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

Authors A. M. Zagatto[1], R. Bertuzzi[2], W. E. Miyagi[1,3], J. Padulo[4,6], M. Papoti[5]

Affiliations Affiliation addresses are listed at the end of the article

Key words
- blood lactate concentration
- glycolytic metabolic pathway
- phosphagen metabolic pathway
- supramaximal efforts

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 subjects 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 $i\dot{V}O_{2MAX}$ ($52.4 \pm 1.7\,mL \cdot kg^{-1}$) 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 path-

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Correspondence
Prof. Alessandro Moura Zagatto, PhD
Department of Physical Education
Sao Paulo State University
Av. Eng. Luiz Edmundo Carrijo Coube
14–01, Bauru
Brazil 17033-360
Tel.: +55/14/31036 82
Fax: +55/14/31036 82
azagatto@yahoo.com.br

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 according to the procedure of Bertuzzi et al. [3].
Althougth these findings suggest that $MAOD_{ALT}$ 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 $MAOD_{ALT}$ 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 $i\dot{V}O_{2MAX}$. Craig et al. [7] reported the effects of bout duration (i. e., 70s, 120s, 300s and 115 % $i\dot{V}O_{2MAX}$) 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 $MAOD_{ALT}$. 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 $MAOD_{ALT}$ remains unknown.
Taking together the limitations of these previous studies, it is possible to note that there are some emergent questions regarding $MAOD_{ALT}$: Is it a valid and reliable procedure to assess conventional MAOD and consequently anaerobic capacity? Is there any ideal supramaximal exercise intensity to determine $MAOD_{ALT}$? To answer these questions, 2 separate studies were performed. The purpose of study A was to verify the effects of supramaximal exercise intensity on $MAOD_{ALT}$ determined in treadmill running, comparing $MAOD_{ALT}$ 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 $MAOD_{ALT}$ since fatigue occurs principally due to decrement of enzymes activity engaged on energy resynthesis, and that exercise intensities between 100 and 120 % $i\dot{V}O_{2MAX}$ would not be modified. The purpose of study B was to verify the test-retest reliability of the $MAOD_{ALT}$ method, and it was hypothesized that $MAOD_{ALT}$ 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 Committee 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 $\dot{V}O_{2MAX}$ 51.8 ± 4.7 $mL \cdot kg^{-1} \cdot min^{-1}$). 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 $\dot{V}O_{2MAX}$ 56.1 ± 5.0 $mL \cdot kg^{-1} \cdot min^{-1}$) – 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 (ATL, Inbramed, Inbrasport, 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 $MAOD_{ALT}$ 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 % CO_2 and 16.02 % O_2; 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 ($\dot{V}O_2$) and blood lactate concentration ($[La^-]$) at rest (baseline values). After each supra-maximal effort test, the $\dot{V}O_2$ was measured for 7 min for determination of the fast component of excess post-exercise oxygen consumption ($EPOC_{FAST}$).
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 $MAOD_{ALT}$

The participants underwent the following tests: a) a maximal graded exercise test (GXT) to assess $i\dot{V}O_{2MAX}$ and the intensity associated with $\dot{V}O_2$ ($i\dot{V}O_{2MAX}$); b) ten 10-min submaximal efforts (30, 35, 40, 45, 50, 55, 60, 65, 70 and 80% of $i\dot{V}O_{2MAX}$) to measure the $\dot{V}O_2$ demand, and c) 8 exhaustive supramaximal efforts (100, 105, 110, 115, 120, 130, 140 and 150% of $i\dot{V}O_{2MAX}$). The submaximal efforts were applied as a warm-up before the supramaximal efforts (i.e., 5-min of recovery between tests), except for the 70 and 80% of $i\dot{V}O_{2MAX}$ 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 $i\dot{V}O_{2MAX}$ 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 $i\dot{V}O_{2MAX}$ and $i\dot{V}O_{2MAX}$

