


**Artigo original**

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## POLYNOMIAL ADJUSTMENT AS A NEW TECHNIQUE FOR DETERMINATION OF LACTATE MINIMUM VELOCITY WITH BLOOD SAMPLING REDUCTION

### AJUSTE POLINOMIAL COMO NOVA TÉCNICA PARA DETERMINAÇÃO DA VELOCIDADE DO LACTATO MÍNIMO COM REDUÇÃO DE COLETAS SANGUÍNEAS

#### ABSTRACT

The purpose of this study was to analyze the possibility of identifying the lactate minimum velocity (LM) and estimating the maximal lactate steady state intensity (MLSS) by applying a polynomial function to just three stages of the LM test. Seventeen physically active males ( $24.1 \pm 4.0$  years;  $23.8 \pm 2.2 \text{ kg} \cdot \text{m}^{-2(-1)}$  BMI;  $11.7 \pm 3.8\%$  body fat) performed: 1) a 1600m time trial (1600mV); 2) a 150m sprint to induce hyperlactatemia, and then an incremental test (InT) consisting of 6 x 800m at intensities of 78, 81, 84, 87, 90 and 93% of 1600mV; 3) 2 to 3 sessions of constant 30 min running tests to identify MLSS. Blood lactate [lac] was determined by an electrochemical method (YSI - 2700 SELECT). The LM was identified visually (LMv) as well by applying polynomial function to the [lac] responses at all 6 stages (LMp), to the 1st, 3rd and 5th stages (LMp135), to the 1st, 3rd and 6th stages (LMp136) and to the 1st, 4th and 6th stages (LMp146) of InT. The ANOVA detected no differences between the velocities ( $\text{m} \cdot \text{min}^{-1}$ ) identified by LMv ( $196.0 \pm 17.8$ ) LMp ( $198.0 \pm 17.6$ ), LMp135 ( $197.7 \pm 17.6$ ), LMp136 ( $200.0 \pm 17.2$ ), LMp146 ( $199.7 \pm 18.1$ ) and MLSS ( $198.7 \pm 16.6$ ) ( $p > 0.05$ ), with a high correlation among each other ( $p < 0.01$ ). The polynomial function identified LM even when applied to just 3 stages of the incremental test, enabling for prediction of MLSS intensity with a reduced number of blood samples being collected during testing.

**Key words:** Lactate; Maximal lactate steady state; Running; Polynomial function.

#### RESUMO

O propósito do estudo foi analisar a possibilidade de identificação da velocidade de lactato mínimo (LM) e de se estimar a máxima fase estável de lactato (MFEL), aplicando a função polinomial de segunda ordem a partir de apenas três estágios do teste do LM. Participaram do estudo 17 homens fisicamente ativos ( $24,1 \pm 4,0$  anos;  $23,8 \pm 2,2 \text{ kg} \cdot \text{m}^{-2(-1)}$  IMC;  $11,7 \pm 3,8\%$  gordura corporal) realizaram: 1) corrida de 1600m no menor tempo possível para cálculo da velocidade média (1600mV); 2) "sprint" de 150m para indução de hiperlactatemia e um teste incremental (Tin) consistindo de 6x800m a intensidades de 78, 81, 84, 87, 90 e 93% do 1600mV; 3) 2 a 3 sessões de 30 min de corrida em intensidade constante para determinação da MFEL. O lactato sanguíneo [lac] foi determinado pelo método eletroenzimático (YSI - 2700 SELECT). O LM foi identificado visualmente (LMv) bem como aplicando ajuste polinomial usando todos os 6 estágios (LMp), o 1º, 3º e 5º estágios (LMp135), o 1º, 3º e 6º estágios (LMp136) e o 1º, 4º e 6º estágios (LMp146) do test incremental. A ANOVA evidenciou não haver diferenças entre as velocidades ( $\text{m} \cdot \text{min}^{-1}$ ) identificadas pelo LMv ( $196,0 \pm 17,8$ ), LMp ( $198,0 \pm 17,6$ ), LMp135 ( $197,7 \pm 17,6$ ), LMp136 ( $200,0 \pm 17,2$ ), LMp146 ( $199,7 \pm 18,1$ ) e MFEL ( $198,7 \pm 16,6$ ) ( $p > 0,05$ ), e alta correlação entre os métodos estudados ( $p < 0,01$ ). A identificação do LM aplicando a função polinomial em apenas 3 estágios, demonstrou ser válida em estimar a MFEL e útil por possibilitar redução no número de coletas de [lac] durante o teste.

