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ARTICLE

Lactate minimum underestimates the maximal lactate steady-state in swimming mice

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Abstract: The intensity of lactate minimum (LM) has presented a good estimate of the intensity of maximal lactate steady-state (MLSS); however, this relationship has not yet been verified in the mouse model. We proposed validating the LM protocol for swimming mice by investigating the relationship among intensities of LM and MLSS as well as differences between sexes, in terms of aerobic capacity. Nineteen mice (male: 10, female: 9) were submitted to the evaluation protocols for LM and MLSS. The LM protocol consisted of hyperlactatemia induction (30 s exercise (13% body mass (bm)), 30 s resting pause and exhaustive exercise (13% bm), 9 min resting pause and incremental test). The LM underestimated MLSS (mice: 17.6%; male: 13.5%; female: 21.6%). Pearson's analysis showed a strong correlation among intensities of MLSS and LM (male (r = 0.67, p = 0.033); female (r = 0.86, p = 0.003)), but without agreement between protocols. The Bland–Altman analysis showed that bias was higher for females (1.5 (0.98) % bm; mean (MLSS and LM): 4.4%–6.4% bm) as compared with males (0.84 (1.24) % bm; mean (MLSS and LM): 4.5%–7.5% bm). The error associated with the estimated of intensity for males was lower when compared with the range of means for MLSS and LM. Therefore, the LM test could be used to determine individual aerobic intensity for males (considering the bias) but not females. Furthermore, the females supported higher intensities than the males. The differences in body mass between sexes could not explain the higher intensities supported by the females.

Key words: blood lactate, maximal lactate steady state, anaerobic threshold, aerobic capacity.

Résumé : L'intensité au minimum de lactate (« LM ») est une bonne estimation de l'intensité au maximum de lactate en régime stable (« MLSS »); toutefois, il n'y a pas d'études sur cette relation dans le modèle de la souris. Nous proposons de valider le protocole LM chez des souris à la nage par l'examen de la relation entre LM et MLSS à diverses intensités et entre les différences liées au sexe en ce qui concerne la capacité aérobie. On administre les protocoles d'évaluation de LM et de MLSS à 19 souris (10 mâles et 9 femelles). Le protocole LM consiste en une induction d'une hyperlactatémie (30 s d'exercice (13 % masse corporelle (mc)), 30 s de repos et exercice épuisant (13 % mc), 9 min de repos et un test d'effort progressif). Le LM sous-estime MLSS (souris : 17,6 %, mâles : 13,5 %, femelles : 21,6 %). L'analyse de Pearson révèle une corrélation élevée entre les intensités de MLSS et LM (mâles (r = 0,67, p = 0,033); femelles (r = 0,86, p = 0,003)), mais sans la concordance entre les protocoles. D'après l'analyse de Bland–Altman, le biais est plus important chez les femelles (1,5 % (0,98) mc; moyenne (MLSS et LM): 4,4–6,4 % mc) comparativement aux mâles (0,84 % (1,24) mc; moyenne (MLSS et LM): 4,5–7,5 % mc). L'erreur associée à l'estimation de l'intensité chez les mâles est plus faible quand on compare l'étendue des moyennes de MLSS et de LM. En conséquence, on pourrait utiliser le test LM pour déterminer l'intensité aérobie chez les mâles (biais pris en compte), mais pas chez les femelles. De plus, les femelles tolèrent de plus fortes intensités que les mâles. Les différences de masse corporelle n'expliquent pas les plus fortes intensités tolérées par les femelles. [Traduit par la Rédaction]

Mots-clés : lactate sanguin, maximum de lactate en régime stable, seuil anaérobie, capacité aérobie.

Introduction

Physical exercise promotes many benefits for improving health and preventing chronic diseases (Schjerve et al. 2008; Fisher et al. 2015; Smith-Ryan et al. 2015), in addition to enhanced physical fitness (Little et al. 2011; Yan et al. 2012; Kang et al. 2013). Rodent models allow for the investigation of the exercise response associated with these benefits, using controlled manipulations, minimal environmental variations, and invasive analysis, which provide in-depth information about cellular and molecular pathways (Levin 2008; Stanford et al. 2015; Garton et al. 2016). Furthermore, the physiological responses elicited from exercise in rodents have been similar to that found in humans (Wisloff et al. 2001; Kemi et al. 2002; Billat et al. 2003; Garton et al. 2016).