The GXT began at 8 km·h^{-1} with stage increments of 1.5 km·h^{-1} 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 $i\dot{V}O_{2MAX}$ 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 $\dot{V}O_2$ average (i.e., $\dot{V}O_2$ average measured during the final 30 s of each stage) measured during the test was assumed as $\dot{V}O_{2MAX}$ [17], considering the verification of at least 2 of the following criteria: the plateau in $\dot{V}O_2$ (variation in $\dot{V}O_2 < 2.1$ mL·kg^{-1}·min^{-1} between the last and penultimate stage of exercise); maximal HR (HR_{MAX}) ≥ 90% of predicted HR_{MAX} (220-age); respiratory exchange ratio (RER) ≥ 1.10 and peak lactate ≥ 8.0 mmol·L^{-1} [17]. If at least 2 criteria were not observed, a new test was applied. The exercise intensity at which the subject reached $\dot{V}O_{2MAX}$ $i\dot{V}O_{2MAX}$ was considered as $i\dot{V}O_{2MAX}$. If the final stage had not been completed, the $i\dot{V}O_{2MAX}$ was calculated according to the equation $i\dot{V}O_{2MAX} = V_{complete} + (\text{Increment} \cdot t/T)$, in which $V_{complete}$ is the running speed of the last complete stage, Inc the speed increment (i.e., 1.5 km·h^{-1}), 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% $i\dot{V}O_{2MAX}$ [26]. $\dot{V}O_2$ was measured throughout each 10-min exercise period as previously described, and the $\dot{V}O_2$ values measured during the final 30-s were averaged and used as the steady-state $\dot{V}O_2$ 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% $i\dot{V}O_{2MAX}$. Each supramaximal trial was separated by a minimum interval of 48 h.

Assessment of maximal accumulated oxygen deficit (MAOD)

Submaximal velocity data and respective $\dot{V}O_2$ ($\dot{V}O_2$ steady state) were fitted in linear regression [23, 26, 35], with the y-intercept fixed at the individual $\dot{V}O_2$ baseline value measured over 10-min at rest prior to exercise [39]. The linear regression was extrapolated to measure estimated oxygen demand at 110% $i\dot{V}O_{2MAX}$ [26, 38]. MAOD was calculated as the difference between estimated $\dot{V}O_2$ demand area (estimated $\dot{V}O_2$ demand multiplied by time to exhaustion) and $\dot{V}O_2$ 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^{-1}) and lean mass (mL·kg^{-1} lean mass).

Assessment of maximal accumulated oxygen deficit alternative ($MAOD_{ALT}$)

The $MAOD_{ALT}$ 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% $i\dot{V}O_{2MAX}$). The $EPOC_{FAST}$ was used to estimate the contribution of the phosphagen metabolic pathway ($W_{[PCR]}$), which was calculated using a bi-exponential fit (Equation 1) in OriginPro 8.0 software (OriginLab Corp., Microcal, Massachusetts, USA) [3].

$$\dot{V}O_{2(t)} = \dot{V}O_{2baseline} + A_1[e^{-(t-\delta)/\tau_1}] + A_2[e^{-(t-\delta)/\tau_2}] \qquad \text{(Equation 1)}$$

Where $\dot{V}O_{2(t)}$ is the oxygen uptake at time t, $\dot{V}O_{2baseline}$ is the oxygen uptake at baseline, A is the amplitude, δ is the time delay and τ is the time constant. 1 and 2 represent the fast and slow components, respectively, and the $EPOC_{FAST}$ was calculated by the product of A_1 and τ1.

The contribution of the glycolytic metabolic pathway ($W_{[La]}$) was estimated by the difference between the quantities of $[La^-]_{PEAK}$ and rest ($[La^-]_{REST}$) ($\Delta[La^-]$), considering each 1 mmol·L^{-1} lactate equivalent to 3 mL O_2·kg^{-1} [8]. ▶ **Fig. 1** shows the responses of oxygen uptake ($\dot{V}O_2$) and blood lactate concentration ($[La^-]$) during one supramaximal effort at 115% of $i\dot{V}O_{2MAX}$ and after the end of the exercise to measure the fast component of EPOC through a bi-exponential fit.

