

# Influence of the selection from incremental stages on lactate minimum intensity: a pilot study

## *Influência da seleção dos estágios incrementais sobre a intensidade de lactato mínimo: estudo piloto*

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**Abstract** – The purposes of this study were to assess the influence of stage selection from the incremental phase and the use of peak lactate after hyperlactatemia induction on the determination of the lactate minimum intensity (iLACmin). Twelve moderately active university students (23±5 years, 78.3±14.1 kg, 175.3±5.1 cm) performed a maximal incremental test to determine the respiratory compensation point (RCP) (initial intensity at 70 W and increments of 17.5 W every 2 minutes) and a lactate minimum test (induction with the Wingate test, the incremental test started at 30 W below RCP with increments of 10 W every 3 minutes) on a cycle ergometer. The iLACmin was determined using second order polynomial adjustment applying five exercise stage selection: 1) using all stages (iLACmin<sub>p</sub>); 2) using all stages below and two stages above iLACmin<sub>p</sub> (iLACmin<sub>A</sub>); 3) using two stages below and all stages above iLACmin<sub>p</sub> (iLACmin<sub>B</sub>); 4) using the largest and same possible number of stages below and above the iLACmin<sub>p</sub> (iLACmin<sub>I</sub>); 5) using all stages and peak lactate after hyperlactatemia induction (iLACmin<sub>D</sub>). No differences were found between the iLACmin<sub>p</sub> (138.2±30.2 W), iLACmin<sub>A</sub> (139.1±29.1 W), iLACmin<sub>B</sub> (135.3±14.2 W), iLACmin<sub>I</sub> (138.6±20.5 W) and iLACmin<sub>D</sub> (136.7±28.5 W) protocols, and a high level of agreement between these intensities and iLACmin<sub>p</sub> was observed. Oxygen uptake, heart rate, rating of perceived exertion and lactate corresponding to these intensities was not different and was strongly correlated. However, the iLACmin<sub>B</sub> presented the lowest success rate (66.7%). In conclusion, stage selection did not influence the determination of iLACmin but modified the success rate.

**Key words:** Aerobic capacity; Lactate-intensity relationship; Lactatemia; Mathematical model.

**Resumo** – Os objetivos foram verificar a influência da seleção de estágios da fase incremental e o uso do lactato pico após indução hiperlactacídêmica na determinação da intensidade de lactato mínimo (iLACmin). Doze universitários moderadamente ativos (23±5 anos, 78,3±14,1 kg, 175,3±5,1 cm) realizaram um teste incremental máximo para determinação do ponto de compensação respiratório (PCR) (início a 70 W e incrementos de 17,5 W a cada 2 minutos) e um teste de lactato mínimo (indução com Wingate, fase incremental iniciado a 30 W abaixo do PCR e incrementos de 10 W a cada 3 minutos) em cicloergômetro. A iLACmin foi determinada utilizando ajuste polinomial de segunda ordem, aplicando cinco seleções de estágios da fase incremental: 1) Utilizando todos os estágios obtidos (iLACmin<sub>p</sub>); 2) Utilizando todos os estágios antes e dois após à iLACmin<sub>p</sub> (iLACmin<sub>A</sub>); 3) Utilizando dois estágios antes e todos após à iLACmin<sub>p</sub> (iLACmin<sub>B</sub>); 4) Utilizando o maior e mesmo número possível de estágios anteriores e posteriores à iLACmin<sub>p</sub> (iLACmin<sub>I</sub>); 5) Utilizando todos os estágios e o lactato pico após indução (iLACmin<sub>D</sub>). Não foram encontradas diferenças entre iLACmin<sub>p</sub> (138,2±30,2 W), iLACmin<sub>A</sub> (139,1±29,1 W), iLACmin<sub>B</sub> (135,3±14,2 W), iLACmin<sub>I</sub> (138,6±20,5 W), iLACmin<sub>D</sub> (136,7±28,5 W) e verificou-se alta concordância entre essas intensidades e iLACmin<sub>p</sub>. O consumo de oxigênio, frequência cardíaca, percepção subjetiva de esforço e lactato nessas intensidades não diferiram e foram fortemente correlacionadas. Entretanto, a iLACmin<sub>B</sub> apresentou o menor índice de sucesso (66,7%). Conclui-se que a seleção de estágios não influenciou na determinação da iLACmin, mas alterou o índice de sucesso.