Evaluation protocols have been validated for rodent, mostly rats, to determine intensity of anaerobic threshold (AT) (Faude et al. 2009), which allows the control and the prescription of intensity in acute sessions of exercise and training (de Araujo et al. 2012, 2013; Almeida et al. 2014; Petriz et al. 2015). Studies regarding evaluation protocols have reported that variations in the individual intensity of AT or blood lactate concentration can be related to different species of animals (Billat et al. 2003; Abreu et al. 2016), strains (Billat et al. 2005; Almeida et al. 2013), ergometers (Manchado et al. 2006*b*), ages (Cunha et al. 2009), and sexes (Billat et al. 2005). Concerning the sexes, the variations phenotypic and genetic have been linked to the different cardiovascular adaptations and aerobic capacity (Konhilas et al. 2004; De Bono et al. 2006), which are

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important factors in exercise performance (Konhilas et al. 2004). However, the mechanisms for these differences remain unknown, which makes it important to propose evaluation methods for mice that consider both sexes.

Among the main evaluation protocols for measuring AT, maximal lactate steady-state (MLSS) is considered to be the gold-standard test for measuring aerobic capacity (Beneke 2003). At MLSS intensity occurs at the balance between production and removal of blood lactate, and is an important marker of energy expended during the efforts (Beneke 2003; Billat et al. 2003; Faude et al. 2009). Furthermore, the MLSS has been applied with success in rats (Manchado et al. 2006*a*; Cunha et al. 2009; Gobatto et al. 2009; Almeida et al. 2013) and mice (Gobatto et al. 2001; Ferreira et al. 2010; Almeida et al. 2011; de Araujo et al. 2015), with benefical training effects on MLSS intensity (Almeida et al. 2014; Petriz et al. 2015). However, the determination of the MLSS intensity requires several effort sessions on different days and many blood samples, which limits the application of this test according the experimental design.

On the other hand, based on the theory of MLSS and blood lactate clearance, Tegtbur et al. (1993) proposed the lactate minimum (LM) test, which could estimate in a single day of effort the intensity related to intensity of MLSS. This test consists of a supramaximal exercise for hyperlactatemia induction, rest period, and incremental phase during metabolic acidosis. The minimum point of the curve between blood lactate concentrations versus intensities is known as intensity of LM. The LM has been applied with success for humans (Jones and Doust 1998; MacIntosh et al. 2002; Pardono et al. 2008; Sotero et al. 2009; Knoepfli-Lenzin and Boutellier 2011), horses (Gondim et al. 2007; Miranda et al. 2014), and rats (Voltarelli et al. 2002; de Araujo et al. 2007, 2012), though a mouse protocol remains to be established.

Considering the importance of evaluation methods to determine the individual intensity of AT for controlling the load (product intensity by volume) (Mujika et al. 1995) during exercise, and several applications of mouse models in research on health and metabolism (Levin 2008; Stanford et al. 2015; Garton et al. 2016), we sought to validate the LM protocol for swimming mice. The analysis consisted of investigating the relationship of LM and MLSS intensities and the differences between sexes with respect to aerobic capacity.

Materials and methods

Animals

Nineteen Swiss mice (male, $n = 10$; female, $n = 9$) provided by the University of Campinas Animal Breeding Center (CEMIB, Brazil) were submitted to swimming exercise tests. The experimental procedures started with untrained adult mice at 12 weeks old, which are not affected by senescence (Flurkey et al. 2007), ranging from 33–40 g (male) and 27–33 g (female) at the beginning, and 35–42 g (male) and 29–35 g (female) at the end of the study. Animals remained on a 12-h light12-h dark cycle and had access to food (commercial rodent chow) and water ad libitum. Groups of 5 animals were housed indoors (22 ± 1 °C) in propylene cages maintained on ventilated shelves. Body mass was determined daily before exercise protocol. All experiments followed the *Guide for the Care and Use of Laboratory Animals* published by National Institute of Health and the guidelines of the Brazilian College for Animal Experimentation. The study was approved by Ethics Committee on the Use of Animals (University of Campinas 3913-1).

Experimental protocol

Adaptation to water

For all procedures, the load was expressed as the equivalent percentage of body mass (% bm) for each animal. This load was built with lead and latex elastic that was tied to the animal's back. All mice were adapted to water (31 ± 1 °C) before the beginning of the experiment during a period of 14 uninterrupted days. For first 3 days, mice were initially inserted in shallow water (3 cm) for 15 min. At fourth day, each mouse swam in deep water (100 cm) for 2 min with an increment of 2 min/day until the eight day of procedure. The intensity with which mice started to swim was considered with respect to body mass (% bm). On the ninth day, the animals were submitted to swimming exercise for 5 min to support an intensity equivalent to 3% bm. Following that, at each day the animals were submitted to different intensity and periods of swimming exercise (5% bm for 3 min, 9% bm for 1 min, 11% bm for 1 min, 13% bm for 30 s, and 15% bm for 30 s, respectively), always followed by another session of 5 min without intensity. The adaptation features are designed so that the animal is familiar with manipulation, imposed intensity and water, to reduce the stress caused by these factors during the evaluation. A similar adaptation protocol has been used in rodents in previous studies carried out in our laboratory, and it was suggested that it reduces the stress without promoting physical training adaptations (Gobatto et al. 2001; Manchado et al. 2006*a*).

