

# Maximal lactate steady state estimated by different methods of anaerobic threshold

## *Máximo estado estável de lactato estimado por diferentes métodos de determinação do limiar anaeróbio*

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**Abstract** – The aim of this study was to compare anaerobic threshold (AT) as determined by four different methods with maximal lactate steady state (MLSS) in endurance runners. Nine moderately trained runners performed the following tests on different days: a maximal incremental exercise test to determine maximal oxygen uptake ( $\text{VO}_2\text{max}$ ), velocity at  $\text{VO}_2\text{max}$  ( $v\text{VO}_2\text{max}$ ), and blood lactate response; and two to five 30-min constant load tests to determine MLSS. Based on the incremental test, four methods of AT determination were used: AT1 – velocity at  $3.5 \text{ mmol.L}^{-1}$  blood lactate; AT2 – velocity corresponding to the minimum lactate-velocity equivalent plus  $1.5 \text{ mmol.L}^{-1}$ ; AT3 – velocity at  $D_{\text{max}}$ ; and AT4 – velocity before the second consecutive blood lactate increase greater than  $0.5 \text{ mmol.L}^{-1}$ . There were no significant differences between MLSS and AT as determined by four different methods. However, the Bland-Altman analysis showed the extent of disagreement between variables when the subjects were analyzed individually. MLSS was significantly correlated with AT1 ( $r=0.68$ ;  $p=0.04$ ) and AT2 ( $r=0.79$ ;  $p=0.01$ ). Thus, although no significant differences were found between AT methods and MLSS, one should be cautious about using these methods interchangeably.

**Key words:** Aerobic exercise; Blood lactate; Running.

**Resumo** – O objetivo deste estudo foi determinar e comparar o limiar anaeróbio (LAN) obtido por quatro diferentes métodos com o máximo estado estável de lactato (MLSS) em corredores de endurance. Nove corredores moderadamente treinados realizaram, em diferentes dias, os seguintes testes: um teste incremental máximo para determinação do consumo máximo de oxigênio ( $\text{VO}_2\text{max}$ ), velocidade correspondente ao  $\text{VO}_2\text{max}$  ( $v\text{VO}_2\text{max}$ ) e resposta do lactato sanguíneo e; dois a cinco testes de intensidade constante, com 30 min de duração, para determinação do MLSS. A partir do teste incremental, foram utilizados quatro métodos de determinação do LAN: LAN1 - velocidade correspondente a  $[\text{La}]$  fixa de  $3,5 \text{ mmol.L}^{-1}$ ; LAN2 - velocidade referente a  $[\text{La}]$  do menor equivalente  $[\text{La}]$ -velocidade somado com  $1,5 \text{ mmol.L}^{-1}$ ; LAN3 - velocidade correspondente ao  $D_{\text{max}}$ ; LAN4 - velocidade anterior ao segundo incremento consecutivo de  $[\text{La}]$  maior que  $0,5 \text{ mmol.L}^{-1}$ . Não existiram diferenças significativas entre o MLSS e o LAN determinado pelos quatro métodos estudados. Entretanto, a análise de Bland-Altman expressou a extensão da discordância entre as variáveis quando os sujeitos foram analisados individualmente. Houve correlações significativas entre MLSS e LAN1 ( $r = 0,68$ ;  $p = 0,04$ ) e entre MLSS e o LAN2 ( $r = 0,79$ ;  $p = 0,01$ ). Assim, apesar de não haver diferença significativa entre os métodos de determinação do LAN com o MLSS, deve-se ter cautela para utilizá-los de forma intercambiável.

**Palavras-chave:** Corrida; Exercício aeróbio; Lactato sanguíneo.

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## INTRODUCTION

Maximal lactate steady state (MLSS) is the highest blood lactate concentration and submaximal work load that can be maintained over time without continual blood lactate accumulation<sup>1</sup>. MLSS is the gold standard method for determination of aerobic capacity. According to some authors, it establishes the physiological transition threshold between heavy and severe exercise domains<sup>2</sup>. To determine the MLSS it is necessary to perform, on different days, two to five 30-minute test sessions of constant-load exercise<sup>3,4</sup>.