**Palavras-chave:** Capacidade Aeróbia; Relação lactato-intensidade; Lactacidemia; Modelo matemático

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## INTRODUCTION

The lactate minimum (LACmin) test, as well as individual anaerobic threshold<sup>1</sup>, anaerobic threshold<sup>2</sup>, respiratory compensation point<sup>3</sup> and others<sup>4</sup>, have been shown to be reproducible<sup>5-7</sup>, valid<sup>8-10</sup> and reliable<sup>11-13</sup> for estimating maximal lactate steady state intensity (iMLSS), which is considered as the gold standard procedure in the evaluation of aerobic capacity. The LACmin consists of an effort to induce hyperlactatemia followed by an incremental test and it has advantages over other protocols that aim to estimate the iMLSS, for example by not using fixed lactate values, such as the 4 mmol•L<sup>-1</sup> proposed by Heck et al.<sup>2</sup>. In addition, during the LACmin test, it is possible to assess aerobic (aerobic capacity) and anaerobic (anaerobic power and capacity) parameters in only one experimental session<sup>14-16</sup>.

Blood lactate concentrations ([lac]) measured during the incremental phase of the LACmin test have a U-shaped profile, which is the lactate minimum intensity (iLACmin), corresponding to the lowest lactate value (minimum point); this is considered to be the highest workload, at which a dynamic balance occurs between lactate production and removal<sup>7</sup>. The minimum lactate point in the lactate-exercise intensity relationship for iLACmin determination can be obtained visually<sup>17</sup> or by mathematical adjustments<sup>11,16,18</sup>.

The use of mathematical procedures enables better adjustments to the lactate-intensity relationship, increasing the accuracy of determining iLACmin<sup>15</sup>. Therefore, analysis of the lactate-intensity response can be performed using a lower number of points (i.e., stages of exercise applied at incremental phases of the LACmin test), reducing application time<sup>9,15,19</sup>. However, in most studies, iLACmin determination is performed without detailed mathematical analysis of the adjustment applied to obtain the U-shaped fit, such as the analysis of an accurate polynomial fit. One of the few studies that did use this analysis was the study of De Araujo and colleagues<sup>20</sup>, which suggested adding a success rate in polynomial model application, only when considering values that had a determination coefficient (R<sup>2</sup>) greater than 0.80 in second order polynomial adjustment.

However, there is no consensus about the influence of these distribution points on mathematical adjustments and in determining iLACmin. Sotero et al.<sup>9</sup> investigated the influence of points selection on mathematical adjustment. The authors determined the iLACmin in standard mode using all the points obtained during the incremental phase and then selecting only three points (1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> points, 1<sup>st</sup>, 3<sup>rd</sup> and 6<sup>th</sup> points and 1<sup>st</sup>, 4<sup>th</sup> and 6<sup>th</sup> points). They did not find differences between the four iLACmin protocols determined in the study, but they did not describe clearly the exercise intensity corresponding to each arrangement of three point iLACmin compared to standard iLACmin or any other physiological index, which would allow examination as to whether any of the arrangements included more points above or below the standard iLACmin. In addition, there is a similarity in the distribution of selected stages in the three arrangements tested, i.e.,

they are all composed of the first point, a point near the iLACmin and a point close to exhaustion.

Pardono et al.<sup>15</sup> based the arrangement of points on the rating of perceived exertion (RPE), determining the iLACmin with all the points obtained in the test and using three arrangements made up of three points each, which corresponded to the first stage, RPE 13 point, RPE 16 point or RPE associated with the last stage of exercise, and adopting the three stages with the lowest lactate values. The iLACmin determined, using all points and the selection of three points including the last stage of exercise intensity, underestimated the iMLSS. However, the iLACmin determined by selecting three points including the value corresponding to RPE 16 was not statistically different from iMLSS.

Therefore, the analysis of point arrangement in mathematical adjustment indicates that a shift may occur on the lactate-intensity curvature due to the location of these points, resulting in errors in iLACmin determination. In general, six stages have been used at the incremental phase of the LACmin test, with the iLACmin identified between the third and fourth stages<sup>21</sup>. However, is it necessary to use a similar number of stages before and after the zero derivative (iLACmin)? What are the effects, on iLACmin determination using mathematical adjustments, of using fewer points (one or two) before or after the derivative zero? It is believed that the iLACmin will not be dependent on the number of points, considering that the lactate-intensity behavior is adjusted by the mathematical function. However, the selection of different arrangements of these points could deface or shift the adjustment curvature, resulting in errors in the iLACmin determination.