**Palavras-chave:** Lactato; Máxima fase estável de lactato; Corrida; Função polinomial.

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## INTRODUCTION

Davis and Gass<sup>1,2</sup> and Davis et al.<sup>3</sup> first observed an equilibrium point between blood lactate production and removal during an incremental test performed after induction of hyperlactatemia. In 1993, Tegtbur et al.<sup>4</sup> proposed the lactate minimal (LM) protocol as an incremental test after maximal effort to identify the intensity corresponding to the lowest [lac]. The lactate minimum intensity (LMI) has been related to the maximal lactate steady state (MLSS), as the highest exercise intensity at which the [lac] variation is lower than 0.05mM.min<sup>-1</sup> between the 10<sup>th</sup> and 30<sup>th</sup> min of constant load exercise<sup>5,6,7</sup>.

Many studies have investigated the validity of the LM protocol, analyzing the effect of variations in the method of inducing hyperlactatemia<sup>8-12</sup>, in the recovery mode and duration after induction of hyperlactatemia<sup>13</sup> and in the intensity and duration of the incremental stages<sup>4,14</sup>. The LM test has also been applied using different ergometers<sup>4,15,17</sup> and athletic modalities<sup>4,16,17</sup> as well as in laboratory and field tests<sup>9,12,18</sup>. In general, the LM protocol has been shown to be valid for predicting the anaerobic threshold and MLSS in both humans<sup>8,9</sup> and rats<sup>20</sup>.

Traditionally, LMI has been identified by visual inspection of the [lac] response during the incremental part of the test.<sup>5,8,15,19</sup> However, mathematical models have been employed to adjust the [lac] kinetics to better identify LMI. During the LM test, the [lac] exhibits a U shape, allowing for the application of mathematical adjustments to calculate the vertice of the abscises from the equation generated. Adjustments that have commonly been applied include the cubic function,<sup>21</sup> the second order polynomial function<sup>9,16,17,22</sup>, and the Spline function<sup>4,5,20</sup>. In order to determine LMI, 5 to 10 incremental stages have often been employed. However, if mathematical adjustments could be applied to the [lac] response during the test (i.e. a polynomial function), a reduction in the number of stages and therefore in blood samples would also be possible.

Therefore, the present study aimed to investigate the possibility of identifying LMI by applying the polynomial function to just three stages during the incremental portion of the LM test, and to analyze its validity for predicting the exercise intensity corresponding to MLSS.

## METHODS

The Human Research Ethics Committee at the Catholic University of Brasília approved this study (n° 019/2004). After being informed about the risks and benefits, 17 physically active males (Table 1) were subjected to the following running tests on a 400m outdoor track, with intervals of at least 48 h. All participants were instructed to avoid physical exercise, alcoholic drinks and caffeine during the 24 h preceding the test. The running velocity during the LM and MLSS tests was controlled by an audible stimulus every 100m.

**Table 1.** Anthropometric characteristics and results of 150m and 1600m test for all volunteers (n=17).

	Age (years)	Weight (kg)	Height (m)	Body Fat (%)	150m (sec)	150m [lac] (m.min <sup>-1</sup> )	1600mV (m.min <sup>-1</sup> )	HR 1600m (bpm)
Mean	24.1	73.4	1.76	11.7	20.6	7.2	237.2	184.8
(±SD)	(4.0)	(7.1)	(0.1)	(3.8)	(1.3)	(1.6)	(22.3)	(10.4)

150m [lac]- peak blood lactate after 150m sprint; 150m (sec)- time taken for the 150m sprint; 1600mV- mean velocity for 1600m running; HR 1600m- heart rate at the end of 1600m test.

### 1600m Test

In this test, the volunteers ran 1600m as fast as possible to determine mean velocity (1600mV) and to calculate the individual velocities of the incremental stages of the LM test, as has been described by others<sup>9,12,16,18,19,24</sup>.

