MAOD Determined in a Single Supramaximal Test: a Study on the Reliability and Effects of Supramaximal Intensities

Abstract

The main barrier to the wide use of maximal accumulated oxygen deficit (MAOD) is the considerable time required to apply several sub- and supra-maximal exercise sessions. The main question of this study was whether the determination of MAOD using a single supramaximal exercise session (MAOD_{ALT}) is valid and reliable in running. We investigated the effects of the supramaximal exercise intensity (A) and the reliability of a single supramaximal exercise session (B) to assess MAOD in treadmill running. For this aim 29 participants participated in A & B studies with single allocation “A” (n = 15) and “B” (n = 14). The conventional MAOD and 8 MAOD_{ALT} were determined in exhaustive efforts varying between 100–150% at an intensity associated with maximal oxygen uptake (i\(\dot{V}O_{2MAX}\)). In B study 2 supramaximal efforts were applied to analyze the test-retest reliability. Non-significant differences were found between MAOD and the 8 values of MAOD_{ALT}. Despite the MAOD being statistically correlated with the MAOD_{ALT} 100% i\(\dot{V}O_{2MAX}\) (0.49 < r > 0.59), MAOD_{ALT} determined at 115% of i\(\dot{V}O_{2MAX}\) (52.4 ± 1.7 mL·kg\(^{-1}\)) presented the higher correlation values (0.65 < r > 0.77) and concordance. In addition, the MAOD at 115% of i\(\dot{V}O_{2MAX}\) presented high test-retest reliability. MAOD_{ALT} determined at 115% of i\(\dot{V}O_{2MAX}\) was a valid and reliable method to assess MAOD in running.

Introduction

Traditionally, anaerobic capacity (i.e., maximal energy amount of adenosine triphosphate that can be resynthesized via the phosphagen and glycolytic metabolic pathways) has been considered one of the most important performance parameters in sports involving exhaustive short-duration efforts [11, 21]. Although several methods have been proposed in the last few decades, the maximal accumulated oxygen deficit (MAOD) is currently the most accepted procedure for estimating anaerobic capacity [23, 26]. Indeed, MAOD has been used to determine training status [34], the metabolic profile of high intensity exercises [32] and to validate anaerobic tests [25, 30]. However, the wide application of MAOD in sports is considered unfeasible due to the excessive number of exercise trials to satisfy the aim of the sport scientist [26]. MAOD is measured by the difference between estimated oxygen uptake (\(\dot{V}O_2\)) demand area and \(\dot{V}O_2\) integrated over time during the supramaximal exercise, with the \(\dot{V}O_2\) demand estimated by a linear regression using at least ten submaximal efforts [23, 26].

In order to clarify this barrier, Bertuzzi et al. [3] proposed an alternative method (MAOD_{ALT}) for estimating MAOD using a single supramaximal exercise session corresponding to 110% intensity associated to maximal oxygen uptake (i\(\dot{V}O_{2MAX}\)). Assuming that the MAOD_{ALT} corresponding the sum of the oxygen demands from glycolytic and phosphagen metabolic pathways. MAOD_{ALT} was determined by the sum of the fast component of excess post-exercise oxygen consumption (EPOC_{FAST}) and the energetic equivalent of oxygen for blood lactate accumulation, which were measured during a single supramaximal test. Particularly, these authors [3] did not find a statistical difference between MAOD and MAOD_{ALT} in addition to which the values were statistically correlated (r = 0.78, P = 0.014). Recently, Brisola et al. [5] reported that MAOD_{ALT} was improved in treadmill running after acute sodium bicarbonate supplementation, evidencing that MAOD_{ALT} is also sensitive to detect modifications in the phosphagen and/or glycolytic metabolic pathways.

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way. Similar results were also described by Zagatto and Gobatto [39], who verified that MAOD did not differ from the sum of the phosphagen and glycolytic pathways during a specific test for table tennis, in accordance to the procedure of Bertuzzi et al. [3]. Although these findings suggest that MAODALT provides a satisfactory estimate of MAOD with a reduced number of testing sessions, it is important to note that the influence of the duration of the supramaximal test on MAODALT determination remains unknown. This seems especially relevant since previous findings have suggested that the duration of the supramaximal test can influence the determination of the anaerobic capacity. Medbo et al [23] reported that MAOD values increase over time in bouts lasting <2 min, while Gastin et al. [12] did not find statistical differences for MAOD assessed in all-out isokinetic and during constant intensity exercise at 100 and 125% of iVO2MAX. Craig et al. [7] reported the effects of bout duration (i.e., 70s, 120s, 300s and 115% iVO2MAX) on MAOD assessment in endurance and sprint cyclists. Despite existing assumptions that the amount of energy resynthesized from non-mitochondrial sources during short-lasting exhaustive exercise is independent of the duration of the bout [12,23], these studies highlight the need to investigate the influence of supramaximal bout duration on MAODALT. In addition, it should be mentioned that before using a new physiological parameter, it is important to determine its reliability. It is well recognized that a reliable test is one that presents small changes in mean values, a small within-individual variation, and a high test-retest correlation [33]. However, despite its practical attractiveness due to the low time required for determination, the reliability of MAODALT remains unknown.