Study B: $MAOD_{ALT}$ test and retest reliability analysis

In study B the participants performed a GXT to assess $\dot{V}O_{2MAX}$ and $i\dot{V}O_{2MAX}$ using the same procedure described in study A and 2 supramaximal exhaustive efforts to verify the $MAOD_{ALT}$ test and retest reliability. The supramaximal exercise intensity that

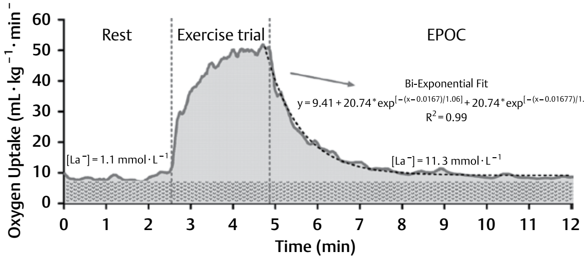

Fig. 1 Responses of oxygen uptake ($\dot{V}O_2$) and blood lactate concentration ($[La^-]$) at rest, the supramaximal effort at 115% of $\dot{V}O_2$ and after the end of exercise to measure the fast component of EPOC through a bi-exponential fit. The curved solid line corresponds to the $\dot{V}O_2$ response and the dashed line corresponds to the bi-exponential fit used in $\dot{V}O_2$ to measure the amplitude and time constant for estimating the phosphagen metabolism. The gray area corresponds to the $\dot{V}O_2$ area during the effort and EPOC whereas the dashed area corresponds to the $\dot{V}O_{2MAX}$ baseline area.

Table 1 GXT parameters.

Variable	
Exercise duration (min)	10.5 ± 0.3 (9.2 to 11.8)
$\dot{V}O_{2MAX}$ (L · min^{-1})	3.62 ± 0.13 (3.34 to 3.90)
$\dot{V}O_{2MAX}$ (mL · kg^{-1} · min^{-1})	51.8 ± 1.2 (49.2 to 54.4)
i$\dot{V}O_{2MAX}$ (km · h^{-1})	14.4 ± 0.2 (13.9 to 14.9)
HR_{MAX} (bpm)	188 ± 4 (184 to 192)
RER	1.16 ± 0.02 (1.11 to 1.20)
RPE (a. u.)	18 ± 0 (17 to 198)
$[La^-]_{PEAK}$ (mmol · L^{-1})	10.5 ± 0.6 (8.9 to 12.3)

Values are mean ± SEM (95 %CI)

resulted in a $MAOD_{ALT}$ 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 $\dot{V}O_2$-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 (η^2) obtained in each statistical analysis is also presented and interpreted as proposed by Hopkins (www.sportsci.org/resource/stats), with effect size < 0.2 considered as trivial, small between 0.2–0.5, moderate between 0.6–1.1, large between 1.2–1.9 and very large > 2.0 [16]. The comparison between MAOD and $MAOD_{ALT}$ values were completed with 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 $MAOD_{ALT}$ 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]. Study B: For the test and retest analysis the paired "t" test, confidence interval estimation for the Bland-Altman limits (95 %LoA), typical error and intra-class correlation test (ICC) were used. In all cases, a significance level of 5 % was assumed.

Results

Comparison between MAOD and the eight $MAOD_{ALT}$ (Study A)

All subjects reached the exhaustion criteria to attain $\dot{V}O_{2MAX}$ 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 $\dot{V}O_2$ at exhaustion observed with increases in exercise intensity in the supra-maximal efforts. $\dot{V}O_{2MAX}$ and $\dot{V}O_2$ at exhaustion for 100 to 115 % of i$\dot{V}O_{2MAX}$ did not differ, a statistical difference being found for $\dot{V}O_2$ at exhaustion (i. e., peak of $\dot{V}O_2$) at exercise intensities higher than 115 % of i$\dot{V}O_{2MAX}$ ($P < 0.002$) (**Table 2**). In addition, significant correlations ($r = 0.58$ to 0.69) were found between $\dot{V}O_{2MAX}$ and $\dot{V}O_2$ at exhaustion during the supra maximal efforts.