Therefore, the purposes of this study were to assess the utilization of different arrangements and numbers of stages from the incremental phase in second order polynomial adjustment, and to investigate the use of lactate peak value after hyperlactatemia induction on the determination of iLACmin. Based on the results of Pardono et al.<sup>15</sup>, who investigated the influence of the number of points standardizing the intensities using RPE, our hypothesis is that point arrangement will not affect iLACmin determination, when applying one test with the intensities and increments selected by means of previously determined aerobic capacity.

## METHODS

### Participants

The sample was composed of twelve moderately active and healthy male students from the physical education course (recreational practitioners of futsal, volleyball, handball, running, cycling, in addition to physical activities realized in the undergraduate classes) and their characteristics are presented in Table 1. Highly trained individuals, smokers or people who had a musculoskeletal injury were not included in the study. The sample size was based on sample calculation taking into consideration the similarity between iLACmin and iMLSS, as their 0.85 correlation (Pardono et al.<sup>15</sup>)

and a test power of 90% that resulted in a minimum sample size of eight participants (software G\*Power 3.0.10, Franz Faul, Germany). All volunteers were informed about possible risks and benefits of experimental procedures and provided written informed consent prior to their participation in the study. All the procedures applied were approved by the Ethics Committee in Human Research of the Federal University of Mato Grosso do Sul (CAAE 0056.0.049.000-11) and were conducted in accordance with the Declaration of Helsinki. Subjects were instructed to refrain from strenuous exercise and to abstain from alcohol and caffeine for at least 24 hours before each test.

### Experimental procedures

According to the experimental design of the study, during the first visit, a maximal incremental test was applied on a mechanical braking cycle ergometer (Biotec 2100 CEFISE, Brazil) to determine the exercise intensity associated with the respiratory compensation point (RCP)<sup>3,22</sup>, followed by a LACmin test adapted from Tegtbur et al.<sup>11</sup> on an electromagnetic cycle ergometer (ErgoCycle 167, Ergo-Fit, Pirmasens, Germany) which was applied after approximately 48 hours. In both procedures a warm-up of five minutes on a cycle ergometer at 70 W was standard, with the tests being started five minutes after the warm-up. .

During both tests, the oxygen uptake ( $\dot{V}O_2$ ), carbon dioxide production ( $\dot{V}CO_2$ ) and pulmonary ventilation ( $\dot{V}_E$ ) were measured continuously, breath by breath, through a stationary gas analyzer True-One 2400 (ParvoMedics, East Sandy, Utah, USA). The gas analyzer was calibrated immediately before each test using known sample gas (3.98%  $CO_2$  e 16.02%  $O_2$ , Air Gas Puritan Medical, USA) and the ventilometer was calibrated using a three liter syringe (Hans Rudolf, Kansas City, Missouri, USA). Heart rate (HR) was measured by a transmitter belt (T31, Polar Electro, Kempele, Finland) and rating of perceived exertion (RPE) was measured using the Borg scale 6-20<sup>23</sup>.

For the determination of  $[La^-]$ , blood samples were collected from the ear lobe (25  $\mu$ L) at the end of each stage and also 5 and 7 minutes after each test, as well as after the Wingate test, which was applied to induce hyperlactatemia in the LACmin test. Blood was collected using a heparinized capillary and transferred to Eppendorf tubes containing 50  $\mu$ L of sodium fluoride (NaF) at 1%, being analyzed in an electrochemical lactimeter SPORT YSI 1500 (Yellow Springs Instruments, Ohio, USA)

### Maximal incremental test

The test started with an intensity corresponding to 70 W, with increments of 17.5 W at each 2 minute stage of the exercise until voluntary exhaustion or until the inability of the individual to maintain a cadence of 70 revolutions per minute (rpm).

The intensity of exercise associated with RCP corresponded to the intensity at which there was a concomitant increase in both ventilatory equivalent of oxygen ( $\dot{V}_E/\dot{V}O_2$ ) and carbon dioxide ( $\dot{V}_E/\dot{V}CO_2$ ).

