



## Original research

# Comparison of different test protocols to determine maximal lactate steady state intensity in swimming

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## ABSTRACT

**Objectives:** This study compared step test, lactate minimum (LM) test and reverse lactate threshold (RLT) test protocols with maximal lactate steady state (MLSS) in free-swimming. All test protocols used fixed duration increments and high work-rate resolution ( $\leq 0.03 \text{ m}\cdot\text{s}^{-1}$ ) to ensure high sensitivity.

**Design:** Validation study.

**Methods:** 23 swimmers or triathletes (12 male and 11 female) of different ages ( $19.0 \pm 5.9$  yrs) and performance levels (400 m personal best  $1.38 \pm 0.13 \text{ m}\cdot\text{s}^{-1}$ , FINA points  $490 \pm 118$ ) completed an incremental step test ( $+0.03 \text{ m}\cdot\text{s}^{-1}$  every 3 min) to determine speed at  $4 \text{ mmol}\cdot\text{L}^{-1}$  and at modified maximal distance method, a LM test, a RLT test and two to five 30 min tests ( $\pm 0.015 \text{ m}\cdot\text{s}^{-1}$ ) to determine MLSS. Following a 200 m all-out and a 5 min rest, LM was determined during an incremental segment ( $+0.03 \text{ m}\cdot\text{s}^{-1}$  every 2 min) as the nadir of the speed-lactate curve. After a priming segment with four increments ( $+0.06 \text{ m}\cdot\text{s}^{-1}$ ), RLT was determined as the lactate apex during a reverse segment ( $-0.03 \text{ m}\cdot\text{s}^{-1}$ ) every 3 min.

**Results:** The mean differences ( $\pm$  limits of agreement) to speed at MLSS were  $+1.0 \pm 4.1\%$  (speed at  $4 \text{ mmol}\cdot\text{L}^{-1}$ ),  $+1.5 \pm 3.5\%$  (modified maximum distance method),  $-0.2 \pm 4.7\%$  (LM) and  $2.0 \pm 3.1\%$  (RLT). All threshold concepts showed good agreement with MLSS pace (intraclass correlation coefficient  $\geq 0.886$ ).

**Conclusions:** Test protocols with a fixed step duration and fine increments allowed high accuracy in estimating MLSS pace. With similar criterion agreement to the LM and RLT tests, incremental step tests appear more practicable due to less prior knowledge required and derivation of individual training zones.

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## Practical Implications

- Narrow speed and time resolution enable sensitive MLSS determination in swimming.
- Test protocols with fixed step duration and fine increments allow high accuracy in estimating MLSS pace.
- ST is more practicable than LMT and RLT test because little prior knowledge is required and individual training zones can be derived.

## 1. Introduction

In swimming, the spectrum of submaximal speeds is narrow due to the nonlinear relationship between active drag and swimming speed. Thus, slight increases in external workload (i.e., speed) may lead to

marked alterations of internal workload, e.g., physiological alterations including blood lactate concentration (bLa), heart rate (HR) and oxygen uptake ( $\text{VO}_2$ ).<sup>1,2</sup> For example, a speed increase from the first rise in bLa above baseline (LT1) of only  $0.06 \text{ m}\cdot\text{s}^{-1}$  may already mark the maximal metabolic steady state (MMSS),<sup>3</sup> which can be sustained almost entirely by oxidative phosphorylation and therefore still yields stable  $\text{VO}_2$  and bLa.<sup>4</sup> Therefore, precise and sensitive testing measures are required to accurately determine training zones and performance development for each athlete.<sup>3,5</sup>

MMSS has often been estimated as the highest speed that can be maintained at a constant bLa, i.e., maximal lactate steady state (MLSS). When swimming slightly above the MLSS pace, marked physiological alterations, like increases in bLa,<sup>1,2,6</sup> minute ventilation,<sup>1</sup> and  $\text{VO}_2$  approaching maximal oxygen uptake ( $\text{VO}_{2\text{peak}}$ )<sup>2</sup> have been reported. However, the MLSS concept has been criticized due to methodological aspects (e.g., arbitrary temporal criteria together with cutoff values for the permitted increase in bLa) and limited test sensitivity due to the inevitable selection of discrete workloads, which may lead to an underestimation of the 'true' MMSS.<sup>4,7,8</sup> Since direct MLSS determination is

Abbreviations: LMT, Lactate minimum test; RLT, Reverse lactate threshold.