The slope, y-intercept and coefficient of determination (R^2) of the $\dot{V}O_2$-velocity relationship were 3.5 ± 0.1 mL · kg^{-1} · min^{-1} km · h^{-1}, 4.7 ± 0.2 mL · kg^{-1} · min^{-1} and 0.92 ± 0.01, respectively. The MAOD and all the $MAOD_{ALT}$ values are presented in **Fig. 2**, and the values did not differ statistically for absolute ($P = 0.56$; $F_{(8,112)} = 0.666$, $\eta^2 = 0.045$), normalized by total body mass ($P = 0.78$; $F_{(8,112)} = 0.595$, $\eta^2 = 0.041$) or lean mass ($P = 0.154$; $F_{(8,112)} = 1.943$, $\eta^2 = 0.122$) (mL · kg^{-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 $MAOD_{ALT}$ determined at 100 and 115 % of i$\dot{V}O_{2MAX}$ (for absolute values, relative to body mass and lean mass), but the $MAOD_{ALT}$ at 115 % of i$\dot{V}O_{2MAX}$ demonstrated better concordance based on effect size, 95 %LoA and typical error. Only the $MAOD_{ALT}$ values presented for lean mass showed a high effect size, 95 %LoA and typical error.

Reliability of $MAOD_{ALT}$ (Study B)

The $\dot{V}O_{2MAX}$ corresponded to 4.10 ± 0.10 L · min^{-1} (95 %CI 3.88 to 4.32 L · min^{-1}) and 56.1 ± 1.3 mL · kg^{-1} · min^{-1} (95 %CI 53.2 to 59.0 mL · kg^{-1} · min^{-1}), whereas the i$\dot{V}O_{2MAX}$ 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 i$\dot{V}O_{2MAX}$ (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 $MAOD_{ALT}$ 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 tlim, $\dot{V}O_2$ at exhaustion and other parameters engaged in the determination of the metabolic pathways of glycolytic (i. e., blood lactate responses and $W_{[La]}$) 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 $MAOD_{ALT}$ measured using different supramaximal intensities were not statistically different (**Fig. 2**). In addition, $MAOD_{ALT}$ measured at 115 % of i$\dot{V}O_{2MAX}$ presented the higher correlations and concordance with MAOD.

Study A

The establishment of $MAOD_{ALT}$ by Bertuzzi et al. [3] is based on previous studies indicating that the resynthesis of high-energy

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 $MAOD_{ALT}$ (n = 15).

	Exercise intensity at $i\dot{V}O_{2MAX}$										
	100%	**105%**	**110%**	**115%**	**120%**	**130%**	**140%**	**150%**	**$F_{(7;98)}$**	**p-value**	**η^2**
Velocity ($km \cdot h^{-1}$)	$14.4 \pm 0.0.23^{abcdefg}$	15.1 ± 0.2^{bcdefg}	15.9 ± 0.3^{cdefg}	16.5 ± 0.3^{defg}	17.3 ± 0.3^{efg}	18.7 ± 0.3^{fg}	20.2 ± 0.3^{g}	21.6 ± 0.3	3723.8	0.000	0.996
tlim (s)	316.9 ± 17.0^{abcdefg}	239.1 ± 10.8^{bcdefg}	197.0 ± 12.0^{cdefg}	156.8 ± 7.6^{defg}	134.1 ± 7.4^{efg}	92.4 ± 4.6^{fg}	67.1 ± 3.8^{g}	52.7 ± 4.0	189.15	0.000	0.931
$\dot{V}O_2$ at exhaustion ($mL \cdot kg^{-1} \cdot min^{-1}$)	51.1 ± 5.0^{deg}	51.6 ± 6.0^{deg}	50.5 ± 4.2^{deg}	49.6 ± 5.5	47.7 ± 3.6	46.5 ± 4.8	47.6 ± 4.8	45.0 ± 4.9	8.212	0.000	0.370
$W_{[La]}$ ($mL \cdot kg^{-1}$)	27.9 ± 1.7	28.8 ± 1.5	30.7 ± 1.6	30.7 ± 1.7	30.5 ± 1.6	30.7 ± 1.4	30.0 ± 1.7	31.9 ± 1.8	1.40	0.213	0.091
$[La^-]_{PEAK}$ ($mmol \cdot L^{-1}$)	10.4 ± 0.6	10.7 ± 0.5	11.4 ± 0.5	11.4 ± 0.6	11.4 ± 0.5	11.4 ± 0.5	11.2 ± 0.6	11.9 ± 0.5	1.661	0.128	0.106
$\Delta[La^-]$ ($mmol \cdot L^{-1}$)	9.3 ± 0.6	9.6 ± 0.5	10.2 ± 0.5	10.2 ± 0.6	10.2 ± 0.5	10.2 ± 0.5	10.0 ± 0.6	10.6 ± 0.6	1.403	0.213	0.091
W_{PCR} ($mL \cdot kg^{-1}$)	21.7 ± 1.0	22.1 ± 0.9	22.8 ± 0.7	21.7 ± 0.7	21.5 ± 1.0	22.0 ± 0.9	24.2 ± 1.2	18.9 ± 1.6	0.673	0.428	0.046