## Lactate minimum test

The Wingate Test (Biotec 2100 CEFISE, Brazil) was applied to induce hyperlactatemia with a load corresponding to 7.5% of the body mass. After eight minutes of passive recovery, the subjects performed an incremental test with an initial intensity corresponding to 30 W below at RCP intensity and staged increments of 10 W every 3 min until voluntary exhaustion.

The iLACmin corresponded to zero derivative of second order polynomial adjust, where only tests that showed a value of  $a > 0$  and a determination coefficient ( $R^2$ )  $> 0.80$  were considered valid, as proposed by De Araujo et al.<sup>20</sup>. The success rate was calculated using the relationship between the tests that showed these criteria and the total sample size.

Five different iLACmin were determined which differ according to arrangements between intensities and lactate, and these corresponded to: 1) the determination of iLACmin using all stages obtained in the test, which was considered in this study as the standard iLACmin (iLACmin<sub>p</sub>), 2) iLACmin determined using all stages before and just two stages after iLACmin<sub>p</sub> (iLACmin<sub>A</sub>); 3) iLACmin determined using only two stages before and all stages after iLACmin<sub>p</sub> until exhaustion (iLACmin<sub>B</sub>), 4) iLACmin determined using the same number of points and the highest possible number of stages before and after iLACmin<sub>p</sub> (iLACmin<sub>I</sub>); 5) iLACmin determined using all points and also the value of the peak concentration of lactate obtained after hyperlactatemia induction (iLACmin<sub>D</sub>). The iLACmin<sub>D</sub> was determined by the relationship between lactate and exercise time, where the lowest lactate value corresponded to a particular exercise time, which enabled, through linear regression between time-intensity, the determination of iLACmin<sub>D</sub>.

## Statistical analysis

Data are presented as means, standard deviation (SD) and 95% confidence intervals (95%CI). The Shapiro-Wilk's test was applied to verify the data normality. The ANOVA one-way with repeated measures was used to compare the iLACmin determined by different arrangements of stages and the values of  $VO_2$ , HR and RPE corresponding to LACmin intensities. The sphericity was verified by the Mauchly's test, in case of significance, the value of the Greenhouse-Geisser adjustment was considered. The effect size (ES) was classified as negligible ( $< 0.35$ ), small (0.35 to 0.80), moderate (0.80 to 1.5) and large ( $> 1.5$ )<sup>24</sup>. The Pearson correlation test was used to search for associations between arrangements of stages, where the correlation coefficient 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)<sup>25</sup>. The Bland-Altman plot was used to check the agreement level between the intensities corresponding to the mathematical adjustments. In all cases a significance level of  $p < 0.05$  was assumed.

## RESULTS

The characteristics of the participants are presented in Table 1.

**Table 1.** Age, total body mass, height, body fat percentage and maximal oxygen uptake (VO<sub>2</sub>MAX) of study participants.

N=12	Age (years)	Body mass (kg)	height (cm)	Body fat (%)	VO <sub>2</sub> max (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )
Mean±SD	23±5	78.3±14.1	175.3±5.1	21.6±6.5	40.2±5.6

The intensity corresponding to RCP was 112.9±26.9 W (95%CI= 95.7-129.9 W). The [La<sup>-</sup>] after Wingate test was 11.1±1.6 mmol·L<sup>-1</sup> (95%CI= 10.1-12.1 mmol·L<sup>-1</sup>).

In the LACmin determination using polynomial adjustment, the success rate for iLACmin<sub>p</sub>, iLACmin<sub>A</sub> and iLACmin<sub>D</sub> was 100%, whereas it was 66,7% for iLACmin<sub>B</sub> and 83,3% for iLACmin<sub>I</sub>. The R<sup>2</sup> values and the number of points used in mathematical adjustment were, respectively, 0.91±0.06 (95%CI=0.87-0.95) and 9.7±2.1 (95%CI=8.4-11.0) for iLACmin<sub>p</sub>, 0.95±0.03 (95%CI=0.93-0.97) and 7.8±1.8 (95%CI=6.6-8.9) for iLACmin<sub>A</sub>, 0.92±0.06 (95%CI=0.88-0.97) and 5.9±1.6 (95%CI=4.6-7.2) for iLACmin<sub>B</sub>, 0.92±0.05 (95%CI=0.88-0.95) and 7.4±2.5 (95%CI=5.6-9.2) for iLACmin<sub>I</sub>, 0.93±0.05 (95%CI=0.90-0.96) and 10.3±2.4 (95%CI=8.7-11.8) for iLACmin<sub>D</sub>.

