

Research article

## Maximal Oxygen Uptake cannot be Determined in the Incremental Phase of The Lactate Minimum Test on a Cycle Ergometer

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### Abstract

The aim of this study was to investigate the maximal oxygen uptake ( $VO_{2MAX}$ ) determined using the incremental phase of the lactate minimum test (LM) on a cycle ergometer. Fifteen trained men were submitted to a graded exercise test (GXT) to evaluate the  $VO_{2MAX}$  and LM. The total durations of the GXT and LM were  $11.2 \pm 1.8$  minutes (CI95%:10.2-12.3 minutes) and  $25.3 \pm 3.2$  minutes (CI95%:23.5-27.0), respectively. For the variables measured at exhaustion in both the GXT and LM, the oxygen uptake ( $54.6 \pm 8.1$  ml·kg<sup>-1</sup>·min<sup>-1</sup> vs  $50.0 \pm 7.7$  ml·kg<sup>-1</sup>·min<sup>-1</sup>), carbon dioxide production ( $66.1 \pm 7.5$  ml·kg<sup>-1</sup>·min<sup>-1</sup> vs  $50.4 \pm 8.0$  ml·kg<sup>-1</sup>·min<sup>-1</sup>), ventilation ( $153.9 \pm 19.0$  L·min<sup>-1</sup> vs  $129.9 \pm 22.9$  L·min<sup>-1</sup>), respiratory exchange ratio ( $1.22 \pm 0.10$  vs  $1.01 \pm 0.05$ ), maximal power output achieved ( $331.6 \pm 45.8$  W vs  $242.4 \pm 41.0$  W), heart rate ( $183.1 \pm 6.9$  bpm vs  $175.9 \pm 10.6$  bpm) and lactate ( $10.5 \pm 2.3$  mmol·L<sup>-1</sup> vs  $6.6 \pm 2.2$  mmol·L<sup>-1</sup>) were statistically lower in the LM ( $p < 0.05$ ). However, the values of rating of perceived exertion ( $17.6 \pm 2.5$  for GXT and  $17.2 \pm 2.3$  for LM) did not differ (ES = 0.12 and CV = 7.8%). There was no good agreement between the values of the  $VO_{2MAX}$  from the GXT and  $VO_{2PEAK}$  from the LM, as evidenced in the Bland-Altman plot ( $4.7$  ml·kg<sup>-1</sup>·min<sup>-1</sup> and  $0.34$  L·min<sup>-1</sup> of mean differences, respectively), as well as the high values of the upper and lower limits of agreement. We conclude that the  $VO_{2PEAK}$  values obtained in the incremental phase of the LM underestimate the  $VO_{2MAX}$ .

**Key words:** Maximal aerobic power, aerobic capacity, aerobic and anaerobic fitness.

### Introduction

The lactate minimum test (LM) is considered a valid protocol for estimating the maximal lactate steady state (MLSS) intensity in only one test session (Bacon and Kern, 1999; MacIntosh et al., 2002; Tegtbur et al., 1993; Simoes et al., 1999; Svedahl and MacIntosh, 2003). The LM is comprised of an intense effort to hyperlactatemia induction, followed by a brief recovery period (i.e., ~ 8 min) and finally an incremental exercise phase. Therefore, during the hyperlactatemia induction phase, the LM allows the use of either an anaerobic test and consequently measurement of anaerobic power/capacity (Dantas De Luca et al., 2003; Zagatto et al., 2004; Pardonon et al., 2008; Zagatto et al., 2014) or a graded exercise test (GXT) and the measurement of maximal oxygen uptake ( $VO_{2MAX}$ ) and maximal aerobic power (Johnson and

Sharpe, 2011; Johnson et al., 2009). However, some studies report that one should be cautious in the choice of the hyperlactatemia induction mode (Johnson et al., 2009; Zagatto et al., 2014), as it can influence determination of lactate minimum intensity and physiological responses at lactate minimum intensity.

In addition, some studies have related the possibility of obtaining indices of maximal aerobic power (i.e.,  $VO_{2MAX}$  and the intensity associated with  $VO_{2MAX}$ ) during the incremental phase of the LM (Dantas De Luca et al., 2003; Simoes et al., 2003). In this way, the application of a procedure for assessing anaerobic fitness using hyperlactatemia induction would be possible, as well as the determination of  $VO_{2MAX}$  and the lowest intensity where the  $VO_{2MAX}$  is reached ( $iVO_{2MAX}$ ) during the incremental phase of the LM. This possibility makes the LM even more attractive for application in the training routines of athletes, because it would enable determination of anaerobic fitness during the induction phase and aerobic endurance,  $VO_{2MAX}$  and  $iVO_{2MAX}$ , during the incremental exercise phase.