### LM Test

The LM test started with a 150m sprint to induce hyperlactatemia. It has been demonstrated in previous studies that such an effort effectively raises [lac] to values above 8mM and is therefore adequate for the LM test<sup>12</sup>. After a 10 min period to recover from the 150m sprint, participants performed 6 x 800 m series<sup>4</sup> with 1 min intervals for blood sampling. The 800 m series were performed at intensities of 78, 81, 84, 87, 90 and 93% of 1600mV. The [lac] for the 150m sprint was obtained between the 9<sup>th</sup> and 10<sup>th</sup> minutes of the recovery period.

### Identification of LMI by visual inspection and by polynomial adjustment

The running velocity corresponding to the lowest [lac] concentration during the incremental test was determined by visual inspection (LMv) and by applying a second order polynomial function (LMP)<sup>16</sup>.

The polynomial adjustment of [lac] response in relation to running velocity was applied to all 6 incremental stages (LMP) and also to a selection of combinations of three stages, as follows: the 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> stages (LMP135); the 1<sup>st</sup>, 3<sup>rd</sup> and 6<sup>th</sup> stages (LMP136); and the 1<sup>st</sup>, 4<sup>th</sup> and 6<sup>th</sup> stages (LMP146) (Figure 1). The polynomial function was applied using the software program Excel (Microsoft Office), and LM velocity was identified from the derivate of the second grade equation, as follows:

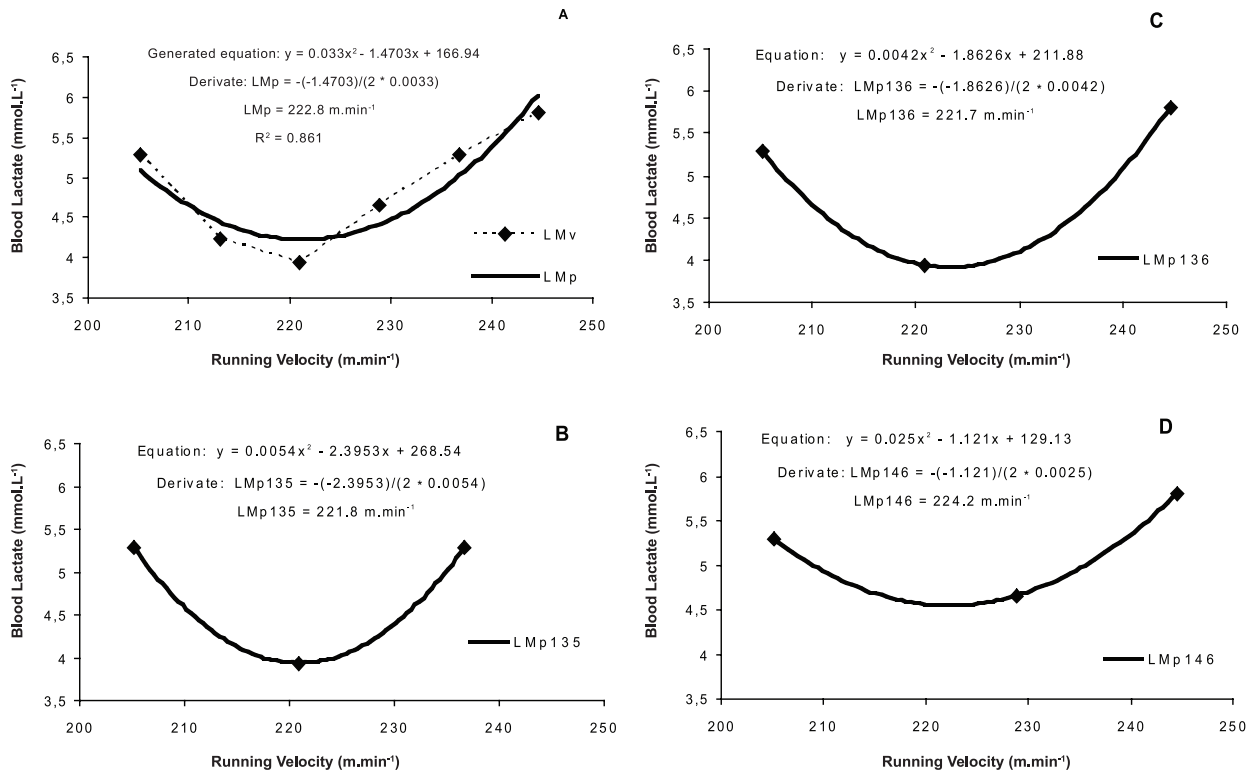
$$y = ax^2 + bx + c$$

$$\text{Derivate of the equation: } 0 = 2ax + b$$

$$x = \frac{-b}{2.a} \quad \text{or} \quad \text{LM velocity} = \frac{-b}{2.a}$$

Where a and b are constants and x is the velocity corresponding to the parabolic vertice, or lactate minimum velocity as represented in figure 1.

### MLSS Tests



**Figure 1.** Determination of LM velocity by different methods in one subject. LM was identified by visual inspection (LMv = 220.9 m.min<sup>-1</sup>) and by second order polynomial function using 6 stages (LMp = 222.8 m.min<sup>-1</sup>) (A), the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> stages (LMp135 = 221.8 m.min<sup>-1</sup>) (B), the 1<sup>st</sup>, 3<sup>rd</sup> and 6<sup>th</sup> stages (LMp136 = 221.7 m.min<sup>-1</sup>) (C) and the 1<sup>st</sup>, 4<sup>th</sup> and 6<sup>th</sup> stages (LMp146 = 224.2 m.min<sup>-1</sup>) (D).

The MLSS was determined in 8 of the 17 participants. The participants were randomly subjected, on different days, to 2 to 4 constant load tests of 30 min, at the velocity corresponding to LMv, and 3% above or below it and so on until reaching the MLSS. During the 30min constant load test a 1 min rest was allowed after each 10-min of exercise for blood collection and [lac] measurement. The MLSS was considered as the highest velocity at which the [lac] did not increase more than 0.05mM.min<sup>-1</sup> between the 10<sup>th</sup> and 30<sup>th</sup> min of exercise, according to Beneke et al. <sup>6,25</sup>.

#### Blood collections and analysis

A 25µl blood sample was taken after each stage of the LM tests as well as at each 10-min during the 30-min constant load tests. The samples were collected from the ear lobe in heparinized glass capillary tubes and deposited in Eppendorf tubes containing 50µl of NaF 1%. Blood lactate concentrations were then determined by an electrochemical method (YSI - 2700 SELECT). The heart rate was also measured during all tests (Polar Sport Tester, Finland).

#### Statistical Analysis

Data were analyzed and expressed as mean ± standard deviation (±SD). Analysis of variance for repeated measures (ANOVA) was applied (SPSS 11.5 for Windows) to compare velocities corresponding to LMv, LMp, LMp135, LMp136, LMp146 and MLSS, with multiple comparisons between pairs subsequently conducted and

adjusted by Bonferroni. Pearson's correlation coefficient was applied to test relationships between study variables and the level of significance adopted was  $p < 0.05$ . The limits of agreement between variables were also analyzed by the Bland and Altman technique<sup>27</sup>.

## RESULTS

The mean values of the intensities identified by visual inspection (vLM) and through the application of the polynomial function to 6 stages (LMp) or to only 3 stages (LMp135, LMp136 and LMp146) for the 17 individuals are presented in Table 2. Table 4 presents the intensities for the 8 participants who also performed the MLSS test.

As presented in Table 2, the repeated measures ANOVA showed no differences between the LM velocities ( $p > 0.05$ ). A high correlation was observed between LMv and LMp ( $r = 0.98$ ), LMp135 ( $r = 0.99$ ), LMp136 ( $r = 0.98$ ) and LMp146 ( $r = 0.96$ ) ( $p < 0.01$ ).