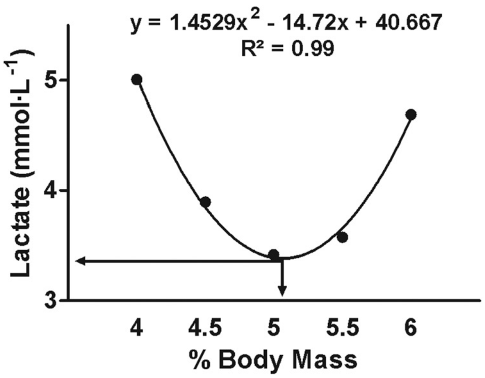
LM test

For determination of LM intensity in mice, the standardized protocol of LM was used in accordance with the methods proposed for rats by de Araujo et al. (2007). The LM tests were performed in individual cylindrical tanks (30 cm diameter × 120 cm depth), filled with water (100 cm). The supramaximal exercise for hyperlactatemia induction comprised 30 s of exercise (13% bm), 30 s of passive recovery, and an exercise bout until exhaustion (13% bm). This protocol of hyperlactatemia induction presented a higher success rate for the determination of individual intensity of LM (de Araujo et al. 2007). Exhaustion was assumed when the animal was unable to stay on the water surface for 10 s (McArdle and Montoye 1966; de Araujo et al. 2007). During rest, the mice stayed in shallow water to avoid thermal stress. At that point, a blood sample was collected, from a small cut at the extremity of the tail, at more than 9 min postexercise. The 9-min recovery time was tested by Voltarelli et al. (2002) as the period that peak of blood lactate occurs. This was followed by an incremental test that was performed with intensities of 4.0%, 4.5%, 5.0%, 5.5%, 6.0%, and 7.0% bm. Each stage lasted 5 min, separated by 30 s for blood sampling. The relationship among blood lactate concentrations ($mmol \cdot L^{-1}$) versus intensities (% bm) of incremental phase was determined by a second-order polynomial. The LM intensity corresponded to the minimum point or derived zero of the curve (Fig. 1). The criterion to determine the success rate of the LM was the presence of the U-shaped ($a > 0$) and coefficient of determination (R^2) of the curve higher than 0.8 (de Araujo et al. 2007, 2012; Voltarelli et al. 2002).

MLSS

The MLSS intensity was defined as the highest intensity that can be maintained during the exercise with blood lactate stabilization (Heck et al. 1985; Beneke 2003). All mice were submitted individually to 30 min of continuous exercise. The first intensity tested corresponded to individual intensities of LM and subsequently, for other efforts, the load increments were of 0.5% bm. The blood samples (15 μL collected from the tail) were collected at rest and 10th and 30th min of exercise; however, the mice were removed from the water every 5 min for 30 s to mimic previous studies in which the blood samples were collected in these moments (Gobatto et al. 2001, 2009; Voltarelli et al. 2002). It was necessary to follow this procedure for at least 2 days and for a maximum of 5 days, with 48 h between them. The criterion to determine the MLSS intensity was the highest intensity in which blood lactate concentration increases less than 1 $mmol \cdot L^{-1}$ between the 10th and 30th min of exercise (Beneke 2003).

Fig. 1. Example of determination of the intensity of lactate minimum (LM) of a mouse during incremental phase. Each point of the curve represents the relationship among blood lactate concentration (mmol·L^{-1}) versus intensity (percentage of body mass (% bm)) during the stages (5 min) of the incremental phase. In this case, the lactate kinetic (U-shaped) and coefficient of determination (R^2) attended to the criterions of success required. The intensity of LM represents the minimum point or derived zero of the curve and it was estimated using a second-order polynomial fit. The arrows indicates the intensity of LM (5.06% bm) and its blood lactate concentration (3.38 mmol·L^{-1}).