Table 2 demonstrates the iLACmin determined using different arrangements, as well as the [La<sup>-</sup>], VO<sub>2</sub>, HR and RPE corresponding to these intensities. There was not a statistical difference (p>0.05) between these parameters, and a very low effect size was observed (Table 2). Strong and significant correlations were also found between the parameters analyzed (Table 3).

The Bland-Altman plot shows good agreement between the iLACmin, with a mean close to zero for the comparison of intensities determined using different arrangements, and iLACmin<sub>p</sub> (Figure 1). However, the comparison between iLACmin<sub>p</sub> and iLACmin<sub>D</sub> showed the best level of agreement with the smallest individual errors identified by upper and lower limits (Figure 1D).

**Table 2.** Mean±standard deviation (95%CI) corresponding to intensity, [La<sup>-</sup>], VO<sub>2</sub>, HR e RPE determined by different arrangements of stages, as well as the effect size (ES).

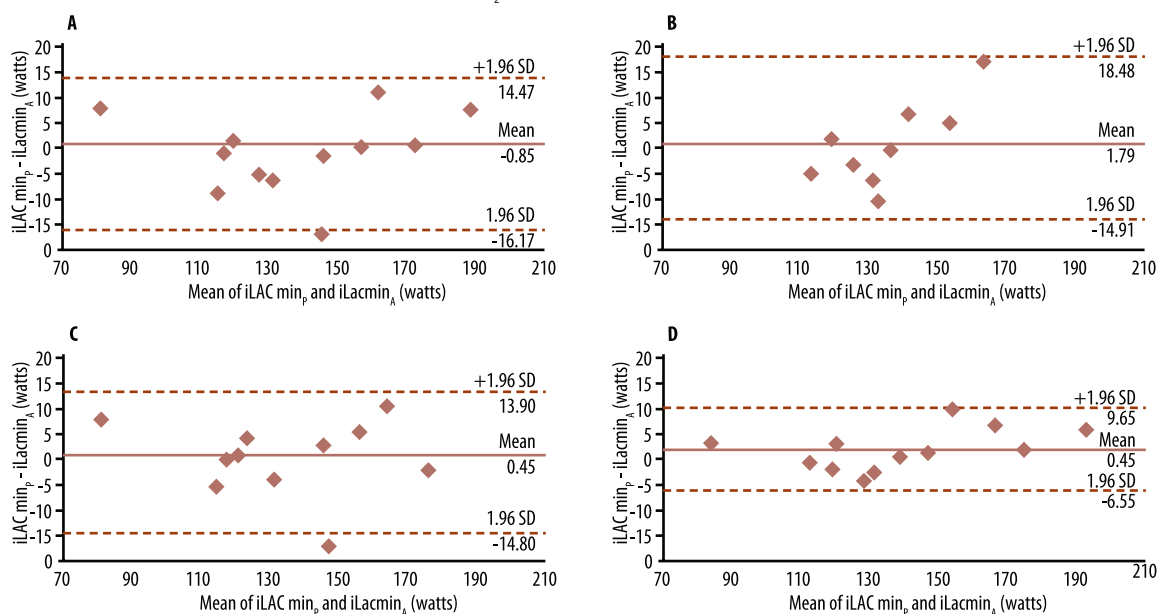
	iLACmin <sub>p</sub> (n=12)	iLACmin <sub>A</sub> (n=12)	iLACmin <sub>B</sub> (n=8)	iLACmin <sub>I</sub> (n=10)	iLACmin <sub>D</sub> (n=12)	ES
Intensity (Watts)	138.2±30.3 (119.0-157.4)	139.1±29.1 (120.6-157.5)	135.3±14.2 (123.4-147.2)	138.6±20.5 (123.9-153.2)	136.7±28.5 (118.6-154.7)	0.227
[La <sup>-</sup> ] (mmol·L <sup>-1</sup> )	5.3±1.7 (4.2-6.4)	5.1±1.7 (4.0-6.2)	5.1±1.6 (3.7-6.4)	5.0±1.4 (4.0-6.0)	5.3±1.7 (4.3-6.4)	0.108
VO <sub>2</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	28.8±3.8 (26.4-31.2)	29.2±4.2 (26.5-31.9)	28.7±3.1 (26.1-31.2)	29.3±4.2 (26.3-32.3)	28.5±3.3 (26.3-30.6)	0.238
HR (bpm)	152.7±16.9 (142.0-163.4)	153.2±15.4 (143.5-163.0)	144.6±14.1 (132.8-156.4)	148.0±12.6 (139.0-157.0)	152.2±16.2 (141.9-162.5)	0.231
RPE	12.8±2.8 (11.0-14.6)	13.0±2.8 (11.2-14.8)	13.1±3.1 (10.5-15.7)	13.1±2.9 (11.0-15.2)	12.9±2.8 (11.1-14.6)	0.153

