SR and decreased SL.
- XII. Higher hip and knee flexion were evidenced at heavy and severe intensity domains.
- XIII. Runners exhibited a consistent gait pattern within 1st and 2nd 400 m laps of the step corresponding to each intensity domain.

Future recommendations

Based on the main findings, it would be important to continue researching this topic, with a specific focus on:

- I. Perform two incremental protocols (with and without the respiratory snorkel), to compare the ventilatory responses and understand their impact on swimming performance.
- II. Inclusion of shorter more intense exertions above $\dot{V}O_{2\max}$ to understand the impact of these high-intensity exertions on exercise economy profile.
- III. Include electromyography analysis to better describe the increase of respiratory muscles activity and their effect on the increase of C during increasing exercise velocity.
- IV. Characterize the effect of velocity and energy contribution on exercise economy estimation also in other cyclic exercise modes.
- V. Evaluate the metabolic and ventilatory responses of an incremental protocol until exhaustion in running, swimming and cycling performed by triathletes to enhance the sport performance.
- VI. Characterize the breathing patterns together with biomechanical responses in road cycling, with similar bike configurations (or clustering based on specific bike characteristics).
- VII. Extend the breathing patterns along the intensity domains in a longitudinal way, including tests based on spirometer and/or plethysmography, to understand the effect of specific training adaptation in breathing mechanics and exercise economy.

Chapter 11

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Chapter 1

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Chapter 2

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