However, none of these studies (Dantas De Luca et al., 2003; Simoes et al., 2003) performed analysis to confirm whether the individuals actually reached  $VO_{2MAX}$  during the GTX, such as attaining the oxygen uptake plateau ( $VO_2$ ), maximum heart rate achieved (HR), respiratory exchange ratio (RER) or peak lactate values (Mier et al., 2012; Midgley and Carroll, 2009; Howley et al., 1995). In addition, it has been recommended that progressive protocols for measuring the  $VO_{2MAX}$  should have a length of around 10-12 minutes. This recommendation is due to evidence of a possible underestimation of  $VO_{2MAX}$  values when using protocols of shorter or longer duration (Buchfuhrer et al., 1983; Yoon et al., 2007). Thus, the possibility of measuring the  $VO_{2MAX}$  in the incremental phase of the LM is still not well established and needs further comparisons.

Therefore, the aim of this study was to investigate  $VO_{2MAX}$  determination using the incremental phase of the LM on a cycle ergometer. Considering the findings of the studies that verified the possibility that  $VO_{2MAX}$  can be obtained in a progressive test even after induction of lactic acidosis (Simoes et al., 2003; Dantas De Luca et al., 2003), we compared the highest  $VO_2$  value measured in the incremental phase of LM with the  $VO_{2MAX}$  determined during the GXT. As a hypothesis, we expected similar  $VO_2$  values from both tests as reported in the literature.

## Methods

### Subjects

Fifteen healthy trained men (twelve cyclists and three triathletes, aged  $31 \pm 6$  years, height of  $1.74 \pm 0.07$  m, body weight of  $74.5 \pm 9.9$  kg, body mass index of  $24.4 \pm 2.6$  kg·m<sup>-2</sup>), with a weekly training volume of between 200 and 400 km, voluntarily participated in the study. The sample size was calculated based on the similarity between values of peak oxygen consumption ( $\text{VO}_{2\text{-PEAK}}$ ) obtained in the incremental test performed with and without an effort to induce hyperlactatemia, as well as their correlation of 0.80 and 95% of test power, which resulted in a minimum sample size of ten participants. The sample size was calculated using G\*Power 3.0.10 software, (G\*Power, Franz Faul, Germany). All participants were informed about the possible risks and benefits of the study procedures and were only eligible for participation in the study after signing a written consent form. All procedures were approved by the Ethics Committee in Research of the Federal University of Mato Grosso do Sul (UFMS) (Process no. 1979/201) and were conducted according to the Declaration of Helsinki.

### Experimental design

The subjects were instructed to avoid caffeine and alcohol during the evaluation period and not to perform strenuous exercise for at least 24 hours prior to each session.

All procedures were performed on an electromagnetic cycle ergometer (Ergoline ER 900, JAEGER, Germany), except for the Wingate test, which was conducted on a mechanically braked cycle ergometer (Biotec, CEFISE, Brazil). Participants performed two visits to the laboratory and were submitted to the graded exercise test (GXT) and lactate minimum test (LM), respectively. Prior to the two tests, a warm up lasting five minutes was performed at 75W, where the subjects were instructed to choose a cadence of their choice (75-90 rpm), which was standardized for both tests. These procedures were applied with a minimum interval of 48 hours.

During the GXT and the LM, the oxygen uptake ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), pulmonary ventilation ( $V_E$ ) and respiratory exchange ratio (RER) were measured breath-to-breath through a stationary gas analyzer (Quark PFT, COSMED, Rome, Italy). The gas analyzer was calibrated using known sample gases (3.98%  $\text{CO}_2$  and 16.02%  $\text{O}_2$ ) and a pneumotachograph through a 3-liter syringe (Hans Rudolf, Kansas City, Missouri, USA), as recommended by the manufacturer. For the analysis of respiratory variables, data were smoothed each 5 points and interpolated each 1 second for the elimination of outlying data, as suggested by Ozyener et al. (2001). The heart rate (HR) was measured using a transmitter belt coupled to the gas analyzer. The Borg scale (6-20) was used to assess the rating of perceived exertion (RPE) which was measured at the end of each exercise stage of the incremental phase of the LM and GXT. In order to determine the starting values of  $\text{VO}_2$ ,  $\text{VCO}_2$ ,  $V_E$ , HR and RER before the two tests ( $\text{VO}_{2\text{-START}}$ ,  $\text{VCO}_{2\text{-START}}$ ,  $V_{E\text{-START}}$ ,  $\text{HR}_{\text{START}}$  and  $\text{RER}_{\text{START}}$ , respectively), the average of 20 seconds immediately prior to the onset testing