Values are mean ± SEM. F, p-value and η^2 values were obtained by one-way repeated measures Analysis of Variance

[a] Significantly different from 105 % ($p < 0.05$)

[b] Significantly different from 110 % ($p < 0.05$)

[c] Significantly different from 115 % ($p < 0.05$)

[d] Significantly different from 120 % ($p < 0.05$)

[e] Significantly different from 130 % ($p < 0.05$)

[f] Significantly different from 140 % ($p < 0.05$)

[g] Significantly different from 150 % ($p < 0.05$)

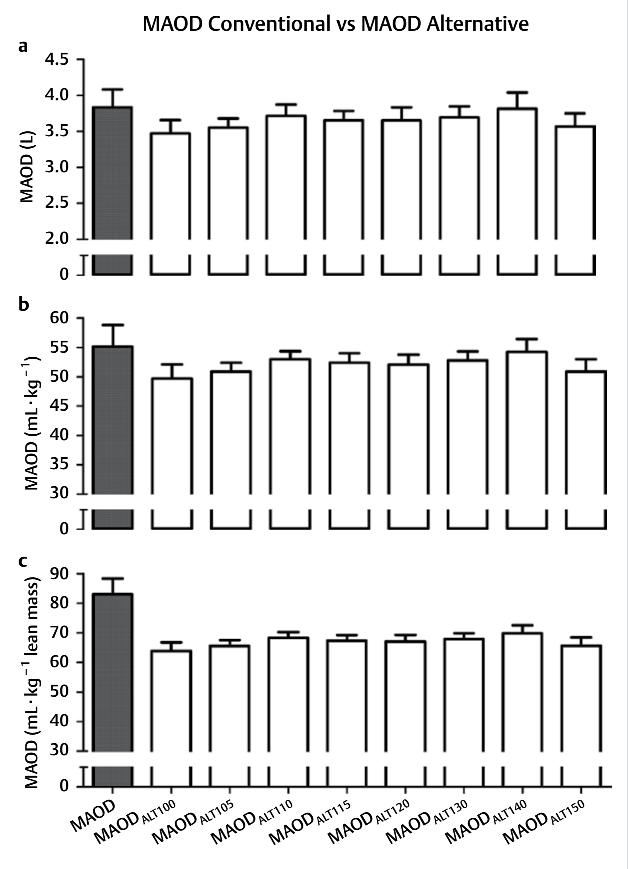

Fig. 2 Maximal accumulated oxygen deficit determined by the conventional method (MAOD) and by the single supra maximal effort method ($MAOD_{ALT}$) and expressed in absolute **a**, relative to total body mass **b** and relative to lean mass **c** values. The $MAOD_{ALT}$ was determined in 8 different supra maximal intensities corresponding to 100 ($MAOD_{ALT100}$), 105 ($MAOD_{ALT105}$), 110 ($MAOD_{ALT110}$), 115 ($MAOD_{ALT115}$), 120 ($MAOD_{ALT120}$), 130 ($MAOD_{ALT130}$), 140 ($MAOD_{ALT140}$) and 150 % ($MAOD_{ALT150}$) of $i\dot{V}O_{2MAX}$.