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already time-consuming (i.e., at least two 30 min trials on separate days are necessary) and a higher resolution to refine MLSS determination would require even more sessions, various single-session tests have been introduced in swimming research to estimate MLSS more feasibly.<sup>5,9</sup>

Incremental step tests (ST) are particularly popular in swimming for determining breakpoints in the speed-lactate curve as practicable MLSS derivatives.<sup>5,9,10</sup> Yet some of the ST protocols have not been validated against physiological criteria<sup>11,12</sup> and are limited by their resolution and a fixed step distance. In detail, most of the ST protocols use speed increments of  $\geq 0.05 \text{ m}\cdot\text{s}^{-1}$ ,<sup>5,9,10,12,13</sup> which can hardly meet the requirements of a high resolution of intensity zones as depicted above.<sup>3</sup> In addition, unlike tethered swimming, in which athletes are attached to a load cell with a cord and are required to maintain their position against gradually increasing resistance,<sup>14</sup> most free-swimming protocols use increments with fixed distances, particularly often 200 m.<sup>5,9,10,12,13</sup> Despite high practicability (i.e., blood sampling always at the same place), this fixed distance approach bears the risk of overestimating breakpoints and derived training zones due to decreasing step durations with increasing speeds.<sup>14–16</sup>

Besides these methodological issues, lactate thresholds derived from STs have been criticized in various sports for their empirical/mathematical rather than physiological association with MLSS.<sup>8,17</sup> In consequence, two single-session tests based on the blood lactate accumulation-elimination-equilibrium, namely the lactate minimum (LM) and reverse lactate threshold (RLT) tests, have been compared to MLSS in cycling and running, showing a high agreement, e.g.,<sup>17–19</sup>. Interestingly, the lactate minimum test (LMT) has already been used in swimming in some investigations,<sup>16,20,21</sup> but unlike tethered swimming, free-swimming protocols have so far lacked aspects similar to those already described for ST protocols (i.e., fixed step distance and coarse intensity resolution). In contrast, the RLT has not yet been implemented in swimming at all.

Therefore, this study aimed to test the level of agreement of different protocols and derived threshold concepts with MLSS established with a narrow speed and temporal resolution in free-swimming. To overcome some of the mentioned limitations, all tests used a higher resolution of the speed gradation and increments with a fixed duration.

## 2. Methods

Participants underwent five to eight separate test sessions: a ST, a LMT, a RLT test and two to five 30 min constant speed trials for MLSS determination. After completing their individual routine warm-up procedure on land and in the water, athletes performed all tests using the front crawl technique, in-water starts and flip turns. All sessions took place simultaneously for a maximum of two participants on a side lane (separated by a lane rope) in the same 50 m pool for each participant. The velocity (calculated by total lap time) was set by a visual pacing device on the bottom of the pool equipped with three flashing LED lights every 10 cm (Virtual Swim Trainer, Indico Technologies, Torino, Italy; precision: 0.02 s). Due to higher speeds after the turns, athletes were instructed to swim as close to the lights as possible by performing smooth turns at submaximal speeds. All tests, including athlete instruction, speed monitoring and data collection, were always performed by at least two experienced diagnosticians, at least one of whom was one of the authors. All athletes were instructed to avoid intense exercise for 24 h preceding each test and instead to perform low to moderate intensity work only when needed. In addition, the single test sessions were separated by at least 24 h, limited to a maximum of three tests per week and the testing time for each participant was kept constant within a time frame of  $\pm 2$  h (only 3 of 23 participants completed one of the tests outside this time frame due to training time constraints).

All testing protocols consisted of stages with a fixed duration and short resting periods (30 s) in-between for blood sampling. Thus, as speed increased, larger distances were covered to keep the time for physiological responses constant. This was achieved by programming

the pacing lights to indicate to the athletes the start and end of each stage at the corresponding point wherever in the pool with a 5 s start-stop countdown. Before the first test, the 400 m personal best ( $v_{400\text{m}}$ ) was requested from the athletes or coaches to define individual initial speeds.<sup>3,6</sup> However, since pilot testing revealed difficulties in selecting appropriate initial intensities for the LMT and RLT test based on  $v_{400\text{m}}$  alone, the order of the tests was kept constant (i.e., ST, LMT and RLT) to incorporate prior knowledge from the ST. During all sessions, HR was recorded continuously (HRM-Swim™, Garmin Deutschland GmbH, Garching) and 20  $\mu\text{l}$  of capillary blood for lactate analysis (Biosen C-line; EKF Diagnostic Sales, Magdeburg, Germany) was taken from the dried earlobe.