Blood samples and analysis

For determination of the blood lactate concentrations, blood samples (15 μL) were collected using heparinized capillary tubes and transferred to microcentrifuge tubes (1.5 mL) containing trichloroacetic acid at 4% (240 μL). The samples were centrifuged for 3 min (3000 r·min^{-1} (1000g)) and 50 μL of plasma was placed onto a microplate. The following reagent (250 μL) was added: glycine/ethylenediaminetetraacetic acid, hydrazine hydrate 24%, lactate dehydrogenase, and b-nicotinamide adenine dinucleotide. The homogenized sample and reagent were incubated for 60 min, and absorbance was determined by spectrophotometer analysis (Asys Expert Plus) at 340 nm. The enzymatic analysis for determining blood lactate concentration was described by Engel and Jones (1978).

Statistical analysis

The results are presented as means ± SD. The statistic procedure consisted of 2-way ANOVA for repeated measures (factors: sex versus protocol) to compare LM and MLSS and 1-way ANOVA to compare body mass. The Scheffe post hoc comparison was used to identify differences among intensities and blood lactate concentrations. The effect size (ES) was calculated based on Cohen's *d* and used to identify the magnitude of the differences. The ES obtained in the statistical analysis was interpreted as proposed by Hopkins et al. (2009), with ES < 0.2 considered to be trivial, 0.2–0.5 considered to be small, 0.6–1.1 considered to be moderate, 1.2–1.9 considered to be large, and > 2.0 considered to be very large. The Bland–Altman analysis (bias (±95 confidence interval)) was used to identify the differences between MLSS and LM (Bland and Altman 1999).

The variability of the intensities was determined using box-plot analysis. On each box, the central mark is the median (50th percentile), the edges of the box are the quartiles (25th and 75th percentiles), and the whiskers extremes are maximum and minimum values. The dispersion of the intensities was calculated by the difference between quartiles. Pearson's correlations (*r*) were used to determine the relationship between the intensities and a 95% confidence interval for linear regression was performed. Correlations were interpreted based on Cohen's scale of magnitudes (Cohen 1988) comprising 0.1 (small), 0.3 (moderate), and 0.5 (large), and were augmented (Hopkins et al. 2009) to include 0.7 (very large) and >0.9 (nearly perfect). In all cases, the statistical significance was set at $P \leq 0.05$.

Results

The LM presented a success rate of 89.5% for all mice (n = 17) from criterions determined (R^2 > 0.8 and U-shaped curve), while males were 90% (n = 9) and females were 88% (n = 8). Differences were found among intensities of LM and MLSS for all mice (Table 1). The intensities of LM were lower than intensities of MLSS (5.18% bm vs. 6.34% bm, $P < 0.001$, F = 78.96), with a large ES (ES = 1.34), underestimating MLSS in 17.6% for all mice (male: 13.5%; female: 21.6%). Nonetheless, correlation analysis between protocol tests showed a very large and significant relationship (r = 0.78, $P < 0.001$) (Fig. 3). The same results were obtained when the protocols were analyzed separately for each sex (Table 1). The intensities of LM were lower than intensity of MLSS for males and females (P = 0.002; $P < 0.001$), and the magnitudes of ES emphasized these differences among intensities. The differences among females were large (ES = 1.76); the differences among males were moderate (ES = 1.13). The blood lactate concentrations were lower in the LM test as compared with the MLSS test for all groups of mice (P = 0.01) as well as for females (P = 0.01). This trend did not hold for male mice (P = 0.23).

Mice presented a large dispersion of intensities in both evaluations protocols (Fig. 2). The dispersion of intensities of males were of 0.6% bm (LM) and 1.19% bm (MLSS), while females were of 1.01% bm (LM) and1.02% bm (MLSS). Overall, a higher dispersion of the intensities occurred below of median values of both protocols. The extreme points (maximum and minimum) provide the range of intensities supported by animals. The range of LM intensity was of 4.00% to 6.20% bm (male) and 4.38% to 6.39% bm (female), while the range of MLSS intensity was of 4.24% to 7.0% bm (male) and 5.38% to 8.39% bm (female). Comparisons between sexes for a given evaluation (e.g., intensities of LM male vs. LM female and MLSS male vs. MLSS female) showed no significant difference (Fig. 2). Significant differences were only observed among LM intensities for males versus MLSS intensities for females, with higher intensities supported by females versus males ($P < 0.001$).

Furthermore, the ANOVA analysis showed an effect of sex over protocol tests with a significant interaction among them (P = 0.022, F = 6.28); thus, different intensities relationships are expected for male and female. Small magnitude was observed in relation to body mass (g) and intensities (LM and MLSS) and the correlation did not present significant difference (Table 2). Nevertheless, when correlations among intensities of LM and MLSS were separately tested to each sex (Fig. 3), a significant and large relationship for male (r = 0.67, P = 0.033) and very large relationship for female (r = 86, P = 0.003) was determined.