was determined. In the same way, the values at exhaustion for  $\text{VO}_2$ ,  $\text{VCO}_2$ ,  $V_E$ , HR and RER ( $\text{VO}_{2\text{-PEAK}}$ ,  $\text{VCO}_{2\text{-PEAK}}$ ,  $V_{E\text{-PEAK}}$ ,  $\text{HR}_{\text{PEAK}}$  and  $\text{RER}_{\text{PEAK}}$ ) were considered as the highest average of the last 20 seconds of each incremental exercise stage (Poole et al., 2008).

For determination of blood lactate concentrations during the LM, blood samples from the earlobe were collected immediately after each stage of the incremental phase using a heparinized capillary and transferred to Eppendorf tubes containing 50 $\mu$ l of 1% sodium fluoride (NaF) for further analysis in an electrochemical lactate analyzer YSI 1500 Sport (Yellow Springs Instruments, USA). Blood samples were collected immediately prior to the GXT and LM for determination of lactate concentrations ( $\text{LAC}_{\text{START}}$ ), and also at 5 and 7 minutes after the GXT and LM to determine the peak lactate concentration ( $\text{LAC}_{\text{PEAK}}$ ).

### Graded Exercise Test (GXT)

The GXT was performed five minutes after the warm up, with an initial intensity of 75W and 25W increments each minute until volitional exhaustion or until the subject could not maintain the pre-stipulated cadence.

The first ventilatory threshold ( $\text{VT}_1$ ) corresponded to the intensity at which the ventilatory equivalent of oxygen ( $V_E/\text{VO}_2$ ) systematically increased without a concomitant increase in the ventilatory equivalent of carbon dioxide ( $V_E/\text{VO}_2$ ), while the second ventilatory threshold ( $\text{VT}_2$ ) corresponded to the intensity at which there was a concomitant increase in both  $V_E/\text{VO}_2$  and  $V_E/\text{VCO}_2$  (Dekerle et al., 2003).

The highest average  $\text{VO}_2$  of the last 20 seconds of each stage was considered as  $\text{VO}_{2\text{MAX}}$ , as long as at least two of the following criteria were observed: the  $\text{VO}_2$  plateau (variation in the  $\text{VO}_2 < 2.1$  ml·kg<sup>-1</sup>·min<sup>-1</sup> between the last and the penultimate exercise stage); HR maximal ( $\text{HR}_{\text{MAX}}$ )  $\geq 90\%$  of the  $\text{HR}_{\text{MAX}}$  predicted for age. (220 - age); RER  $\geq 1.10$  and  $\text{LAC}_{\text{PEAK}} \geq 8.0$  mmol·L<sup>-1</sup>, as suggested by Howley and colleagues (Howley et al., 1995). If at least two  $\text{VO}_{2\text{MAX}}$  criteria were not observed, the GXT was applied again. The maximum power output achieved ( $\text{PO}_{\text{PEAK}}$ ) in the GXT was considered as the greatest intensity performed in a completed stage. When exhaustion occurred prior to the completion of a full exercise stage; the  $\text{PO}_{\text{PEAK}}$  was calculated as suggested by Kuipers et al. (1985). The  $\text{PO}_{\text{PEAK}}$  of GXT was used to calculate the initial intensity and rate of incremental effort in the LM.

### Lactate minimum test (LM)

Initially, the Wingate test was used as the hyperlactatemia induction mode, which corresponded to a maximal effort lasting 30 seconds with a workload equivalent to 7.5% of body mass (Bar-Or, 1987). The power peak (PP), PP relative to body mass, mean power (MP), MP relative to body mass and fatigue index (FI) were calculated.

After eight minutes of passive recovery, the incremental phase was initiated with an initial intensity corresponding to 35% of the  $\text{PO}_{\text{PEAK}}$  measured during the GXT with increments of 5% of the  $\text{PO}_{\text{PEAK}}$  of GXT every 3 minutes until exhaustion (Zagatto et al., 2014). The highest intensity of exercise performed in the incremental

phase of the LM was calculated using the same procedure described for the GXT.