23 healthy front crawl swimmers or triathletes of different ages and performance levels (recreational to regional level) volunteered to participate in the study (Table 1). Inclusion criteria were cardiovascular health, systematic training and competition experience for at least 3 years and a target  $v_{400\text{m}}$  of  $>1.33 \text{ m}\cdot\text{s}^{-1}$ . These rather broad criteria were chosen to validate the test protocols for a wide range of athletes. The participants and their parents (when necessary) were informed about the benefits and risks of the investigation and provided written informed consent. The study was conducted according to the Declaration of Helsinki and was approved by the university's ethical committee (124/2020).

The 3 min fixed duration incremental ST started at 88% of  $v_{400\text{m}}$  minus four increments ( $0.12 \text{ m}\cdot\text{s}^{-1}$ ),<sup>3,6</sup> and increased by  $0.03 \text{ m}\cdot\text{s}^{-1}$  until exhaustion (average number of steps  $8.6 \pm 1.0$ ). The swimming speed in the last stage ( $v_{\text{peak}}$ ) was recorded and corrected for the effective duration if the 3 min were not completed. For threshold determination, bLa was plotted against swimming speed and fitted by a third-order polynomial ( $R^2 \geq 0.98$ ).<sup>22</sup> The speed corresponding to a bLa of  $4 \text{ mmol}\cdot\text{L}^{-1}$  (OBLA), a widely used threshold concept in swimming,<sup>5,23</sup> and the modified maximum distance method (mDmax), recently shown to be valid in swimming,<sup>9</sup> were determined as estimates of MLSS pace. mDmax was determined as the point on the third-order polynomial with the maximal perpendicular distance to the straight line formed by the LT1 and the final data point. Based on the method proposed by Bishop et al.,<sup>24</sup> LT1 was defined as the point on the fitted curve with a slope equal to 13.3, which corresponds to an increase in bLa of  $0.4 \text{ mmol}\cdot\text{L}^{-1}$  but allows for higher resolution.<sup>22</sup>

The LMT comprised a priming segment to assess  $\text{VO}_{2\text{peak}}$  and to induce hyperlactatemia and an incremental segment to determine the LM. The 300 m priming segment consisted of 100 m at progressively increased speed directly followed by a 200 m all-out.<sup>20</sup> Immediately after striking the wall, the spirometry mask was firmly applied to the participant ( $< 60$  s) who rested in the water in an upright position immersed to the mid-sternum.<sup>25</sup> The measured  $\text{VO}_2$  was corrected by the post-exercise HR decline and the highest 5 s average value was considered the  $\text{VO}_{2\text{peak}}$ .<sup>25</sup> Following a resting period of 5 min (including  $\text{VO}_2$  assessment), the second segment started at the average of the three values 88% of  $v_{400\text{m}}$ ,<sup>3,6</sup> OBLA and mDmax minus four increments ( $0.12 \text{ m}\cdot\text{s}^{-1}$ ) and speed was increased by  $0.03 \text{ m}\cdot\text{s}^{-1}$  every 2 min until exhaustion (average number of steps  $8.8 \pm 1.2$ ). The shorter stage duration compared to the ST was chosen after pilot tests revealed underestimation of speed at MLSS with longer durations (3 min), a known weakness of the LMT design, attributable to the greater cumulative time below the MMSS that does not necessarily lead to the highest possible lactate equilibrium.<sup>8</sup> Following previous work,<sup>16,17</sup> swimming speed at LM was calculated by the first derivative of a third-order polynomial fitting of the speed-lactate curve ( $R^2 \geq 0.98$ ).

The RLT test comprised an incremental priming segment with four coarse speed increments ( $0.06 \text{ m}\cdot\text{s}^{-1}$ ) starting at the average of the three values 88% of  $v_{400\text{m}}$ ,<sup>3,6</sup> OBLA and mDmax minus  $0.12 \text{ m}\cdot\text{s}^{-1}$  and increasing up to the average of the three values plus  $0.06 \text{ m}\cdot\text{s}^{-1}$ . The reverse segment immediately followed with a speed reduced by  $0.03 \text{ m}\cdot\text{s}^{-1}$ , which decreased by another  $0.03 \text{ m}\cdot\text{s}^{-1}$  every 3 min until the initial speed was reached again (total number of steps ten). Analogous

**Table 1**  
Descriptive characteristics of the participants ( $n = 23$ ) presented as mean  $\pm$  standard deviation along with the range for the whole group and separated by sex.