**Table 2.** Mean velocities corresponding to LMv, LMp, LMp135, LMp136 and LMp146 (n=17).

	LMv (m.min <sup>-1</sup> )	LMp (m.min <sup>-1</sup> )	LMp135 (m.min <sup>-1</sup> )	LMp136 (m.min <sup>-1</sup> )	LMp146 (m.min <sup>-1</sup> )
Mean	199.4	200.3	199.8	202.0	200.9
(±SD)	(19.4)	(19.1)	(19.4)	(18.5)	(19.0)

**Table 3.** Mean  $\pm$ SD of the running velocities corresponding to LMv, LMp, LMp135, LMp136, LMp146 and MLSS (n=8).

	LMv (m.min <sup>-1</sup> )	LMp (m.min <sup>-1</sup> )	LMp135 (m.min <sup>-1</sup> )	LMp136 (m.min <sup>-1</sup> )	LMp146 (m.min <sup>-1</sup> )	MLSS (m.min <sup>-1</sup> )
Mean	196.0	198.0	197.7	200.0	199.7	198.7
( $\pm$ SD)	(17.8)	(17.6)	(17.6)	(17.2)	(18.1)	(16.6)

When the results of the eight individuals that performed the MLSS test were analyzed, no statistical differences were observed between MLSS and any running velocity corresponding to LM identified by different methods ( $p > 0.05$ ; Table 3). A high correlation was also obtained between MLSS and LMv ( $r = 0.92$ ), LMp ( $r = 0.91$ ), LMp135 ( $r = 0.90$ ), LMp136 ( $r = 0.90$ ) and LMp146 ( $r = 0.90$ ) ( $p < 0.01$ ).

The bias  $\pm 95\%$  limits of agreement for comparisons between the speed obtained in MLSS and LMv [-2 (11.6) m.min<sup>-1</sup>], MLSS and LMp [-0.6 (13.6) m.min<sup>-1</sup>], MLSS and LMp135 [-0.9 (14) m.min<sup>-1</sup>], MLSS and LMp136 [1.2 (14.8) m.min<sup>-1</sup>], MLSS and LMp146 [1,0 (15,7) m.min<sup>-1</sup>], suggest a good agreement between the MLSS and the LM determined by different methods (Figure 2).