Additionally, the Bland–Altman analysis showed a lower bias for males than females and for both was found a wide limit of agreement (Fig. 4). The comparison among intensities of MLSS and LM for males (Fig. 4) presented values of bias of 0.84 (1.24) % bm, while for females (Fig. 4) the values were 1.5 (0.98) % bm. For all mice (Fig. 4), the value of bias was 1.15 (1.27) %. Furthermore, for males the distribution of points was close to mean intensity, which was different than what occurred for females.

Discussion

The main finding of this study was that intensity obtained in the LM protocol underestimated the intensity of MLSS by 17.6% for all mice (male: 13.5%; female: 21.6%). The intensities of LM and MLSS presented strong correlations for both sexes (male (r = 0.67); female (r = 0.86)); however, the LM value was not in agreement with the MLSS value. Nevertheless, the analysis of agreement showed that bias was higher for females (1.5 (0.98) % bm): this bias represents a magnitude about 50% within of the range of mean intensities observed between protocols (4.5%–7.5% bm). For males,

Table 1. Intensities of evaluation protocols.

Groups	LM (% bm)	MLSS (% bm)	ES (Cohen's *d*)	LM_C (mmol·L^{-1})	$MLSS_C$ (mmol·L^{-1})	ES (Cohen's *d*)
All mice	5.18±0.67*	6.34±1.02	1.34	3.03±0.77*	3.49±0.86	0.56
Male	5.02±0.61*	5.86±0.85	1.13	3.33±0.87	3.74±1.09	0.41
Female	5.38±0.72*	6.88±0.96	1.76	2.70±0.51*	3.22±0.39	1.14

Note: Intensities and blood lactate concentration of evaluation protocols (lactate minimum (LM) and maximal lactate steady-state (MLSS)) are expressed as means ± SD. LM_C, blood lactate concentration; $MLSS_C$, blood lactate concentration; ES, effect size.

*Significant difference of MLSS or $MLSS_C$ ($P < 0.05$).

Fig. 2. Variability of the intensities (percentage of body mass (% bm)) of lactate minimum (LM) and maximal lactate steady-state (MLSS). a, Differences among intensities of LM and MLSS for males ($P < 0.05$); b, differences among intensities of LM and MLSS for females ($P < 0.05$); and c, differences between sexes ($P < 0.05$). On each box plot, the central mark is the median (50th percentile), the edges of the box represent the quartiles' 25th and 75th percentiles. The whiskers extend to the most extremes points (minimum and maximum).

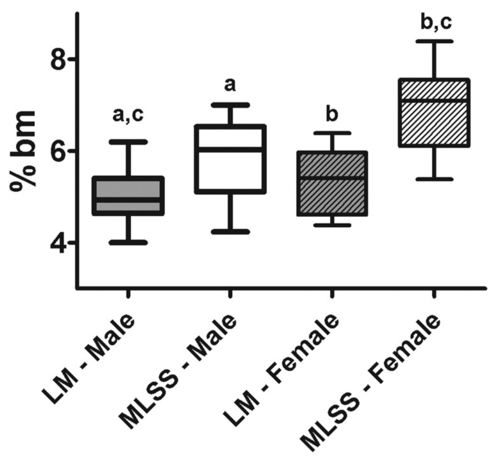


Table 2. Body mass values for each sex (mean ± SD) and Pearson's correlation analysis (*r*) results among protocol intensities (LM and MLSS; % bm) vs. body mass (g).

Groups	Body mass, g	LM vs. body mass, *r* (*p* value)	MLSS vs. body mass, *r* (*p* value)
Male	40.23±1.95*	−0.13 (0.71)	−0.11 (0.76)
Female	31.90±2.08	0.12 (0.74)	−0.23 (0.54)

Note: % bm, percentage of body mass; LM, lactate minimum; MLSS, maximal lactate steady-state.

*Significant difference of female. All cases $P < 0.05$.

a lower bias was found (0.84 [1.24] % bm), and this bias represents a magnitude about 42% within of the range of mean intensities between protocols (4.4%–6.4% bm). Based on these results, the LM protocol tested could be used to estimate individual intensity of aerobic capacity for male mice, with lower error associated with the intensities of MLSS as compared with females.

