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The influence of testing modality on lactate threshold and the velocity associated with VO_{2max}

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Chapter I

1. General introduction

Physiological variables, such as maximum work rate or maximal oxygen uptake ($\dot{V}O_{2\,max}$), together with other submaximal metabolic inflection points (e.g. the lactate threshold, LaT or the pulmonary ventilation threshold, VET) are regularly quantified by sports scientists during incremental exercise testing to exhaustion. These variables have been shown to correlate with endurance performance, have been used to prescribe exercise training loads and are useful to monitor adaptation to training. It is generally regarded that *performance velocity* (the average speed in an endurance event) will be dictated by the *performance power* (the average work performed during an endurance task) and *performance oxygen uptake* (% $\dot{V}O_{2\,max}$ maintained during an endurance task), which is, in turn influenced by the percentage of $\dot{V}O_2$ at LaT, as well as $\dot{V}O_{2\,max}$ (Coyle 1995). These important physiological characteristics are usually determined from incremental exercise testing and are considered to be necessary for athletes to successfully perform in endurance events (Coyle 1995; Hawley et al. 1997).

Incremental exercise testing is a standard procedure for determining submaximal and maximal physiological variables. However, a key variant in most scientific research and performance diagnosis is the type of incremental test. An incremental protocol can be modified on the basis of the starting work rate, as well as, the duration and the magnitude of work rate increments. Currently, there is no consensus on the methods used to measure maximal and submaximal physiological parameters from such tests (Bentley et al. 2007).

1.1 VO_{2max} and the velocity associated with VO_{2max} (vVO_{2max})

Maximum rate of oxygen uptake ($\dot{V}O_{2\,max}$) is one of the most utilized parameters in basic and applied physiology, as it reflects the maximum capacity of the cardiorespiratory and musculoskeletal systems during exercise in sport, as well as in work and clinical environment (Kirkeberg et al. 2011; Levine 2008). $\dot{V}O_{2\,max}$ was initially defined by Hill and Lupton in 1923 as the oxygen uptake attained during maximal exercise intensity that could not be increased despite further increases in exercise workload (Hill and Lupton 1923). In their pioneering work, Hill and Lupton (1923) stated also that "considering the case of running, there is clearly some critical speed for each individual at which there is a genuine dynamic equilibrium… above which the maximum oxygen intake is inadequate", thus starting to point out the importance of determining also the velocity associated with $\dot{V}O_{2\,max}$ (the so-called $v\dot{V}O_{2\,max}$) (Daniels and Scardina 1984) or maximum aerobic speed, MAS (Hill and Rowell 1996)). Nowadays, $v\dot{V}O_{2\,max}$ is largely utilized to give a practical evaluation of aerobic demands during running activities and to plan specific training workloads (Denadai et al. 2006; Smith et al. 2003; Buchheit and Laursen 2013). Indeed, it provides an integrated measure of both $\dot{V}O_{2\,max}$ and the energetic cost of running into a single factor, hence, being directly representative of an athletes' locomotor ability (Billat and Koralsztein 1996; Daniels and Scardina 1984). Moreover, $v\dot{V}O_{2\,max}$ is able to explain individual differences in runners' aerobic performance that $\dot{V}O_{2\,max}$ or running economy alone are not (Daniels and Scardina 1984). The precise determination of this variable is therefore of crucial importance.

1.2 The history of vVO_{2max}: definitions and description

Although $\dot{V}O_{2\,max}$ has been generally accepted as physiological variable that best described the capacities of the cardiovascular and respiratory systems, the $v\dot{V}O_{2\,max}$ was assessed only 50 years later to give a practical assessment of aerobic demands and capability during running performance.

Several definitions, methods and abbreviations were used to define $v\dot{V}O_{2\,max}$ over the years. Volkov et al. (1975) proposed the critical speed as the running speed corresponding to $\dot{V}O_{2\,max}$. This parameter was determined through a ramp exercise from the baseline level to all-out running through stepwise increments of 1 m/s every three minutes. Thereafter, the maximal aerobic speed (MAS), as the maximal speed sustainable in an incremental track test was utilized (Leger and Boucher 1980; Berthoin et al. 1994; Lacour JR 1989). Based on the works of di Prampero and colleagues (di Prampero 1986; di Prampero et al. 1986), some authors proposed the term V_{amax} as the lower velocity that elicits a $\dot{V}O_2$ equal to $\dot{V}O_{2\,max}$ (Billat and Koralsztein 1996; Billat et al. 1994; Lacour et al. 1990). Daniels et al. (1984) were the first to introduce the term velocity at $\dot{V}O_{2\,max}$ ($v\dot{V}O_{2\,max}$). This parameter was calculated using a square-wave protocol of four 6 min incremental sub-maximal workloads from the regression curve relating running velocity and $\dot{V}O_2$ to $\dot{V}O_{2\,max}$, with the velocity of running that corresponds to $\dot{V}O_{2\,max}$ being identified (Daniels J. 1984). After that, other authors used similar methods to assess $v\dot{V}O_{2\,max}$ (Morgan et al. 1989; Daniels J. 1984; Abe et al. 1998). Subsequently, the term $v\dot{V}O_{2\,max}$ has been commonly accepted and used to define the minimal velocity that elicited $\dot{V}O_{2\,max}$ in incremental exercise tests (Billat and Koralsztein 1996; Billat 2000; Billat et al. 1999; Billat et al. 2000; Billat et al. 1996; Billat et al. 2003; Buchheit et al. 2012; Buchheit and Laursen 2013). Instead, the term maximal aerobic speed (MAS) or peak treadmill velocity (PTV) are used to define the higher speed maintained during a maximal track or treadmill test independently of $\dot{V}O_{2\,max,}$ while the term V_{max} was proposed by Kuipers et al. when the maximal running speed was calculated from a stage couldn't be completed to the full length (Dupont et al. 2004; McLaughlin et al. 2010; Kuipers et al. 2003; Dupont et al. 2002).

1.3 Protocols for VO_{2max} and vVO_{2max} assessment

Although definitions may not vary greatly between authors, the protocol of determining $\dot{V}O_{2\,max}$ and the speed chosen to calculate the oxygen cost of running can influence the value of $v\dot{V}O_{2\,max}$. The velocities of the stages and increases in velocity used by different authors are factors responsible for the different values of $v\dot{V}O_{2\,max}$ found in the same athlete.

An inaccurate method for measuring $v\dot{V}O_{2\,max}$ on treadmill in laboratory presents a certain number of problems in the subsequent use of the results, for example in the elaborating training programs. In studies conducted using trained and untrained populations, a shorter exercise protocol (<60-seconds stage increments) is typically used to measure $\dot{V}O_{2\,max}$ and then, on a second day, a submaximal test is used to quantify the submaximal parameters (Coyle 1995). However, on cycle ergometer, it is also popular to use a single test comprising ≥3-minutes stage durations to assess trained subjects (Bishop et al. 1998b; Bentley et al. 1998; Padilla et al. 2000). However, a sport scientist can approach incremental exercise testing with a variety of protocols aimed at determining a number of different physiological variables; modification of the exercise testing protocol can have implication for the variable measured and, hence, the use of these variables in longitudinal analysis and performance diagnostics (Bentley and McNaughton 2003).

In treadmill testing, two main protocols are generally utilized in $\dot{V}O_{2\,max}$ and $v\dot{V}O_{2\,max}$ assessment: continuous ramp and discontinuous square-wave (SW) incremental protocols. In continuous ramp incremental protocols, work rate increments are administered without any resting period in between. Ramp protocols may differ for slope of running velocity *vs* time relationship (ramp slope) (Smith et al. 2003; Billat et al. 1996) and/or treadmill incline (Duncan et al. 1997) (see Figure 1 A and B). While $\dot{V}O_{2\,max}$ was found to be independent of the protocol adopted (Duncan et al. 1997; McConnell and Clark 1988; Davies et al. 1984; Kirkeberg et al. 2011; Kuipers et al. 2003; Billat et al. 1996), ramp slope was often claimed to be responsible for different $v\dot{V}O_{2\,max}$ assessments during ramp protocols (Billat and Koralsztein 1996; Kuipers et al. 2003; Berthon and Fellmann 2002). Instead, in discontinuous SW incremental protocol, also known as incremental intermittent Astrand-type test (Astrand et al. 2003), each work load lasts 3-6 minutes, with resting periods in between. This testing modality is still preferred by some investigators to allow the cardiorespiratory system to better adjust to the work rate administered (Bernard et al. 1998; Duncan et al. 1997). Indeed, during ramp protocols, the increments in running velocity with time can be faster than the cardiorespiratory and metabolic adjustments (Gravelle et al. 2012), thus challenging the anaerobic metabolism to a different extent, at least above a certain exercise intensity.

On cycle ergometer, it should be noted that Adami et al. (2013) compared six different continuous incremental ramp tests with a discontinuous incremental SW test. Although no differences in $\dot{V}O_{2\,max}$ among protocols were observed, the peak power attained during the incremental ramp tests was always significantly higher than that attained in the Astrand-type test. In addition, during the ramp tests the peak power was lower, the longer the step duration.

Figure 1A

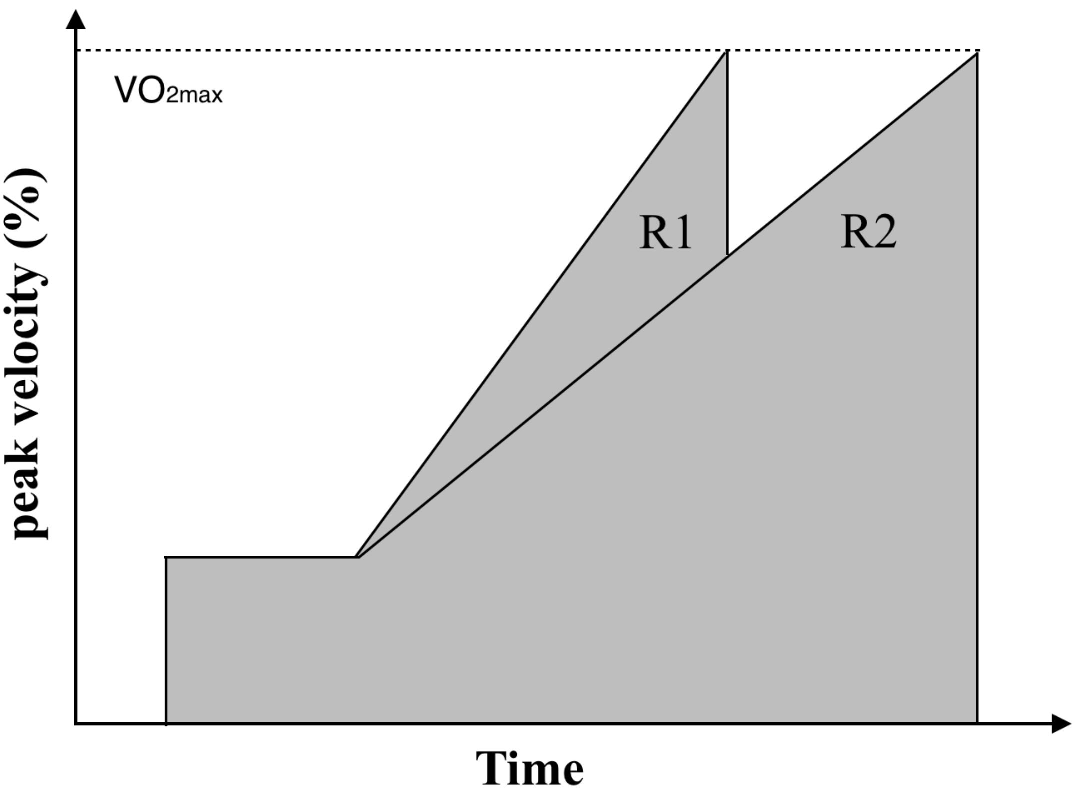

Fig. 1A: graphical representation for two continuous incremental ramp protocols with different velocity vs time slope (R1, the steepest slope, R2, the protocol with the less steep slope).

Figure 1B

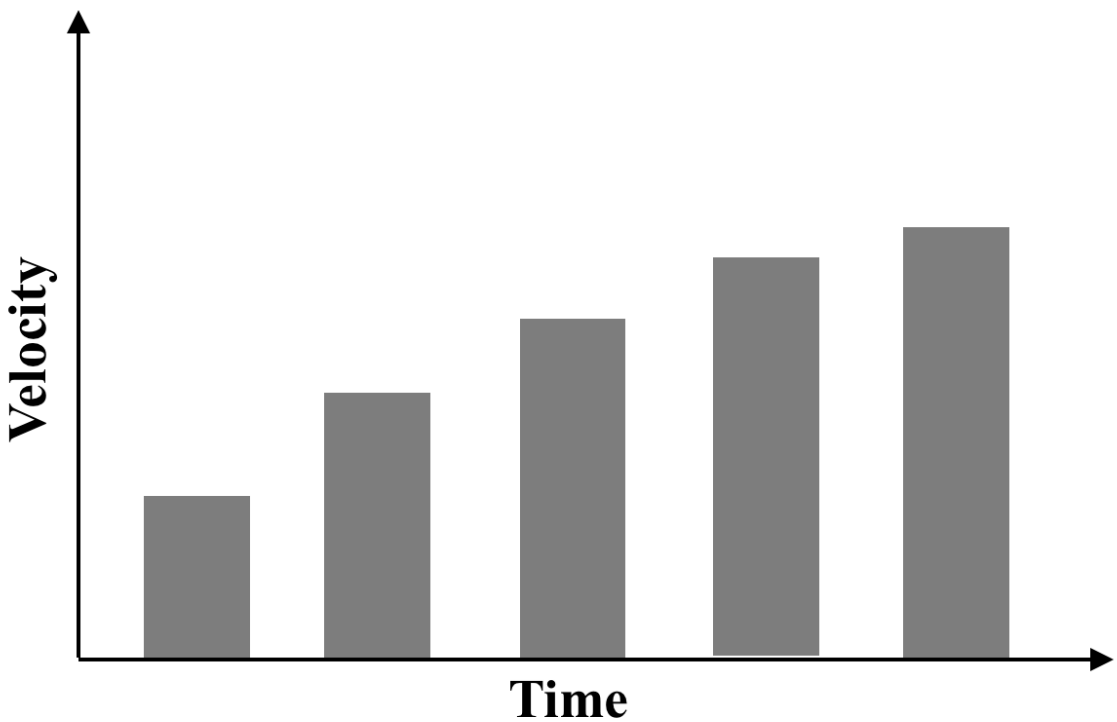

Fig. 1B: graphical representation for discontinuous incremental square-wave protocol (SW).

1.4 Oxygen uptake kinetics

Muscular exercise requires transitions to and from metabolic rates often exceeding an order of magnitude above resting and places prodigious demands on the oxidative machinery and O_2-transport pathway. The science of kinetics seeks to characterize the dynamic profiles of the respiratory, cardiovascular, and muscular systems and their integration to resolve the essential control mechanisms of muscle energetics and oxidative function: a goal not feasible using the steady-state response. Essential features of the $\dot{V}O_2$-kinetics response are highly interesting in athlete's population. For a given metabolic demand, fast $\dot{V}O_2$-kinetics mandates a smaller O_2 deficit, less substrate-level phosphorylation and high exercise tolerance. By the same token, slow $\dot{V}O_2$-kinetics incurs a high O_2 deficit, present a greater challenge to homeostasis and presages poor exercise tolerance. Compelling evidence supports that, in healthy individuals walking, running, or cycling upright, $\dot{V}O_2$-kinetics controls resides within the exercising muscle(s) and is therefore not dependent upon, or limited by, upstream O_2-transport systems. However, the balance of $\dot{V}O_2$-kinetics control may change with different mode (e.g. leg vs. arm) and intensity (moderate and heavy vs. severe) of exercise, within different fiber-type populations.

As early as 1922, Hill, colleagues and other sever authors (Hill 1940; Henry 1951; Poole and Jones 2012; Poole et al. 2012) demonstrated that, following the onset of moderate intensity exercise, pulmonary $\dot{V}O_2$ as a function of time, *t*, increases as an exponential process (see Figure 2): where *t* is the time elapsed from exercise onset and $\Delta\dot{V}O_2ss$ is the steady-state increase of $\dot{V}O_2$ above baseline. The rate constant, *k*, is independent of $\Delta\dot{V}O_2$ ss across a broad range of metabolic demands. This relationship can also be expressed as:

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2 ss(1 - e^{-t/\tau}),$$

where τ is the time constant (i.e., 1/k denoting the time to reach 63% $\Delta\dot{V}O_2$ ss) which may span a broad range from ~10 to >100s (see Figure 3). Importantly, at these exercise intensities, the off transient is symmetrical to the on-transient:

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2(0)e^{-t/\tau}.$$

Thus, $\tau\dot{V}O_2$ is a fundamental parameter of aerobic performance (Whipp et al. 1970; Whipp et al. 1981) and differences in $\tau\dot{V}O_2$ (i.e., the speed of $\dot{V}O_2$-kinetics) may help explain the broad range of physical/athletic capabilities and exercise tolerance across populations (Jones and Burnley 2009). Accordingly, trained endurance athletes exhibit extremely fast $\dot{V}O_2$-kinetics whereas untrained slow $\dot{V}O_2$-kinetics. Indeed, a slow $\dot{V}O_2$-kinetics is associated with a grater depletion of intramuscular high-energy phosphates and accumulation of lactate and hydrogen ions. Furthermore, $\dot{V}O_2$-kinetics was appreciably faster in men with a high $\dot{V}O_{2\,max}$ versus their counterparts with a lower $\dot{V}O_{2\,max}$ (Poole and Jones 2012).

Figure 3

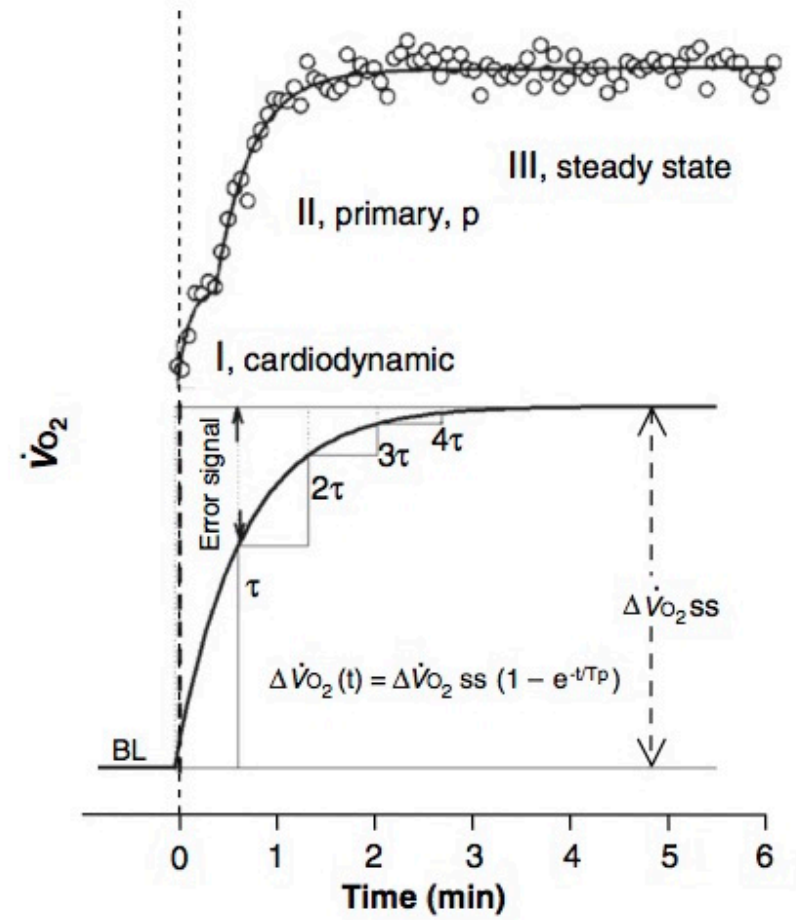

Fig. 3: Top: breath-by-breath alveolar $\dot{V}O_2$ response following the onset of moderate intensity cycle ergometer exercise. Phase I (cardiodynamic), II (primary), and III (steady-state) are designated and fit by an appropriate exponential model. Bottom: schematic demonstrating fundamental properties of the single component exponential response. The rate of $\dot{V}O_2$ increase is quantified by the time constant (τ) of the exponential (~40s for this example) where BL signifies baseline $\dot{V}O_2$ and Δ the increase or amplitude of $\dot{V}O_2$ above baseline. For each multiple of τ $\dot{V}O_2$ increases by 63% of the difference between that value at the previous τ and the required steady-state. Poole and Jones (2012).