Taking together the limitations of these previous studies, it is possible to note that there are some emergent questions regarding MAODALT: Is it a valid and reliable procedure to assess conventional MAOD and consequently anaerobic capacity? Is there any ideal supramaximal exercise intensity to determine MAODALT? To answer these questions, 2 separate studies were performed. The purpose of study A was to verify the effects of supramaximal exercise intensity on MAODALT determined in treadmill running, comparing MAODALT determined using 8 different supramaximal exercise intensities with traditional MAOD [26]. It was hypothesized that an exercise intensity that led to a time to exhaustion of less than 2 min could affect MAODALT since fatigue occurs principally due to decrement of enzymes activity engaged on energy resynthesis, and that exercise intensities between 100 and 120% iVO2MAX would not be modified. The purpose of study B was to verify the test-retest reliability of the MAODALT method, and it was hypothesized that MAODALT is a reliable method to assess MAOD. In study B the best supramaximal effort determined in study A based on correlation and concordance analysis was considered as the exercise intensity.

Methods

Participants

In both studies, the subjects were instructed to avoid alcohol and caffeine during the evaluation period and not to perform strenuous exercise for at least 24 h prior to each session. The subjects were informed about the possible risks and benefits of the study prior to signing an informed consent, and all procedures were conducted respecting the declaration of Helsinki. The experimental procedures used in both studies, as well as the informed consent, were approved by the Research Ethics Com-

mittee of the University (Protocol number 645.784/2014). This study was performed in accordance with the ethical standards of this journal [13].

15 healthy and moderately active men participated in study A, (mean ± SD, age 24 ± 4 years; body mass 69.8 ± 7.6 kg; height 174.2 ± 5.4 cm; body fat 16.3 ± 4.4 % and VO2MAX 51.8 ± 4.7 mL kg⁻¹·min⁻¹). The subjects were not trained, but performed frequent physical activity such as soccer, running and cycling. 14 men, recreationally trained and experienced in running participated in the follow-up study (Study B) (mean ± SD, age 28 ± 5 years; total body mass 73.5 ± 8.5 kg; height 178.7 ± 5.6 cm; body fat 14.3 ± 5.3 % and VO2MAX 56.1 ± 5.0 mL kg⁻¹·min⁻¹) – none of whom participated in study A. These subjects performed at least 3 running training sessions per week, but were not competitive athletes.

Experimental design

All effort tests in both studies were performed on a motorized treadmill (ATI, Inbramed, Inbraspport, Porto Alegre, RS, Brazil) with a fixed treadmill incline of 1% [19,29]. To eliminate any influence of circadian variation, each subject completed all trials at the same time period of day in controlled environmental conditions regarding temperature (22.9 ± 1.3 °C) and relative humidity (43.8 ± 6.3 %). All sessions were separated by a minimum interval of 48 h. In all the supramaximal efforts the subjects were verbally encouraged, and the participants wore a safety belt attached to their chest to ensure maximal effort. Prior to each exercise trial, the subjects responded to the profile of mood states (POMS) scale to measure their motivation for the effort. If a state of fatigue, low vigor, or stress was detected, a new date for the test was scheduled. Prior to the exercise tests in each study, the body composition of the participants was assessed by means of a whole-body dual-energy X-ray absorptiometry scan (DXA) (Hologic QDR, Discovery, Bedford, USA). This analysis was used to measure the body lean mass and then to equalize the MAODALT by lean mass. On the same day as the DXA analysis, each participant performed a familiarization session on the treadmill.

Physiological analysis

In all procedures, the gas-exchange responses were measured breath-by-breath using a stationary gas analyzer (Quark PFT, COSMED, Rome, Italy). The gas analyzer was calibrated using ambient air and a sample of known gases (5.06% CO₂ and 16.02% O₂; White Martins, Osasco, Brazil) and the spirometer with a 3-L syringe (Hans Rudolf, Kansas City, Missouri, USA), according to the manufacturer’s recommendations. For analysis of respiratory variables, the data were smoothed every 5 points and interpolated every 1 s [28]. Heart rate (HR) was measured using a transmitter belt coupled to the gas analyzer. Before each effort trial the participants remained seated for 10 min to measure the oxygen uptake (VO₂) and blood lactate concentration ([La⁻]) at rest (baseline values). After each supra-maximal effort test, the VO₂ was measured for 7 min for determination of the fast component of excess post-exercise oxygen consumption (EPOCFAST). Blood samples were collected 3, 5 and 7 min after all tests to determine peak blood lactate concentration ([La⁻]peak). Blood samples were collected from the earlobe (25 µL) using heparinized capillaries and transferred to Eppendorf tubes containing 50 µL of sodium fluoride 1 %. The samples were analyzed in an electrochemical lactimeter YSI 2300 STAT (Yellow Spring Instruments, Yellow Spring, Ohio, USA).
Study A: Effect of exercise intensity on MAOD<sub>ALT</sub>