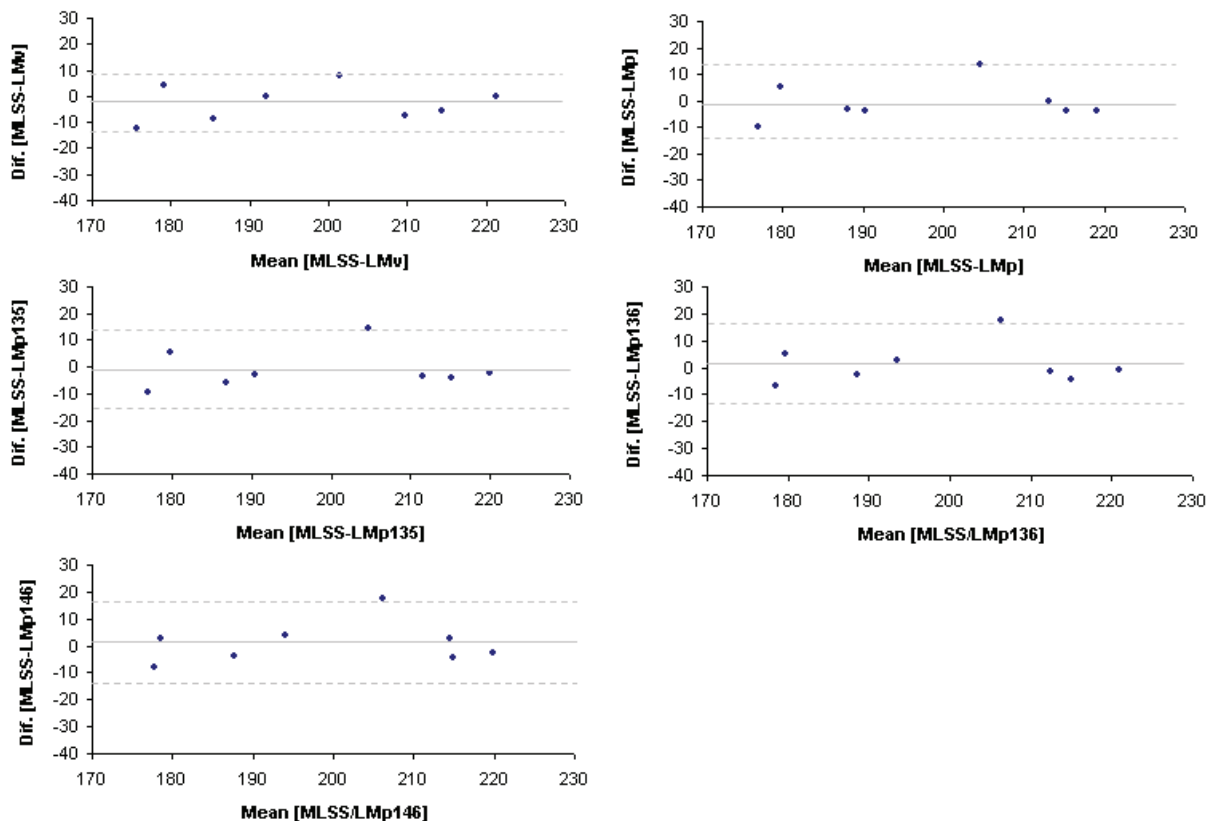
## DISCUSSION

The present study analyzed the possibility of identifying LM velocity by applying the polynomial function to a reduced number of incremental stages. The main finding was that the methods for determining

LM from either 6 or just 3 stages resulted in LM velocities that did not differ from each other, and were valid to estimate MLSS for running.

The exercise intensity corresponding to MLSS is considered a gold-standard among protocols for functional evaluation using [lac] responses. However, its applicability is difficult since many constant load exercise sessions are necessary<sup>26</sup>. The LM test protocols, such as LMv and LMp used in the present study, have shown themselves to be practical and valid for predicting MLSS. In recent decades, many studies have proposed protocols to determine MLSS intensity in a single testing session. The onset of [lac] accumulation (OBLA or 4mM lactate threshold)<sup>28</sup>, the individual anaerobic threshold<sup>29,30</sup> and the LM test are examples of such protocols. The LM protocol is more recent<sup>4</sup>, and has been shown to be valid for the estimation of anaerobic threshold and MLSS<sup>8,9,16,24</sup>.

The application of polynomial function during LM testing is possible due to the parabolic (U shaped) [lac] response. This mathematical approach has been applied in recent studies and has been demonstrated to be efficient for estimating the intensity corresponding to MLSS<sup>9,16,17,22</sup>, corroborating the present study. However, the main contribution of the present study was the identification of LM and prediction of MLSS from just 3 stages of the LM test. The application of this technique in order to reduce the number of collections and/or incremental stages, and increase its usefulness to estimate MLSS, had not been attempted before. Preliminary studies in our laboratory<sup>22</sup> had already indicated the possibility of using this model



**Figure 2.** Limits of agreement between MLSS and the LMI determined by different methods (n=8).

to reduce the number of stages and blood collections during incremental tests on cycling, with incremental stages being selected based on the rate of perceived exertion, which is extremely interesting in practical and economical terms, besides the reducing the risks associated with multiple blood sampling.

In the present study, while performing the LM protocol on a track with a reduced number of stages, no differences were identified between LM and MLSS ( $p > 0.05$ ), providing evidence of the possibility of using just three collection points to determine LM and estimate the MLSS.

Our evidence of the validity of LM for estimating MLSS are in agreement with other authors<sup>9,16,24</sup>. In the present study, no significant differences were found between the different methods of LM determination (Table 2) or between them and MLSS (Table 3). Finally, a high correlation was observed between the study protocols, and the Bland and Altman technique<sup>27</sup> detected a high level of concordance between MLSS and LM determined by different methods (Figure 2).

## CONCLUSION

It is concluded that the application of a polynomial function with a reduced number of blood collections appears to be valid for identifying LM and for predicting MLSS, allowing material to be saved and the discomfort and risks associated with several blood collections to be minimized. Therefore, we suggest that the application of the polynomial function to the 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> stages, that corresponded to 78, 84, 90% of 1600mV should be preferred, since it does not require the last incremental stage, allowing for a sub maximal instead maximal exercise test which, in turn, could be applied to sedentary people. However, more studies are necessary to investigate the application of polynomial function to the LM test using just three incremental stages in other exercise modes and populations. Furthermore, the application of the rate of perceived exertion scale to select the intensities of the incremental stages should be analyzed.

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