1.5 The use of vVO_{2max} in high-intensity interval training

High-intensity interval training (HIT), in a variety of form, is today one of the most effective means of improving cardiorespiratory and metabolic function and, in turn, physical performance of athletes. HIT involves repeated short-to-long bouts of rather high-intensity exercise interspersed with recovery periods. For team sport players, the inclusion of sprints and all-out efforts into HIT programmes has also been shown to be an effective practice. It is believed that an optimal stimulus to elicit both maximal cardiovascular and peripheral adaptations is one where athletes spend at least several minutes per sessions at intensities higher than 90% of their $\dot{V}O_{2\,max}$. While use of HIT is not only approach to improve physiological parameters and performance, there has been a growth in interest by the sport science community for characterizing training protocols that allow athletes to maintain long periods of time above 90% of $\dot{V}O_{2\,max}$. Prescription for HIT consists of the manipulation of many variables which include the work interval intensity, exercise modality, number of repetitions, number of series, a swell as the between-series duration and recovery intensity. Furthermore, the sport that the athletes is involved in (i.e. training specificity) should first be considered in relation to the desired long-term training adaptations. The manipulation of any of these variables can affect the acute physiological responses to HIT. The attraction of the $v\dot{V}O_{2\,max}$ use to 'shape' the HIT session is that the entire locomotor profile can be performed in accordance with the athlete's maximal potential. In team sport, due to the technical/tactical requirements, and following the important principle of training specificity, game- (i.e. so called small-sided game, SSG) or skill-based conditioning has received an exponential grown in interest. While understanding of the $\dot{V}O_2$ responses to SSG is limited and the overall load cannot be precisely standardized, the SSGs have limitation that support the use of less specific (i.e. run based) but more controlled HIT formats at certain times of the season or for specific player needs. In these cases, the $v\dot{V}O_{2\,max}$ method represents an integrated measure of both $\dot{V}O_{2\,max}$ and the energetic cost of running into a single factor and permit to induce highly controlled physiological responses during HIT.

During a single constant-load exercise or intermittent exercise, a work intensity close to $v\dot{V}O_{2\,max}$ is required to elicit maximal $\dot{V}O_2$ responses. Several studies had used $v\dot{V}O_{2\,max}$ (% of $v\dot{V}O_{2\,max}$) in an attempt to determine the % of $v\dot{V}O_{2\,max}$ that determines the longer time at $v\dot{V}O_{2\,max}$ (T@ $v\dot{V}O_{2\,max}$). Not surprisingly, time to exhaustion at $\dot{V}O_{2\,max}$, was inversely related to running intensity if 90%, 100%, 120% and 140% of their $v\dot{V}O_{2\,max}$ was used (Byrnes et al. 1985; Midgley et al. 2006; Billat et al. 2000). In another study, middle-distance runners did not manage to reach $\dot{V}O_{2\,max}$ while running at 92% of $v\dot{V}O_{2\,max}$ (Gollnick et al. 1974). Furthermore, the ability to reach $\dot{V}O_{2\,max}$ during a single run between LaT and $v\dot{V}O_{2\,max}$ is likely fitness dependent with highly trained athletes unlikely to reach their $\dot{V}O_{2\,max}$(Altenburg et al. 2007). Lastly, work intensity of ≥95% $v\dot{V}O_{2\,max}$ are therefore recommended for maximizing T@ $v\dot{V}O_{2\,max}$ during a single isolated run. However, in practice, athletes do not exercise to exhaustion, but use intervals or set.

Slightly lower intensity (≥90% $v\dot{V}O_{2\,max}$) can also be used when considering repeated exercise bouts (as during HIT sessions), since interval $\dot{V}O_2$ is likely to increase with repetitions with the development of a $\dot{V}O_2$ slow component (Iaia and Bangsbo 2010; Buchheit and Laursen 2013). Lastly, the correct $v\dot{V}O_{2\,max}$ determination and the differences in $v\dot{V}O_{2\,max}$ calculated using different protocols are very important issue to determine workloads on the field both in individual and team sports athletes.

1.6 Long distance runners vs soccer players assessment

As said before, nowadays, $v\dot{V}O_{2\,max}$ is largely utilized to give a practical evaluation of aerobic demands during running activities (Denadai et al. 2006; Smith et al. 2003; Buchheit and Laursen 2013) because it provides an integrated measure of both $\dot{V}O_{2\,max}$ and the energetic cost of running into a single factor. Moreover, $v\dot{V}O_{2\,max}$ is considered to be necessary for athletes to successfully perform in endurance events (Coyle 1995; Hawley et al. 1997). However, this type of testing procedure is usually utilized also with other athletes, involved in running but in a different way than runners.

For example, soccer players are often evaluated by different testing protocols for $\dot{V}O_{2\,max}$ and $v\dot{V}O_{2\,max}$ assessment, because these parameter are adopted to induce high intensity training on the field (Buchheit and Laursen 2013).

The game of soccer has many complex characteristics when it comes to performance assessment. Different patterns of movements, combined with a large range of physiological demand, lead to highly variable individual activity patterns throughout a soccer match. Among the most important elements of fitness, when assessing the performance of soccer players, are aerobic and anaerobic power, muscle strength, flexibility, speed and agility. However, high level of aerobic fitness is key, particularly at elite level (Svensson and Drust 2005). Soccer incorporates periods of high-intensity exercise interspersed with periods of lower-intensity exercise. The physiological demands require players to be competent in several aspects of fitness, which include aerobic and anaerobic aspects; these fitness components often vary with the individual player, the positional role in the team and the team's style of play (Bangsbo and Lindquist 1992). Despite this, it is important that the player and coach obtain objective information about the players' physical performance to clarify the objective of training, plan short- an long-term training programmes, provide objective feedback and motivate the player to train harder.

On one hand, the sport scientist can, though physiological testing of the participants, analyse physiological factors and use the information to provide individual profile of their respective strengths and weaknesses. These data can form the basis for the development of optimal training strategies (Svensson and Drust 2005). On the other hand, the physiological assessment may be used to determine training load and the intensity of effort during skill-conditioning drills measured by time-motion analysis technologies.

Furthermore, long distance runners and soccer player can be both tested using different protocols that could determine different physiological variables at maximal and submaximal exercise; additionally, these athletes have a different physiological profile, more aerobic (with a higher $\dot{V}O_{2\,max}$) for long distance runners than soccer players, which could have a slower $\dot{V}O_2$-kinetic and a different capacity to adjust the oxygen transport system at each workload.

1.7 The lactate threshold (LaT) assessment

As mentioned above, since the early work of Hill and Lupton (Hill and Lupton 1923), the success in aerobic performance has been associated with a high peak oxygen uptake ($\dot{V}O_{2\,peak}$) and $v\dot{V}O_{2\,max}$. However, it has been suggested that also parameters at submaximal exercise can provide a useful prediction of endurance performance (Bosquet et al. 2002; Farrell et al. 1979; Yoshida et al. 1987). For instance, the lactate threshold (LaT), the work rate at which blood lactate concentration ($[La^-]_b$) starts to increase above resting levels (Brooks 1985), has been shown to be strictly related to endurance performance both in trained (Farrell et al. 1979) and untrained individuals (Yoshida et al. 1987). LaT is commonly used to assess the effects of a training intervention, evaluate physical fitness, and determine the workload intensity during aerobic activities (Bishop et al. 1998b; Allen et al. 1985).

Several different methods have been proposed to determine LaT over the years, among which fixed $[La^-]_b$ levels, such as 4 mM (Sjodin and Jacobs 1981), or the work rate at which the first increase in $[La^-]_b$ of 1 mM (Δ1 mM) above resting levels occurs (Thoden 1991a). To date, though, no generally accepted fitting procedure has been established (Bentley et al. 2007). However, the analysis of the whole $[La^-]_b$ curve is considered more appropriate to assess LaT compared to fixed levels methods (Faude et al. 2009). Cheng et al. (Cheng et al. 1992) proposed the D_{MAX} method to determine the point on the regression curve that yielded the maximal perpendicular distance to the straight line formed by the two end data points. To minimize the influence of the starting point of the incremental protocol, Bishop and colleagues (Bishop et al. 1998b) utilized a modified D_{MAX} threshold ($D_{MAX\ MOD}$) considering the point on the polynomial regression curve that yielded the maximal perpendicular distance to the straight line formed by the first increase in $[La^-]_b$ and the final lactate point as LaT. Beaver and coworkers(Beaver et al. 1985) proposed the Log-Log model, in which the pattern of $[La^-]_b$ was studied using a transformation defined by plotting $\log([La^-]_b)$ *vs* $\log(\dot{V}O_2)$. A plot of this function exhibits a phase of very slow increase followed by a phase of rapid increase, defining a transition in the underlying relationship between $[La^-]_b$ and $\dot{V}O_2$. A linear regression analysis was therefore used to locate the LaT (Beaver et al. 1985). Taking into account $\dot{V}O_2$, this approach is independent of the protocol adopted when LaT is expressed as $\dot{V}O_2$ or $\%\dot{V}O_{2\,peak}$, as also demonstrated by some authors (Yoshida 1984; McLellan 1985).

Therefore, also LaT can be influence by the method and different testing modality used (incremental continuous ramp protocols with different velocity vs time slope).

2. Aims

In training, relative $v\dot{V}O_{2\,max}$ submaximal and supra-maximal work rates are largely utilized to manipulate the acute physiological responses to exercise and to administer training workloads (Buchheit and Laursen 2013). However, several protocols with different velocity *vs* time slope as utilized and a $v\dot{V}O_{2\,max}$ misestimate may consequently affect relative training workloads calculation, thus correct $v\dot{V}O_{2\,max}$ assessment is a crucial issue.

Despite a relatively large number of studies had focused on the comparison among different types of running protocols for $\dot{V}O_{2\,max}$ assessment (Kirkeberg et al. 2011; Kuipers et al. 2003), no studies compared continuous *vs* discontinuous incremental protocols for $v\dot{V}O_{2\,max}$ determination on the treadmill; furthermore, a comparisons among protocols between long distance runners and soccer players for both $\dot{V}O_{2\,max}$ and $v\dot{V}O_{2\,max}$ was performed. Moreover, a systematic study investigating which method for LaT assessment on the treadmill is less affected by ramp slope is still lacking.

Therefore, the aim of this study was to compare a discontinuous SW incremental test with two continuous incremental ramp tests, differing in ramp slopes, for $v\dot{V}O_{2\,max}$ assessment on the treadmill in physically active participant and among RUN and SOC. We hypothesized that, due to the faster increase in running velocity with time, $v\dot{V}O_{2\,max}$ would be higher in the continuous incremental ramp tests compared to SW. In addition, $v\dot{V}O_{2\,max}$ values closer to that obtained in SW should be achieved in the ramp protocol with the less steep ramp slope. Moreover, this difference should be higher in soccer players than in runners, due to a different capacity to adjust the oxygen transport system at each workload.

In the third study, the aim was to determine which of the methods that are commonly utilized to assess LaT (D_{MAX}, $D_{MAX\ MOD}$, 4 mM, Δ1 mM and Log-Log) would be less sensitive to differences in ramp slope.

Chapter II

1. General Abstract

Aim: The velocity associated with maximum aerobic power (v$\dot{V}O_{2\,max}$) and lactate threshold (LaT) are important physiological parameters, which are utilized to determine relative workloads on the field. The testing modality adopted to evaluate it, though, may cause differences in v$\dot{V}O_{2\,max}$ and LaT assessment and, in turn, in training intensity. Long distance runners (RUN) and soccer players (SOC) are both athletes involved with running. However, the physiological demands are different: in RUN are continuous while in SOC are discontinuous, with an alternation of aerobic and anaerobic tasks. Therefore, the aim of the studies was to compare two different testing modalities (continuous incremental ramp and discontinuous square wave (SW) protocols) for v$\dot{V}O_{2\,max}$ assessment on the treadmill in physically active male, RUN and SOC. Hypothesis is that due to the faster increase in running velocity with time, *v*$\dot{V}O_{2\,max}$ would be higher in the continuous incremental ramp tests compared to SW and this difference should be higher in SOC than in RUN, due to a different capacity to adjust the oxygen transport system at each workload. Moreover, we studied how the slope of the increase in running velocity with time (ramp slope) during continuous incremental ramp protocol, though, may affect LaT assessment measured with different methods.

Methods: Seventeen physically active participants, eight RUN and nine SOC performed three maximum incremental tests on a treadmill: two continuous ramp protocols, with different ramp slopes (R1, 1 km·h^{-1} per min; and R2, 1 km·h^{-1} every 2 min), and one discontinuous SW protocol, in random order, for maximum oxygen uptake ($\dot{V}O_{2\,max}$) and v

$\dot{V}O_{2\,max}$ determination. Cardiorespiratory and metabolic parameters were collected breath-by-breath at rest and during exercise. Blood lactate concentration $[La^-]_b$ was measured at rest, during, and at peak exercise. In both protocols, LaT was calculated by D_{MAX}, $D_{MAX\,MOD}$, 4 mM, Δ1 mM and Log-Log methods.

Results: v$\dot{V}O_{2\,max}$ was significantly higher in R1 and R2 compared to SW (16.8±0.6, 20.7±0.5, 18.6±0.4 km·h^{-1} for SW, R1, R2, respectively; P<0.001). No significant differences were found among protocols for $\dot{V}O_{2\,max}$ (4018±111, 4039±110, 4003±100 ml·min^{-1} for SW, R1, R2, respectively) as well as for expiratory ventilation, carbon dioxide production, blood lactate concentration, and heart rate. In the second study, no significant differences between groups and protocols were found in $\dot{V}O_{2\,max}$ as well as in VE, VCO_2, $[La^-]_{peak}$ and HR at maximum exercise. However, *v*$\dot{V}O_{2\,max}$ was significantly higher in R1 and R2 compared to SW in SOC, while only R1 was significantly higher than SW in RUN. A higher difference between R1 *vs* SW and in R2 *vs* SW was found in SOC than RUN for both ramps (+29% and 16% *vs* SW for R1 and R2 in SOC and +16% and 6% *vs* SW for R1 and R2 in RUN). Moreover, LaT had higher velocities in R1 for D_{MAX} (16.5±0.4 *vs* 15.1±0.4 km·h^{-1}, *P*=0.002, ES: 3.17, CI: 2.16/4.18), $D_{MAX\,MOD}$ (17.7±0.5 *vs* 15.6±0.4 km·h^{-1}, *P*<0.001, ES: -0.90, IC: -1.61/-0.20), 4 mM (17.0±0.6 *vs* 15.5±0.5 km·h^{-1}, *P*<0.001, ES: -0.57; IC: -1.18/0.18), Δ1 mM (17.1±0.5 *vs* 15.1±0.4 km·h^{-1}, *P*<0.001, ES: -0.57, IC: -1.26/0.12), but not for Log-Log.

Conclusion: In spite of similar $\dot{V}O_{2\,max}$ values, v$\dot{V}O_{2\,max}$ was higher during continuous incremental ramp tests compared to SW due to the longer time for cardiorespiratory and metabolic adjustments, suggesting different aerobic and anaerobic metabolism involvement. However, the difference was significantly higher in SOC than RUN, possibly due to a slower capacity to adjust the oxygen transport system to a given workload in SOC. Even though the three protocols can be used to assess $\dot{V}O_{2\,max}$, the $v\dot{V}O_{2\,max}$ differences between protocols must be acknowledged to prescribe correctly high intensity training, especially for soccer players. Lastly, the testing modality influenced also LaT assessment. Indeed, with the only exception of Log-Log, all the other methods presented significantly higher velocities at LaT when the steeper ramp slope (R1) was utilized.

2. Materials and methods

2.1 Participants

We selected seventeen voluntary physically active, male participants (age: 22.6 ± 1.8 years; stature: 1.75 ± 0.04 m; body mass: 68.7 ± 4.0 kg; mean ± standard deviation), eight soccer players (age 22.1 ± 1.8 years; stature 1.75 ± 0.05 m; body mass 70.3 ± 3.7 kg; mean ± standard deviation) and eight runners (age 23.0 ± 1.8 years; stature 1.76 ± 0.03 m; body mass 66.7 ± 4.0 kg; mean ± standard deviation) to participate in the study. Participants were all familiar with running activities, clinically healthy, with no recent history of musculoskeletal injuries. The ethics committee of the local university approved the study (protocol #102/14) which was performed in accordance with the principles of the 1964 Declaration of Helsinki. All participants gave their written consent after full explanation of the purpose of the study and the experimental design.

2.2 Experimental procedures

All tests were conducted approximately at the same time of the day in a climate-controlled laboratory (constant temperature of 22 ± 1 °C and relative humidity of 50 ± 5 %). After the familiarization visit, each participant performed one discontinuous SW test and two continuous incremental ramp tests (with different ramp slopes, as described below) in random order for $v\dot{V}O_{2\,max}$ determination. All tests were conducted on a motorized treadmill ergometer (RAM s.r.l., mod. 770 S, Padua, Italy) with 1% incline. At rest and during exercise, expiratory ventilation ($\dot{V}E$), $\dot{V}O_2$, CO_2 production ($\dot{V}CO_2$), and other gas exchange parameters were measured by a metabolimeter (Cosmed, mod. Quark *b*2, Rome, Italy) on a breath-by-breath basis. The system was calibrated prior to each test with a 3-L syringe (Hans Rudolf, Shawnee, Kansas, USA) and gas mixtures of known concentration (O_2 16%, CO_2 5%, balance N_2). Heart rate (f_H) was monitored continuously (Polar Electro Oy, mod. S810i, Kempele, Finland). Arterial O_2 saturation (SaO_2) was determined by a fingertip infrared oximeter (NONIN Medical, mod. 3011, Minneapolis, MN). The lactameter was precisely calibrated before and after each test to guarantee consistent data (Baldari et al. 2009). At the end of the test, the rate of perceived exertion (RPE) was determined on a Borg scale (6-20) for general, respiratory and muscular fatigue. During tests, each participant was verbally encouraged and strongly motivated by operators to reach his maximum exercise capacity.