In the past few decades, other methods have been proposed to make MLSS determination simpler. The methods use blood lactate ([La]) concentrations from a single incremental test<sup>5</sup>. Heck et al.<sup>5</sup> proposed that MLSS could be estimated from a single incremental graded exercise using fixed [La] of 4.0 or 3.5 mmol.L<sup>-1</sup> for five and three-minute long protocols, respectively. However, the use of fixed [La] does not allow for the evaluation of individual blood lactate kinetic curve during incremental exercise. Consequently, the method may underestimate or overestimate MLSS<sup>6-10</sup>.

But other methods have been proposed for determination of MLSS that to take into consideration the individual variation in [La] kinetics and the exponential increase in [La] as exercise intensity increases<sup>6-10</sup>. Before Heck et al.<sup>5</sup>, Stegmann et al.<sup>6</sup> proposed a method for determination of MLSS using individual lactate kinetics and incremental exercise. The method used both the effort stage and the subsequent and immediate recovery stage (individual anaerobic threshold – IAT). Due to the complex characteristics of determination of IAT<sup>6</sup>, Berg et al.<sup>7</sup> proposed that MLSS can be generally estimated adding 1.5 mmol.L<sup>-1</sup> to the [La] associated with the lowest lactate/intensity equivalent.

Cheng et al.<sup>8</sup> suggested that the Dmax model, which evaluates the behavior of the whole [La] curve during incremental exercise, is an objective and reliable method for determination of aerobic threshold. Others suggested that a protocol using the individual lactate minimum allows for the determination of MLSS in a single incremental exercise session<sup>9</sup>. Baldari and Guidetti<sup>10</sup> showed that the MLSS may be defined based on the intensity prior to the second lactate increase of at least 0.5 mmol.L<sup>-1</sup> in an incremental test.

Several studies have tested the validity of methods for MLSS determination in endurance runners<sup>5,9,11,12</sup>, cyclists<sup>11,13-16</sup>, rowers<sup>17</sup>, team sports<sup>9,18</sup>, physically active individuals<sup>19-22</sup> and untrained individuals<sup>4</sup>. In the specific case of runners, studies have yet to directly investigate the relationship between MLSS and anaerobic threshold (AT) determined by the methods proposed by Berg et al.<sup>7</sup>, Cheng et al.<sup>8</sup> and Baldari and Guidetti<sup>10</sup>. Based on the different approach of each method, it is possible to hypothesize that AT and MLSS measured directly will not always agree. A disagreement may indicate that different exercise intensities will be used to identify the same physiological phenomenon. This difference may interfere in prescribing and controlling aerobic training. Faude et al.<sup>23</sup> and Figueira et al.<sup>24</sup> reported a

lack of studies using the Bland-Altman diagram to assess agreement between MLSS measured directly and AT from incremental tests with runners.

The objective of the present study is to determine and compare the AT using the methods proposed by Heck et al.<sup>5</sup>, Berg et al.<sup>7</sup>, Cheng et al.<sup>8</sup> and Baldari and Guidetti<sup>10</sup> for MLSS in moderately-trained endurance runners.

## MATERIALS AND METHODS

### Participants

The study included nine moderately-trained runners with at least two years of experience with endurance training. The participants' average characteristics ( $\pm$  SD) were: age 29.2 ( $\pm$  10.9) years; weight: 64.2 ( $\pm$  8.5) kg; height: 171.8 ( $\pm$  5.7) cm; and body fat percentage: 11.3 ( $\pm$  3.7%). The average distance in the weekly training of participants was 70.0 km. All procedures in the present study were approved by the Ethics Committee of Research with Human Beings, Universidade Federal de Santa Catarina (UFSC). All participants signed an informed consent form (UFSC, protocol 222/08).

### Design and procedures

Participants were asked to arrive at the laboratory fully hydrated and fed. They were asked not to perform any intense training up to 48 hours prior to the test. The experiment was carried out in two weeks and all tests were carried out during the same period of time of the day. Initially, participants were submitted to an incremental test to determine maximum oxygen uptake ( $\text{VO}_2\text{max}$ ), speed at  $\text{VO}_2\text{max}$  ( $v\text{VO}_2\text{max}$ ), and blood lactate response. We carried out two to five 30-minute constant-load exercise sessions to determine MLSS. All tests were performed at 20 to 22° C and 60.0 % relative air humidity. An interval of at least 48h was observed between each test.