The intensity of the LM was considered as the point where the lowest lactate value was observed using a second order polynomial function fitting blood lactate to power. In the second order polynomial function, fitting the blood lactate versus power output curve for the lactate minimum intensity determination, the fits were only considered valid with an  $a$  value  $> 0$  (i.e., second order polynomial equation) and determination coefficients ( $R^2$ ) higher than 0.80, as suggested by De Araujo et al. (2007).

### Statistical analysis

Data are presented as means, standard deviations (SD) and 95% confidence intervals (CI 95%). In order to verify data normality, the *Shapiro-Wilk's* test was used. For comparison of respiratory variables measured immediately before the two tests (GXT and LM) and at the moment of exhaustion the paired *t*-test, effect size (ES) (Cohen, 1988) and coefficient of variation (CV) of the difference were used. The analysis of variance (ANOVA) for repeated measures was used to compare the intensities of  $VT_1$ ,  $VT_2$  and LM. The *Pearson* correlation was adopted to verify the level of association and the *Bland-Altman* plot was used to verify the level of agreement between the  $VO_2$  values obtained at the exhaustion moment of the GXT ( $VO_{2MAX}$ ) and LM ( $VO_{2-PEAK}$ ). In all cases a significance level of  $p < 0.05$  was assumed. Statistical analyses were performed using the software package SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

### Results

The total duration of the GXT was  $11.2 \pm 1.8$  minutes (CI95%: 10.2-12.3 minutes), while the incremental phase of LM was  $25.3 \pm 3.2$  minutes (CI95%: 23.5-27.0). In all subjects at least two criteria for confirmation of the  $VO_{2MAX}$  were verified in the GXT, hence the application of a further test was not required.

The PP, PP relative to body mass, MP, MP relative to body mass and FI from the Wingate test were  $916.5 \pm 139.5$  W (CI95%: 839.2-993.7 W),  $12.3 \pm 0.9$  W·kg<sup>-1</sup> (CI95%: 11.8-12.7 W),  $708.8 \pm 119.4$  W (CI95%: 642.6-774.9 W),  $9.5 \pm 0.8$  W·kg<sup>-1</sup> (CI95%: 9.0-9.9 W) and  $44.6 \pm 8.1\%$  (CI95%: 40.1-49.0%), respectively. The LM intensity corresponded to  $189.0 \pm 26.7$  W (CI95%: 174.2-203.8 W) and expressed relative to the  $PO_{PEAK}$  of the GXT corresponded to  $57.0 \pm 2.3\%$  (CI95%: 55.7-58.3%).

The LM intensity was higher than the  $VT_1$  ( $173.2 \pm 30.2$  W; CI95%: 155.8-190.6 W) but lower than the  $VT_2$  ( $259.7 \pm 51.7$  W; CI95%: 231.1-288.3 W) ( $F_{(2,26)} = 81.771$ ;  $p < 0.0001$ ;  $ES = 0.86$  and statistical power = 100%), although it was statistically correlated with both  $VT_1$  and  $VT_2$  ( $r = 0.86$  and  $0.92$ , respectively;  $p < 0.001$ ).

The values of the respiratory parameters ( $VO_2$ ,  $VCO_2$ ,  $V_E$ , HR and RER and RER), heart rate and blood lactate concentration measured immediately before the GXT and LM are presented in Table 1. The  $VO_{2-START}$ ,  $VCO_{2-START}$ ,  $V_{E-START}$ ,  $HR_{START}$ , HR expressed relative to  $HR_{MAX}$  from the GXT ( $HR_{\%MAX-START}$ ) and  $LAC_{START}$  were higher at the beginning of the LM compared with the same period of the GXT ( $p < 0.05$ ), except for  $RER_{START}$  which did not differ. In addition, in general the effect sizes verified were considered very large (ES around 0.66 to 3.00), as well as high coefficients of variation (CV  $> 11\%$ ). No correlations were observed between the resting values of the GXT and LM measured immediately before the GXT and LM (Table 1).