\*p<0.05. iLACmin<sub>p</sub> = lactate minimum intensity (iLACmin) determined using all stages; iLACmin<sub>A</sub> = iLACmin determined using all stages below and two stages above iLACmin<sub>p</sub>; iLACmin<sub>B</sub> = iLACmin determined using two stages below and all stages above iLACmin<sub>p</sub>; iLACmin<sub>I</sub> = iLACmin determined using largest and same possible number of stages below and above the iLACmin<sub>p</sub>; iLACmin<sub>D</sub> = iLACmin determined using all stages and peak lactate after hyperlactatemia induction; [La<sup>-</sup>]: blood lactate concentration, VO<sub>2</sub>: oxygen uptake, HR: heart rate; RPE: rating of perceived exertion; ES: effect size.

**Table 3.** Coefficients of correlation (r) between intensity, lactate, VO<sub>2</sub>, HR and RPE corresponding to iLACminP, iLACminA, iLACminB, iLACminC and iLACminD.

	Intensity	[La <sup>-</sup> ]	VO <sub>2</sub>	HR	RPE
iLACmin <sub>p</sub> vs iLACmin <sub>A</sub>	r=0.95 <sup>†</sup>	r=0.95 <sup>†</sup>	r=0.90 <sup>†</sup>	r=0.97 <sup>†</sup>	r=0.96 <sup>†</sup>
iLACmin <sub>p</sub> vs iLACmin <sub>B</sub>	r=0.94 <sup>†</sup>	r=0.81 <sup>#</sup>	r=0.88 <sup>†</sup>	r=0.96 <sup>†</sup>	r=0.97 <sup>†</sup>
iLACmin <sub>p</sub> vs iLACmin <sub>I</sub>	r=0.94 <sup>†</sup>	r=0.91 <sup>†</sup>	r=0.85 <sup>†</sup>	r=0.96 <sup>†</sup>	r=0.97 <sup>†</sup>
iLACmin <sub>p</sub> vs iLACmin <sub>D</sub>	r=0.98 <sup>†</sup>	r=0.97 <sup>†</sup>	r=0.95 <sup>†</sup>	r=0.99 <sup>†</sup>	r=0.99 <sup>†</sup>
iLACmin <sub>A</sub> vs iLACmin <sub>B</sub>	r=0.93 <sup>†</sup>	r=0.76 <sup>#</sup>	r=0.86 <sup>†</sup>	r=0.96 <sup>†</sup>	r=0.95 <sup>†</sup>
iLACmin <sub>A</sub> vs iLACmin <sub>I</sub>	r=0.98 <sup>†</sup>	r=0.89 <sup>†</sup>	r=0.97 <sup>†</sup>	r=0.99 <sup>†</sup>	r=0.98 <sup>†</sup>
iLACmin <sub>A</sub> vs iLACmin <sub>D</sub>	r=0.95 <sup>†</sup>	r=0.92 <sup>†</sup>	r=0.91 <sup>†</sup>	r=0.97 <sup>†</sup>	r=0.97 <sup>†</sup>
iLACmin <sub>B</sub> vs iLACmin <sub>I</sub>	r=0.90 <sup>†</sup>	r=0.83 <sup>#</sup>	r=0.83 <sup>†</sup>	r=0.94 <sup>†</sup>	r=0.93 <sup>†</sup>
iLACmin <sub>B</sub> vs iLACmin <sub>D</sub>	r=0.93 <sup>†</sup>	r=0.89 <sup>†</sup>	r=0.90 <sup>†</sup>	r=0.97 <sup>†</sup>	r=0.98 <sup>†</sup>
iLACmin <sub>I</sub> vs iLACmin <sub>D</sub>	r=0.93 <sup>†</sup>	r=0.90 <sup>†</sup>	r=0.89 <sup>†</sup>	r=0.96 <sup>†</sup>	r=0.95 <sup>†</sup>