The participants underwent the following tests: a) a maximal graded exercise test (GXT) to assess iVO<sub>2MAX</sub> and the intensity associated with VO<sub>2</sub>(iVO<sub>2MAX</sub>); b) ten 10-min submaximal efforts (30, 35, 40, 45, 50, 55, 60, 65, 70 and 80% of iVO<sub>2MAX</sub>) to measure the VO<sub>2</sub> demand, and c) 8 exhaustive supramaximal efforts (100, 105, 110, 115, 120, 130, 140 and 150% of iVO<sub>2MAX</sub>). The submaximal efforts were applied as a warm-up before the supramaxial efforts (i.e., 5-min of recovery between tests), except for the 70 and 80% of iVO<sub>2MAX</sub> that were applied in a single test. The sub and supra efforts were allocated 30–150%, 35–140%, 40–130%, 45–120%, 50–115%, 55–110%, 60–105% and 65–100% of iVO<sub>2MAX</sub> were applied in random order. The resting values were also measured before the warm-up to ensure the baseline measurement.

Graded exercise test (GXT) to assess iVO<sub>2MAX</sub> and iVO<sub>2MAX</sub>

The GXT began at 8 km·h<sup>-1</sup> with stage increments of 1.5 km·h<sup>-1</sup> every 2 min until exhaustion, given voluntarily by the participant or by the inability to perform the effort at the pre-determined speed [5]. The GXT was based on the guidelines of Howley et al. [17] for iVO<sub>2MAX</sub> and was designed to last 8–12 min. The Borg scale (6–20) [4] was used to assess the rating of perceived exertion (RPE) at the end of each stage of the GXT. The highest VO<sub>2</sub> average (i.e., VO<sub>2</sub> average measured during the final 30 s of each stage) measured during the test was assumed as Vo<sub>2MAX</sub> [17], considering the verification of at least 2 of the following criteria: the plateau in VO<sub>2</sub> (variation in VO<sub>2</sub> < 2.1 mL·kg<sup>-1</sup>·min<sup>-1</sup> between the last and penultimate stage of exercise); maximal HR (HR<sub>MAX</sub>=90% of predicted HR<sub>MAX</sub> (220-age); respiratory exchange ratio (RER)≥1.10 and peak lactate ≥ 8.0 mmol·L<sup>-1</sup> [17]. If at least 2 criteria were not observed, a new test was applied. The exercise intensity at which the subject reached VO<sub>2MAX</sub> iVO<sub>2MAX</sub> was considered as iVO<sub>2MAX</sub>. If the final stage had not been completed, the iVO<sub>2MAX</sub> was calculated according to the equation $i\text{VO}_{2\text{MAX}} = V_{\text{complete}} + ([\text{Increment} 	imes t] / T)$, in which $V_{\text{complete}}$ is the running speed of the last complete stage, Inc the speed increment (i.e., 1.5 km·h<sup>-1</sup>), t the time in seconds sustained during the incomplete stage and T the time in seconds required to complete a stage (i.e., 120 s) using the method proposed by Kuipers et al. [20].

Submaximal and supra-maximal exercises

10 submaximal exercise sessions were performed over a 10-min period corresponding to 30, 35, 40, 45, 50, 55, 60, 65, 70 and 80% iVO<sub>2MAX</sub> [26]. VO<sub>2</sub> was measured throughout each 10-min exercise period as previously described, and the VO<sub>2</sub> values measured during the final 30-s were averaged and used as the steady-state VO<sub>2</sub> for the corresponding velocity. 5 min after the submaximal efforts, exhaustive supramaximal exercises were applied at 100, 105, 110, 115, 120, 130, 140 and 150% iVO<sub>2MAX</sub>. Each supramaximal trial was separated by a minimum interval of 48 h.

Assessment of maximal accumulated oxygen deficit (MAOD)

Submaximal velocity data and respective VO<sub>2</sub> (VO<sub>2</sub> steady state) were fitted in linear regression [23,26,35], with the y-intercept fixed at the individual VO<sub>2</sub> baseline value measured over 10-min at rest prior to exercise [39]. The linear regression was extrapolated to measure estimated oxygen demand at 110% iVO<sub>2MAX</sub> [26,38]. MAOD was calculated as the difference between estimated VO<sub>2</sub> demand area (estimated VO<sub>2</sub> demand multiplied by time to exhaustion) and VO<sub>2</sub> integrated over time in the maximal exercise [23]. Absolute MAOD values were reduced by 10% to correct for the contribution of body oxygen stores to the energy supply [3,23]. The MAOD was presented in absolute values (liters) and normalized by total body mass (mL·kg<sup>-1</sup>) and lean mass (mL·kg<sup>-1</sup> lean mass).