<u>Continuous incremental ramp test 1 (R1)</u>. After 5 minutes of baseline measurements, participants warmed up at 10 km·h^{-1} for 5 minutes. Then, running velocity was increased progressively by 1 km·h^{-1} per minute, until volitional exhaustion. At rest and during exercise, the cardiorespiratory and gas exchange variables were collected on a breath-by-breath modality. $[La^-]_b$ was determined at rest, at the end of each step and at minute 1, 3 and 5 over recovery. To determine the achievement of $\dot{V}O_{2\,peak}$, the plateauing of $\dot{V}O_2$ (< 2.1ml·kg-1·min-1 decrease) despite an increase in workload was utilized. If the stated criterion was not fulfilled, participants were asked to perform a constant load test to the limit of tolerance at a work rate above the highest achieved on the incremental ramp test, as suggested by Rossiter and coworkers (Rossiter et al. 2006). Secondary criteria to establish

$\dot{V}O_{2\,peak}$ were not used to avoid possible significant underestimation of the value (Poole et al. 2008).

Continuous incremental ramp test 2 (R2). R2 followed the same experimental procedures as R1, but with an increase in treadmill running velocity of 1 km·h^{-1} every two minutes.

Discontinuous SW incremental test protocol. SW involved five workloads of 4 min each, with at least 5 min of rest in between. After 5 min of baseline measurements while standing still on the treadmill, the first two work rates were set at 8 and 10 km·h^{-1}, respectively, for all participants. The other three workloads were administered according to individual cardiorespiratory response to the first two workloads and the theoretical maximum f_H calculated with the equation proposed by Tanaka and colleagues (Tanaka et al. 2001). The last workload was administered with a running velocity slightly above that considered associated with $\dot{V}O_{2\,max}$ according to extrapolations from submaximal f_H and running velocity relationship.

2.3 Data analysis

In SW, the $\dot{V}O_2$ and the other cardiorespiratory and metabolic variables were determined as the average value of the last (fourth) minute during each workload. In R1 and R2, the cardiorespiratory and metabolic responses to exercise data were averaged during the last 30 s of each step at submaximal workload and over the last 30 s before exhaustion.

In SW, R1 and R2, individualized lactate thresholds (LaT) were determined by the D_{MAX} method (Cheng et al. 1992), according to which LaT was identified as the point on the third order polynomial curve that yielded the maximal perpendicular distance to the straight line formed by the two end data points. Similarly to another study (Bernard et al. 2000), LaT calculated from R1 was utilized to limit the range of exercise during which the $\dot{V}O_2$ *vs* running velocity relationship at submaximal exercise was considered. During SW, $v\dot{V}O_{2\,max}$ was extrapolated from the regression analysis equation of $\dot{V}O_2$ as a function of running velocity at submaximal workloads below the LaT (Ferretti 2014). During R1 and R2, $v\dot{V}O_{2\,max}$ was determined as the minimal running velocity that elicited $\dot{V}O_{2\,max}$ over a period of 30 s (Billat et al. 1996).

In the third study, during continuous incremental ramp protocols, the individualized LaTs were determined by five different methods. i) In D_{MAX} method LaT was identified as the points on the third order polynomial curve that yielded the maximal perpendicular distance to the straight line formed by the two end data points (Cheng et al. 1992); ii) $D_{MAX\,MOD}$, a modified D_{MAX} method, identified as the point on the third order polynomial curve that yielded the maximal perpendicular distance to the straight line formed by the point preceding an increase of lactate concentration greater than 0.4 mM and the final lactate point (Fabre et al. 2010); iii) The 4 mM, is the fixed point at which blood lactate reach a concentration of 4 mM (Heck et al. 1985); iv) The Δ1 mM is the velocity at which blood lactate increases to 1 mM above resting value (Coyle et al. 1983); and v) the Log-Log model, in which the pattern of $[La^-]_b$ was studied using a transformation defined by plotting log([La$^-$]$_b$) *vs* log($\dot{V}O_2$). A linear regression analysis was used to identify LaT (Beaver et al. 1985). With all methods, we expressed LaT in terms of running velocity, $\dot{V}O_2$ and % $\dot{V}O_{2\,peak}$.

2.4 Statistical analysis

Statistical analysis was performed using a statistical software package (Sigma Plot for Windows, v 12.5, Systat Software Inc., San Jose, CA, USA). To check the normal distribution of the sampling, a Kolgomorov-Smirnov test was applied. A sample size of seventeen participants was selected to ensure a statistical power higher than 0.80. To assess significant differences in $v\dot{V}O_{2\,max}$, cardiorespiratory, metabolic and perceptual variables among tests, we utilized one-way analysis of variance (ANOVA) for repeated measures. Also to assess significant differences in LaT between both protocols and methods a one-way analysis of variance (ANOVA) for repeated measures was performed. For all pairwise multiple comparisons, a post hoc Shapiro-Wilk test was applied. A regression analysis was used to assess the relationship between $\dot{V}O_2$ and running velocity at submaximal exercise. A Student's t-test determined the differences in slope in the comparison between protocols for both $v\dot{V}O_{2\,max}$ and $\dot{V}O_{2\,max}$ (Prism 5, GraphPad Software Inc., La Jolla, CA, USA). Among $v\dot{V}O_{2\,max,}$ $\dot{V}O_{2\,max}$, and LaT the magnitude of the changes was assessed using effect size (ES) statistics with 95% confidence intervals (95% CI) or partial eta square (η^2_P), as appropriate. ES was classified as trivial for ES values (0-0.19), small between (0.20-0.49), medium (0.50-0.79), large (0.80 and greater). Pearson's product moment and 95% CI were utilized to assess the relationship among protocols for $v\dot{V}O_{2\,max}$ and $\dot{V}O_{2\,max}$. The correlation coefficients were interpreted as follows: r <0.1 trivial; 0.1≤ r <0.3 small; 0.3≤ r <0.5 moderate; 0.5≤ r <0.7 large; 0.7≤ r <0.9 very large; 0.9≤ r <1 nearly perfect. Statistical significance was set at an α level of 0.05. Unless otherwise stated, all values are presented as mean ± standard error (SE).

Chapter III

1st study

Comparison between continuous and discontinuous incremental treadmill test to assess the velocity at VO_{2max}

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1. Abstract

Aim: The velocity associated with maximum aerobic power (v$\dot{V}O_{2\,max}$) is an important physiological parameter, which is utilized to determine relative workloads on the field. The testing modality adopted to evaluate it, though, may cause differences in v$\dot{V}O_{2\,max}$ assessment and, in turn, in training intensity. The aim of the study was to compare two different testing modalities (continuous incremental ramp and discontinuous square wave (SW) protocols) for v$\dot{V}O_{2\,max}$ assessment on the treadmill.

Methods: Seventeen physically active participants performed three maximum incremental tests on a treadmill: two continuous ramp protocols, with different ramp slopes (R1, 1 $km \cdot h^{-1}$ per min; and R2, 1 $km \cdot h^{-1}$ every 2 min), and one discontinuous SW protocol, in random order, for maximum oxygen uptake ($\dot{V}O_{2\,max}$) and v$\dot{V}O_{2\,max}$ determination. Cardiorespiratory and metabolic parameters were collected breath-by-breath at rest and during exercise.

Results: v$\dot{V}O_{2\,max}$ was significantly higher in R1 and R2 compared to SW (16.8±0.6, 20.7±0.5, 18.6±0.4 $km \cdot h^{-1}$ for SW, R1, R2, respectively; P<0.001). No significant differences were found among protocols for $\dot{V}O_{2\,max}$ (4018±111, 4039±110, 4003±100 $ml \cdot min^{-1}$ for SW, R1, R2, respectively) as well as for expiratory ventilation, carbon dioxide production, blood lactate concentration, and heart rate.

Conclusion: In spite of similar $\dot{V}O_{2\,max}$ values, v$\dot{V}O_{2\,max}$ was higher during continuous incremental ramp tests compared to SW possibly due to the longer time for

cardiorespiratory and metabolic adjustments, suggesting different aerobic and anaerobic metabolism involvement. The differences among protocols should be considered when *v* $\dot{V}O_{2\,max}$ is used for training purposes.

2. Aim of the study

Despite a relatively large number of studies had focused on the comparison among different types of running protocols for $\dot{V}O_{2\,max}$ assessment (Kirkeberg et al. 2011; Kuipers et al. 2003), no studies compared continuous *vs* discontinuous incremental protocols for $v\dot{V}O_{2\,max}$ determination on the treadmill. Therefore, the aim of this study was to compare a discontinuous SW incremental test with two continuous incremental ramp tests, differing in ramp slopes, for $v\dot{V}O_{2\,max}$ assessment on the treadmill. We hypothesized that, due to the faster increase in running velocity with time, $v\dot{V}O_{2\,max}$ would be higher in the continuous incremental ramp tests compared to SW. In addition, $v\dot{V}O_{2\,max}$ values closer to that obtained in SW should be achieved in the ramp protocol with the less steep ramp slope.

3. Results

The main cardiorespiratory, metabolic, and perceptual parameters at maximum exercise are given in Table 1. As shown in Figure 1, the $v\dot{V}O_{2\,max}$ was significantly higher in both ramps compared to SW (+24 ± 3% for R1 *vs* SW, P<0.001, ES: 2.8, CI: 1.8/3.7; +11 ± 2% for R2 *vs* SW, P<0.001, ES: 1.7, CI: 0.9/2.5). $v\dot{V}O_{2\,max}$ in R1 was significantly higher than in R2 (+11 ± 1% for R1 *vs* R2, P<0.001, ES: 2.5, CI: 1.6/3.4). At peak exercise, no significant differences among the three protocols were found for $\dot{V}O_2$, as well as for f_H, $\dot{V}E$, and [La$^-$]. Only muscular RPE showed significant differences among protocols, with a lower perception of muscular effort in R1 compared to SW (P=0.007, ES: 0.91, CI: -1.6/-0.2).

The relationships between R1 and R2 *vs* SW for $v\dot{V}O_{2\,max}$ and $\dot{V}O_{2\,max}$ values are shown in Fig. 2 (panel A and B, respectively). The slopes and intercepts of the linear regression analysis of $v\dot{V}O_{2\,max}$ between R1 and SW, and between R2 and SW were significantly different from the identity line (R1 *vs* SW, P= 0.013, ES: 2.7, CI: 1.8/3.7; R2 *vs* SW, P<0.001, ES: 1.1, CI: 0.4/1.9; see Fig. 2, panel A). The two $v\dot{V}O_{2\,max}$ regression lines (R1 *vs* SW, R2 *vs* SW) had different intercepts (P<0.001; see Fig. 2 panel A) but similar slopes. This was not the case for $\dot{V}O_{2\,max}$ regression analysis, where slopes and intercepts were not significantly different from the identity line and from each other (Fig. 2, panel B).

Significant correlations for $v\dot{V}O_{2\,max}$ (Fig. 2, panel A) and $\dot{V}O_{2\,max}$ (Fig. 2, panel B) values between R1 and SW, and between R2 and SW were found. Correlation was significant also between R1 and R2 (r = 0.87, P<0.001 and r = 0.92, P<0.001 for $v\dot{V}O_{2\,max}$ and $\dot{V}O_{2\,max}$ values, respectively; not shown in figure).

LaT occurred at 14.2 ± 0.4, 16.5 ± 0.4, and 15.2 ± 0.4 km·h^{-1} in SW, R1, and R2, respectively (Fig. 3). Therefore, LaT in R1 was on average 2.1 km·h^{-1} (+15%; P<0.001, ES: 1.3, CI: 0.6/2.1) and 1.3 km·h^{-1} (+9%, P<0.001, ES: 0.8, CI: 0.1/1.5) right shifted compared to SW and R2, respectively. LaT in R2 was on average 0.7 km·h^{-1} higher than LaT in SW (+5%, P=0.035, ES: 0.6, CI: -0.1/-1.3).

The $\dot{V}O_2$ *vs* velocity relationship, which was determined below the LaT calculated in R1, is shown in Fig. 4 for each testing modality. The regression analysis highlighted differences in slope between SW and R1 (P<0.001, ES: 1.54, CI: 0.7/2.3) and between R1 and R2 (P<0.001, ES: -1.04, CI: -1.8/-0.3).

Table 1. Cardiorespiratory, metabolic, and perceptual variables at maximum exercise

	SW	R1	R2
$v\dot{V}O_{2\,max}$ ($km \cdot h^{-1}$)	16.8 (0.6)	20.7 (0.5)*	18.6 (0.4)*, #
$\dot{V}O_2$ ($ml \cdot min^{-1}$)	4018 (111)	4039 (110)	4003 (100)
$\dot{V}O_2$ ($ml \cdot kg \cdot min^{-1}$)	56.7 (4.5)	57.3 (4.8)	56.6 (4.8)
$\dot{V}CO_2$ ($ml \cdot min^{-1}$)	4494 (115)	4547 (117)	4394 (105)
RER	1.12 (0.01)	1.13 (0.02)	1.10 (0.01)
SaO_2 (%)	89 (0.9)	91 (0.8)	90 (0.5)
f_H ($beats \cdot min^{-1}$)	187 (1.0)	188 (2.0)	188 (2.0)
$\dot{V}E$ ($l \cdot min^{-1}$)	157 (3.9)	161 (4.8)	160 (4.2)
$[La^-]_{peak}$ (mM)	12.2 (0.7)	12.0 (0.6)	11.2 (0.4)
General RPE (au)	18.5 (0.3)	18.2 (0.3)	18.1 (0.3)
Respiratory RPE (au)	18.4 (0.3)	18.0 (0.4)	17.9 (0.4)
Muscular RPE (au)	18.6 (0.4)	17.1 (0.4)*	17.9 (0.4)

$v\dot{V}O_{2\,max}$, velocity associated with maximum oxygen uptake; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; SaO_2, arterial O_2 saturation; f_H, heart rate; $\dot{V}E$, expiratory ventilation; $[La^-]_{peak}$, peak blood lactate concentration; and rate of perceived exertion (RPE) at general, respiratory, and muscular level. Variables were determined at maximum exercise in the three testing conditions (SW, square wave protocol; R1, ramp 1; R2, ramp 2). SE values are given in brackets. $^*P<0.05$ *vs* SW; $^{\#}P<0.05$ *vs* R1.

Figure 1

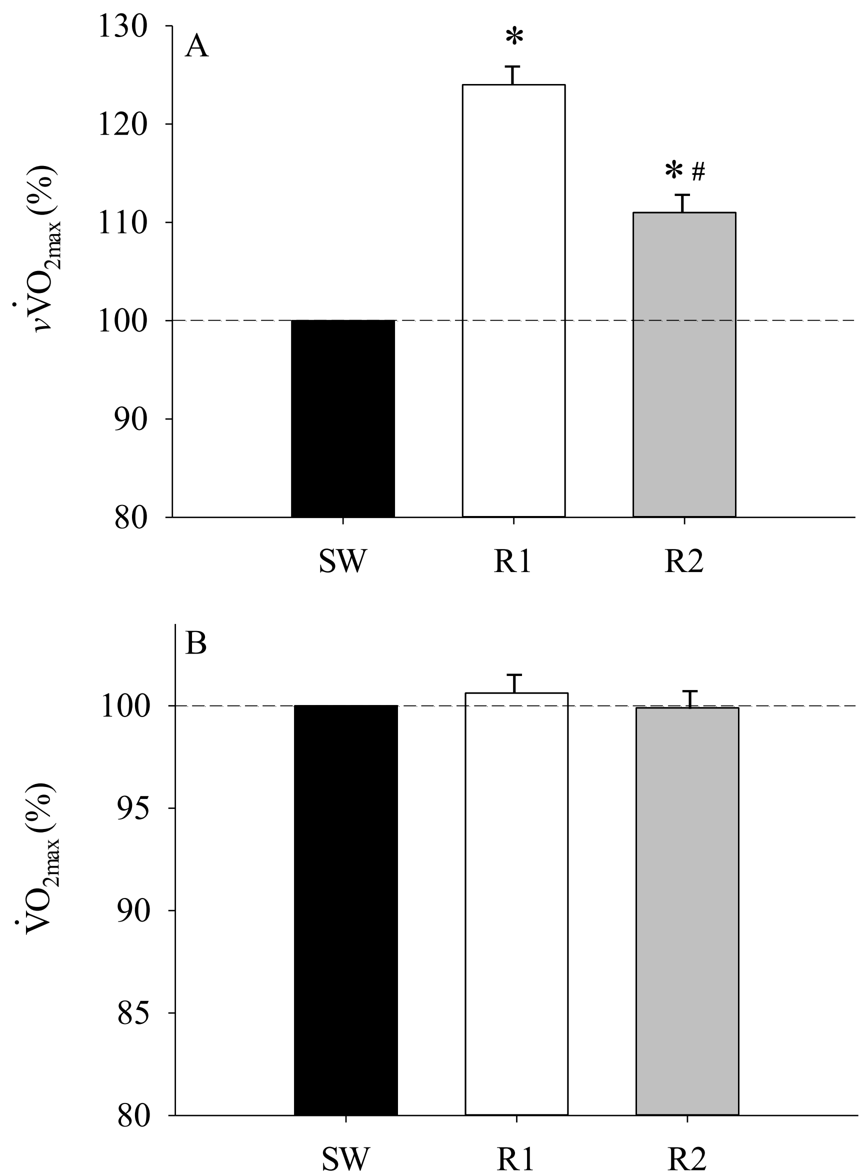
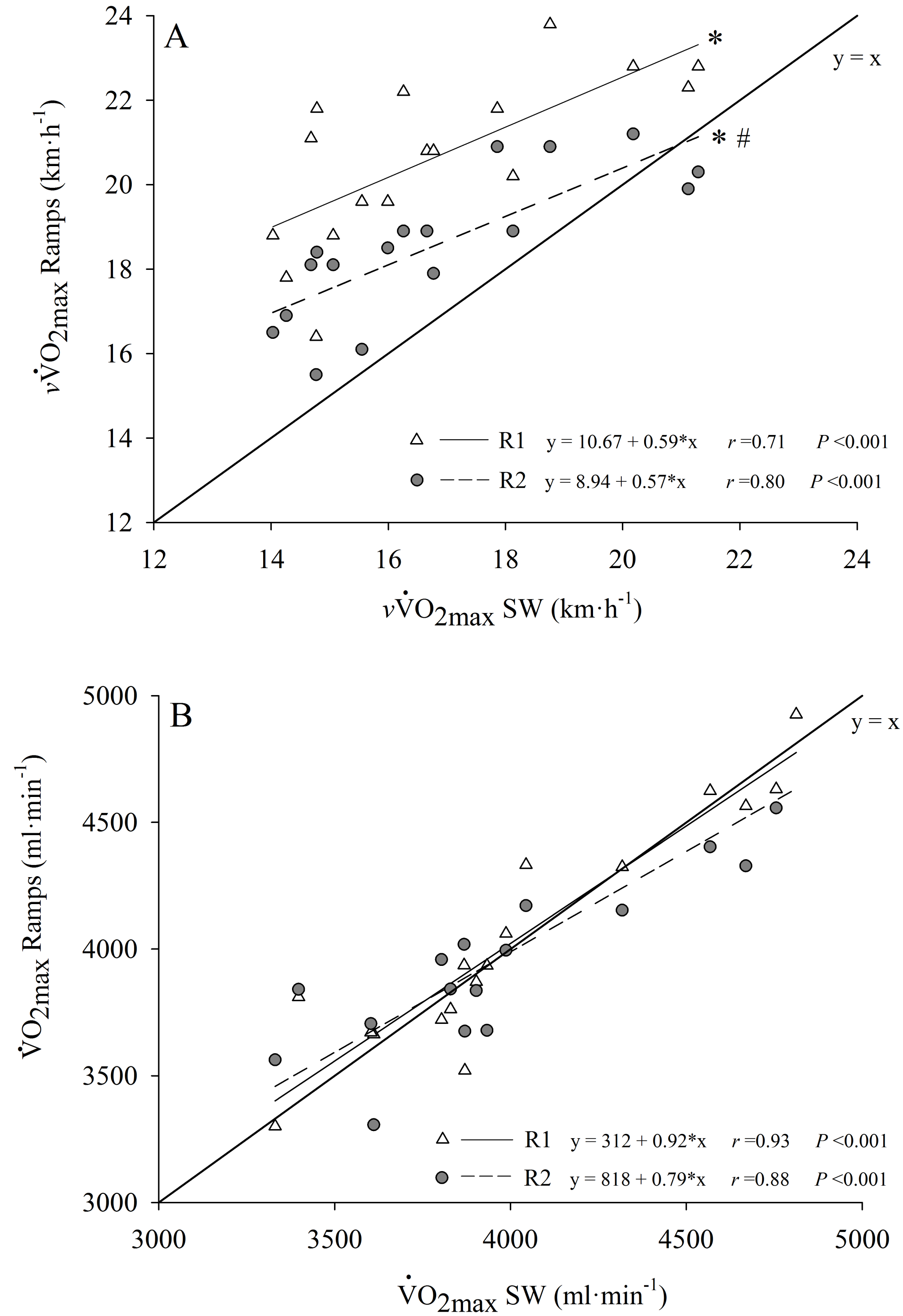


Fig. 3 The velocity associated with maximum oxygen uptake ($v\dot{V}O_{2\,max}$, panel A) and maximum oxygen uptake ($\dot{V}O_{2\,max}$, panel B) calculated during ramp 1 (R1) and ramp 2 (R2) protocols are presented as a percentage of their relative maximal value determined during the discontinuous incremental square-wave (SW) protocol (dashed line). *P<0.05 *vs* SW; $^{\#}P$<0.05 *vs* R1.