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 O_2 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 $i\dot{V}O_{2MAX}$ 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 $i\dot{V}O_{2MAX}$-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 $MAOD_{ALT}$ 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 $MAOD_{ALT}$ assessed at 115 %

of $i\dot{V}O_{2MAX}$ presented the highest concordance and coefficient of correlation with the conventional method. In other words, MAOD is determined using a supramaximal effort varying between 110 and 125% of $i\dot{V}O_{2MAX}$ [3,10,35,38,39]. The application of a supramaximal effort that leads to a time to exhaustion of less than 2-min seems not to be long enough to deplete the anaerobic stores, and therefore the MAOD values are underestimated [23], but this was not observed in the current study (**Fig. 2**). Some studies [23] have described that the ideal supramaximal time to exhaustion seems to be between 2–3 min (i.e., exercise intensity range varying between 110 and 120% of $i\dot{V}O_{2MAX}$). However, Medbo et al. [23] also reported that exercise lasting more than 3-min can increase the statistical error in determining MAOD. The exercise intensity at 115% of $i\dot{V}O_{2MAX}$ was assumed as the best intensity to determine the $MAOD_{ALT}$ as the time to exhaustion verified was 156.8 ± 29.4 s, respecting the range of 2–3 min reported by Medbo et al. [23].

Table 3 Comparison between the conventional MAOD and the 8 values of $MAOD_{ALT}$ expressed in absolute values and relative to body mass and lean mass. (n = 15).

MAOD		$MAOD_{ALT100}$	$MAOD_{ALT105}$	$MAOD_{ALT110}$	$MAOD_{ALT115}$	$MAOD_{ALT120}$	$MAOD_{ALT130}$	$MAOD_{ALT1140}$	$MAOD_{ALT150}$
Absolute values	*r (95% IC)*	0.59 * (0.11 to 0.84)	0.49 (−0.02 to 0.80)	0.38 (−0.16 to 0.75)	0.73 ** (0.34 to 0.90)	0.26 (−0.29 to 0.68)	0.24 (−0.315 to 0.67)	0.30 (−0.25 to 0.70)	0.30 (−0.25 to 0.71)
	Effect size	−0.37 (small)	−0.34 (small)	−0.03 (trivial)	−0.12 (trivial)	−0.17 (trivial)	−0.09 (trivial)	0.15 (trivial)	−0.33 (small)
	95%LoA	−0.26 L; ± 0.43	−0.18 L; ± 0.46	−0.01 L; ± 0.49	−0.08 L; ± 0.39	−0.08 L; ± 0.54	−0.04 L; ± 0.53	−0.08 L; ± 0.56	−0.16 L; ± 0.53 L
	Typical error	0.66 L	0.72 L	0.77 L	0.61 L	0.84 L	0.82 L	0.86 L	0.82 L
Body mass values	*r (95% IC)*	0.57 * (0.08 to 0.84)	0.47 (−0.05 to 0.79)	0.27 (−0.28 to 0.69)	0.77 *** (0.43 to 0.92)	0.10 (−0.44 to 0.58)	0.12 (−0.41 to 0.60)	0.14 (−0.40 to 0.61)	0.21 (−0.34 to 0.65)
	Effect size	−0.41 (small)	−0.40 (small)	−0.13 (trivial)	−0.14 (trivial)	−0.47 (trivial)	−0.24 (small)	0.13 (trivial)	−0.50 (small)
	95%LoA	$-3.95\,mL \cdot kg^{-1}$; ± 6.45	$-2.75\,mL \cdot kg^{-1}$; ± 6.98	$-0.63\,mL \cdot kg^{-1}$; ± 7.53	$-1.26\,mL \cdot kg^{-1}$; ± 5.87	$-1.57\,mL \cdot kg^{-1}$; ± 8.10	$-0.86\,mL \cdot kg^{-1}$; ± 7.96	$0.61\,mL \cdot kg^{-1}$; ± 8.23	$-2.72\,mL \cdot kg^{-1}$; ± 7.91
	Typical error	$10.03\,mL \cdot kg^{-1}$	$10.85\,mL \cdot kg^{-1}$	$11.71\,mL \cdot kg^{-1}$	$9.13\,mL \cdot kg^{-1}$	$12.60\,mL \cdot kg^{-1}$	$12.38\,mL \cdot kg^{-1}$	$12.79\,mL \cdot kg^{-1}$	$12.30\,mL \cdot kg^{-1}$
Lean mass value	*r (95% IC)*	0.49 * (−0.03 to 0.80)	0.33 (−0.22 to 0.72)	0.16 (−0.38 to 0.62)	0.65 ** (0.20 to 0.87)	−0.07 (−0.56 to 0.46)	−0.09 (−0.57 to 0.44)	0.00 (−0.51 to 0.51)	0.11 (−0.43 to 0.59)
	Effect size	−1.48 (large)	−1.98 (large)	−2.37 (very large)	−1.29 (large)	−1.37 (large)	−1.34 (large)	−1.26 (large)	−2.89 (very large)
	95%LoA	$-17.36\,mL \cdot kg^{-1}$; ± 11.80	$-15.67\,mL \cdot kg^{-1}$; ± 12.76	$-12.88\,mL \cdot kg^{-1}$; ± 12.52	$-13.88\,mL \cdot kg^{-1}$; ± 11.34	$-14.15\,mL \cdot kg^{-1}$; ± 14.67	$-13.30\,mL \cdot kg^{-1}$; ± 14.47	$-11.39\,mL \cdot kg^{-1}$; ± 14.76	$-15.58\,mL \cdot kg^{-1}$; ± 14.20
	Typical error	15.07 mL · kg	16.30 mL · kg	17.26 mL · kg	14.47 mL · kg	18.73 mL · kg	18.48 mL · kg	18.85 mL · kg	18.13 mL · kg

* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

Coefficients of correlation (*r*), mean bias; confidence limits as ± value obtained by Bland-Altman concordance analysis

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% $i\dot{V}O_{2MAX}$ 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% (ICC = 0.95) and 120% of peak exercise intensity during GXT in cycling (ICC = 0.97). However, Doherty and Smith [9] questioned the outcomes of Weber and Schneider [35] mainly because of the heterogeneity of the participants, suggesting that inclusion of males and females and individuals of varying fitness levels could facilitate the correlation values. In addition, Doherty et al. [10] in a running study at 125% $i\dot{V}O_{2MAX}$ reported that the MAOD is not a reliable method due to the high limits of agreement of 95% of $15.1\,mL \cdot kg^{-1} \cdot min^{-1}$ 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 $MAOD_{ALT}$ 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 ± $2.9\,mL \cdot kg^{-1}$ (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 $MAOD_{ALT}$ assessed at 115% $i\dot{V}O_{2MAX}$ 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 $i\dot{V}O_{2MAX}$ can be used to estimate the conventional MAOD, but the test-retest reliability was tested only for 115% $i\dot{V}O_{2MAX}$ 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

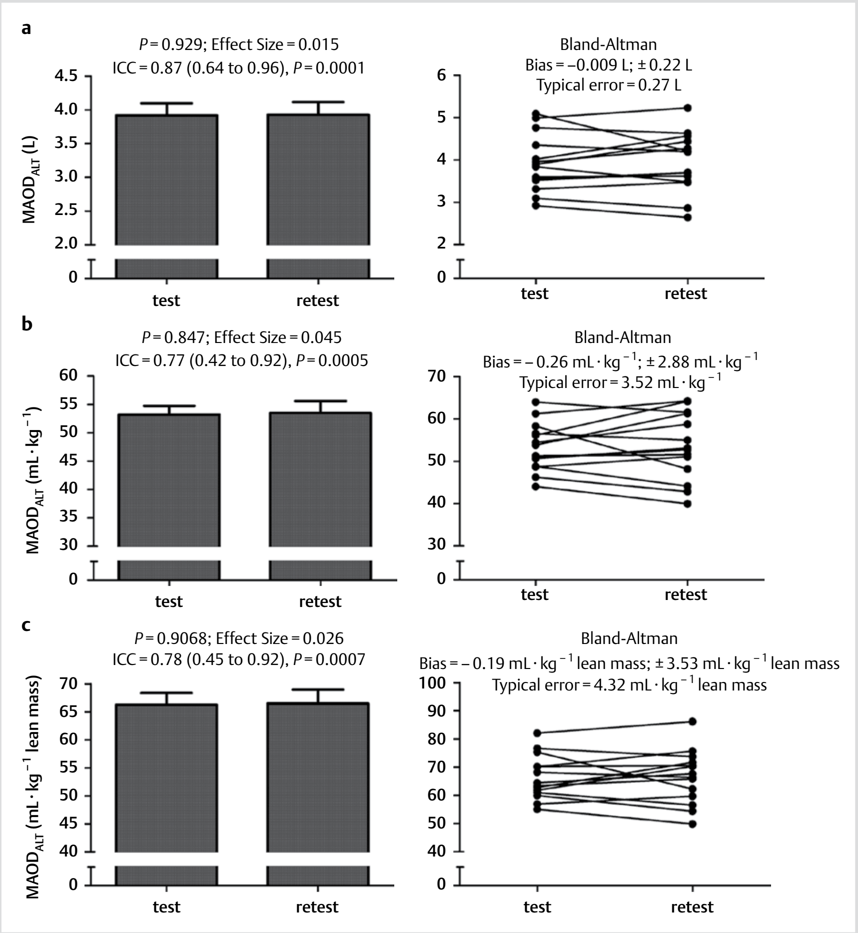

Fig. 3 Values of $MAOD_{ALT}$ measured in test and retest conditions presented in absolute **a**, relative to body mass **b** and relative to lean mass **c** values. (n = 14). The bar values (left panels) correspond to mean ± SEM and the values in lines (right panels) correspond to individual values.

Table 4 Time to exhaustion (tlim), oxygen uptake at exhaustion ($\dot{V}O_2$ exhaustion) and other parameters engaged in the determination of the glycolytic (i. e., blood lactate responses and $W_{[La]}$) and phosphagen (W_{PCR}) metabolic pathways in the test and retest conditions (n = 14).