For the variables measured at exhaustion (Table 2), the respiratory variables,  $PO_{PEAK}$ ,  $HR_{PEAK}$  and HR expressed relative to  $HR_{MAX}$  from the GXT ( $HR_{\%MAX-PEAK}$ ) and  $LAC_{PEAK}$  were statistically lower in the LM compared to the GXT ( $p < 0.05$ ), with very large effect sizes (ES 0.50 to 2.80) and coefficients of variation (CV around 3.1 to 22.2%). However, the values of RPE did not differ (ES = 0.166 and CV = 7.8%). There was no good agreement between the values of the  $VO_{2MAX}$  from the GXT and  $VO_{2-PEAK}$  from the LM both in absolute values and relative to body mass, as evidenced by the distance between zero and the mean of the differences,  $4.7$  ml·kg<sup>-1</sup>·min<sup>-1</sup> and  $0.34$  L·min<sup>-1</sup>, respectively. Moreover, high values of upper and lower limits of agreement were observed, reinforcing the weak agreement between values (Figure 1).

### Discussion

The main finding of this study was the significant difference found between  $VO_{2-PEAK}$  assessed during the incremental phase of the LM and the  $VO_{2MAX}$  measured during a robust GXT, rejecting our initial hypothesis. The GXT protocol used to evaluate the  $VO_{2MAX}$  followed the main recommendations in the literature, refuting previous findings from other authors which suggested the incremental phase of the lactate minimum as being able to measure this physiological variable (Simoes et al., 2003; Dantas De Luca et al., 2003).

**Table 1.** Mean ( $\pm$ SD) and [CI95%] corresponding to oxygen uptake ( $VO_{2-START}$ ), carbon dioxide production ( $VCO_{2-START}$ ), ventilation ( $V_{E-START}$ ), respiratory exchange ratio ( $RER_{START}$ ), heart rate ( $HR_{START}$ ) and percentage of  $HR_{MAX}$  from the GXT ( $HR_{\%MAX-START}$ ) immediately before the GXT and LM.

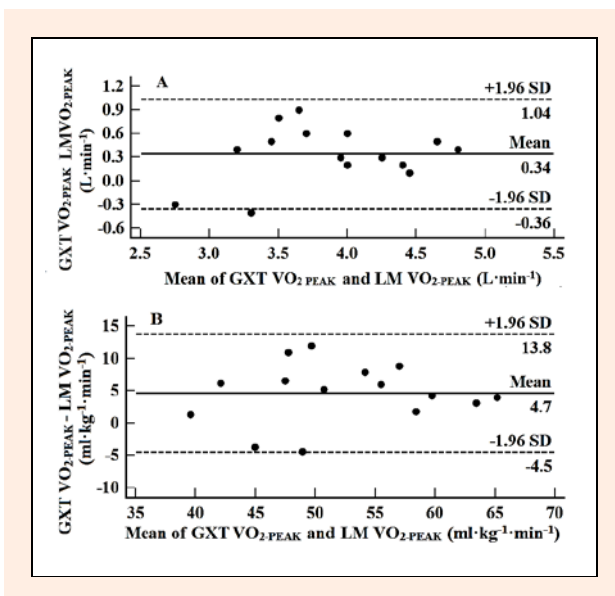
	GXT	LM	ES	CV (%)	CoC (r)
$VO_{2-START}$ (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	7.4 (.9) [6.8-8.0]	9.9 (2.3)* [8.1-11.6]	1.562	19.6	.43
$VO_{2-START}$ (L·min <sup>-1</sup> )	.6 (.1) [.4-.6]	.7 (.2)** [.6-.8]	.666	19.6	.36
$VCO_{2-START}$ (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	7.1 (1.2) [6.3-7.8]	10.4 (2.1)* [8.9-11.9]	2.000	27.4	.21
$VCO_{2-START}$ (L·min <sup>-1</sup> )	.5 (.1) [.4-.5]	.8 (.1)*** [.6-.8]	3.000	27.4	.30
$V_{E-START}$ (L·min <sup>-1</sup> )	17.6 (3.5) [15.3-19.8]	33.8 (8.2)*** [27.9-39.7]	2.769	44.8	-.21
$RER_{START}$	.96 (.12) [.88-1.03]	1.07 (.10) [.99-1.13]	1.000	11.5	-.47
$HR_{START}$ (bpm)	76.6 (12.5) [68.5-84.5]	97.7 (9.3)** [91.0-104.3]	1.935	19.4	.19
$HR_{\%MAX-START}$ (%)	41.8 (6.9) [37.5-46.2]	53.4 (4.7)** [50.1-56.8]	2.012	19.4	.20
$LAC_{START}$ (mmol·L <sup>-1</sup> )	1.64 (0.55) [1.31-1.95]	13.0 (3.1)* ** [11.1-14.7]	1.950	108.3	-.26