Figure 2



A
B
y = x
*
* #
R1 y = 10.67 + 0.59*x r =0.71 P <0.001
R2 y = 8.94 + 0.57*x r =0.80 P <0.001
R1 y = 312 + 0.92*x r =0.93 P <0.001
R2 y = 818 + 0.79*x r =0.88 P <0.001
vVO2max Ramps (km·h-1)
vVO2max SW (km·h-1)
VO2max Ramps (ml·min-1)
VO2max SW (ml·min-1)

Fig. 2 The relationship between $v\dot{V}O_{2\,max}$ (panel A) and $\dot{V}O_{2\,max}$ (panel B) values determined during continuous (R1 and R2) and discontinuous (square wave, SW) incremental protocols are shown. In both panels, the bold solid line represents the identity line (y = x), and the thin solid and dashed lines are the regression lines for the R1 *vs* SW and the R2 *vs* SW relationships, respectively. Regression equations and correlation coefficients are also reported. **P*<0.05 *vs* identity line (slope and intercept); $^{\#}$*P*<0.001 *vs* R1 (intercept).

Figure 3

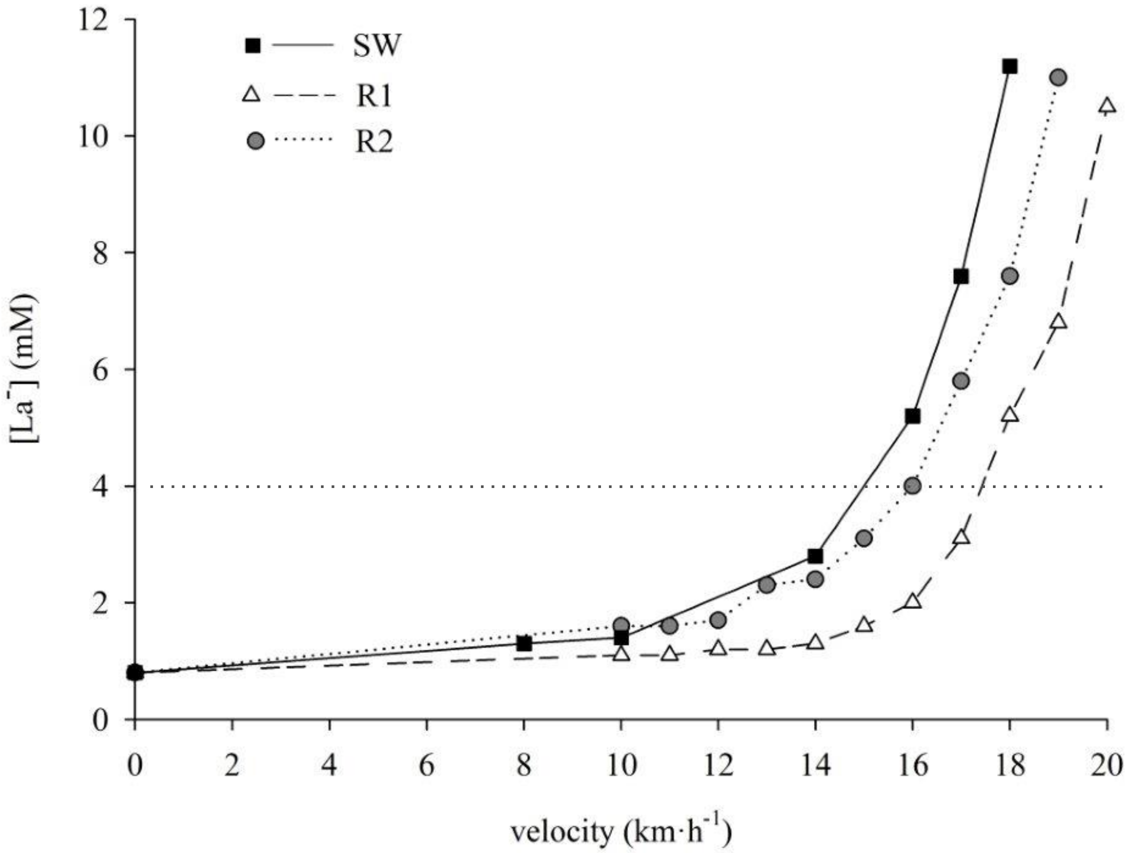


Fig. 3 Blood lactate concentration [La-] as a function of running velocity in a representative participant in all tested conditions (square wave, SW, dashed line; ramp 1, R1, solid line; and ramp 2, R2, dotted line). The horizontal dotted line represent the lactate threshold (LaT) measure by 4mM method for the representative participant.

Figure 4

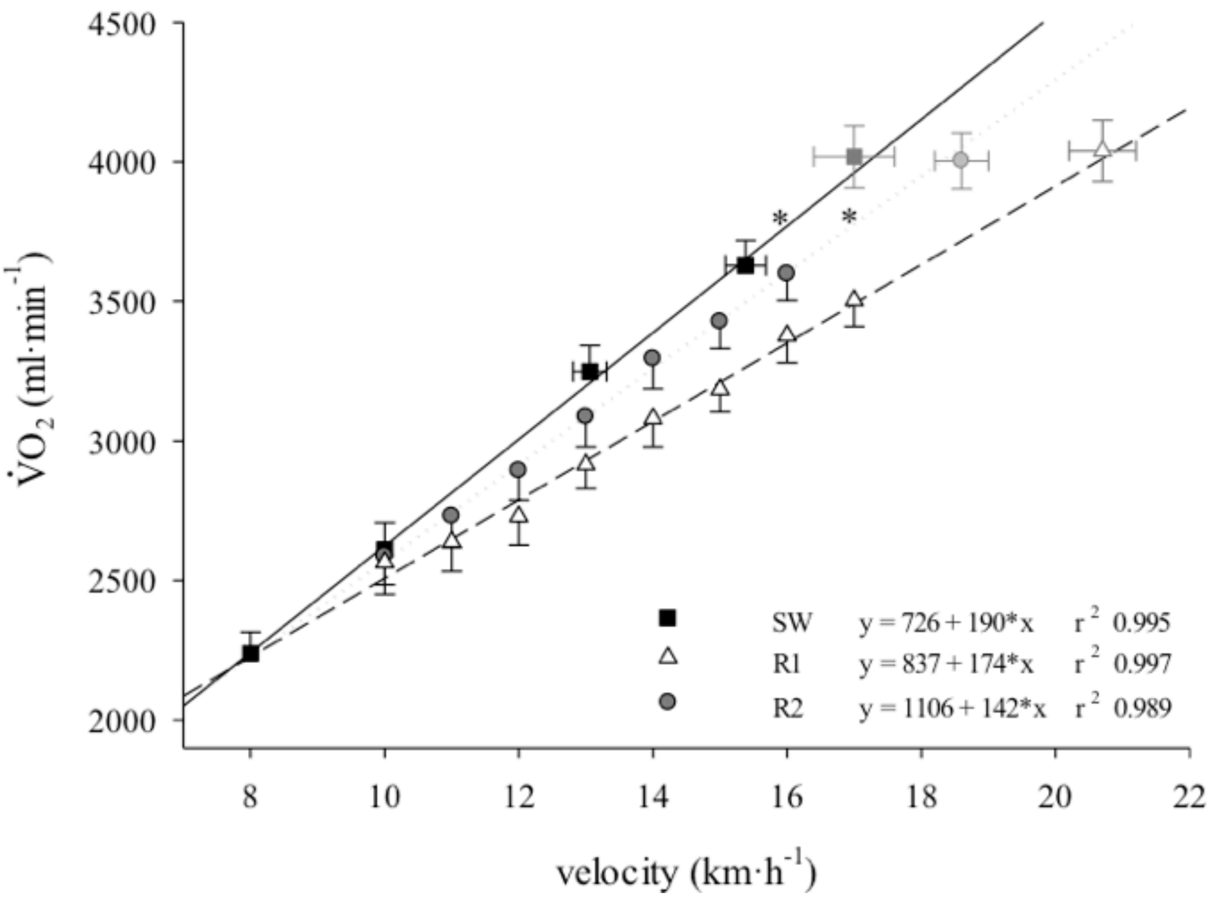


Fig. 4 The $\dot{V}O_2$ as a function of running velocity is presented at submaximal work rates (below the velocity at LaT calculated in R1 condition). The solid, dashed and dotted lines represent the regression lines for the square wave (SW), ramp 1 (R1) and ramp 2 (R2) protocols, respectively. Regression equations and correlation coefficients are also reported. The last three experimental points (dark grey square, triangle and circle) represent the mean value at maximal exercise for SW, R1 and R2, respectively. *P<0.05 *vs* R1 (regression slope).

4. Discussion

The main and novel finding of the present study was the quantification of the $v\dot{V}O_2$ max differences between continuous and discontinuous protocols. Indeed, $v\dot{V}O_{2\,max}$ was higher in the two continuous incremental ramp tests compared to SW, despite similar $\dot{V}O_{2\,max}$ values. Our data may be explained by the increase in workload (running velocity) with time faster than the cardiorespiratory and metabolic adaptations during continuous incremental exercise, while in SW test a better matching of workload and metabolic power could be achieved. Therefore, R1 and R2 may require a larger intervention of the anaerobic pathway to attain $v\dot{V}O_{2\,max}$ compared to SW. To further support this hypothesis, $v\dot{V}O_{2\,max}$ in the continuous incremental ramp test with the lower ramp slope (R2) was closer to that reported in SW.

When considering only the findings on $\dot{V}O_{2\,max}$, the present results are in line with previous reports, where $\dot{V}O_{2\,max}$ was found to be independent from the protocol adopted. In particular, Davies et al. (1984) utilized three treadmill continuous ramp incremental protocols, with constant speed and an increase in incline of 1.5% every 1, 2 and 3 minutes, respectively, and a horizontal treadmill continuous ramp incremental test with an increase in speed of 1 km/h every minute. They found no differences in $\dot{V}O_{2\,max}$ and concluded that all protocols were effective methods to elicit the maximum aerobic power. McConnell and Clark (1988) tested four continuous ramp incremental protocols with different constant speeds and increasing treadmill incline by 2.5% every 1 or 2 minutes, and observed similar

$\dot{V}O_{2\,max}$ values in all tests. Duncan et al. (1997) compared a treadmill discontinuous square-wave protocol, with different workloads, to a continuous ramp incremental protocol with constant speed and increments in incline. Yet again, similar $\dot{V}O_{2\,max}$ values were reported between the two protocols, even though a clear $\dot{V}O_2$ *vs* workload plateau was not evident in the continuous incremental ramp protocol. However, contrary to the present investigation, $v\dot{V}O_{2\,max}$ was not calculated. When moving from running (treadmill) to cycling (cycle ergometer) testing modality, the same phenomenon can be observed. For instance, Zhang et al. (1991) investigated the effects of four different continuous ramp incremental protocols on $\dot{V}O_{2\,max}$. The protocols had the same work rate *vs* time slope but different work rate increase patterns (continuous incremental or stepwise incremental, with different step durations). They observed that $\dot{V}O_{2\,max}$ was the same in all conditions, and therefore independent of the pattern of work rate increase. The lack of significant differences in $\dot{V}O_{2\,max}$ among protocols in our and in the other investigations is suggestive that all the considered testing modalities were able to challenge maximally the aerobic system.

Conversely, the present study revealed a significant effect of testing modality on $v\dot{V}O_{2\,max}$, with a higher value in continuous (R1 and R2) than in discontinuous (SW) incremental protocols.

Kuipers et al. (2003) retrieved similar findings when utilizing three continuous incremental ramp protocols with different ramp slopes (1 $km \cdot h^{-1}$ increment per minute, 2 $km \cdot h^{-1}$

increment every 3 minutes, and 2 km·h^{-1} every 6 minutes). $v\dot{V}O_{2\,max}$ was different among conditions, with the fastest speed for the ramp with the steepest ramp slope. However, Kuipers and co-workers defined $v\dot{V}O_{2\,max}$ as the maximal running speed calculated from the last ramp stage was utilized and not as the minimal velocity that elicits $\dot{V}O_{2\,max}$. Moreover, they did not compare the continuous incremental ramp tests to a discontinuous protocol. Interestingly, when protocols vary in parameters other that ramp slope, no differences in $v\dot{V}O_{2\,max}$ are found. Indeed, Billat et al. (1996) utilized two ramp protocols with different step increments but same ramp slope, and found no differences in $v\dot{V}O_{2\,max}$. This finding may suggest that step increments alone do not affect $v\dot{V}O_{2\,max}$ assessment.

Due to the lack of studies on the treadmill comparing continuous and discontinuous incremental tests for $v\dot{V}O_{2\,max}$ assessment, some insights on the present findings may be obtained from prior works on the cycle ergometer. Some explanations can be inferred from some assumptions about the critical power concept (Morton 1994; Monod and Scherrer 1965; Jones et al. 2010), i.e., the highest power that can be sustained relying exclusively on aerobic metabolism (Ferretti 2015). The aerobic supply is rate- but not capacity-limited, with the limiting threshold coinciding with the critical power. On the contrary, the anaerobic source is not rate- but capacity-limited, with the amount of energy known as the anaerobic work capacity (Morton 2011). Above the critical power, both aerobic and anaerobic energy sources contribute to exercise as work rate increases. Exercise cessation therefore occurs when all the anaerobic work capacity has been utilised. On these bases, Morton (2011) noticed that several studies investigating the cardiorespiratory responses to continuous incremental ramp exercise, with different ramp slopes, showed that the protocols with the steeper slopes led to higher peak work rates at exhaustion. As an explanation, the author proposed algebraic, calculus and geometrical models, all based on whole body bioenergetics involving aerobic and aerobic metabolism and allowing the prediction of peak power output from ramp slope. Experimental evidence to the validity of Morton's model was provided by Adami et al. (2013) who compared six continuous incremental ramp tests and a discontinuous incremental SW test. The peak power attained during the incremental ramp tests was confirmed to be inversely related to the ramp slope and always significantly higher than that attained in the SW, Astrand-type test. To further support this interpretation, we can consider the previously-mentioned work of Billat et al. (1996), who utilized two ramp protocols with the same ramp slope, but different step increments. In that study, indeed, similar $v\dot{V}O_{2\,max}$ (as an index of peak power) were reached, suggesting that when the ramp slope does not change, $v\dot{V}O_{2\,max}$ cannot differ due to the same anaerobic capacity. However, Billat and colleagues did not investigate the differences in $v\dot{V}O_{2\,max}$ between continuous and discontinuous incremental tests on the treadmill. Nevertheless, in line with these observations, $v\dot{V}O_{2\,max}$ in the present study was significantly higher in the continuous incremental ramp protocol with the steepest ramp slope (R1) compared to that in R2.

At submaximal exercise, lower $\dot{V}O_2$ values at the same submaximal running speed were found compared to SW in both ramp protocols (see Fig. 2), suggesting that ramp slope in

R1 and R2 involved an increase in running velocity with time faster than cardiorespiratory and metabolic adjustments. Interestingly, R2 had a higher slope in $\dot{V}O_2$ *vs* velocity relationship than R1, but not different from that in SW, suggesting that when ramp slope is not too steep, the difference in $\dot{V}O_2$ from SW values at the same work rate may become negligible, although differences in $v\dot{V}O_{2\,max}$ still exist. The fast increments in running velocity with time during ramp protocols possibly delayed also blood La^- accumulation, leading to a higher LaT compared to SW. Remarkably, the ramp with the steeper ramp slope (R1) had also the highest LaT value. Similarly to Kuipers et al. (2003), we can suggest that a steep ramp slope delays blood La^- accumulation during exercise because of the scarce time for La^- equilibration between muscle and blood.

It has been postulated that the slow component, which results in a continuous increase in $\dot{V}O_2$ during heavy- and severe-intensity exercise (Poole et al. 1988), especially at high work rates above the critical power (Whipp 2005), implies that the $\dot{V}O_2$ *vs* work rate relationship is non-linear (Zoladz et al. 1998). Therefore, one can argue that the slow component phenomenon may have affected the present findings and calculations. However, while it should be taken into account that the magnitude of the slow component is considerably lower in treadmill running than in cycling of a similar relative intensity (Carter et al. 2000), the running velocities utilized in the present study for $v\dot{V}O_{2\,max}$ extrapolation were below the exercise intensity at which critical power generally occurs (80-88% $\dot{V}O_{2\,max}$) (Poole et al. 1988).