Assessment of maximal accumulated oxygen deficit alternative (MAOD<sub>ALT</sub>)

The MAOD<sub>ALT</sub> was assumed as the sum of the glycolytic metabolic pathway and phosphagen metabolic pathway [2,3,5,24,37,39] and was determined for each supra-maximal effort (i.e., 100, 105, 110, 115, 120, 130, 140 and 150% iVO<sub>2MAX</sub>). The EPOC<sub>FAST</sub> was used to estimate the contribution of the phosphagen metabolic pathway ($W_{\text{PCR}}$), which was calculated using a bi-exponential fit (Equation 1) in OriginPro 8.0 software (OriginLab Corp., Microcal, Massachusetts, USA) [3].

$$\text{VO}_2(t) = \text{VO}_2\text{baseline} + A_1e^{-t/\tau_1} + A_2e^{-t/\tau_2} \quad \text{(Equation 1)}$$

Where $\text{VO}_2(t)$ is the oxygen uptake at time t, $\text{VO}_2\text{baseline}$ is the oxygen uptake at baseline, $A_1$ is the amplitude, $\delta$ is the time delay and $\tau$ is the time constant. 1 and 2 represent the fast and slow components, respectively, and the EPOC<sub>FAST</sub> was calculated by the product of $A_1$ and $\tau_1$.

The contribution of the glycolytic metabolic pathway ($W_{\text{Lac}}$) was estimated by the difference between the quantities of [La]<sub>PEAK</sub> and rest ([La]<sub>REST</sub>) ([La]<sub>REST</sub>), considering each 1 mmol·L<sup>-1</sup> lactate equivalent to 3 mL O<sub>2</sub>·kg<sup>-1</sup> [8]. Fig. 1 shows the responses of oxygen uptake (VO<sub>2</sub>) and blood lactate concentration ([La]) during one supramaximal effort at 115% of iVO<sub>2MAX</sub> and after the end of the exercise to measure the fast component of EPOC through a bi-exponential fit.

Study B: MAOD<sub>ALT</sub> test and retest reliability analysis

In study B the participants performed a GXT to assess VO<sub>2MAX</sub> and iVO<sub>2MAX</sub> using the same procedure described in study A and 2 supramaximal exhaustive efforts to verify the MAOD<sub>ALT</sub> test and retest reliability. The supramaximal exercise intensity that...
resulted in a MAODALT with greater concordance and reliable compared to conventional MAOD in study A was considered as supramaximal intensity in the study B. The procedures during the test and for analysis and determination were the same as described in study A.

Prior to the supramaximal effort, a warm-up was performed at 6 km·h⁻¹ lasting 5 min and the test was applied 5 min after the end of the warm-up.

### Statistical analysis

In both studies, the data are presented as mean ± standard error of the mean (SEM) and confidence interval of 95% (95% CI). The variables were examined using the Shapiro Wilk’s test to verify the normality of the data. Study A: Linear regression analysis was used to determine the VO₂-intensity relationship for the ten submaximal exercise trials. For analysis of the values from the supramaximal effort outcomes and between MAOD determined in the conventional and alternative procedures, the one-way repeated measures Analysis of Variance was used for comparisons. In addition, Mauchly’s sphericity test was applied to the data, and sphericity was assumed to be violated when the F test was significant. In case of sphericity violation, the Greenhouse-Geisser Epsilon correction was used. Analyses were completed using the “Bonferroni” post hoc test. The effect size (η²) obtained in each statistical analysis is also presented and interpreted as very weak to negligible (0 to 0.2), weak (0.2 to 0.4), moderate (0.4 to 0.7), strong (0.7 to 0.9), and very strong (0.9 to 1.0) [31].

Study B: For the test and retest analysis the paired “t” test, confidence interval estimation for the Bland-Altman limits (95%LoA; Bias; confidence limits as ± value) and typical error. In addition, the Pearson’s correlation test was used to verify the association between the MAOD and MAODALT values. The coefficient of correlation was classified as very weak to negligible (0 to 0.2), weak (0.2 to 0.4), moderate (0.4 to 0.7), strong (0.7 to 0.9), and very strong (0.9 to 1.0) [31].

### Results

#### Comparison between MAOD and the eight MAODALT (Study A)

All subjects reached the exhaustion criteria to attain VO₂MAX in the GXT and did not need to repeat the test. The physiological response values at the exhaustion moment in the GXT are shown in Table 1.

Significant differences (P < 0.001) were found for velocity, time to exhaustion (tlim) and VO₂ at exhaustion observed with increases in exercise intensity in the supra-maximal efforts. VO₂MAX and VO₂ at exhaustion for 100 to 115% of VO₂MAX did not differ, a statistical difference being found for VO₂ at exhaustion (i.e., peak of VO₂) at exercise intensities higher than 115% of VO₂MAX (P < 0.002) (Table 2). In addition, significant correlations (r = 0.58 to 0.69) were found between VO₂MAX and VO₂ at exhaustion during the supramaxal efforts.