### Determination of $\text{VO}_2\text{max}$ , $v\text{VO}_2\text{max}$ and AT

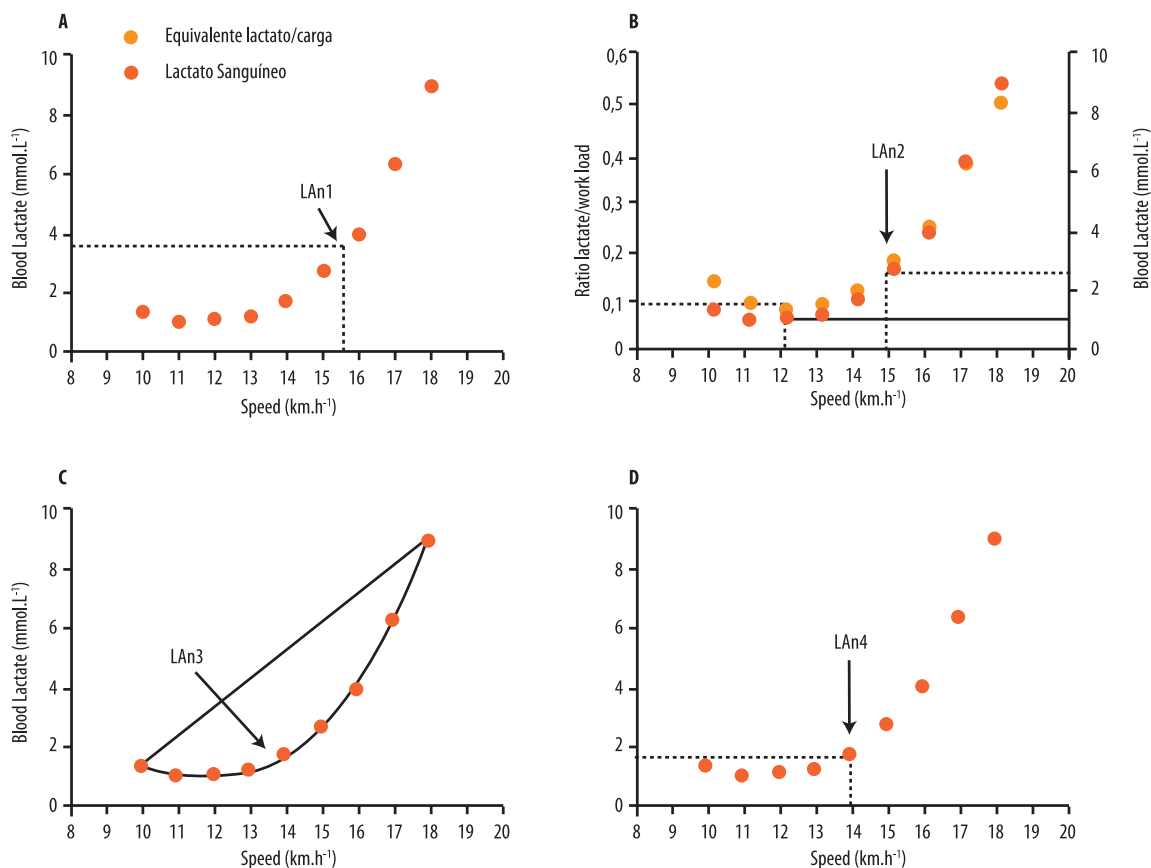
The  $\text{VO}_2\text{max}$  was determined using an incremental exercise protocol on a treadmill (IMBRAMED SUPER ATL, Porto Alegre, Brazil). The initial speed was 10  $\text{km}\cdot\text{h}^{-1}$  (1.0% inclination), with increments of 1  $\text{km}\cdot\text{h}^{-1}$  every three minutes until voluntary exhaustion. Between each stage there was a 30-s interval for collection of a 25  $\mu\text{L}$  blood sample from the ear lobe. The sample was used for determining [La] by an electrochemical method (YSI 2700 STAT, Yellow Springs, OH, USA).

Oxygen uptake ( $\text{VO}_2$ ) was measured using the breath-by-breath analyzer (K4b<sup>2</sup>, Cosmed, Rome, Italy).  $\text{VO}_2$  was measured during the whole test and the data were reduced to 15-s averages.  $\text{VO}_2\text{max}$  was determined based on the highest value obtained during the test in the 15-s intervals. To determine whether participants reached  $\text{VO}_2\text{max}$ , we followed the criteria proposed by Lacour et al.<sup>25</sup>.  $v\text{VO}_2\text{max}$  was determined based on the slowest running speed at which  $\text{VO}_2\text{max}$  was obtained<sup>26</sup>.