\*, \*\* and \*\*\* denote  $p \leq 0.05$ , 0.01 and 0.001 respectively in relation to GXT. CoC = Coefficients of correlation

**Table 2.** Mean (SD) and [CI95%] corresponding to oxygen uptake ( $\text{VO}_{2\text{-PEAK}}$ ), carbon dioxide production ( $\text{VCO}_{2\text{-PEAK}}$ ), ventilation ( $\text{V}_{\text{E-PEAK}}$ ), respiratory exchange ratio ( $\text{RER}_{\text{PEAK}}$ ), heart rate ( $\text{HR}_{\text{PEAK}}$ ), percentage of  $\text{HR}_{\text{MAX}}$  from the GXT ( $\text{HR}_{\% \text{MAX-PEAK}}$ ), rating of perceived exertion ( $\text{RPE}_{\text{PEAK}}$ ) and power output ( $\text{PO}_{\text{PEAK}}$ ) at the exhaustion moment of the GXT and LM.

	GXT	LM	ES	CV (%)	CoC (r)
$\text{VO}_{2\text{-PEAK}}$ ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	54.6 (8.1) [50.1-59.1]	50.0 (7.7)** [45.7-54.3]	.582	7.9	.83 <sup>§</sup>
$\text{VO}_{2\text{-PEAK}}$ ( $\text{L}\cdot\text{min}^{-1}$ )	4.0 (.6) [3.7-4.4]	3.7 (.6)** [3.4-4.0]	.500	8.0	.83 <sup>§</sup>
$\text{VCO}_{2\text{-PEAK}}$ ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	66.1 (7.5) [61.8-70.2]	50.4 (8.0)*** [45.9-54.8]	2.025	19.4	.63 <sup>§</sup>
$\text{VCO}_{2\text{-PEAK}}$ ( $\text{L}\cdot\text{min}^{-1}$ )	4.9 (.6) [4.6-5.2]	3.7 (.5)*** [3.4-4.0]	2.181	19.4	.59 <sup>§</sup>
$\text{V}_{\text{E-PEAK}}$ ( $\text{L}\cdot\text{min}^{-1}$ )	153.9 (19.0) [143.4-164.4]	129.9 (22.9)*** [117.2-142.6]	1.145	13.0	.45
$\text{RER}_{\text{PEAK}}$	1.22 (.10) [1.17-1.28]	1.01 (.05)*** [0.98-1.03]	2.800	13.5	.69 <sup>§</sup>
$\text{HR}_{\text{PEAK}}$ (bpm)	183.1 (6.9) [179.2-186.9]	175.9 (10.6)*** [170.1-181.8]	.822	3.1	.79 <sup>§</sup>
$\text{HR}_{\% \text{MAX-PEAK}}$ (%)	100.0 (.0)	96.0 (3.7)*** [94.0-98.1]	2.054	3.1	-
$\text{LAC}_{\text{PEAK}}$ ( $\text{mmol}\cdot\text{L}^{-1}$ )	10.5 (2.3) [9.3-11.8]	6.6 (2.2)** [5.3-7.8]	1.733	35.0	.74 <sup>§</sup>
$\text{RPE}_{\text{PEAK}}$	17.6 (2.5) [16.3-18.9]	17.2 (2.3) [15.9-18.5]	.166	7.8	.50
$\text{PO}_{\text{PEAK}}$ (W)	331.6 (45.8) [306.2-356.9]	242.4 (41.0)*** [219.7-265.0]	2.055	22.2	.87 <sup>§</sup>

\*, \*\* and \*\*\* denote  $p \leq 0.05$ , 0.01 and 0.001 respectively in relation to GXT. <sup>§</sup>  $p < 0.05$ . CoC = Coefficients of correlation



**Figure 1.** Bland-Altman plot between the values of oxygen uptake obtained at the exhaustion moment ( $\text{VO}_{2\text{-PEAK}}$ ) of the graded exercise test (GXT) and lactate minimum test (LM) in absolute values (A) and relative to body mass (B).