Our study is the first to propose validation of the LM protocol for swimming mice with a consideration towards sex. This rodent model is widely used in research related to health and metabolism associated to exercise, which acts as non-drug intervention able to modulate different responses into the organism (Levin 2008; Stanford et al. 2015; Garton et al. 2016). de Araujo et al. (2007) standardized the LM protocol for swimming rats, comparing 4 protocols for hyperlactatemia induction. The success rates among them were different (58%, 55%, 80%, and 91%) considering the following conditions: polynomial fit ($R^2 > 0.08$) and U-shaped curve (blood lactate concentration vs. intensities). We tested the same protocol that obtained higher success (30 s of exercise (13% bm), 30 s of passive recovery and exercise bout until exhaustion (13% bm)) because of better assurance for the LM identification, finding also a high success rate in the determination of intensity of LM (all mice: 89.5%, male: 90%, female: 88%).

Although our study found that LM underestimates MLSS for mice, de Araujo et al. (2007) found that intensity obtained with the LM protocol (5.06% ± 0.93% bm) accurately represented intensity of MLSS (5.3% ± 0.5%) for rats. The same conclusion was reported earlier by Voltarelli et al. (2002), which found an LM intensity of 4.95% ± 0.1% bm in swimming rats. These authors proposed a hyperlactatemia induction protocol with a 6-min exercise interval (6 × 30-s jump into the water and 30 s of rest); however, this protocol was tested posteriorly to success rate and did not present a suitable percentage for LM identification (de Araujo et al. 2007). Both of studies presented used 9 min of recovery time and the incremental test (4.0%, 4.5%, 5%, 5.5%, 6%, and 7% bm for 5 min in each one), and we kept the same protocols. Nevertheless, the LM intensities obtained in our study were slightly higher than both of the other studies (Table 1).

The MLSS protocol, which is considered to be the gold-standard to determine AT, consisted of subsequent sessions of exercise, adding each trial an intensity increment to find the highest occurring intensity at the balance between production and the removal of blood lactate (Beneke 2003). In our study, the intensity increment used to identify MLSS intensity was 0.5% bm, while in study of Voltarelli et al. (2002), the intensity increment was 1% below and 1.5% above the LM intensity; the choice of a lower intensity increment can improve the accuracy to determine the intensity of AT. Considering that the overall dispersion of the intensities of LM and MLSS found for our study were approximately 1% bm (Fig. 2), if the choice was a higher intensity increment (>0.5% bm), it is possible for alterations in the relationship among the intensities of the protocols to occur because of lack of precision.

The LM had been used as a good evaluation protocol to estimate intensity of MLSS in humans (Jones and Doust 1998; MacIntosh et al. 2002; Pardono et al. 2008; Sotero et al. 2009; Knoepfli-Lenzin and Boutellier 2011), horses (Gondim et al. 2007; Miranda et al. 2014), and as discussed in rats (Voltarelli et al. 2002; de Araujo et al. 2007, 2012). Nevertheless, another studies also found that LM intensity underestimates MLSS intensity (Carter et al. 1999; Ribeiro et al. 2009; Labruyère and Perret 2012; Zagatto et al. 2014), and the reason for this could be associated to specific phases of the LM (hyperlactatemia induction, resting pause, and incremental test).

Specifics phases of the LM could modify the relationship between production and clearance of lactate, promoting a left displacement of the lactate curve and underestimate the LM intensity (Heck et al. 1991; Carter et al. 1999; Ribeiro et al. 2009; Labruyère and Perret 2012; Perret et al. 2012). Recently, Zagatto et al. (2014) verified differences between the intensity of LM determined after different methods of hyperlactatemia induction in cycling, attesting that blood acid-base balance during resting pause can alter the response of the incremental phase. Perret et al. (2012) evaluated athletes using LM and MLSS and they found a strong relationship among them, but also an LM intensity lower than the MLSS intensity. These authors suggest that lower blood lactate concentration