The anaerobic threshold was determined using four different methods. By using the Heck et al.<sup>5</sup> method (Figure 1A), AT was determined based on

the speed corresponding to the fixed [La] of 3.5 mmol.L<sup>-1</sup> (AT1). By using the Berg et al.<sup>7</sup> method (Figure 1B), AT was determined by identifying the [La] that corresponded to the lowest ratio between [La] and running speed. Once the [La] for the lowest ratio is determined, 1.5 mmol.L<sup>-1</sup> is added to the figure to obtain the [La] that represents the AT and its corresponding speed (AT2).

Using the Dmax method (Figure 1C), proposed by Cheng et al.<sup>8</sup>, the AT was determined based on individual diagrams. The [La] was obtained from the end of each stage plotted as a function of the respective running speeds. The plot was fitted to a third order polynomial regression curve; subsequently, we employed a two-segment linear adjustment using the two extremes of the curve (the first and the last speed of the incremental protocol), this allowed us to derive a straight line. The Dmax was defined as the greatest distance obtained perpendicularly to the straight line obtained above. The AT was determined as the speed derived from the [La] corresponding to the Dmax (AT3). Finally, using the Baldari and Guidetti<sup>10</sup> method (Figure 1D), similarly to the previous method<sup>8</sup>, the AT was determined based on individual plots. The [La] was obtained at the end of each stage and plotted as a function of the respective running speeds. However, in this specific case the AT corresponded to the speed reached prior to the second consecutive increment of [La] greater than 0.5 mmol.L<sup>-1</sup> (AT4).



**Figure 1.** Determination of the anaerobic threshold (AT) according to the methods proposed by Heck et al.5 (A), Berg et al.7 (B), Cheng et al.8 (C) and Baldari and Guidetti10 (D). The data shown correspond to a single participant.

## Determination of MLSS

To determine MLSS we carried out five constant-load exercise tests that lasted 30 minutes. In each test we gave participants 30-s intervals at 10 and 30 minutes in order to collect a 25- $\mu$ l blood sample from the ear lobe. The sample was used to determine [La]. The intensity of the first test was 80.0% of  $v\text{VO}_2\text{max}$ . Based on the observation of lactataemia for the first test, the following tests were performed at faster or slower speeds (0.5  $\text{km}\cdot\text{h}^{-1}$ ), on different days, until we reached [La] stability or until stability was lost, respectively. MLSS was defined as the fastest speed at which [La] did not exceed 1  $\text{mmol}\cdot\text{L}^{-1}$  in the last 20 minutes of the test<sup>3</sup>. The [La] that corresponded to MLSS was determined using the average between the 10<sup>th</sup> and the 30<sup>th</sup> minute of exercise<sup>3</sup>.

## Statistical analysis

Data are presented as averages and standard deviation (SD). We used the Shapiro-Wilk test ( $n < 50$ ) to assess whether the population was normally distributed; we also used a one-way ANOVA for repeated measures, together with the Bonferroni method, to evaluate the differences between the methods. Pearson's correlation was used to evaluate the correlation between MLSS and the different methods used. Also, the agreement between the methods and MLSS was investigated using the Bland-Altman diagram. We adopted a statistically significant level of 5.0%.