Simoes et al. (2003) compared the metabolic and ventilatory responses during an LM (hyperlactatemia induction performed with a Wingate test) and individual anaerobic threshold (IAT) tests. The incremental phase of the LM test and the IAT were performed identically and the  $\text{VO}_{2\text{MAX}}$  was not significantly different. With the same goal, Dantas de Luca et al. (2003) investigated the possibility of determining  $\text{VO}_{2\text{-PEAK}}$  in the LM test on a cycle ergometer comparing two identical incremental tests with and without hyperlactatemia induction. Similar values were observed in both the LM and the incremental test without hyperlactatemia induction. However, in neither the study by Simoes et al. (2003) or that of Dantas de Luca et al. (2003), which compared the  $\text{VO}_2$  measured at exhaustion during an incremental test with and without hyperlactatemia induction, was an insightful analysis conducted to confirm the determination of the  $\text{VO}_{2\text{MAX}}$ . In addition, the length of the incremental test without hyperlactatemia induction in both studies was longer than recommended in the literature for progressive tests to evaluate  $\text{VO}_{2\text{MAX}}$  ( $10 \pm 2$  minutes). This long lasting protocol

may have led to underestimated values of  $\text{VO}_{2\text{MAX}}$  (Buchfuhrer et al., 1983) and was probably also observed in the present study since the LM lasted for around 25 minutes. In addition, Zagatto et al. (2014) described that the mode of hyperlactatemia induction modifies the performance and physiological responses in the LM mainly due to the pronounced acidosis, such as found after a Wingate test.

It is noteworthy that the duration of the incremental stages of the LM is at least three minutes due to the necessity of analysis of the slow kinetics of blood lactate, resulting in a long incremental protocol for assessment of the  $\text{VO}_{2\text{MAX}}$ . In longer, compared to shorter progressive protocols for assessment of the  $\text{VO}_{2\text{MAX}}$ , underestimation of the  $\text{VO}_{2\text{MAX}}$  values may occur as a result of a complication in the oxygen transport due to an increase in core temperature, increasing the peripheral vasodilatation and decreasing venous return and the ejection volume (Astorino et al., 2004; Yoon et al., 2007). Thus,  $\text{VO}_{2\text{MAX}}$  determined through the incremental phase of the LM must be evaluated with care and is not possible with this approach.

Similar to the results reported by Dantas de Luca et al. (2003), in the present study higher values of  $\text{VO}_{2\text{START}}$  were observed in the LM compared to the GXT. This high metabolic rate after hyperlactatemia induction might be related to phosphocreatine and ATP resynthesis, replenishment of oxygen from the blood and muscle and lactate removal (Gaesser and Brooks, 1984; Borsheim and Bahr, 2003). In addition, this increase in  $\text{VO}_2$  after an intense effort can also be explained by increased body temperature, ventilation and circulation (Borsheim and Bahr, 2003). These explanations are supported by the highest  $\text{V}_{\text{E-START}}$  and  $\text{HR}_{\text{START}}$  in this study (Table 1), so that the high  $\text{V}_E$  at the beginning of the test could also be related to the mechanism of hyperventilation in response to acidosis caused by the intense effort performed in the hyperlactatemia induction phase (Dantas De Luca et al., 2003), resulting in an increased  $\text{VCO}_2$ .

The LM requires an effort intense enough to induce high levels of blood lactate, which requires a large energy demand of the anaerobic pathways. However, although the effort used in this study (i.e., Wingate test) has a significant contribution from the anaerobic lactic metabolism (Beneke et al., 2002), it appears to be too

short to contribute significantly to the depletion in muscle glycogen stores. Moreover, the difference in the pattern of increments in the two tests (1min vs 3min) requires that the individual supports each stage of exercise for a longer period in the LM compared to the GXT, requiring a greater contribution of the anaerobic lactic metabolism and consequently, affecting ability to endure high intensities in the incremental phase. This information is also supported by the low values of  $RER_{PEAK}$ ,  $HR_{PEAK}$  and  $LAC_{PEAK}$  after LM and similar values for  $RPE_{PEAK}$  (Table 2), indicating that exhaustion occurred prior to obtaining the maximum physiological stress and probably due to depleting energy substrate, which occurs owing to test duration. Furthermore, these factors related to energy substrate depletion and the pattern of increment in the exercise stages could explain the observation of lower  $VO_{2-PEAK}$  in the LM compared to  $VO_{2-PEAK}$  in the GXT and the strong correlations between these variables (Table 2), as well as the low level of reproducibility and agreement (Figure 1).