<sup>†</sup>p<0.05; <sup>#</sup>p<0.001. iLACmin<sub>p</sub> = lactate minimum intensity (iLACmin) determined using all stages; iLACmin<sub>A</sub> = iLACmin determined using all stages below and two stages above iLACmin<sub>p</sub>; iLACmin<sub>B</sub> = iLACmin determined using two stages below and all stages above iLACmin<sub>p</sub>; iLACmin<sub>I</sub> = iLACmin determined using largest and same possible number of stages below and above the iLACmin<sub>p</sub>; iLACmin<sub>D</sub> = iLACmin determined using all stages and peak lactate after hyperlactatemia induction; [La<sup>-</sup>]: blood lactate concentration, VO<sub>2</sub>: oxygen uptake, HR: heart rate; RPE: rating of perceived exertion.



**Figure 1.** Bland-Altman plot between iLACminP and iLACminA (A), iLACminP and iLACminB (B), iLACminP and iLACminI (C), iLACminP and iLACminD (D).

## DISCUSSION

The main finding of this study was that both the iLACmin and the physiological variables corresponding to this intensity were not influenced by the number and arrangement of points used in the application of second order polynomial adjustment, but the success rate in the determination of iLACmin was modified. These results meet the initial assumptions of the study and are consistent with other studies that used the same polynomial adjustment in different arrangements of points and found no differences in the determination of iLACmin<sup>9,26</sup>.

Previous studies have reported aspects that can influence the lactate-intensity mathematical relationship, such as the type of exercise adopted

to induce hyperlactatemia<sup>10,12</sup>, the type of recovery after induction<sup>16</sup>, the use of different initial intensities in the incremental phase<sup>18,27</sup> and the time interval between stages<sup>27</sup>, but have not investigated the influence of the arrangement of points of the incremental phase on the determination of iLACmin. Furthermore, none of these studies adopted a criterion for fit analysis, as used by De Araújo et al.<sup>20</sup>, which considered disabled all the tests that were verified  $R^2$  lower than 0.80 and  $a < 0$  of the polynomial fit, considering these as criteria for the success rate. The inclusion of this success rate strengthens iLACmin determination, excluding values that possibly lead to mistakes.

Although this study did not find differences in the determination of the iLACmin through polynomial fit with different arrangements of stages, studies show controversial findings. With the aim of reducing the number of incremental stages during LMT, Sotero et al.<sup>9</sup> did not find significant differences between iLACmin determined on race track running, by means of selecting different incremental stages, and iMLSS, and strong correlations were observed between all these intensities. However, Pardono et al.<sup>15</sup> found differences between iLACmin and iMLSS on the cycle ergometer where in the first arrangement all stages of the test were used, in the second the first stage, the stage of RPE 13 and the stage of exhaustion were used, the third was composed of three stages corresponding to the lower lactate values obtained in the test and the fourth consisted of the first stage and the stages corresponding to RPE 13 and 16. These authors found that the iLACmin determined by the first two arrangements were statistically different to iMLSS, while the other two did not differ. Thus, the present study corroborates the findings of Sotero et al.<sup>9</sup>, although the selection criteria of the stages was different between the studies.

In the present study, the total number of points in the arrangements used does not seem to influence the success rate, considering that the lowest average for the total number of points used (iLACmin<sub>b</sub>) (table 2) was similar to that usually observed in LACmin protocol tests (about 6 points)<sup>21</sup>. However, selecting more points before the nadir seems to have been a determining factor in achieving high success rates, as observed in iLACmin<sub>A</sub>, iLACmin<sub>p</sub> and iLACmin<sub>D</sub>. Thus, assuming that the number of points before the derivative zero is related to the initial intensity and the overload adopted after each stage during the incremental phase, our results seem to indicate that a greater number of stages before the zero derived allow a better characterization of the lactate-intensity curvature and greater reliability of mathematical fit.