5. Conclusions

While we were not able to find differences among protocols for $\dot{V}O_{2\,max}$, $v\dot{V}O_{2\,max}$ was higher in the two continuous incremental ramp tests with respect to SW. Therefore, while different testing modalities can be used for $\dot{V}O_{2\,max}$ assessment on the treadmill, care should be taken in choosing the correct testing protocol when the $v\dot{V}O_{2\,max}$ needs to be determined. As $v\dot{V}O_{2\,max}$ is commonly utilized to shape the training intensity and to manipulate the acute physiological responses during training session, a precise $v\dot{V}O_{2\,max}$ assessment can allow coaches and trainers to plan training sessions involving running activities at the correct exercise intensity. Due to the longer time for cardiorespiratory and metabolic adjustments, SW protocol or, at least, a continuous protocol with a mild ramp slope seem to be preferential choices for a more precise $v\dot{V}O_{2\,max}$ assessment. These differences among protocols should be considered when $v\dot{V}O_{2\,max}$ is used for training purposes.

Chapter IV

2nd Study

Comparison between continuous incremental ramp test and discontinuous square-wave test for $v\text{VO}_{2\text{max}}$ assessment in long distance runners and soccer players

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2. **A. Riboli**, E. Limonta, S. Rampichini, E. Cè, A. Veicsteinas, F. Esposito. *Effect of testing modality on the assessment of the velocity at maximum aerobic power in runners and soccer players*. pp.661-661. In Book of abstracts of the 19th annual Congress of the European College of Sport Science, 2nd - 5th July 2014, Amsterdam – The Netherlands.

3. **A. Riboli**, E. Limonta, E. Cè, M. Venturelli, G. Alberti, A. Veicsteinas, F. Esposito. *Comparison between continuous incremental ramp test and discontinuous square-wave test for vVO_{2max} assessment in long distance runners and soccer players.* Sport Sci Health (2014), 10 (suppl. 1): S57-S57.

1. Abstract

Aim: In treadmill testing, the running velocity associated with maximum oxygen uptake ($v\dot{V}O_{2\,max}$) is largely utilized for both laboratory testing and training on the field. Differences between two continuous incremental ramps test and a discontinuous square wave tests (SW) in $v\dot{V}O_{2\,max}$ assessment have been already described. Long distance runners and soccer players are both athletes involved with running. However, the physiological demands are different: in runners are continuous while in soccer players are discontinuous, with an alternation of aerobic and anaerobic tasks. Therefore, the aim of the study was to compare the $v\dot{V}O_{2\,max}$ difference between ramps and SW in both these athletes. Hypothesis is that, this difference should be higher in soccer players than in runners, due to a different capacity to adjust the oxygen transport system at each workload.

Method: Eight runners (RUN) and nine soccer players (SOC) reported to the laboratory three times to perform three maximum incremental tests: R1 (1 km/h per min), R2 (1 km/h every two minutes) and SW (workloads of 4 min each, with 5 min of rest in between), in random order, on a motorised treadmill for $\dot{V}O_{2\,max}$ and $v\dot{V}O_{2\,max}$ assessment. At rest and during exercise, cardiorespiratory and metabolic parameters were collected breath-by-breath. Blood lactate concentration $[La^-]$ was measured at rest and at maximum exercise.

Results: No significant differences between groups and protocols were found in $\dot{V}O_{2\,max}$ (SOC: 3892±104 *vs* 3922±423 ml/min; RUN: 4159±115 *vs* 4170±116, for SW and R1, respectively), as well as in VE, VCO_2, $[La^-]_{peak}$ and HR at maximum exercise; as expected,

only $\dot{V}O_{2\,max}$ expressed in millilitres per minute per kilograms (ml/min/kg) was higher in RUN than SOC (P<0.05). However, $v\dot{V}O_{2\,max}$ was significantly higher in R1 and R2 compared to SW in SOC, while only R1 was significantly higher than SW in RUN. A higher difference between R1 *vs* SW and in R2 *vs* SW was found in SOC than RUN for both ramps (+29% and 16% *vs* SW for R1 and R2 in SOC and +16% and 6% *vs* SW for R1 and R2 in RUN).

Conclusion: Despite similar $\dot{V}O_{2\,max}$ values, $v\dot{V}O_{2\,max}$ was higher in R1 than in SW in both groups; in SOC also vVO_{2max} in R2 was higher than in SW. However, the difference was significantly higher in SOC than RUN, possibly due to a slower capacity to adjust the oxygen transport system to a given workload in SOC. Even though the three protocols can be used to assess $\dot{V}O_{2\,max}$, the $v\dot{V}O_{2\,max}$ differences between protocols must be acknowledged to prescribe correctly high intensity training, especially for soccer players.

2. Aim of the study

In treadmill testing, the running velocity associated with $\dot{V}O_{2\,max}$ ($v\dot{V}O_{2\,max}$) is largely utilized to provide a practical evaluation of aerobic demands during exercise and to plan specific training workloads (Buchheit and Laursen 2013). Two different types of running testing modality are mainly used in $\dot{V}O_{2\,max}$ and $v\dot{V}O_{2\,max}$ assessment in the laboratory: continuous and discontinuous incremental protocols (Billat et al. 1996; Daniels J. 1984). In continuous protocols, the increment in running velocity with time can be faster than the cardiorespiratory and metabolic adjustments. On the contrary, in discontinuous square-wave incremental tests, the cardiorespiratory system can reach a metabolic steady-state condition, thus allowing a more precise matching between mechanical demands during exercise and metabolic response, thus minimizing inaccuracy in $v\dot{V}O_{2\,max}$ calculation. Moreover, in long distance running and soccer, the physiological requirements are different, even though both sports involve running. In long distance runners, indeed, the physiological demands are continuous and mainly aerobic. On the contrary, soccer players run in a discontinuous fashion, with an alternation of aerobic and anaerobic tasks.

The aim of the study was to compare a discontinuous square-wave incremental test and a continuous incremental ramp test for $v\dot{V}O_{2\,max}$ assessment in runners and soccer players. Hypothesis can be made that, in spite of similar $\dot{V}O_{2\,max}$ values, $v\dot{V}O_{2\,max}$ would be higher in the continuous incremental ramp test compared to the discontinuous square-wave test, where cardiorespiratory and metabolic steady-state can be achieved, especially in soccer players.

3. Results

The main cardiorespiratory, metabolic, and perceptual parameters at maximum exercise are given in Table 1 and Table 2. As shown in Figure 1, the $v\dot{V}O_{2\,max}$ was significantly higher in both ramps compared to SW in both groups (18.8 ± 0.5, 22.1 ± 0.3, 19.9 ± 0.3. in RUN and 15.1 ± 0.2, 19.4 ± 0.4, 17.4 ± 0.3 in SOC for SW, R1, and R2, respectively; P<0.05). In Figure 3A the $v\dot{V}O_{2\,max}$ for both R1 and R2 for RUN and SOC is shown in respect to identity line. A significantly difference in slope and intercept between R1 *vs* identity line was found for both RUN and SOC, while in R2 slope and intercept were significantly different only for SOC (see Figure 2A)

When $v\dot{V}O_{2\,max}$ is express as percentage of SW protocol, in RUN, $v\dot{V}O_{2\,max}$ in R1 was significantly higher than in SW (+16 ± 3% for R1 *vs* SW; P<0.05), while no significantly difference for R2 *vs* SW (+6 ± 2% for R2 vs SW; ns) was found. In SOC, $v\dot{V}O_{2\,max}$ in both R1 and R2 was significantly higher than in SW (+29 ± 3% for R1 *vs* SW, and +16 ± 2% in R2 than SW; P<0.05) (see Figure 3).

At peak exercise, in both group no significant differences among the three protocols were found for $\dot{V}O_2$, as well as for f_H, $\dot{V}E$, and [La$^-$]. Only relative $\dot{V}O_{2\,max}$ values (ml/min/kg) differed significantly between RUN *vs* SOC (see Table 1, Table 2, and Figure 2B).

In RPE no significantly differences among groups and protocols were found; only muscular RPE showed significant differences between R1 vs SW in SOC, with a lower perception of muscular effort in R1 compared to SW (see Table 2).

Table 1

		SW	R1	R2
$\dot{V}O_2$ (ml/min)	RUN	4159 (115)	4170 (116)	4133 (117)
	SOC	3892 (103)	3922 (102)	3887 (79)
$\dot{V}O_2$ (ml/min/kg)	RUN	59.0 (1.2)	59.2 (1.2)	58.7 (1.3)
	SOC	54.6 (0.6)$^{\#}$	55.0 (0.9)$^{\#}$	54.6 (0.6)$^{\#}$
$\dot{V}CO_2$ (ml/min)	RUN	4665 (107)	4582 (124)	4493 (111)
	SOC	4341 (114)	4515 (117)	4306 (101)
RER	RUN	1.13 (0.01)	1.10 (0.02)	1.09 (0.02)
	SOC	1.12 (0.02)	1.16 (0.02)	1.11 (0.01)
SaO_2 (%)	RUN	90 (0.9)	90 (0.9)	90 (0.6)
	SOC	90 (1.2)	92 (1.1)	91 (0.9)
f_H (bpm)	RUN	186 (2.0)	188 (2.0)	188 (2.0)
	SOC	187 (1.0)	189 (2.0)	187 (2.0)
$\dot{V}E$ (l/min)	RUN	163 (2.6)	167 (4.7)	164 (4.3)
	SOC	151 (4.3)	155 (4.7)	157 (4.7)
$[La^-]_{peak}$ (mM)	RUN	12.5 (0.5)	13.0 (1.0)	11.4 (0.6)
	SOC	11.8 (0.2)	11.3 (0.3)	10.9 (0.7)

$\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; SaO_2, arterial O_2 saturation; f_H, heart rate; $\dot{V}E$, expiratory ventilation; $[La^-]_{peak}$, peak blood lactate concentration. Variables were determined at maximum exercise in the three testing conditions (SW, square wave protocol; R1, ramp 1; R2, ramp 2) for both long distance runners (RUN) and soccer players (SOC). SE values are given in brackets. # P<0.05 SOC *vs* RUN.

Table 2

		SW	R1	R2
General RPE (au)	RUN	18.0 (0.5)	18.2 (0.4)	17.9 (0.4)
	SOC	19.0 (0.3)	18.2 (0.4)	18.3 (0.4)
Respiratory RPE (au)	RUN	17.7 (0.5)	18.5 (0.4)	17.9 (0.5)
	SOC	19.0 (0.3)	17.6 (0.7)	17.9 (0.6)
Muscular RPE (au)	RUN	18.4 (0.5)	17.4 (0.5)	17.9 (0.6)
	SOC	18.7 (0.6)	16.9 (0.6)*	17.9 (0.5)

The rate of perceived exertion (RPE) at general, respiratory, and muscular level. Variables were determined at maximum exercise in the three testing conditions (SW, square wave protocol; R1, ramp 1; R2, ramp 2) for both long distance runners (RUN) and soccer players (SOC). SE values are given in brackets.

Figure 1

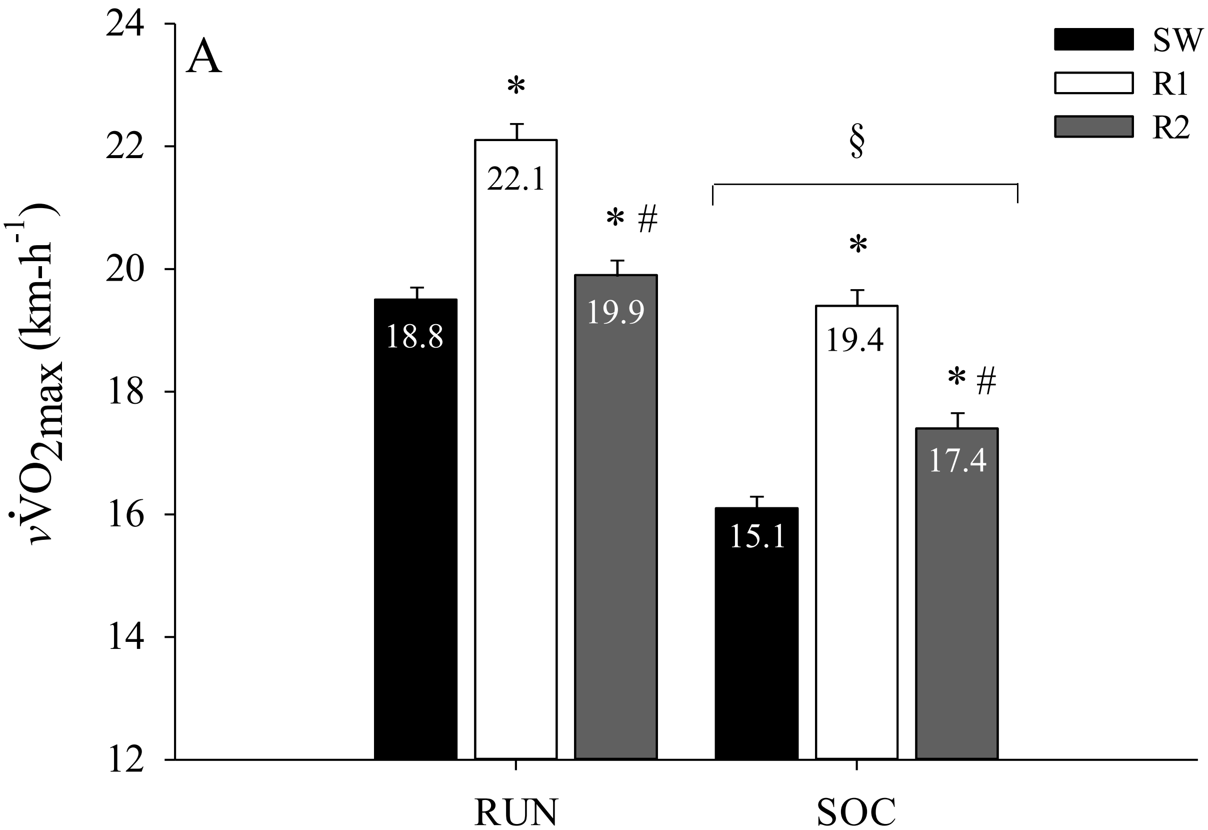
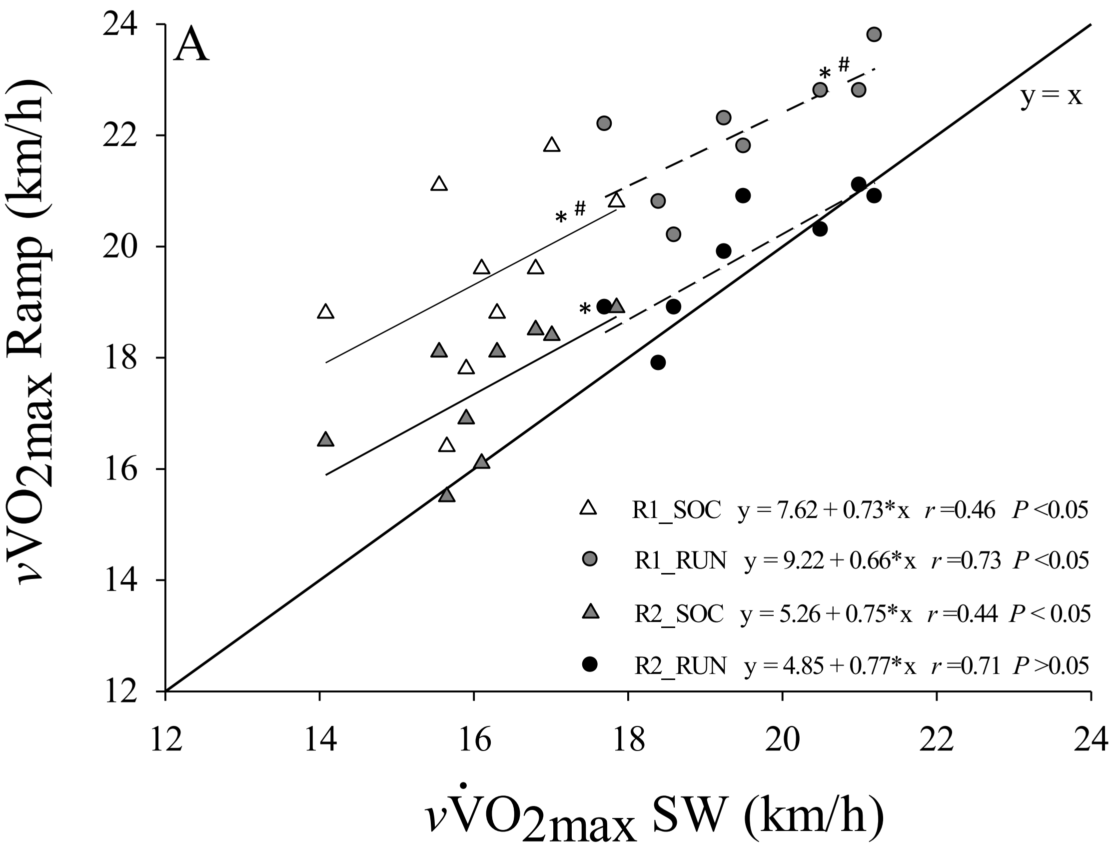


Fig. 1 The velocity associated with maximum oxygen uptake ($v\dot{V}O_{2\,max}$) calculated during square-wave (SW), ramp 1 (R1) and ramp 2 (R2) protocols are presented for both runners (RUN) and soccer players (SOC). *P<0.05 *vs* SW; #P<0.05 *vs* R1; §P<0.05 SOC *vs* RUN

Figure 2A



A
24
22
20
18
16
14
12
12
14
16
18
20
22
24
y = x
* #
* #
*
R1_SOC y = 7.62 + 0.73*x r =0.46 P <0.05
R1_RUN y = 9.22 + 0.66*x r =0.73 P <0.05
R2_SOC y = 5.26 + 0.75*x r =0.44 P < 0.05
R2_RUN y = 4.85 + 0.77*x r =0.71 P >0.05
vV̇O2max Ramp (km/h)
vV̇O2max SW (km/h)

Figure 2B

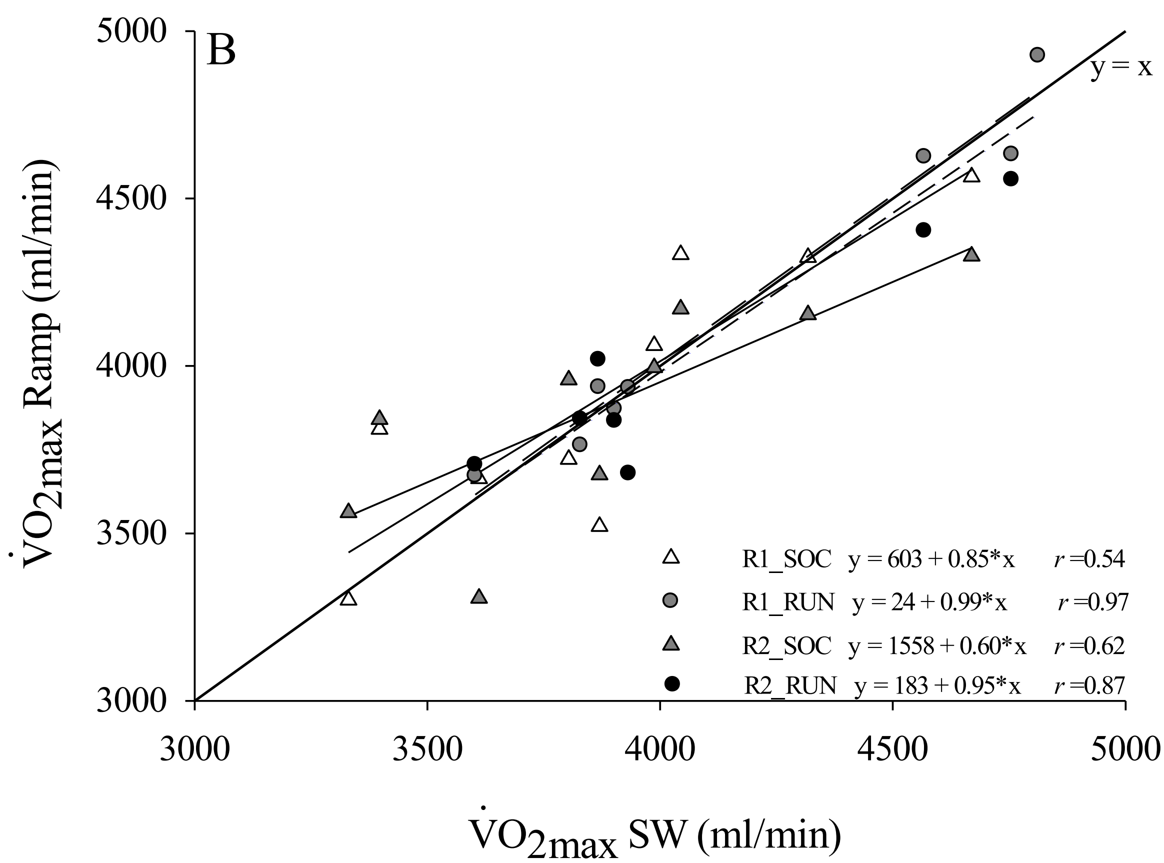


Fig. 2 The relationship between $v\dot{V}O_{2\,max}$ (panel A) and $\dot{V}O_{2\,max}$ (panel B) values determined during continuous (R1 and R2) and discontinuous (square wave, SW) incremental protocols are shown for both RUN and SOC. In both panels, the bold solid line represents the identity line (y = x), the thin solid are the regression lines for the R1 *vs* SW and the R2 *vs* SW for SOC, and the dashed lines are the regression lines for the R1 *vs* SW and the R2 *vs* SW relationships, for RUN. Regression equations and correlation coefficients are also reported. **P*<0.05 *vs* identity line (slope and intercept); $^{\#}P$<0.001 *vs* R2 (intercept).