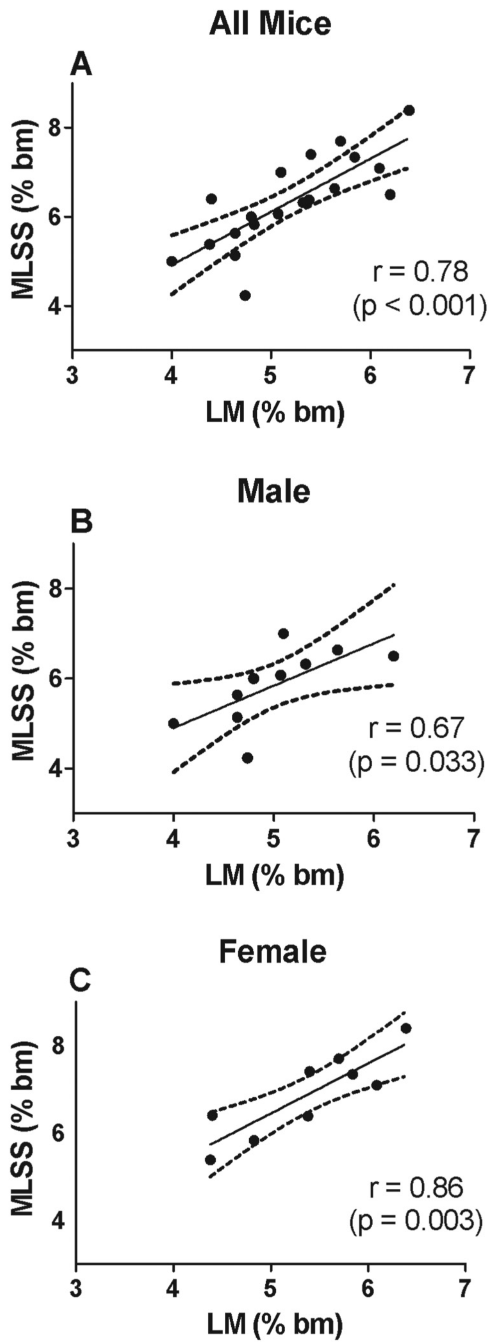


Fig. 3. Pearson's correlation analysis among intensities of lactate minimum (LM; percentage of body mass (% bm)) and maximal lactate steady-state (MLSS) (% bm) to (A) all mice (n = 19), (B) males (n = 10), and (C) females (n = 9). The relationship among intensities was represented by linear regression (line) ± confidence interval (95%: dashed line). All cases are P < 0.05.

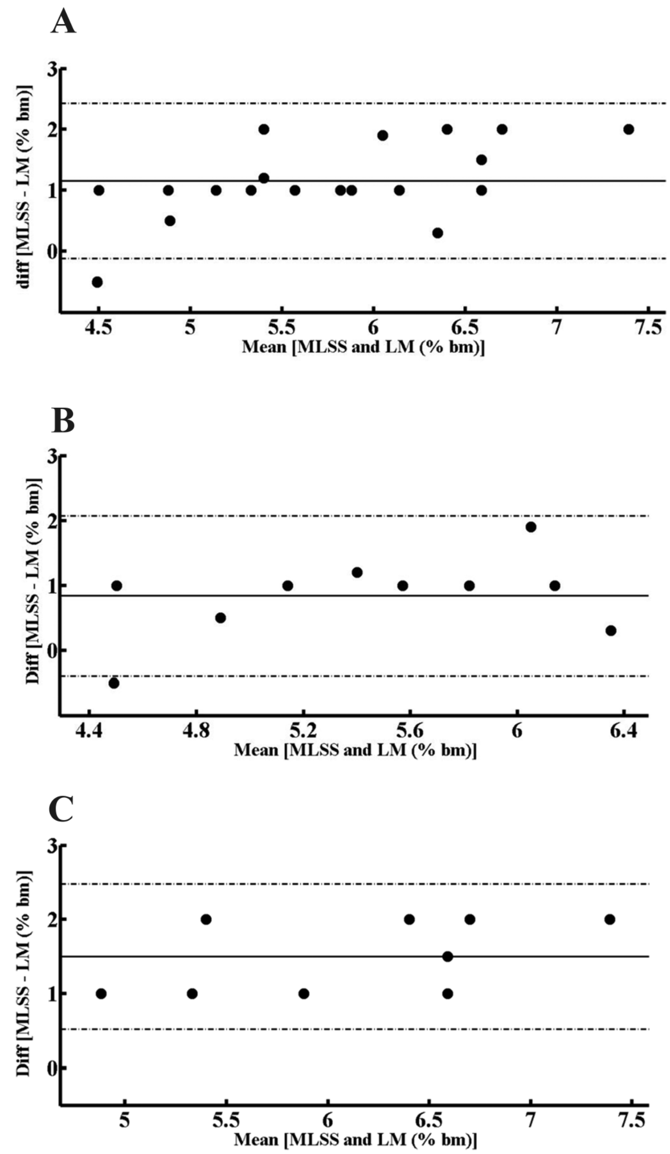


Fig. 4. Limits of agreement among intensities of maximal lactate steady-state (MLSS) and lactate minimum (LM) (A) for all mice, (B) for males, and (C) for females through the Bland and Altman (1999) analysis. % bm, percentage of body mass. Diff, difference among values of MLSS and LM.

before the incremental phase associated with individual characteristics of subject, such as higher aerobic capacity and higher blood lactate clearance, could underestimate LM. Thus, the relationship between LM and MLSS seems controversial, being important to the standardization of evaluation protocols.