The slope, y-intercept and coefficient of determination (R²) of the VO₂-velocity relationship were 3.5 ± 0.1 mL·kg⁻¹·min⁻¹·km⁻¹, 4.7 ± 0.2 mL·kg⁻¹·min⁻¹·km⁻¹ and 0.92 ± 0.01 respectively. The MAOD and all the MAODALT values are presented in Fig. 2, and the values did not differ statistically for absolute (P = 0.56; F(8,112) = 0.666, η² = 0.045), normalized by total body mass (P = 0.78; F(8,112) = 0.595, η² = 0.041) or lean mass (P = 0.154; F(8,112) = 1.943, η² = 0.122) (mL·kg⁻¹·min⁻¹ lean mass). In addition, Table 3 presents the coefficient of correlation (r), effect size, 95%LoA and typical error for the comparison between MAOD assessed in the conventional method and the MAODs assessed in the alternative method. Significant correlations (P < 0.05) were found only for MAODALT determined at 100 and 115% of iVO₂MAX for absolute values, relative to body mass and lean mass), but the MAODALT at 115% of iVO₂MAX demonstrated better concordance based on effect size, 95%LoA and typical error. Only the MAODALT values presented for lean mass showed a high effect size, 95%LoA and typical error.

#### Reliability of MAODALT (Study B)

The VO₂MAX corresponded to 4.10 ± 0.10 L·min⁻¹ (95% CI 3.88 to 4.32 L·min⁻¹) and 56.1 ± 3.1 mL·kg⁻¹·min⁻¹ (95% CI 53.2 to 59.0 mL·kg⁻¹·min⁻¹), whereas the iVO₂MAX was 16.8 ± 0.3 km·h⁻¹ (95% CI 16.1 to 17.5 km·h⁻¹). The supramaximal exercise intensity used in this study corresponded to 115% of iVO₂MAX (finding in study A) and was 19.3 ± 0.4 km·h⁻¹ (95% CI 18.5 to 20.1 km·h⁻¹). The mean and individual values for MAODALT in the test and retest conditions are presented in Fig. 3 and no significant differences were found. In addition, a good concordance between values (95%LoA), low typical error and a high and significant intraclass correlation coefficient (ICC > 0.77, P < 0.001) was observed. Moreover, the trim, VO₂ at exhaustion and other parameters engaged in the determination of the metabolic pathways of glycolytic (i.e., blood lactate responses and W₅₀/L₅₀) and phosphagen did not differ between test and retest conditions (P > 0.05), showed trivial effect size and high and significant intraclass correlation, evidencing the reliability of the method. These data are presented in Table 3, 4.

#### Discussion

The main finding of the study was that the MAOD and MAODALT measured using different supramaximal intensities were not statistically different (Fig. 2). In addition, MAODALT measured at 115% of iVO₂MAX presented the higher correlations and concordance with MAOD.

### Study A

The establishment of MAODALT by Bertuzzi et al. [3] is based on previous studies indicating that the resynthesis of high-energy

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Table 1 GXT parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise duration (min)</th>
<th>VO₂MAX (L·min⁻¹)</th>
<th>VO₂MAX (mL·kg⁻¹·min⁻¹)</th>
<th>iVO₂MAX (km·h⁻¹)</th>
<th>HRMAX (bpm)</th>
<th>RER</th>
<th>RPE (a.u.)</th>
<th>[La⁻]PEAK (mmol·L⁻¹)</th>
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<tr>
<td>Values are mean ± SEM (95% CI)</td>
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Table 2 Comparison between MAOD and the eight MAODALT (Study B).

<table>
<thead>
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<th>Variable</th>
<th>Exercise duration (min)</th>
<th>VO₂MAX (L·min⁻¹)</th>
<th>VO₂MAX (mL·kg⁻¹·min⁻¹)</th>
<th>iVO₂MAX (km·h⁻¹)</th>
<th>HRMAX (bpm)</th>
<th>RER</th>
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Table 3 Reliability of MAODALT (Study B).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise duration (min)</th>
<th>VO₂MAX (L·min⁻¹)</th>
<th>VO₂MAX (mL·kg⁻¹·min⁻¹)</th>
<th>iVO₂MAX (km·h⁻¹)</th>
<th>HRMAX (bpm)</th>
<th>RER</th>
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<th>[La⁻]PEAK (mmol·L⁻¹)</th>
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phosphate stores and the glycolytic energy cost can be accessed by the fast component of the excess post-exercise oxygen consumption [14] and blood lactate accumulation O2 equivalent [8], respectively.