Figure 3

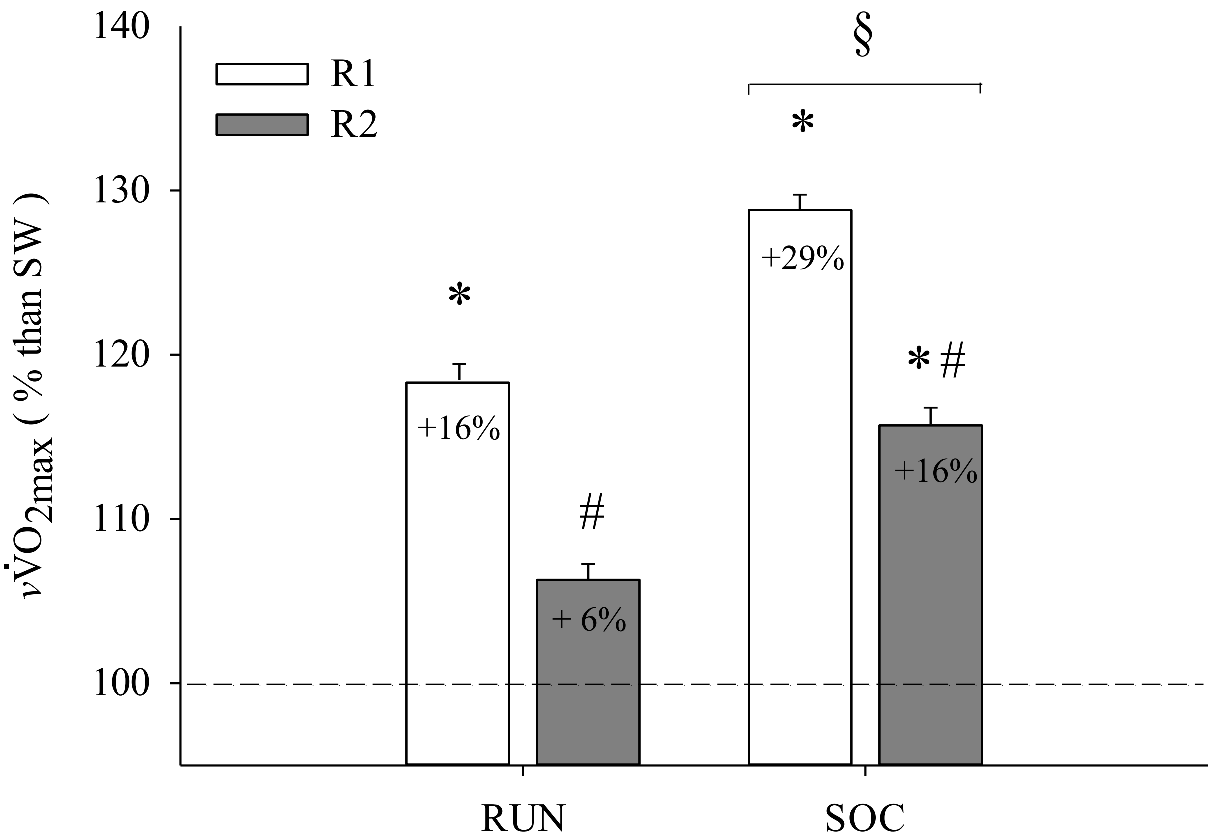


Fig. 3: The velocity associated with maximum oxygen uptake ($v\,\dot{V}O_{2\,max}$) calculated during ramp 1 (R1) and ramp 2 (R2) protocols are presented as a percentage of their relative maximal value determined during the discontinuous incremental square-wave (SW) protocol (dashed line) for both long distance runners (RUN) and soccer players (SOC). $^{*}P<0.05$ *vs* SW; $^{\#}P<0.05$ *vs* R1; $^{\S}P<0.05$ SOC *vs* RUN

4. Discussion

The main and novel finding of the present study was the quantification of the $v\dot{V}O_2$ max differences between continuous and discontinuous protocols in athletes both involved in running but in different way: long distance runners and soccer players.

Despite similar $\dot{V}O_{2\,max}$ values between protocols in both groups, the $v\dot{V}O_{2\,max}$ was significantly higher only in R1 compared to SW in RUN, while the $v\dot{V}O_{2\,max}$ was significantly higher in both ramps compared to SW in SOC. Our data may be explained by the increase in workload (running velocity) with time faster than the cardiorespiratory and metabolic adaptations during continuous incremental exercise, while in SW test a better matching of workload and metabolic power could be achieved. Therefore, R1 and R2 may require a larger intervention of the anaerobic pathway to attain $v\dot{V}O_{2\,max}$ compared to SW, especially in soccer players. To further support this hypothesis, $v\dot{V}O_{2\,max}$ in the continuous incremental ramp test with the lower ramp slope (R2) was significantly higher but closer to that reported in SW in SOC; instead in RUN the $v\dot{V}O_{2\,max}$ wasn't significantly different between R2 compared to SW.

As expected, the present $\dot{V}O_{2\,max}$ results are in line with previous reports on the treadmill, where $\dot{V}O_{2\,max}$ was found to be independent of the protocol adopted (Duncan et al. 1997; McConnell and Clark 1988; Davies et al. 1984; Kirkeberg et al. 2011; Kuipers et al. 2003; Billat et al. 1996). When moving from running (treadmill) to cycling (cycle ergometer), the same findings can be observed (Adami et al. 2013). The lack of significant differences in

$\dot{V}O_{2\,max}$ among protocols in our and in the other investigations is suggestive that all the considered testing modalities were able to challenge maximally the aerobic system.

Even though, no goal standard for $v\dot{V}O_{2\,max}$ assessment is up to now not reported some considerations by other research about $\dot{V}O_{2\,max}$ can be found in literature about SW as a better way to match workload and metabolic power. Astrand PO (2003) considered the discontinuous incremental test with several submaximal, maximal, or supramaximal 5 to 6 min work loads, with or without resting period between each load as the most preferable test to achieve the oxygen uptake increase to a level adequate to the demand. Astrand and Saltin since 1961 don't support the often-quoted statement of a steady state after 1 min of exercise for $\dot{V}O_2$ evaluation. Also Ingham et al. (2013), in rowing, asserted that the association between variable such as $\dot{V}O_{2\,max}$ and the power associated with $\dot{V}O_{2\,max}$ ($w\dot{V}O_{2\,max}$) derived its physiological parameters from discontinuous incremental test. For Bishop et al. (1998a) and Taylor et al. (1955) during $\dot{V}O_2$ assessment, the submaximal work stages should be at least three minutes to allow the athletes to attain $\dot{V}O_2$ steady-state when mapping out aerobic capacity; also other researcher suggested that 3 minutes duration of exercise might be satisfactory for these purposes. (Hawley and Noakes 1992; Padilla et al. 2000). Ingham et al. (2013), based on the works of Thoden (1991b) and Bentley and McNaughton (2003) concluded that one possible rationale for using the longer workloads is that a steady-state of exercise is achieved and thus gas exchange measurements are more indicative of the exercise stress imposed. Therefore, in reasons to the time to attain the cardiorespiratory and metabolic adaptations during exercise, the two continuous ramp protocols could have a increase in workload (running velocity) with time faster than the cardiorespiratory and metabolic adaptations during continuous incremental exercise, especially in soccer players.

Astrand and Saltin since 1961, suggested that two minutes of exercise seem might be sufficient in well-trained subjects to adjust the oxygen transporting system so the $\dot{V}O_{2\,max}$ are attained but it should be emphasized by 4-5 min stage.

The present study revealed a significant effect of testing modality on $v\dot{V}O_{2\,max}$, with a higher value in continuous (R1 and R2) than in discontinuous (SW) incremental protocols.

Kuipers et al. (2003) retrieved similar findings when utilizing three continuous incremental ramp protocols with different ramp slopes (1 $km \cdot h^{-1}$ increment per minute, 2 $km \cdot h^{-1}$ increment every 3 minutes, and 2 $km \cdot h^{-1}$ every 6 minutes). $v\dot{V}O_{2\,max}$ was different among conditions, with the fastest speed for the ramp with the steepest ramp slope, as in this study for both groups. However, Kuipers and co-workers defined $v\dot{V}O_{2\,max}$ as the maximal running speed calculated from the last ramp stage was utilized and not as the minimal velocity that elicits $\dot{V}O_{2\,max}$. Moreover, they did not compare the continuous incremental ramp tests to a discontinuous protocol. Interestingly, when protocols vary in parameters other that ramp slope, no differences in $v\dot{V}O_{2\,max}$ are found. Indeed, Billat et al. (1996) utilized two ramp protocols with different step increments but same ramp slope, and found no differences in $v\dot{V}O_{2\,max}$. This finding may suggest that step increments alone do not affect $v\dot{V}O_{2\,max}$ assessment.

Athletes involved in different running activities had a different time to adjustment at the onset of exercise presenting a faster $\dot{V}O_2$-kinetics higher the aerobic performance ability (Poole and Jones 2012). For a given metabolic demand, fast $\dot{V}O_2$-kinetics mandates a smaller O_2 deficit, less substrate-level phosphorylation and high exercise tolerance (Dupont et al. 2005). The time constant of phase II of $\dot{V}O_2$-kinetics has been shown to be shorter in the trained, compared with the untrained population (Koppo et al. 2004). The adaptations to endurance exercise training enabled an individual to adjust to the energy requirement of exercise more rapidly, resulting in a smaller O_2 deficit (Dupont et al. 2005). In their study, Dupont et al. (2005) found that soccer player with a faster $\dot{V}O_2$-kinetics had a significantly less decrease during repeated sprint and concluded that faster $\dot{V}O_2$-adjustment at the onset of exercise might lead to a greater contribution of oxidative phosphorylation and a smaller O_2 deficit to the total energy expenditure for this kind of exercise.

The $v\dot{V}O_{2\,max}$ in SOC was significantly higher in both ramps (+29 % and +16 % for R1 and R2 *vs* SW, respectively), while in RUN the percentage of the difference among protocols showed a higher $v\dot{V}O_{2\,max}$ only in R1 *vs* SW (+16%). The higher difference between R1 *vs* SW for SOC and RUN (+29% *vs* +16% for SOC *vs* RUN, respectively) can be explained by the different physical capability (see $\dot{V}O_{2\,max}$ expressed as relative values, ml/min/kg, Table 1). Indeed, $\tau\dot{V}O_2$ at the onset of exercise is faster in athletes with higher $\dot{V}O_{2\,max}$ and more involved in aerobic performance; Poole and Jones (2012) illustrated that the $\tau\dot{V}O_2$ is a fundamental parameter of aerobic performance that may help to explain the broad range of physical/athletic capabilities and exercise tolerance across populations. Therefore, less difference between R2 *vs* SW in RUN highlights that continuous incremental ramp protocol with at least 2 min can be sufficient in well-trained aerobic athletes to reach adjustments between workload and metabolic power, while not in SOC.

5. Conclusions

While we were not able to find differences among protocols for $\dot{V}O_{2\,max}$, $v\dot{V}O_{2\,max}$ was higher in the two continuous incremental ramp tests with respect to SW in both groups. Therefore, while different testing modalities can be used for $\dot{V}O_{2\,max}$ assessment on the treadmill, care should be taken in choosing the correct testing protocol when the $v\dot{V}O_{2\,max}$ needs to be determined. Indeed the difference among protocols were higher in SOC than RUN, explained by a different $\tau\dot{V}O_2$ that is faster in well trained athlete. As $v\dot{V}O_{2\,max}$ is commonly utilized to shape the training intensity and to manipulate the acute physiological responses during training session, a precise $v\dot{V}O_{2\,max}$ assessment can allow coaches and trainers to plan training sessions involving running activities at the correct exercise intensity. Due to the longer time for cardiorespiratory and metabolic adjustments, SW protocol or, at least, a continuous protocol with a mild ramp slope seem to be preferential choices for a more precise $v\dot{V}O_{2\,max}$ assessment. These differences among protocols should be considered when $v\dot{V}O_{2\,max}$ is used for training purposes, especially for SOC.

Chapter V

3rd study

Effect of ramp slope on different methods to assess lactate threshold

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1. Abstract

Purpose: The lactate threshold (LaT), an important physiological parameter both in the laboratory and on the field, is usually determined during continuous incremental ramp tests. The slope of the increase in running velocity with time (ramp slope), though, may affect LaT assessment. Therefore, the aim of this study was to determine which of the methods that are commonly utilized to assess LaT would be less sensitive to differences in ramp slope.

Methods: Sixteen participants performed on a treadmill two maximum incremental continuous ramp protocols (1 km·h^{-1} per min, R1, and 2 km·h^{-1} per min, R2) on different days, in random order. At rest and during exercise, cardiorespiratory and metabolic parameters were collected breath-by-breath. Blood lactate concentration $[La^-]_b$ was measured at rest, during, and at peak exercise. In both protocols, LaT was calculated by D_{MAX}, $D_{MAX\ MOD}$, 4 mM, Δ1 mM and Log-Log methods.

Results LaT had higher velocities in R1 for D_{MAX} (16.5±0.4 *vs* 15.1±0.4 km·h^{-1}, *P*=0.002), $D_{MAX\ MOD}$ (17.7±0.5 *vs* 15.6±0.4 km·h^{-1}, *P*<0.001), 4 mM (17.0±0.6 *vs* 15.5±0.5 km·h^{-1}, *P*<0.001), Δ1 mM (17.1±0.5 *vs* 15.1±0.4 km·h^{-1}, *P*<0.001), but not for Log-Log.

Conclusions: Care must be taken with the protocol choice and the method used for LaT determination because testing modality influenced LaT assessment. Indeed, with the only exception of Log-Log, all the other methods presented significantly higher velocities at LaT when the steeper ramp slope (R1) was utilized.

Keywords: Incremental ramp test; testing modality; lactate curve, treadmill

2. Aim of the study

Since the early work of Hill and Lupton (1923), the success in aerobic performance has been associated with a high peak oxygen uptake ($\dot{V}O_{2\,peak}$). However, it has been suggested that also parameters at submaximal exercise can provide a useful prediction of endurance performance (Bosquet et al. 2002; Farrell et al. 1979; Yoshida et al. 1987). For instance, the lactate threshold (LaT), the work rate at which blood lactate concentration ([La^-]) starts to increase above resting levels (Brooks 1985) has been shown to be strictly related to endurance performance both in trained (Farrell et al. 1979) and untrained individuals (Yoshida et al. 1987). LaT is commonly used to assess the effects of a training intervention, evaluate physical fitness, and determine the workload intensity during aerobic activities (Bishop et al. 1998b; Allen et al. 1985).

The variety of testing protocols and criteria to assess LaT may lead to considerable lack of clarity (Faude et al. 2009). Indeed, several types of continuous incremental ramp protocols are generally used to determine the rise in [La^-] with work rate for LaT assessment. However, varying the stage duration or work rate increments, thus determining a different work rate increments *vs* time slope (ramp slope), may lead to possible differences in [La^-] *vs* work rate plots and, in turn, in LaT calculation.(Foxdal et al. 1994) Physiological explanation was given that the time allowed for La^- diffusion in the blood until the next work rate increment may differ with ramp slope (Bentley et al. 2007).

Several different methods have been proposed to determine LaT over the years, among which fixed [La^-] levels, such as 4 mM (Sjodin and Jacobs 1981), or the work rate at which the first increase in [La^-] of 1 mM (Δ1 mM) above resting levels occurs (Thoden 1991a).

To date, though, no generally accepted fitting procedure has been established (Bentley et al. 2007). However, the analysis of the whole [La^-] curve is considered more appropriate to assess LaT compared to fixed levels methods (Faude et al. 2009).

Therefore, a systematic study investigating which method for LaT assessment on the treadmill is less affected by ramp slope is still lacking. On these bases, the aim of the study was to determine which of the methods that are commonly utilized to assess LaT (D_{MAX}, $D_{MAX\ MOD}$, 4 mM, Δ1 mM and Log-Log) would be less sensitive to differences in ramp slope.

3. Results

As shown in table 1, in cardiorespiratory and metabolic parameters ($\dot{V}O_2$, RER, SaO_2, f_H, $\dot{V}E$, $[La^-]_{peak}$) no differences were found between protocols. The average RPE values at the end of the tests are shown in Table 1.