	Test	Retest	Effect Size	95 %LoA	*p*-value	ICC (95 %CI)
tlim (s)	113.4 ± 7.0 (98.3 to 128.4)	112.7 ± 8.0 (95.3 to 130.1)	− 0.02 (trivial)	0.64 (− 9.57 to 10.86)	0.89	0.83 ** (0.55 to 0.94)
$\dot{V}O_2$ at exhaustion (mL · kg^{-1} · min^{-1})	53.7 ± 5.6 (50.4 to 56.9)	54.5 ± 5.0 (51.7 to 57.4)	0.17 (trivial)	− 0.84 (− 2.43 to 0.76)	0.28	0.88 ** (0.68 to 0.96)
$W_{[La]}$ (mL · kg^{-1})	31.0 ± 1.5 (27.7 to 34.2)	29.7 ± 2.2 (25.0 to 34.4)	− 0.21 (small)	1.26 (− 1.76 to 4.28)	0.38	0.75 ** (0.39 to 0.91)
$[La^-]_{PEAK}$ (mmol · L^{-1})	11.7 ± 0.5 (10.5 to 12.8)	11.1 ± 0.7 (9.6 to 12.7)	− 0.23 (small)	0.52 (− 0.49 to 1.53)	0.29	0.75 ** (0.38 to 0.91)
$\Delta[La^-]$ (mmol · L^{-1})	10.3 ± 0.5 (9.2 to 11.4)	9.9 ± 0.7 (8.3 to 11.5)	− 0.18 (trivial)	0.42 (− 0.59 to 1.43)	0.38	0.75 ** (0.39 to 0.91)
W_{PCR} (mL · kg^{-1})	22.2 ± 0.8 (20.5 to 24.0)	23.8 ± 1 (21.6 to 25.9)	0.45 (small)	− 1.53 (− 3.09 to 0.04)	0.06	0.72 ** (0.32 to 0.90)

** $p < 0.01$. Values are mean ± SEM (95 %CI). *p*-value obtained by paired t-test

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 O_2 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 $MAOD_{ALT}$, indicating its ability to predict anaerobic capacity. Our results also revealed that $MAOD_{ALT}$ determined at 115 % of $i\dot{V}O_{2MAX}$ 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].

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Affiliations
[1] Department of Physical Education, UNESP- Univ Estadual Paulista, Bauru, Brazil
[2] School of Physical Education and Sport, University of São Paulo (USP), São Paulo, Brazil
[3] Post-Graduate Program in Motricity Sciences, UNESP - Univ Estadual Paulista, Rio Claro – SP, Brazil
[4] University eCampus, Novedrate (CO), Italy
[5] School of Physical Education and Sport of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[6] Faculty of Kinesiology, University of Split, Split, Croatia

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