However, it is important to note that in both studies from Bertuzzi et al. [3] and Zagatto & Gobatto [39], the conventional MAOD was determined using around 4 and 6 submaximal efforts to fit the \( \dot{V}O_2\) demand-intensity linear regression, which is less than the 10 submaximal trials suggested by Noordhoff et al. [26] for robust MAOD determination. Buck and McNaughton [6] reported that the use of less than 10 submaximal effort to determine MAOD could improve the error during the chosen of the \( \dot{V}O_2\) -intensity. Thus, the current study is the first to compare MAOD ALT with conventional MAOD determined using a more robust method [26]. Our findings reinforce the outcomes from Bertuzzi et al. [3] and Zagatto et al. [39] who reported that MAODALT was a valid method for anaerobic capacity determination in a single supramaximal effort.

The MAOD and all the MAOD ALT did not differ independently of the supramaximal effort (Fig. 2), evidencing that the anaerobic sources seem to be completely depleted during efforts lasting between 52.7 ± 4.0 s and 316.9 ± 17.0 s (Table 2). However, despite being statistically similar, the MAODALT assessed at 115 % relative to lean mass (values) were obtained by one-way repeated measures Analysis of Variance. 

Table 2: Velocity, time to exhaustion (tlim), oxygen uptake (\( \dot{V}O_2 \)), blood lactate concentration at peak ([La]PEAK) and delta lactate (\( \Delta[La] \)), glycolytic metabolism pathway (W[La]) and phosphagen metabolic pathway (W[PCR]) for the 8 supramaximal exercise intensities used to assess the MAODALT (n = 15).

<table>
<thead>
<tr>
<th>Exercise intensity at ( \dot{V}O_2)MAX</th>
<th>F(7,8)</th>
<th>p-value</th>
<th>( \eta^2 )</th>
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<tr>
<td>100 %</td>
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<td>Velocity (km·h⁻¹)</td>
<td>14.4±0.23</td>
<td>0.000</td>
<td>0.996</td>
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<td>tlim (s)</td>
<td>316.9±17</td>
<td>0.000</td>
<td>0.993</td>
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<tr>
<td>( \dot{V}O_2 ) at exhaustion (ml·kg⁻¹·min⁻¹)</td>
<td>51.1±5.0</td>
<td>0.000</td>
<td>0.370</td>
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<td>W[La] (ml·kg⁻¹)</td>
<td>27.9±1.7</td>
<td>0.000</td>
<td>0.370</td>
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<td>[La]PEAK (mmol·L⁻¹)</td>
<td>10.4±0.6</td>
<td>0.000</td>
<td>0.106</td>
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<td>( \Delta[La] ) (mmol·L⁻¹)</td>
<td>9.3±0.6</td>
<td>0.000</td>
<td>0.106</td>
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<tr>
<td>W[PCR] (ml·kg⁻¹)</td>
<td>21.7±1.0</td>
<td>0.000</td>
<td>0.046</td>
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Values are mean ± SEM. F, p-value and \( \eta^2 \) values were obtained by one-way repeated measures Analysis of Variance.

<table>
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<tr>
<th>Exercise intensity at ( \dot{V}O_2)MAX</th>
<th>F(7,8)</th>
<th>p-value</th>
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<td>105 %</td>
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<tr>
<td>150 %</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2: Maximal accumulated oxygen deficit determined by the conventional method (MAOD) and by the single supra maximal method (MAODALT) and expressed in absolute and relative to total body mass and relative to lean mass. The MAODALT was determined in 8 different supramaximal intensities corresponding to 100 % (MAODALT100), 110 % (MAODALT110), 120 % (MAODALT120), 130 % (MAODALT130), 140 % (MAODALT140) and 150 % (MAODALT150) of \( \dot{V}O_2\)MAX. 

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Table 3

<table>
<thead>
<tr>
<th>MAOD</th>
<th>MAODALT 100</th>
<th>MAODALT 110</th>
<th>MAODALT 115</th>
<th>MAODALT 120</th>
<th>MAODALT 130</th>
<th>MAODALT 140</th>
<th>MAODALT 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>r (95% IC)</td>
<td>0.59 * (0.11 to 0.84)</td>
<td>0.49 (−0.02 to 0.80)</td>
<td>0.38 (−0.16 to 0.75)</td>
<td>0.73 ** (0.34 to 0.90)</td>
<td>0.26 (−0.29 to 0.68)</td>
<td>0.24 (−0.31 to 0.65)</td>
<td>0.30 (−0.25 to 0.71)</td>
</tr>
<tr>
<td>Effect size</td>
<td>−0.37 (small)</td>
<td>−0.34 (small)</td>
<td>−0.03 (trivial)</td>
<td>−0.12 (trivial)</td>
<td>−0.17 (trivial)</td>
<td>0.15 (trivial)</td>
<td>−0.33 (small)</td>
</tr>
<tr>
<td>95% LoA</td>
<td>−0.26 L; ± 0.43</td>
<td>−0.18 L; ± 0.46</td>
<td>−0.01 L; ± 0.49</td>
<td>−0.08 L; ± 0.39</td>
<td>−0.08 L; ± 0.54</td>
<td>−0.04 L; ± 0.53</td>
<td>−0.08 L; ± 0.56</td>
</tr>
</tbody>
</table>