Table 1 – Cardiorespiratory, metabolic and perceptual variables at maximum exercise

	R1	R2
$\dot{V}O_2$ (ml·min^{-1})	4070 (112)	4023 (104)
RER	1.13 (0.02)	1.11 (0.02)
SaO_2 (%)	91.3 (0.8)	90.1 (0.5)
f_H (bpm)	188 (2)	188 (2)
$\dot{V}E$ (l·min^{-1})	161 (5)	160 (4)
$[La^-]$ (mM)	12.3 (0.7)	12.1 (0.5)
General RPE (au)	18.3 (0.3)	18.1 (0.3)
Respiratory RPE (au)	18.1 (0.4)	17.8 (0.4)
Muscular RPE (au)	17.2 (0.4)	17.9 (0.4)

$\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; SaO_2, arterial O_2 saturation; f_H, heart rate; $\dot{V}E$, expiratory ventilation; $[La^-]$, blood lactate concentration; and RPE, rate of perceived exertion at general, respiratory and muscular level. Variables were determined at maximum exercise in the two testing conditions (R1, ramp 1; R2, ramp 2). SE values are given in brackets.

In table 2, the comparisons between methods for LaT assessment (D_{MAX}, $D_{MAX\ MOD}$, 4 mM, Δ1 mM, and Log-Log) measured by both incremental continuous ramp protocols (R1 and R2) are shown. Differences among LaT methods were found in R1 (P<0.001, η^2_P = 0.798), while no differences among LaT methods were found in R2 (P = 0.06, η^2_P =0.505). Moreover, some differences in LaT assessment were found between R1 and R2 (P<0.001, η^2_P = 0.709). In particular, LaT measured by Log-Log method in R1 was significantly lower than LaT measured by $D_{MAX\ MOD}$ (P<0.001, ES: -0.90, IC: -1.61/-0.20), 4 mM (P<0.001, ES: -0.57; IC: -1.18/0.18) and Δ1 mM (P<0.001, ES: -0.57, IC: -1.26/0.12) methods (see table 2). The LaT measured by $D_{MAX\ MOD}$ method was significantly higher also than D_{MAX} (see table 2, P<0.001, ES: 0.65, IC: -0.04/1.34). In R1, when LaT is expressed as $\dot{V}O_2$ (ml·min^{-1}), the Log-Log method returned a value significantly lower than $D_{MAX\ MOD}$ (P=0.029, ES: -0.57, IC: -1.26/0.12). However, both in R1 and R2, when LaT is expressed as a percentage of $\dot{V}O_{2\ peak}$ (%$\dot{V}O_{2\ peak}$), no significantly differences among methods and protocols were found.

When comparing LaT determined by the same method, a significantly higher LaT during R1 than R2 was found for D_{MAX} (P=0.002, ES: 3.17, CI: 2.16/4.18), $D_{MAX\ MOD}$ (P<0.001, ES: 1.00, CI: 0.29/1.71), 4 mM (P<0.001, ES: 0.57, CI: -0.11/1.26), and Δ1 mM (P=0.001, ES: 1.02, CI: 0.30/1.73) (see table 2 and Fig. 1). Conversely, when LaT was identified as $\dot{V}O_2$ (ml·min^{-1}) or %$\dot{V}O_{2\ peak}$, no differences between protocols were found.

Table 2 – Lactate thresholds

		D_{MAX}	$D_{MAX\ MOD}$	4 mM	Δ1 mM	Log-Log
R1	Velocity ($km{\cdot}h^{-1}$)	16.5 (0.4)	17.7 (0.5) $^{\#,\circ}$	17.0 (0.6) $^{\#}$	17.1 (0.5) $^{\#}$	15.9 (0.5)
	$\dot{V}O_2$ ($ml{\cdot}min^{-1}$)	3521 (96)	3712 (102)	3642 (112)	3641 (120)	3463 (112)
	$\dot{V}O_2$ (% $\dot{V}O_{2\ peak}$)	86.7 (1.5)	91.3 (1.2)	89.5 (1.3)	89.5 (1.8)	85.2 (2.0)
R2	Velocity ($km{\cdot}h^{-1}$)	15.1 (0.4)*	15.7 (0.5)*	15.7 (0.5)*	15.1 (0.4)*	15.5 (0.5)
	$\dot{V}O_2$ ($ml{\cdot}min^{-1}$)	3479 (107)	3586 (116)	3560 (125)	3491 (115)	3544 (134)
	$\dot{V}O_2$ (% $\dot{V}O_{2\ peak}$)	86.4 (1.3)	89.0 (1.0)	88.2 (1.2)	86.7 (1.6)	87.9 (1.9)

LaT (lactate threshold, $km{\cdot}h^{-1}$), calculated with the five methods (D_{MAX} , $D_{MAX\ MOD}$, 4 mM, Δ1 mM, Log-Log), and the corresponding oxygen uptake ($\dot{V}O_2$, expressed as $ml{\cdot}min^{-1}$ and % $\dot{V}O_{2\ peak}$), were determined in the two testing conditions (R1, ramp 1; R2, ramp 2). SE values are given in brackets.

$^{\#}P<0.05$ *vs* Log-Log; $^{\circ}P<0.001$ *vs* D_{MAX}; $^{*}P<0.001$ *vs* R1.

Figure 1

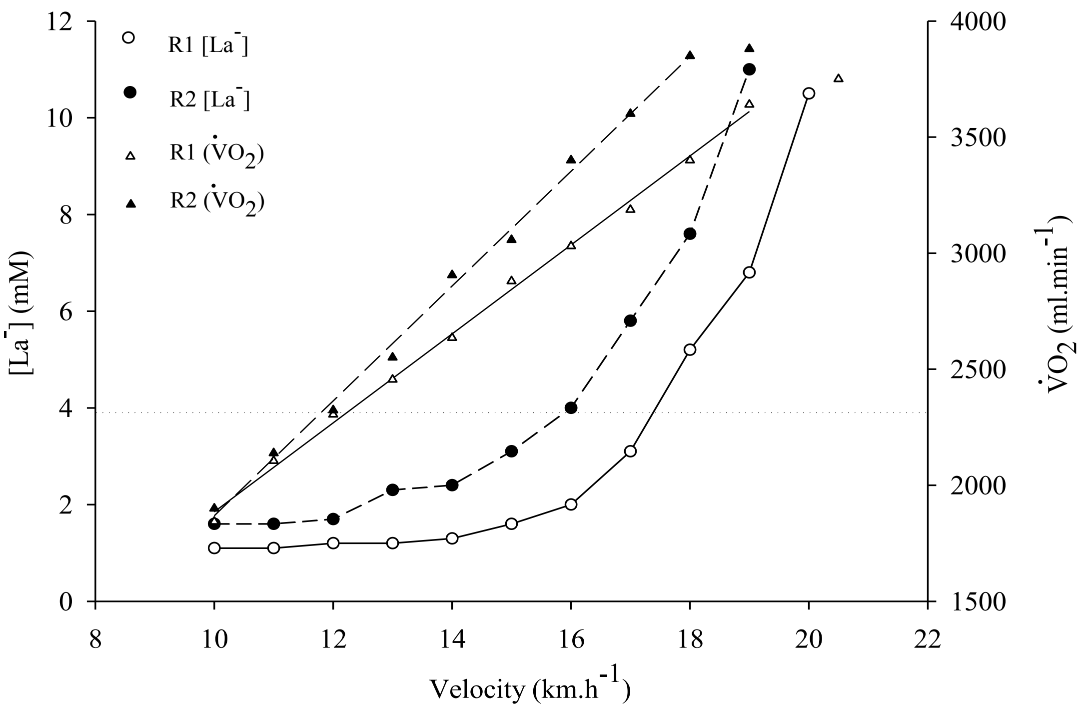

Fig. 1 Blood lactate concentration ([La$^-$]) as a function of running velocity in R1 (ramp 1, open circles with solid line) and R2 (ramp 2, closed circles with dashed line) in a representative participant. $\dot{V}O_2$ as a function of running velocity is also given for R1 (ramp 1, open triangles) and R2 (ramp 2, closed triangles) for the same subject. The regression lines for the $\dot{V}O_2$ vs running velocity at submaximal level are also drawn for R1 (solid line) and R2 (dashed line). The horizontal dotted line shows LaT determined by the 4 mM method.

In Fig. 2, the LaT difference (ΔLaT) measured by the same method between R1 and R2 are shown (see fig. 1). The ΔLaT for Log-Log was significantly lower than the ΔLaT for $D_{MAX\ MOD}$ (P= 0.007, ES: -0.92, CI: -1.62/-0.21) and Δ1mM (P= 0.04, *ES*: -0.81, CI: -1.51/-0.11).

Figure 2

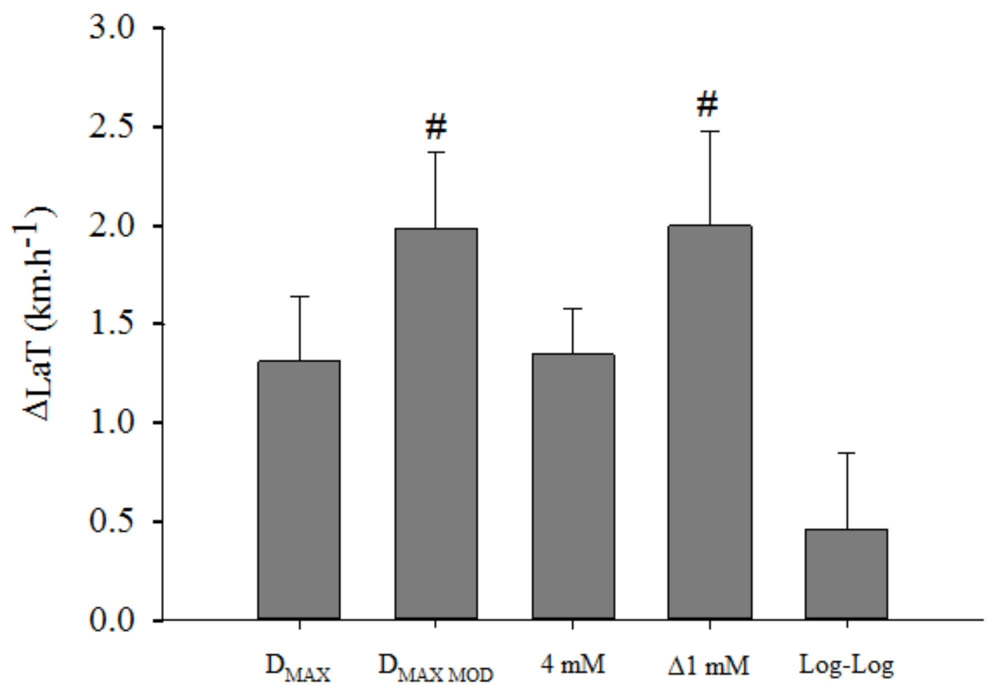

Fig. 2 Difference in LaT (ΔLaT) between R1 and R2 for each threshold detection method (D_{MAX}, $D_{MAX\ MOD}$, 4 mM, Δ1mM, and Log-Log). $^{\#}P$<0.05 *vs* Log-Log.

4. Discussion

The main finding of the present study was that LaT, expressed as running velocity, shifted toward faster velocities in the protocol with the steeper ramp slope (R1) in all methods, with the only exception of Log-Log.

Faster velocities at LaT were observed in R1 for D_{MAX}, $D_{MAX\ MOD}$, 4 mM, and Δ1 mM. This finding well agrees with previous studies reporting that LaT might be overestimated when steeper ramp slopes are used (Kuipers et al. 1985; Foxdal et al. 1996; Yoshida 1984; Bentley et al. 2007) since La^- is time-dependently produced from skeletal muscle (Davis et al. 1982; Whipp et al. 1981; Yoshida 1984). Proven that a valid estimation of [La-] from arterialized blood samples for a given workload could be obtained if blood sampling is performed under a steady state condition (Forster et al. 1972), a possible explanation for the present findings may come from the faster changes in intramuscular La^- accumulation rate when a steeper ramp slope is adopted. Indeed, the fast increments in running velocity with time during R1 may have delayed La^- accumulation in the blood and therefore prevented the equilibrium between muscle and blood [La^-] (Gavin et al. 2014). Interestingly, LaT differences among protocols disappeared with R2, suggesting that when the ramp slope allows a greater attainment of equilibrium between muscle and blood [La^-], all methods provided similar LaT values.

However, there was no protocol effect in none of the LaT methods investigated when LaT was expressed as $\dot{V}O_2$. Previous studies found that $\dot{V}O_2$ at LaT is independent of the protocol adopted (Davies et al. 1984; Kirkeberg et al. 2011; Kuipers et al. 2003; Billat et al. 1996) even though the relative workloads are higher for the ramp with the steeper ramp slope (Bentley et al. 2007). These results suggest that the protocol with shorter steps involve an increase in workload with time faster than cardiorespiratory and metabolic adjustments, thus inducing higher workloads but similar $\dot{V}O_2$ at LaT. In previous studies, several authors suggested that LaT was independent of the protocol adopted when expressed as $\dot{V}O_2$ or % $\dot{V}O_{2\,peak}$ (Weston et al. 2002; Yoshida 1984; McLellan 1985).

This is probably why the Log-Log was the only method not affected by ramp slope. In the Log-Log model, indeed, the pattern of $[La^-]_b$ changes is obtained using a transformation defined by plotting $\log([La^-]_b)$ *vs* $\log(\dot{V}O_2)$. LaT is therefore determined using a linear regression analysis and defined by the transition phase in the underlying relationship between $[La^-]_b$ and $\dot{V}O_2$.

Noticeably, ΔLaT between R1 *vs* R2 was significantly different from that calculated for Log-Log only for $D_{MAX\ MOD}$ and Δ1 mM (see Figure 2). This finding suggests that D_{MAX} and 4 mM, although affected by ramp slope, nevertheless provide LaT values in terms of running velocity not very far from Log-Log.

5. Practical Applications

Differences in ramp slope protocols and methods for LaT determination should be taken into account when testing. Indeed, misestimating LaT may lead to an inappropriate planning of workloads when using LaT as a reference to determine training intensity. Differences between protocols are not a matter of problem when $\dot{V}O_2$ is measured and

Log-Log- method can be therefore used. However, when $\dot{V}O_2$ assessment is not available, D_{MAX} and 4 mM methods should be preferably chosen.

6. Conclusions

In conclusion, care must be taken with the protocol choice and the method used for LaT determination because testing modality influenced significantly LaT assessment. Indeed, with the only exception of Log-Log method, all the other methods presented significantly higher velocities at LaT when the steeper slope (R1) of the velocity *vs* time relationship was utilized. Furthermore, differences among LaT methods were retrieved in R1 but not in R2, suggesting that a ramp protocol with a less steep ramp slope could be a better choice for LaT determination due to a reasonable greater attainment of equilibrium between muscle and blood [La^-].

General conclusions

The $\dot{V}O_{2\,max}$ and $v\dot{V}O_{2\,max}$ at maximum exercise, and LaT at submaximal exercise are parameters largely utilized to determine individual physiological profile and work rate on the field both in individual and team sports. However the protocol used in laboratory introduces significantly differences in both $v\dot{V}O_{2\,max}$ and LaT, while all testing modality can be utilized for $\dot{V}O_{2\,max}$ determination. Comparing continuous incremental ramp protocols and discontinuous incremental square-wave (SW) protocol, a significantly higher $v\dot{V}O_{2\,max}$ was found in ramps than SW. Moreover, also comparing ramps each other, steeper the running velocity versus time slope, higher the $v\dot{V}O_{2\,max}$. Similar results in LaT determination were found, due to a not reach equilibrium between muscle and blood lactate accumulation; a higher LaT in the protocol with the steeper velocity *vs* time slope was found.

When long distance runners (RUN) and soccer players (SOC) are compare, a higher $\dot{V}O_{2\,max}$ per kilogram and per minute was found in RUN than SOC, while no other differences in cardiorespiratory and metabolic parameters at maximum exercise were measured. Using the three different testing modality, in both groups the protocol with the steeper velocity *vs* time slope determine a higher $v\dot{V}O_{2\,max}$; a significantly higher different between R1 *vs* SW and R2 *vs* SW in SOC than RUN were found. In SOC each protocols differ each other in $v\dot{V}O_{2\,max}$ determination. Instead, in RUN no significantly difference between R2 *vs* SW was found possibly due to a faster capacity to adjust the cardiorespiratory and metabolic system at each workload than SOC.

Our data may be explained by the increase in workload (running velocity) with time faster than the cardiorespiratory and metabolic adaptations during continuous incremental exercise, while in SW test a better matching of workload and metabolic power could be achieved. Therefore, R1 and R2 may require a larger intervention of the anaerobic pathway to attain $v\dot{V}O_{2\,max}$ compared to SW. To further support this hypothesis, $v\dot{V}O_{2\,max}$ in the continuous incremental ramp test with the lower ramp slope (R2) was closer to that reported in SW, and not different than SW when RUN was considered. Indeed, more aerobic athletes, probably have a faster $\dot{V}O_2$ - kinetics connected with a faster capacity to adjust cardiorespiratory and metabolic at each submaximal workload.

Lastly, even though the three protocols can be used to assess $\dot{V}O_{2\,max}$ in both RUN and SOC, the $v\dot{V}O_{2\,max}$ differences between protocols must be acknowledged to prescribe correctly high intensity training in each athlete, but especially for soccer players.

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References

Abe D, Yanagawa K, Yamanobe K, Tamura K (1998) Assessment of middle-distance running performance in sub-elite young runners using energy cost of running. European journal of applied physiology and occupational physiology 77 (4):320-325. doi:10.1007/s004210050340

Adami A, Sivieri A, Moia C, Perini R, Ferretti G (2013) Effects of step duration in incremental ramp protocols on peak power and maximal oxygen consumption. European journal of applied physiology 113 (10):2647-2653. doi:10.1007/s00421-013-2705-9

Allen WK, Seals DR, Hurley BF, Ehsani AA, Hagberg JM (1985) Lactate threshold and distance-running performance in young and older endurance athletes. Journal of applied physiology 58 (4):1281-1284

Altenburg TM, Degens H, van Mechelen W, Sargeant AJ, de Haan A (2007) Recruitment of single muscle fibers during submaximal cycling exercise. Journal of applied physiology 103 (5):1752-1756. doi:10.1152/japplphysiol.00496.2007

Astrand PO RK, Dahl HA, Stromme SB (2003) Textbook of work physiology. Physiological bases, fourth ed.