The wide variability of the individual intensities can be observed in our study for both evaluation protocols (Fig. 2). In a study with swimming mice, Gobatto et al. (2009) found a mean MLSS intensity of 4.6% bm; however, intensities varied from 3% to 7% bm, with approximately 44% of the mice supporting intensities of MLSS ≥ 5% bm. This large range of intensities (Gobatto et al. 2009) supports our results for male and female mice; however, the females presented higher intensities for both evaluation protocols (reaching about 8% bm with the MLSS protocol). Nonetheless, the comparison between sexes did not reveal differences between males and females for a given evaluation protocol, probably because of the broad dispersion of the intensities measured. For practical applications, the significance (P values) of these findings does not seem to represent real differences between sexes (Baker 2016), since higher intensities could be observed when we evaluated females.

Another important finding of this study is the significant interaction of sexes between protocols, wherein the relationship of the intensities obtained in both protocols changes when it is evaluated for males and females. It is possible that sex-based differences in body composition may have affected the intensity values; however, there was no significant correlation among intensities of LM and MLSS and body mass (Table 2). In contrast, Almeida et al.

(2011) used MLSS to compare mouse strains (ob/ob vs. ob/OB) with regard to swimming exercise. They found a mean intensity of 4.3% bm versus 3.2% bm for ob/ob and ob/OB, respectively. The difference in the body composition is related to a large amount of adipose tissue found in ob/ob mice that present leptin deficiency. Thus, the authors discuss the facilitation of fluctuation associated with the body composition that does not necessarily represent a greater physical condition relative to aerobic capacity.

The study of Hoydal et al. (2007) showed that female mice presented higher weekly rates of maximal oxygen uptake compared with male mice (4.8 vs. 2.9 mL/kg$^{0.75}$) and higher running speeds. De Bono et al. (2006) also found that female mice ran 40% more than male mice. Moreover, others evidence suggest that female mice present a better aerobic condition related to oxygen consumption, mitochondrial adaptations, signaling pathways (e.g., MAPK), and cardiac adaptations than male mice (Konhilas et al. 2004; Dworatzek et al. 2014). Based on the results presented here, it appears that females present higher aerobic capacity than males; however, additional investigations should be carried out to understand sex-based differences during exercise.

Different blood lactate concentrations are observed depending on the species (rats or mice), type of exercise (running or swimming), and type of method (exhaustive or nonexhaustive). Our study presented lower values of blood lactate concentration at aerobic capacity intensity compared with other studies that evaluated aerobic capacity. For swimming rats others observed approximately 5 mmol·L^{-1} of blood lactate concentration at aerobic capacity intensity (Gobatto et al. 2001; Manchado et al. 2006*a*; de Araujo et al. 2007) and approximately 3 to 4 mmol·L^{-1} of blood lactate concentration at aerobic capacity in running rats (Manchado et al. 2005; Ferreira et al. 2007; Almeida et al. 2014; Petriz et al. 2015). For swimming mice, the range of aerobic capacity varied between 4 to 6.5 mmol·L^{-1} (Gobatto et al. 2009; de Araujo et al. 2015), while for running mice aerobic capacity was 3 mmol·L^{-1} (Ferreira et al. 2007). These different results can be related to the ergometer (Manchado et al. 2006*b*) and individual characteristics of the species (Abreu et al. 2016). Factors such as the skeletal muscle recruited, type of fibers, and oxidative capacity of the muscle, as well body size and composition, oxygen consumption, and trainability, suggest different stabilization intensities and clearance rates of blood lactate (Billat et al. 2003; Hoydal et al. 2007; Brooks 2009).

Thus, the LM protocol tested in our study underestimated the intensity of MLSS for both sexes. Future studies should investigate another protocol with changes in the specific phases (hyperlactatemia induction, resting pause, and incremental test) to characterize the relationship to LM intensity. The present study began the investigation into sex-based differences in exercise intensity. It remains to be determined whether these sex-based differences are due exclusively to body mass.

In summary, the present study proposed to validate the LM protocol for swimming mice because of the possibility to estimate in 1 session the intensity of MLSS. However, the LM protocol tested underestimated the MLSS for both sexes. For practical applications, the bias associated was lower for males versus females; thus, the LM protocol could be used to determine individual aerobic capacity of exercise for male mice. More studies about the standardization of evaluation protocols for mice are necessary, considering several applications such as nutrition, pathologies, and metabolism.

Conflict of interest statement

The authors have no conflict of interest associated with this manuscript.

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