Body mass

<table>
<thead>
<tr>
<th>MAOD</th>
<th>MAODALT 100</th>
<th>MAODALT 110</th>
<th>MAODALT 115</th>
<th>MAODALT 120</th>
<th>MAODALT 130</th>
<th>MAODALT 140</th>
<th>MAODALT 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>r (95% IC)</td>
<td>0.49 * (−0.03 to 0.80)</td>
<td>0.33 (−0.22 to 0.72)</td>
<td>0.16 (−0.38 to 0.62)</td>
<td>0.65 (0.20 to 0.87)</td>
<td>0.09 (−0.57 to 0.51)</td>
<td>−0.08 (−0.57 to 0.44)</td>
<td>−0.26 (−0.57 to 0.05)</td>
</tr>
<tr>
<td>Effect size</td>
<td>−0.41 (small)</td>
<td>−0.40 (small)</td>
<td>−0.04 (small)</td>
<td>−0.39 (small)</td>
<td>−0.08 (small)</td>
<td>−0.08 (small)</td>
<td>−0.16 (small)</td>
</tr>
<tr>
<td>95% LoA</td>
<td>−3.95 mL · kg⁻¹; ± 6.45</td>
<td>−2.75 mL · kg⁻¹; ± 6.98</td>
<td>−0.63 mL · kg⁻¹; ± 7.53</td>
<td>−1.26 mL · kg⁻¹; ± 5.87</td>
<td>−1.57 mL · kg⁻¹; ± 8.10</td>
<td>−1.64 mL · kg⁻¹; ± 7.96</td>
<td>−1.92 mL · kg⁻¹; ± 7.91</td>
</tr>
</tbody>
</table>

Lean mass

<table>
<thead>
<tr>
<th>MAOD</th>
<th>MAODALT 100</th>
<th>MAODALT 110</th>
<th>MAODALT 115</th>
<th>MAODALT 120</th>
<th>MAODALT 130</th>
<th>MAODALT 140</th>
<th>MAODALT 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>r (95% IC)</td>
<td>0.57 * (0.43 to 0.92)</td>
<td>0.10 (−0.44 to 0.58)</td>
<td>0.22 (−0.51 to 0.93)</td>
<td>0.67 ** (0.20 to 0.90)</td>
<td>−0.07 (−0.56 to 0.46)</td>
<td>−0.09 (−0.57 to 0.44)</td>
<td>0.00 (−0.51 to 0.51)</td>
</tr>
<tr>
<td>Effect size</td>
<td>−0.39 (small)</td>
<td>−0.38 (small)</td>
<td>−0.50 (small)</td>
<td>−0.37 (small)</td>
<td>−0.07 (−0.56 to 0.46)</td>
<td>−0.12 (−0.57 to 0.44)</td>
<td>−0.26 (−0.57 to 0.05)</td>
</tr>
<tr>
<td>95% LoA</td>
<td>−19.36 mL · kg⁻¹; ± 11.80</td>
<td>−14.88 mL · kg⁻¹; ± 12.76</td>
<td>−19.88 mL · kg⁻¹; ± 12.52</td>
<td>−13.30 mL · kg⁻¹; ± 14.47</td>
<td>−14.15 mL · kg⁻¹; ± 14.76</td>
<td>−14.47 mL · kg⁻¹; ± 14.76</td>
<td>−14.15 mL · kg⁻¹; ± 14.76</td>
</tr>
</tbody>
</table>

Study B

Because of the difficulty in determining the conventional MAOD, studies that investigate the test and retest reliability are very poor [10, 18, 35]. Jacobs et al. [18] evaluated the reliability of the conventional MAOD method equivalent to 125% \( \dot{V} \text{O}_2 \text{MAX} \) using 2 trials and found a correlation coefficient of 0.97, concluding that MAOD is a reliable method. Weber and Schneider [35] also found similar findings in males and females at 110% of ICC=0.95 and 120% of peak exercise intensity during GXT in cycling (ICC=0.97). However, Doherty et al. [10] in a running study at 125% \( \dot{i} \text{V} \text{O}_2 \text{MAX} \) reported that the MAOD is not a reliable method due to the high limits of agreement of 95% of 15.1 mL · kg⁻¹ · min⁻¹ found, despite a high intra-class coefficient correlation (ICC=0.91).

It should be mentioned that before using a new physiological parameter, it is important to determine its reliability. A reliable test is one that presents small changes in mean values, a small within-individual variation, and a high test-retest correlation [33]. The current study is the first to investigate the reliability of MAODALT and our findings lead to highly repeatable values based on a trivial effect size in the test-retest (≤0.45), intra-class correlations (≥0.77) [36], limits of agreement of 95% of ±2.9 mL · kg⁻¹ (i.e., value relative to body mass) [1], a trivial effect and low typical error [15], which are the recommended procedures to evaluate the reliability of a test-retest method [1, 15]. Therefore, our results revealed that MAODALT assessed at 115% \( \dot{V} \text{O}_2 \text{MAX} \) seems to be reliable.