In cases in which it was not possible to determine the iLACmin<sub>b</sub>, the incremental phase point resulted in a profile in [La<sup>-</sup>] different to U-shaped. Carter, Jones and Doust<sup>18</sup> found that when the initial intensity of the incremental phase corresponded to the lactate threshold (LT) or 1 km·h<sup>-1</sup> above the LT, it was not possible to determine the iLACmin. Furthermore, when the incremental phase was initiated with an intensity of 0.5 km·h<sup>-1</sup> below the LT, the iLACmin overestimated the LT. It is possible that the use of



initial intensities very close to the iLACmin represent a state very close to the balance between production and removal of lactate and, the point at which the accumulation of this metabolite is achieved rapidly occurs, resulting in an [La<sup>-</sup>] which is apparently linear.

Voltarelli, Mello and Gobatto<sup>26</sup> proposed the use of lactate peak value of induction through the relationship between lactate vs. time of exercise and differences in the iLACmin determined with and without the addition of lactate peak value of induction in swimming with rats were not found. Our study is consistent with the work of these authors, considering that the iLACmin<sub>D</sub> was not different from iLACmin determined using different arrangements and showed the highest level of agreement with iLACmin<sub>P</sub> (Figure 1D) and the smaller individual errors identified by the lower and upper limits (UL: -6.55 W; LL: 9.65 W).

Another important point to investigate in the mathematical adjustment of lactate-intensity behavior is that the [lac] during the incremental phase in the LACmin test represents not only the metabolic demands of exercise intensity<sup>27</sup>, considering that some factors involved in the transport of blood lactate and muscle can also cause changes in lactate-intensity. Among these factors, the participation of monocarboxylic transporters (MCT's) is quite relevant, considering that studies have shown that high intensity training increases the content of MCT1 in human skeletal muscle<sup>28</sup>, so that individuals who are more trained aerobically have a greater capacity to remove blood lactate during rest and exercise compared to untrained individuals<sup>29</sup>. Thus, it is believed that the adjustment in the lactate-intensity relationship could be influenced by the training status of the individual, especially the removal rate of lactate after induction to hyperlactatemia.

Accordingly, the [lac] at intensities below the iLACmin during the incremental phase could be influenced by several factors related to methodological variations. However, at intensities above iLACmin, the [lac] are basically dependent on the intensity and duration of the exercise stage, considering that after iLACmin greater production than removal of lactate occurs. However, aspects related to the points obtained after iLACmin which may perhaps influence the mathematical adjustment have not been investigated. Some authors have used, as a criterion to indicate the end of the test, the observation of three consecutive increases in [lac]<sup>7</sup>, always being obtained after iLACmin.

Therefore, several factors may have been responsible for the lowest coefficient determination values in the mathematical adjustment in cases where it was not possible to determine the iLACmin<sub>P</sub>. In other studies prior evaluations have been used to select the exercise intensities of the incremental phase, where it is possible to apply approximately the same number of stages before and after iLACmin. However, in this study this arrangement (iLACmin<sub>D</sub>) presented a success rate of 83.3%.

### Limitations of the study

A possible limitation of the study was that there was no comparison of the

variables of intensity,  $\text{VO}_2$ , HR or RPE from different intensities of lactate minimum with the iMLSS. Another limitation of the study was the sole application of a LACmin test and performing arrangements of points obtained only from this test. In addition, because the study sample consisted of moderately active students and not cyclists, the findings of this study are limited to this population, and its extrapolation to the population of cyclists should be performed with caution.

### Practical applications

The results of this study indicate that a greater number of exercise stages before the derivate zero allows a better fit in the behavior of the lactate-intensity response, and consequently, has higher success in the determination of iLACmin. Furthermore, one should be cautious when using only a few exercise stages before the iLACmin, such as when applying very high initial intensities, considering that there is a high probability of error in the determination of iLACmin.

### CONCLUSION

In sum, according to our findings, iLACmin determination through second order polynomial adjustment in this study was not influenced by different numbers and arrangements of stages or by the use of the peak lactate value after hyperlactatemia induction. However, the success rate was dependent on the arrangement of stages used in the mathematical model.

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