## RESULTS

The  $\text{VO}_2\text{max}$  and  $v\text{VO}_2\text{max}$  averages were  $63.9 \pm 4.0 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  and  $17.6 \pm 0.9 \text{ km}\cdot\text{h}^{-1}$  respectively. Table 1 shows the [La] that correspond to MLSS and the AT calculated by the four different methods investigated. There was a significant difference between the [La] for MLSS for AT2 and AT4. The absolute ( $\text{km}\cdot\text{h}^{-1}$ ) and relative ( $\%v\text{VO}_2\text{max}$ ) values are presented in table 1. There were no significant differences between the MLSS and the AT determined by the different methods. However, the  $\pm 95\%$  bias in the limits of agreement for the comparisons between MLSS and AT1 ( $-0.4 \pm 2.1 \text{ km}\cdot\text{h}^{-1}$ ;  $-2.3 \pm 15.9\%$ ), MLSS and AT2 ( $-0.1 \pm 1.2 \text{ km}\cdot\text{h}^{-1}$ ;  $-0.3 \pm 8.5\%$ ), MLSS and AT3 ( $-0.3 \pm 1.9 \text{ km}\cdot\text{h}^{-1}$ ;  $-1.9 \pm 13.0\%$ ) and MLSS and AT4 ( $0.3 \pm 2.7 \text{ km}\cdot\text{h}^{-1}$ ;  $2.6 \pm 18.3\%$ ) shows the disagreement between the variables when subjects are analyzed individually (Figure 2). There were participants who were above (overestimated MLSS) and below (underestimated MLSS) the limits of agreement for AT1 and AT4, respectively (Figure 2). The subjects whose MLSS showed good accuracy (within  $0.5 \text{ km}\cdot\text{h}^{-1}$ ), were underestimated ( $0.5 \text{ km}\cdot\text{h}^{-1}$  below) or overestimated ( $0.5 \text{ km}\cdot\text{h}^{-1}$  above) are presented in Table 2. The data shows that AT2 had the best results for participants with more accurate estimation of MLSS. The correlation between MLSS and AT was significant only between MLSS and AT1 ( $r = 0.68$ ;  $p = 0.04$ ) and MLSS and AT2 ( $r = 0.79$ ;  $p = 0.01$ ).

**Table 1.** Speed and corresponding [La] for MLSS and AT according to the four methods investigated

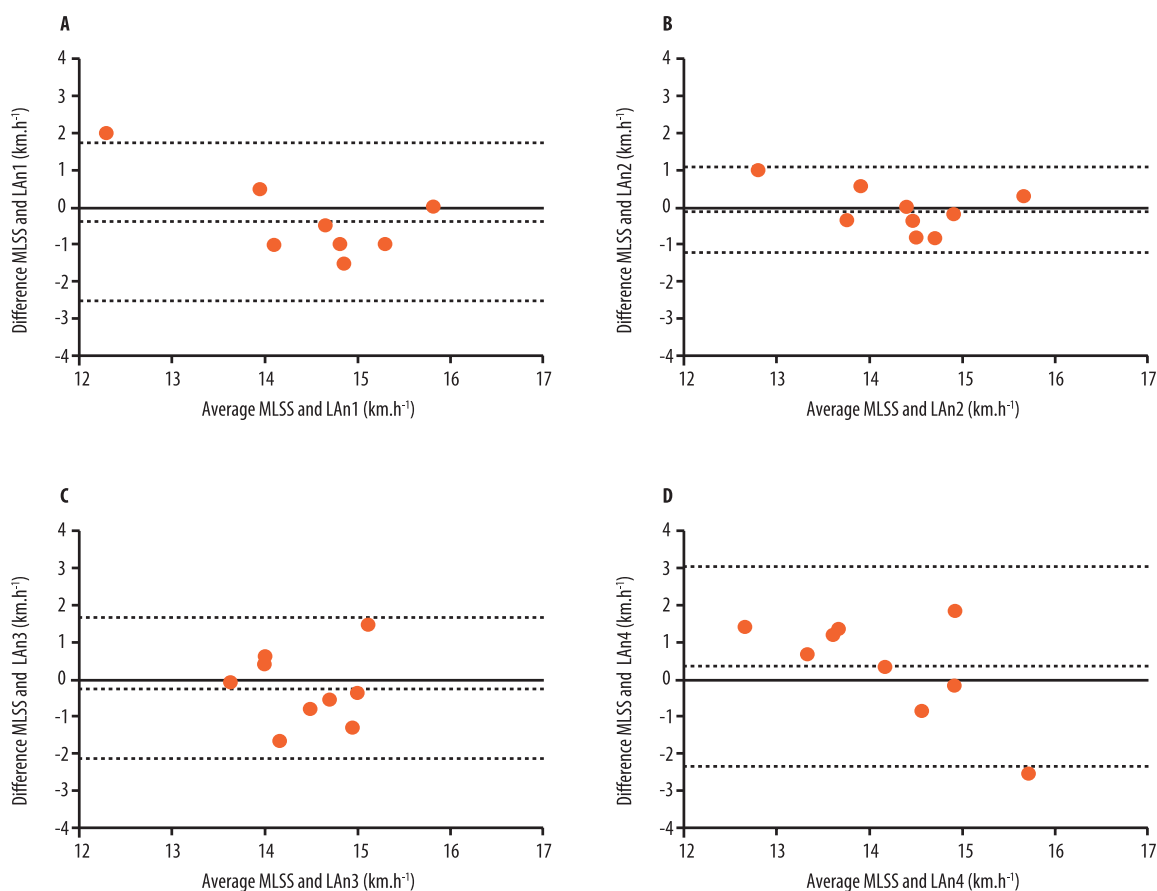
	MLSS	AT1	AT2	AT3	AT4
[La] (mmol.L <sup>-1</sup> )	4.3 ± 1.1	3.5 ± 0.0	3.1 ± 0.6*	3.4 ± 1.4	2.7 ± 1.0*
Speed (km.h <sup>-1</sup> )	14.3 ± 0.7	14.7 ± 1.4	14.4 ± 1.0	14.6 ± 0.7	14.0 ± 1.5
%vVO <sub>2</sub> max (%)	81.7 ± 5.2	83.8 ± 8.5	81.9 ± 5.8	83.2 ± 3.3	79.7 ± 7.4