Astrand PO, Rodahl K, Dahl HA, Stromme SB (2003) Textbook of work physiology. Physiological bases of Exercise (fourth ed.). Human Kinetics, Champain (IL, USA)

Baldari C, Bonavolonta V, Emerenziani GP, Gallotta MC, Silva AJ, Guidetti L (2009) Accuracy, reliability, linearity of Accutrend and Lactate Pro versus EBIO plus analyzer. European journal of applied physiology 107 (1):105-111. doi:10.1007/s00421-009-1107-5

Bangsbo J, Lindquist F (1992) Comparison of various exercise tests with endurance performance during soccer in professional players. International journal of sports medicine 13 (2):125-132. doi:10.1055/s-2007-1021243

Beaver WL, Wasserman K, Whipp BJ (1985) Improved detection of lactate threshold during exercise using a log-log transformation. Journal of applied physiology 59 (6):1936-1940

Bentley DJ, McNaughton LR (2003) Comparison of W(peak), VO2(peak) and the ventilation threshold from two different incremental exercise tests: relationship to endurance performance. Journal of science and medicine in sport / Sports Medicine Australia 6 (4):422-435

Bentley DJ, Newell J, Bishop D (2007) Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes. Sports medicine 37 (7):575-586

Bentley DJ, Wilson GJ, Davie AJ, Zhou S (1998) Correlations between peak power output, muscular strength and cycle time trial performance in triathletes. The Journal of sports medicine and physical fitness 38 (3):201-207

Bernard O, Maddio F, Ouattara S, Jimenez C, Charpenet A, Melin B, Bittel J (1998) Influence of the oxygen uptake slow component on the aerobic energy cost of high-intensity submaximal treadmill running in humans. European journal of applied physiology and occupational physiology 78 (6):578-585. doi:10.1007/s004210050464

Bernard O, Ouattara S, Maddio F, Jimenez C, Charpenet A, Melin B, Bittel J (2000) Determination of the velocity associated with VO2max. Medicine and science in sports and exercise 32 (2):464-470

Berthoin S, Gerbeaux M, Turpin E, Guerrin F, Lensel-Corbeil G, Vandendorpe F (1994) Comparison of two field tests to estimate maximum aerobic speed. Journal of sports sciences 12 (4):355-362. doi:10.1080/02640419408732181

Berthon P, Fellmann N (2002) General review of maximal aerobic velocity measurement at laboratory. Proposition of a new simplified protocol for maximal aerobic velocity assessment. The Journal of sports medicine and physical fitness 42 (3):257-266

Billat LV, Koralsztein JP (1996) Significance of the velocity at VO2max and time to exhaustion at this velocity. Sports medicine 22 (2):90-108

Billat V, Renoux JC, Pinoteau J, Petit B, Koralsztein JP (1994) Reproducibility of running time to exhaustion at VO2max in subelite runners. Medicine and science in sports and exercise 26 (2):254-257

Billat VL (2000) VO2 slow component and performance in endurance sports. British journal of sports medicine 34 (2):83-85

Billat VL, Blondel N, Berthoin S (1999) Determination of the velocity associated with the longest time to exhaustion at maximal oxygen uptake. European journal of applied physiology and occupational physiology 80 (2):159-161. doi:10.1007/s004210050573

Billat VL, Bocquet V, Slawinski J, Laffite L, Demarle A, Chassaing P, Koralsztein JP (2000) Effect of a prior intermittent run at vVO2max on oxygen kinetics during an all-out severe run in humans. The Journal of sports medicine and physical fitness 40 (3):185-194

Billat VL, Hill DW, Pinoteau J, Petit B, Koralsztein JP (1996) Effect of protocol on determination of velocity at VO2 max and on its time to exhaustion. Archives of physiology and biochemistry 104 (3):313-321. doi:10.1076/apab.104.3.313.12908

Billat VL, Lepretre PM, Heubert RP, Koralsztein JP, Gazeau FP (2003) Influence of acute moderate hypoxia on time to exhaustion at vVO2max in unacclimatized runners. International journal of sports medicine 24 (1):9-14. doi:10.1055/s-2003-37251

Bishop D, Jenkins DG, Mackinnon LT (1998a) The effect of stage duration on the calculation of peak VO2 during cycle ergometry. Journal of science and medicine in sport / Sports Medicine Australia 1 (3):171-178

Bishop D, Jenkins DG, Mackinnon LT (1998b) The relationship between plasma lactate parameters, Wpeak and 1-h cycling performance in women. Medicine and science in sports and exercise 30 (8):1270-1275

Bosquet L, Leger L, Legros P (2002) Methods to determine aerobic endurance. Sports medicine 32 (11):675-700

Brooks GA (1985) Anaerobic threshold: review of the concept and directions for future research. Medicine and science in sports and exercise 17 (1):22-34

Buchheit M, Hader K, Mendez-Villanueva A (2012) Tolerance to high-intensity intermittent running exercise: do oxygen uptake kinetics really matter? Frontiers in physiology 3:406. doi:10.3389/fphys.2012.00406

Buchheit M, Laursen PB (2013) High-intensity interval training, solutions to the programming puzzle: Part I: cardiopulmonary emphasis. Sports medicine 43 (5):313-338. doi:10.1007/s40279-013-0029-x

Byrnes WC, Clarkson PM, White JS, Hsieh SS, Frykman PN, Maughan RJ (1985) Delayed onset muscle soreness following repeated bouts of downhill running. Journal of applied physiology 59 (3):710-715

Carter H, Jones AM, Barstow TJ, Burnley M, Williams CA, Doust JH (2000) Oxygen uptake kinetics in treadmill running and cycle ergometry: a comparison. Journal of applied physiology 89 (3):899-907

Cheng B, Kuipers H, Snyder AC, Keizer HA, Jeukendrup A, Hesselink M (1992) A new approach for the determination of ventilatory and lactate thresholds. International journal of sports medicine 13 (7):518-522. doi:10.1055/s-2007-1021309

Coyle EF (1995) Integration of the physiological factors determining endurance performance ability. Exercise and sport sciences reviews 23:25-63

Coyle EF, Martin WH, Ehsani AA, Hagberg JM, Bloomfield SA, Sinacore DR, Holloszy JO (1983) Blood lactate threshold in some well-trained ischemic heart disease patients. Journal of applied physiology: respiratory, environmental and exercise physiology 54 (1):18-23

Daniels J. SN Elite and subelite female middle- and long-distance runners. In: Landers DM e (ed) 1984 Olympic Scientific Congress, Oregon. Champaign (IL) Human Kinetics, Jul 19-23 1984. pp 57-72

Daniels JT, Scardina N Elite and subelite female middle- and long-distance runners. In: Landers DM e (ed) 1984 Olympic Scientific Congress, Oregon. Champaign (IL) Human Kinetics, Jul 19-23 1984. pp 57-72

Davies B, Daggett A, Jakeman P, Mulhall J (1984) Maximum oxygen uptake utilising different treadmill protocols. British journal of sports medicine 18 (2):74-79

Davis JA, Whipp BJ, Lamarra N, Huntsman DJ, Frank MH, Wasserman K (1982) Effect of ramp slope on determination of aerobic parameters from the ramp exercise test. Medicine and science in sports and exercise 14 (5):339-343

Denadai BS, Ortiz MJ, Greco CC, de Mello MT (2006) Interval training at 95% and 100% of the velocity at VO2 max: effects on aerobic physiological indexes and running performance. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 31 (6):737-743. doi:10.1139/h06-080

di Prampero PE (1986) The energy cost of human locomotion on land and in water. Int J Sports Med 7:55-72

di Prampero PE, Atchou G, Bruckner JC, Moia C (1986) The energetics of endurance running. European journal of applied physiology and occupational physiology 55 (3):259-266

Duncan GE, Howley ET, Johnson BN (1997) Applicability of VO2max criteria: discontinuous versus continuous protocols. Medicine and science in sports and exercise 29 (2):273-278

Dupont G, Blondel N, Lensel G, Berthoin S (2002) Critical velocity and time spent at a high level of VO2 for short intermittent runs at supramaximal velocities. Canadian journal of applied physiology = Revue canadienne de physiologie appliquee 27 (2):103-115

Dupont G, Millet GP, Guinhouya C, Berthoin S (2005) Relationship between oxygen uptake kinetics and performance in repeated running sprints. European journal of applied physiology 95 (1):27-34. doi:10.1007/s00421-005-1382-8

Dupont G, Moalla W, Guinhouya C, Ahmaidi S, Berthoin S (2004) Passive versus active recovery during high-intensity intermittent exercises. Medicine and science in sports and exercise 36 (2):302-308. doi:10.1249/01.MSS.0000113477.11431.59

Fabre N, Balestreri F, Pellegrini B, Schena F (2010) The modified Dmax method is reliable to predict the second ventilatory threshold in elite cross-country skiers. Journal of strength and conditioning research / National Strength & Conditioning Association 24 (6):1546-1552. doi:10.1519/JSC.0b013e3181dc450a

Farrell PA, Wilmore JH, Coyle EF, Billing JE, Costill DL (1979) Plasma lactate accumulation and distance running performance. Medicine and science in sports 11 (4):338-344

Faude O, Kindermann W, Meyer T (2009) Lactate threshold concepts: how valid are they? Sports medicine 39 (6):469-490. doi:10.2165/00007256-200939060-00003

Ferretti G (2014) Maximal oxygen consumption in healthy humans: theories and facts. European journal of applied physiology 114 (10):2007-2036. doi:10.1007/s00421-014-2911-0

Ferretti G (2015) Energetics of muscular exercise. Springer, Heidelberg (Germany)

Forster HV, Dempsey JA, Thomson J, Vidruk E, DoPico GA (1972) Estimation of arterial PO2, PCO2, pH, and lactate from arterialized venous blood. J Appl Physiol 32 (1):134-137

Foxdal P, Sjodin A, Sjodin B (1996) Comparison of blood lactate concentrations obtained during incremental and constant intensity exercise. International journal of sports medicine 17 (5):360-365. doi:10.1055/s-2007-972861

Foxdal P, Sjodin B, Sjodin A, Ostman B (1994) The validity and accuracy of blood lactate measurements for prediction of maximal endurance running capacity. Dependency of analyzed blood media in combination with different designs of the exercise test. International journal of sports medicine 15 (2):89-95. doi:10.1055/s-2007-1021026

Gavin JP, Willems ME, Myers SD (2014) Reproducibility of lactate markers during 4 and 8 min stage incremental running: a pilot study. Journal of science and medicine in sport / Sports Medicine Australia 17 (6):635-639. doi:10.1016/j.jsams.2013.08.006

Gollnick PD, Piehl K, Saltin B (1974) Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. The Journal of physiology 241 (1):45-57

Gravelle BM, Murias JM, Spencer MD, Paterson DH, Kowalchuk JM (2012) Adjustments of pulmonary O2 uptake and muscle deoxygenation during ramp incremental exercise and constant-load moderate-intensity exercise in young and older adults.

Journal of applied physiology 113 (9):1466-1475. doi:10.1152/japplphysiol.00884.2011

Hawley JA, Myburgh KH, Noakes TD, Dennis SC (1997) Training techniques to improve fatigue resistance and enhance endurance performance. Journal of sports sciences 15 (3):325-333. doi:10.1080/026404197367335

Hawley JA, Noakes TD (1992) Peak power output predicts maximal oxygen uptake and performance time in trained cyclists. European journal of applied physiology and occupational physiology 65 (1):79-83

Heck H, Mader A, Hess G, Mucke S, Muller R, Hollmann W (1985) Justification of the 4-mmol/l lactate threshold. International journal of sports medicine 6 (3):117-130. doi:10.1055/s-2008-1025824

Henry FM (1951) Aerobic oxygen consumption and alactic debt in muscular work. J Appl Physiol 3 (7):427-438

Hill AV, Lupton H (1923) Muscular exercise, lactic acid and the supply and utilisation of oxygen. Q J Med 16:135-171

Hill DK (1940) The time course of the oxygen consumption of stimulated frog's muscle. The Journal of physiology 98 (2):207-227

Hill DW, Rowell AL (1996) Running velocity at VO2max. Medicine and science in sports and exercise 28 (1):114-119

Iaia FM, Bangsbo J (2010) Speed endurance training is a powerful stimulus for physiological adaptations and performance improvements of athletes. Scandinavian journal of medicine & science in sports 20 Suppl 2:11-23. doi:10.1111/j.1600-0838.2010.01193.x

Ingham SA, Pringle JS, Hardman SL, Fudge BW, Richmond VL (2013) Comparison of step-wise and ramp-wise incremental rowing exercise tests and 2000-m rowing ergometer performance. International journal of sports physiology and performance 8 (2):123-129

Jones AM, Burnley M (2009) Oxygen uptake kinetics: an underappreciated determinant of exercise performance. International journal of sports physiology and performance 4 (4):524-532

Jones AM, Vanhatalo A, Burnley M, Morton RH, Poole DC (2010) Critical power: implications for determination of V O2max and exercise tolerance. Medicine and science in sports and exercise 42 (10):1876-1890. doi:10.1249/MSS.0b013e3181d9cf7f

Kirkeberg JM, Dalleck LC, Kamphoff CS, Pettitt RW (2011) Validity of 3 protocols for verifying VO2 max. International journal of sports medicine 32 (4):266-270. doi:10.1055/s-0030-1269914

Kuipers H, Rietjens G, Verstappen F, Schoenmakers H, Hofman G (2003) Effects of stage duration in incremental running tests on physiological variables. International journal of sports medicine 24 (7):486-491. doi:10.1055/s-2003-42020

Kuipers H, Verstappen FT, Keizer HA, Geurten P, van Kranenburg G (1985) Variability of aerobic performance in the laboratory and its physiologic correlates. International journal of sports medicine 6 (4):197-201. doi:10.1055/s-2008-1025839

Lacour JR MA, Dormios D, et al. (1989) Validation of the UMTT test in a group of elite middle-distance runners. Sci Mot 7:3-8

Lacour JR, Padilla-Magunacelaya S, Barthelemy JC, Dormois D (1990) The energetics of middle-distance running. European journal of applied physiology and occupational physiology 60 (1):38-43

Leger L, Boucher R (1980) An indirect continuous running multistage field test: the Universite de Montreal track test. Canadian journal of applied sport sciences Journal canadien des sciences appliquees au sport 5 (2):77-84

Levine BD (2008) VO2max: what do we know, and what do we still need to know? The Journal of physiology 586 (1):25-34. doi:10.1113/jphysiol.2007.147629

McConnell TR, Clark BA (1988) Treadmill protocols for determination of maximum oxygen uptake in runners. British journal of sports medicine 22 (1):3-5

McLaughlin JE, Howley ET, Bassett DR, Jr., Thompson DL, Fitzhugh EC (2010) Test of the classic model for predicting endurance running performance. Medicine and science in sports and exercise 42 (5):991-997. doi:10.1249/MSS.0b013e3181c0669d

McLellan TM (1985) Ventilatory and plasma lactate response with different exercise protocols: a comparison of methods. International journal of sports medicine 6 (1):30-35. doi:10.1055/s-2008-1025809

Midgley AW, McNaughton LR, Wilkinson M (2006) Is there an optimal training intensity for enhancing the maximal oxygen uptake of distance runners?: empirical research findings, current opinions, physiological rationale and practical recommendations. Sports medicine 36 (2):117-132

Monod H, Scherrer J (1965) The work capacity of a synergic muscular group. Ergonomics 8 (3):329-338. doi:10.1080/00140136508930810

Morgan DW, Baldini FD, Martin PE, Kohrt WM (1989) Ten kilometer performance and predicted velocity at VO2max among well-trained male runners. Medicine and science in sports and exercise 21 (1):78-83

Morton HR (1994) Critical power test for ramp exercise. European journal of applied physiology 69:435-438

Morton RH (2011) Why peak power is higher at the end of steeper ramps: an explanation based on the "critical power" concept. Journal of sports sciences 29 (3):307-309. doi:10.1080/02640414.2010.534809

Padilla S, Mujika I, Orbananos J, Angulo F (2000) Exercise intensity during competition time trials in professional road cycling. Medicine and science in sports and exercise 32 (4):850-856

Poole DC, Hirai DM, Copp SW, Musch TI (2012) Muscle oxygen transport and utilization in heart failure: implications for exercise (in)tolerance. American journal of physiology Heart and circulatory physiology 302 (5):H1050-1063. doi:10.1152/ajpheart.00943.2011

Poole DC, Jones AM (2012) Oxygen uptake kinetics. Comprehensive Physiology 2 (2):933-996. doi:10.1002/cphy.c100072

Poole DC, Ward SA, Gardner GW, Whipp BJ (1988) Metabolic and respiratory profile of the upper limit for prolonged exercise in man. Ergonomics 31 (9):1265-1279. doi:10.1080/00140138808966766

Poole DC, Wilkerson DP, Jones AM (2008) Validity of criteria for establishing maximal O2 uptake during ramp exercise tests. European journal of applied physiology 102 (4):403-410. doi:10.1007/s00421-007-0596-3

Rossiter HB, Kowalchuk JM, Whipp BJ (2006) A test to establish maximum O2 uptake despite no plateau in the O2 uptake response to ramp incremental exercise. Journal of applied physiology 100 (3):764-770. doi:10.1152/japplphysiol.00932.2005

Sjodin B, Jacobs I (1981) Onset of blood lactate accumulation and marathon running performance. International journal of sports medicine 2 (1):23-26. doi:10.1055/s-2008-1034579

Smith TP, Coombes JS, Geraghty DP (2003) Optimising high-intensity treadmill training using the running speed at maximal O(2) uptake and the time for which this can be maintained. European journal of applied physiology 89 (3-4):337-343. doi:10.1007/s00421-003-0806-6

Svensson M, Drust B (2005) Testing soccer players. Journal of sports sciences 23 (6):601-618. doi:10.1080/02640410400021294

Tanaka H, Monahan KD, Seals DR (2001) Age-predicted maximal heart rate revisited. Journal of the American College of Cardiology 37 (1):153-156

Taylor HL, Buskirk E, Henschel A (1955) Maximal oxygen intake as an objective measure of cardio-respiratory performance. J Appl Physiol 8 (1):73-80

Thoden J (1991a) Physiological testing of the high-performance athlete, vol Champaign (IL): Human Kinetics.

Thoden JS (1991b) Testing aerobic power. In: Physiological testing of the high-performance athlete.

Volkov NI, Shirkovets EA, Borilkevich VE (1975) Assessment of aerobic and anaerobic capacity of athletes in treadmill running tests. European journal of applied physiology and occupational physiology 34 (2):121-130

Weston SB, Gray AB, Schneider DA, Gass GC (2002) Effect of ramp slope on ventilation thresholds and VO2peak in male cyclists. International journal of sports medicine 23 (1):22-27. doi:10.1055/s-2002-19267

Whipp BJ (2005) The kinetics of oxygen uptake. In: Jones AM, Poole DC (eds) Oxygen Uptake Kinetics. Routledge, New York, pp 62-94

Whipp BJ, Davis JA, Torres F, Wasserman K (1981) A test to determine parameters of aerobic function during exercise. Journal of applied physiology: respiratory, environmental and exercise physiology 50 (1):217-221

Whipp BJ, Seard C, Wasserman K (1970) Oxygen deficit-oxygen debt relationships and efficiency of anaerobic work. J Appl Physiol 28 (4):452-456

Yoshida T (1984) Effect of exercise duration during incremental exercise on the determination of anaerobic threshold and the onset of blood lactate accumulation. European journal of applied physiology and occupational physiology 53 (3):196-199

Yoshida T, Chida M, Ichioka M, Suda Y (1987) Blood lactate parameters related to aerobic capacity and endurance performance. European journal of applied physiology and occupational physiology 56 (1):7-11

Zhang YY, Johnson MC, 2nd, Chow N, Wasserman K (1991) Effect of exercise testing protocol on parameters of aerobic function. Medicine and science in sports and exercise 23 (5):625-630

Zoladz JA, Duda K, Majerczak J (1998) Oxygen uptake does not increase linearly at high power outputs during incremental exercise test in humans. European journal of applied physiology and occupational physiology 77 (5):445-451