Similar to the physiological variables, in the present study the largest values for  $PO_{PEAK}$  were observed in the GXT compared to the LM and the strongest correlations between them (Table 2). The exercise intensity peak obtained in the incremental test has been shown to be highly reproducible and has the ability to predict the average power in both time-trial cycling (Balmer et al., 2000) and running performances over 5 and 10 km (Machado et al., 2013). However, the reproducibility and validity of this parameter as a performance indicator is likely to be dependent on the duration of the exercise stages in the incremental protocol (Machado et al., 2013; Bentley and McNaughton, 2003). In our study, the tests (GXT vs LM) had different stage durations (1min vs 3min), so that they were similar to other protocols used in studies that found no differences in  $VO_{2-PEAK}$ , but the intensity peak of the incremental test was higher in shorter protocols (Bentley and McNaughton, 2003; Bishop et al., 1998; Roffey et al., 2007). Furthermore, as previously reported, our results indicate that the individuals do not appear to have reached a maximum physiological stress in the LM and therefore, the assessment of higher intensity reached in the incremental test of the LM should be interpreted and used with caution.

The LM intensity was statistically higher than the  $VT_1$  but lower than the  $VT_2$ . The LM is considered to represent the  $VT_2$  and therefore a value higher than  $VT_1$  is expected. However, a value lower than  $VT_2$  could be attributed to differences in the incremental protocol. In the current study, the GXT was designed to accurately measure the  $VO_{2MAX}$ . There are several studies in the literature affirming that the design of the graded exercise test can affect  $VT_2$  (Bentley and McNaughton 2003), such as Bentley and McNaughton (2003) who described that  $VT_2$  intensity is altered according to stage duration of the graded exercise test in cycling. In addition, LM intensity allows estimation of the maximal exercise intensity at which a blood lactate equilibrium occurs, and therefore the comparison of LM with MLSS seems to be more highly recommended, mainly based on the significant difference between MLSS intensity and  $VT_2$ . To reinforce this affirmation, recently Keir et al. (2015) reported that MLSS

intensity and  $VT_2$  intensity were statistically different but that oxygen uptake at MLSS and  $VT_2$  did not differ, suggesting that they may manifest similar physiological phenomenon.

A possible limitation of this study is the duration of the incremental phase of the LM employed ( $25.3 \pm 3.2$  minutes). In order to develop the LM protocol the success rate found for determining lactate minimum intensity with a greater number of points before the derived zero was taken into account (Miyagi et al., 2013), which generated the expected number of points. However, the increment in the LM is lower than in the GXT (25W vs 5% of the  $PO_{PEAK}$  from GXT), which together with the length of each stage (3 min), results in a large difference in the metabolic demand of each stage of exercise, influencing the maximum intensity achieved and also in obtaining a maximum physiological stress in the LM. Thus, further investigations on the modification in the pattern of increments in LM (i.e., shorter protocols) are required to verify the possibility of determining the  $VO_{2MAX}$  in the incremental phase, although such changes do not influence the main objective of this evaluation protocol, which was aerobic capacity determination.

As a practical application, the LM can be considered advantageous because it allowed the aerobic (i.e., lactate minimum intensity) and anaerobic indices (i.e., peak power, mean power and fatigue index) to be obtained in the same session. In athletes, simultaneous evaluation of aerobic and anaerobic parameters in training routines provides an insight into performance and adaptation to training (Hasanli et al., 2014). The literature has shown that the evaluator can choose either an evaluation of anaerobic fitness or GXT to determine  $VO_{2MAX}$  during the hyperlactatemia induction phase of the LM test (Pardono et al., 2008; Johnson et al., 2009). However, our findings suggest that the incremental phase will only allow the determination of aerobic capacity (i.e., lactate minimum intensity) and not  $VO_{2MAX}$ .

## Conclusion

Therefore, from the results of the present study it is concluded that the  $VO_{2-PEAK}$  values obtained in the incremental phase of the LM underestimate  $VO_{2MAX}$ . Therefore, it is not possible to evaluate this indicator of aerobic power in the incremental phase of the LM.

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### Key points

- The VO<sub>2</sub>MAX is not attained during the incremental phase of the lactate minimum test;
- The physiological responses at exhaustion during LM are not similar to physiological responses measured during GXT;
- There is a weak agreement between the peak VO<sub>2</sub> measured at exhaustion during LM and the VO<sub>2</sub>MAX measured during GXT.

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