[La] = blood lactate concentration; MLSS = Maximal lactate steady state; AT1 = AT using Heck et al.<sup>5</sup>; AT2 = AT using Berg et al.<sup>7</sup>; AT3 = AT using Cheng et al.<sup>8</sup>; AT4 = AT using Baldari and Guidetti<sup>10</sup>; %vVO<sub>2</sub>max = percentage of vVO<sub>2</sub>max for the AT and MLSS; \* p < 0.05 in relation to the [La] for MLSS.

**Table 2.** Number of subjects who presented speed corresponding to AT within, below and above 0.5 km.h<sup>-1</sup> of the MLSS.

	Within 0.5 km.h <sup>-1</sup>	0.5 km.h <sup>-1</sup> below	0.5 km.h <sup>-1</sup> above
AT1	3	1	5
AT2	5	2	2
AT3	3	2	4
AT4	2	5	2

MLSS = Maximal lactate steady state; AT1 = AT using Heck et al.<sup>5</sup>; AT2 = AT using Berg et al.<sup>7</sup>; AT3 = AT using Cheng et al.<sup>8</sup>; AT4 = AT using Baldari and Guidetti<sup>10</sup>.



**Figure 2.** Bland-Altman plots showing the bias and limits of agreement (dashed lines) for the comparisons between MLSS and AT1 (A), MLSS and AT2 (B), MLSS and AT3 (C), and MLSS and AT4 (D). The filled line represents bias = 0.

## DISCUSSION

The results show that there were no significant differences between the MLSS and AT calculated by the four different methods investigated (Table 1). Though each of the techniques investigated suggests different approaches for the analysis of the [La] curve in an incremental test, the average values were similar to the MLSS. However, the  $\pm 95\%$  bias in the limits of agreement for the comparisons between MLSS and the different ATs shows the disagreement between the variables when subjects are analyzed individually (Figure 2). Moreover, there were significant correlations ( $p < 0.05$ ) between MLSS and AT1 ( $r = 0.68$ ) and MLSS and AT2 ( $r = 0.79$ ). To our knowledge, this is the first study to investigate the agreement between MLSS measured directly by the AT according to the methods by Heck et al.<sup>5</sup>, Berg et al.<sup>7</sup>, Cheng et al.<sup>8</sup> and Baldari and Guidetti<sup>10</sup>. Other studies<sup>20-22</sup> investigated the agreement between MLSS and AT measured by the individual lactate minimum<sup>9</sup>.

AT1 was not significantly different from MLSS and there was a moderately significant correlation between the variables ( $r = 0.68$ ). This result is in agreement with previous studies carried out with endurance runners<sup>5,12</sup>, cyclists<sup>4,13-16</sup>, team sports<sup>18</sup>, physically active individuals<sup>19</sup> and untrained individuals<sup>4</sup>. Jones and Doust<sup>12</sup> showed that endurance runners' AT1 and MLSS were significantly similar and highly correlated ( $r = 0.93$ ). Likewise, Denadai et al.<sup>18</sup> and Figueira et al.<sup>19</sup> showed that AT1 was not different from MLSS in soccer players and in individuals who are physically active, respectively. The studies reported correlations of  $r = 0.80$  and  $r = 0.94$ , respectively.