Limitations of study

The anaerobic capacity is an index associated with high-intense and short duration activities [27], and therefore the use of non-trained or active participants instead of sprint runners could be considered a limitation of the study. In addition, in study A, it was observed that exercise intensities at 100 and 115% of \( \dot{i} \text{V} \text{O}_2 \text{MAX} \) can be used to estimate the conventional MAOD, but the test-retest reliability was tested only for 115% \( \dot{i} \text{V} \text{O}_2 \text{MAX} \) which can also be considered as a limitation of the study. Moreover, some restrictions on the use of whole-body physiological variables to determine anaerobic capacity have been reported. For example, the contribution of the glycolysis metabolism pathway...
contribution could be underestimated as a portion of the lactate released into the blood may be oxidized in active skeletal muscle [22] and other tissues such as heart during exercise. In addition, the O2 equivalent for blood lactate accumulation used in the present study does not represent the exact stoichiometric relationship between lactate formation and ATP resynthesis.

In conclusion, the findings of the current study demonstrated that the intensity of the supramaximal exercise did not affect the determination of MAODALT, indicating its ability to predict anaerobic capacity. Our results also revealed that MAODALT determined at 115% of i ˙VO2Max was a valid, reliable and reproducible method for assessing anaerobic capacity in a single supramaximal effort in running.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was performed in accordance with the ethical standards of this journal [13].

Acknowledgements

The authors would like to thank the FAPESP, process numbers 2013/12940-8 and 2014/02829-5.

Table 4  Time to exhaustion (tlim), oxygen uptake at exhaustion (VO2 exhaustion) and other parameters engaged in the determination of the glycolytic (i.e., blood lactate responses and W[La]) and phosphagen (WPCR) metabolic pathways in the test and retest conditions (n = 14).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>Retest</th>
<th>Effect Size</th>
<th>95 %LoA</th>
<th>p-value</th>
<th>ICC (95 %CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tlim (s)</td>
<td>113.4 ± 7.0 (98.3 to 128.4)</td>
<td>112.7 ± 8.0 (95.3 to 130.1)</td>
<td>−0.02 (trivial)</td>
<td>0.64 (− 9.57 to 10.86)</td>
<td>0.89</td>
<td>0.83 ** (0.55 to 0.94)</td>
</tr>
<tr>
<td>VO2 at exhaustion (mL · kg⁻¹ · min⁻¹)</td>
<td>53.7 ± 5.6 (50.4 to 56.9)</td>
<td>54.5 ± 5.0 (51.7 to 57.4)</td>
<td>0.17 (trivial)</td>
<td>−0.84 (−2.43 to 0.76)</td>
<td>0.28</td>
<td>0.88 ** (0.68 to 0.96)</td>
</tr>
<tr>
<td>W[La] (mL · kg⁻¹)</td>
<td>31.0 ± 1.5 (27.7 to 34.2)</td>
<td>29.7 ± 2.2 (25.0 to 34.4)</td>
<td>−0.21 (small)</td>
<td>1.26 (−1.76 to 4.28)</td>
<td>0.38</td>
<td>0.75 ** (0.39 to 0.91)</td>
</tr>
<tr>
<td>[La]PEAK (mmol · L⁻¹)</td>
<td>11.7 ± 0.5 (10.5 to 12.8)</td>
<td>11.1 ± 0.7 (9.6 to 12.7)</td>
<td>−0.23 (small)</td>
<td>0.52 (−0.49 to 1.53)</td>
<td>0.29</td>
<td>0.75 ** (0.38 to 0.91)</td>
</tr>
<tr>
<td>Δ[La] (mmol · L⁻¹)</td>
<td>10.3 ± 0.5 (9.2 to 11.4)</td>
<td>9.9 ± 0.7 (8.3 to 11.5)</td>
<td>−0.18 (trivial)</td>
<td>0.42 (−0.59 to 1.43)</td>
<td>0.38</td>
<td>0.75 ** (0.39 to 0.91)</td>
</tr>
<tr>
<td>WPCR (mL · kg⁻¹)</td>
<td>22.2 ± 0.8 (20.5 to 24.0)</td>
<td>23.8 ± 1 (21.6 to 25.9)</td>
<td>0.45 (small)</td>
<td>−1.53 (−3.09 to 0.04)</td>
<td>0.06</td>
<td>0.72 ** (0.32 to 0.90)</td>
</tr>
</tbody>
</table>

*p < 0.01. Values are mean ± SEM (95 %CI). p-value obtained by paired t-test.
Conflict of interest: The authors have no conflict of interest to declare.

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