Studies with exercises on stationary bicycles for both trained cyclists and untrained individuals showed that the intensity that corresponded to the AT and the fixed [La] of  $3.5 \text{ mmol.L}^{-1}$  was not different from the MLSS<sup>4,13-15</sup>. Though the AT for the fixed [La] was similar to MLSS, other studies with running<sup>5,12,18,19</sup> and cycling<sup>4,13-16</sup>, and others with different exercise modalities, have shown a difference between the two variables<sup>11,17,19</sup>. The main criticism in relation to the use of this method is that it does not evaluate the individual kinetics of the lactate curve during an incremental test. Therefore, it may under or overestimate the MLSS<sup>10</sup> since there is significant individual variability in the [La] of MLSS<sup>6,17</sup>.

Stegmann et al.<sup>6</sup> proposed that the AT could be identified based on the individual [La] values determined during an incremental test. Considering the significant individual variability in the results ([La] between 1.4 and  $7.5 \text{ mmol.L}^{-1}$ ), the authors introduced a method to estimate MLSS individually (*individual anaerobic threshold* - IAT), which did not use the fixed [La]<sup>6</sup>. Urhausen et al.<sup>11</sup> corroborated this model and showed that the IAT did not differ from the MLSS in endurance runners and trained cyclists. Similarly, Figueira and Denadai<sup>13</sup> did not find differences between IAT and MLSS in trained cyclists. Conversely, despite the similarities in runners<sup>11</sup> and cyclists<sup>11,13</sup>, in rowers the MLSS was significantly overestimated<sup>17</sup>.



Due to the complex characteristics of determination of IAT<sup>6</sup>, Berg et al.<sup>7</sup> proposed a new method to make MLSS identification simpler. Using regression analyses, the authors observed that AT is generally identified at 1.5 mmol.L<sup>-1</sup> above the [La] of the lowest lactate/speed ratio for endurance runners<sup>7</sup>. The AT2 in the present study was similar to the MLSS and showed the highest correlation coefficient ( $r = 0.79$ ;  $p = 0.01$ ). In addition, this method showed the best agreement with MLSS according to the Bland-Altman plots. Table 2 also shows that five out of nine subjects had their AT2 within a 0.5 km/h<sup>-1</sup> variation of MLSS. The high level of agreement observed between AT2 and MLSS in comparison with the other methods analyzed in the study may be explained in part by two relevant factors. First, the determination of a minimum value based on the relationship between [La] and speed of exercise ensures appropriate evaluation of individual kinetics in the lactate curve during an incremental test. Second, the method presents the simplest calculation of AT, i.e., adding 1.5 mmol.L<sup>-1</sup> to the [La] that corresponds to the ratio, using individual values. This simplicity is evident in comparison with the complexity of the other methods<sup>6,8</sup>, which may more easily result in errors in AT calculation. Thus, differently from the original IAT<sup>6</sup> method, the Berg et al.<sup>7</sup> method does not require individuals to perform incremental tests to exhaustion. The test may end at submaximal exercise intensities if the only objective is to determine the AT.

With the goal of allowing for more individualized calculations than simply using fixed [La], and allowing for less subjective methods than the more simplified analyses, Cheng et al.<sup>8</sup> suggested the Dmax model (which evaluates the behavior of the whole [La] curve during an incremental test) may be an appropriate method for determining physiological variables associated with aerobic capacity. In addition to the lack of concrete evidence about the validity of the model<sup>16</sup>, there are scarce reports of the use of the method (proposed originally with stationary bicycles<sup>8</sup>) with other exercise modalities in the scientific literature. The lack of studies that have validated the method underscores the importance of the present study and its application with runners.

Though there were no differences between the AT3 and MLSS averages, there was also no significant correlation between the two. To explain these results, two important points in relation to the application of the method must be highlighted. First, differently from the other methods analyzed in the present study<sup>5,7,10</sup> and similarly to the method proposed by Stegmann et al.<sup>6</sup>, the AT determined using Cheng et al.'s<sup>8</sup> method also requires participants to perform the incremental test until maximum voluntary exhaustion. The determination of AT is possible only with the analysis of the entire [La] curve. Second, since the Dmax model is dependent on the behavior of the entire [La] curve, some issues arise in relation to its applicability in the determination of AT: while some authors applied the method using polynomial regression<sup>27</sup>, others used exponential functions<sup>28</sup>. The difference in mathematical functions may generate distortions in the intensity determined by the model.



Recently, and based on the criticism related to the use of stages shorter than six minutes in incremental tests to determine AT, Baldari and Guidetti<sup>10</sup> proposed a method for identification of the AT. The method uses individual kinetics and the time necessary for [La] to reach a steady state. The central hypothesis of the authors is that [La], at a given stage of three minutes during an incremental test, is the [La] for the speed of the previous stage, in a constant-load exercise<sup>10</sup>.

The AT4, similarly to the AT determined by other methods<sup>5,7,8</sup>, did not show significant differences in comparison with the MLSS. This result is in agreement with those reported by Baldari and Guidetti<sup>10</sup>. The [La] steady state was observed during 30 minutes of exercise for AT4. When the exercise was carried out with the intensity of the stage immediately above (increases of 1 km.h<sup>-1</sup> and 2 km.h<sup>-1</sup>, respectively, for men and women) we did not observe a stable [La]. In some cases, participants showed exhaustion and the exercise had to be terminated early. Some considerations must be made in relation to the method. One important issue is the absolute intensity in which AT4 is determined. The original method<sup>10</sup> indicates that AT is always determined based on the load that corresponds to a specific stage; never to an intermediary load between two stages (Figure 1D).

In addition, the authors did not determine the MLSS in a clear manner. To verify the validity of AT, the authors submitted their participants to exercise at their AT and at the speed corresponding to the stage immediately above<sup>10</sup>. Therefore, they observed that during the exercise at AT, the [La] was stable for all participants evaluated; during the exercise at maximum speed, in turn, the [La] did not stabilize. The authors' interpretation is that the method is reliable for precise estimation of MLSS<sup>10</sup>. However, the procedure may indicate an intensity of exercise which despite a stable [La] state is not necessarily the MLSS. The procedure may also neglect intermediary intensities which may be the real MLSS intensity. In the present study, the method showed the lowest AT, thus underestimating the MLSS for most subjects (Table 2).

Billat et al.<sup>29</sup> and Philp et al.<sup>30</sup> recently reported the use of MLSS as an efficient stimulus for training. The studies reported significant effects of MLSS training on the physiological variables associated with aerobic metabolism; they also reported changes in performance, in time to exhaustion in MLSS, and in the substrate used during the exercise at this intensity level<sup>29,30</sup>. However, since exercise intensities at  $\pm 5.0\%$  of MLSS show physiological responses and adaptation significantly different than those found for MLSS<sup>30</sup>, the question arises whether the methods for determination of AT evaluated in the study are valid estimates of MLSS (according to the Bland-Altman plot analyses)?

The disagreement shown in the confidence intervals of the Bland-Altman diagrams for MLSS and AT1, AT3, and AT4 ( $\pm 15.9$ ; 13.0; 18.3% respectively) may lead individuals to different physiological and metabolic adaptations: the individuals may be exercising different physiological domains. Consequently, in situations that require precise determination

of aerobic capacity (for example, prescription of training intensity; experimental studies), it is preferable that MLSS be determined directly.

The present study has some limitations that should be considered. First, there is the sample size. From an operational point of view, there is the difficulty of recruiting well-trained athletes that have time available to participate in a study that requires up to seven visits to a laboratory in just two weeks. Of course, a larger sample would have given us more robust results and statistical power in the analyses, and would also allow for a more precise quantification of the relationship between the methods for determination of AT and the MLSS measured directly. To our knowledge, with the exception of the AT and fixed  $[La]^5$ , there are no studies that have verified the relationship between AT identified by the other methods<sup>7,8,10</sup> and the MLSS determined directly during running. This is both an advantage and a limitation, since it limits the comparison and discussion of our results.

## CONCLUSIONS

Though there were no differences between the average speeds for the four methods used for determination of AT and MLSS, we should proceed with caution if the methods are to be used interchangeably with endurance runners. The Bland-Altman diagram showed that there were limitations in the agreement between individuals. Despite the reduced number of participants, in the present study the method proposed by Berg et al.<sup>7</sup> showed the best agreement with the MLSS. Further studies should be conducted to validate the method, especially in relation to other sports modalities.

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