Sex	n	Age [yrs]	Height [cm]	Mass [kg]	$\text{VO}_{2\text{peak}}$ [ $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ]	$v_{400\text{m}}$ [ $\text{m} \cdot \text{s}^{-1}$ ]	FINA points [AU]	Training volume [ $\text{h} \cdot \text{wk}^{-1}$ ]
Male	12	20.2 $\pm$ 5.7 (13.2–33.3)	179 $\pm$ 7 (166–194)	67.9 $\pm$ 10.0 (51.9–84.0)	60.9 $\pm$ 7.2 (47.2–74.4)	1.41 $\pm$ 0.15 (1.14–1.59)	484 $\pm$ 142 (247–669)	14 $\pm$ 6 (7–21)
Female	11	17.7 $\pm$ 6.2 (13.1–30.0)	169 $\pm$ 7 (154–176)	57.4 $\pm$ 5.7 (48.6–68.0)	52.6 $\pm$ 6.3 (38.7–59.2)	1.34 $\pm$ 0.08 (1.21–1.46)	499 $\pm$ 89 (368–636)	14 $\pm$ 5 (5–20)
All	23	19.0 $\pm$ 5.9 (13.1–33.3)	174 $\pm$ 8 (154–194)	62.9 $\pm$ 9.7 (48.6–84.0)	56.9 $\pm$ 7.9 (38.7–74.4)	1.38 $\pm$ 0.13 (1.14–1.59)	490 $\pm$ 118 (247–669)	14 $\pm$ 5 (5–21)

Abbreviations:  $\text{VO}_{2\text{peak}}$ : maximal oxygen uptake (assessed after a 200 m all-out);  $v_{400\text{m}}$ : 400 m personal best.

to the LM, swimming speed corresponding to the lactate apex reached during the reverse segment (RLT) was determined using the first derivative of a third-order polynomial fitting of the speed-lactate curve ( $R^2 \geq 0.99$ ).<sup>8</sup>

MLSS was determined by two to five  $6 \times 5$  min constant speed tests, the first one set at the average of OBLA, mDmax, LM and RLT. The 5 min intervals were chosen to allow determination of additional temporal criteria (i.e., between the 15th and 30th and 20th and 30th min) besides the traditional interval (i.e., between the 10th and 30th min) for the permitted  $\leq 1 \text{ mmol} \cdot \text{L}^{-1}$  increase in bLa by blood sampling every 5 min during the 30 s resting phases as suggested recently.<sup>4</sup> A narrow speed gradation, i.e.,  $0.015 \text{ m} \cdot \text{s}^{-1}$  steps, allowed high sensitivity in the detection of the ‘true’ MMSS.

For the ST, LMT and RLT test, bLa and HR corresponding to the respective thresholds were calculated by third-order polynomial and linear interpolation, respectively. For the MLSS tests, bLa and HR were averaged over the respective time intervals.

Homoscedasticity and normality were checked by visual inspection of residual histograms, residual plots and Q-Q-plots. Linear mixed-effects models were constructed to examine differences between speeds corresponding to each threshold concept using the *lme4* package in R.<sup>26</sup> As fixed effects, threshold concepts (five levels), sex (two levels) and age (covariate) were entered into the model (with and without interaction plus random intercepts for participants) and retained if maximum likelihood ratio test indicated a significant alteration ( $p < 0.05$ ). Consequently, multiple post-hoc pairwise comparisons were conducted with the ‘Bonferroni’ adjustment method using the *emmeans* package. Cohen's effect sizes (ES) along with 95% confidence intervals (CI) to indicate the accuracy of ES estimates were determined using the *effsize* package to assess the magnitude of differences between threshold concepts. ES estimates were interpreted as follows:  $\text{ES} < 0.2 = \text{trivial}$ ,  $0.2 \leq \text{ES} < 0.6 = \text{small}$ ,  $0.6 \leq \text{ES} < 1.2 = \text{moderate}$  and  $\text{ES} \geq 1.2 = \text{large}$ .<sup>27</sup>

A Bland-Altman analysis was performed using the *BlandAltmanLeh* package to check the agreement (bias along with limits of agreement [LoA], i.e., 1.96-fold standard deviation [SD]) between the measures presented in [ $\text{m} \cdot \text{s}^{-1}$ ] and in [%] of MLSS pace. To account for correlation and agreement between measurements, intraclass correlation coefficients (ICC) along with 95% CI were calculated using the *icc* package with single measure two-way mixed-effects models and the ‘absolute agreement’ as the type of analysis.<sup>19,28</sup> The agreement was interpreted as follows:  $\text{ICC} \leq 0.50 = \text{poor}$ ,  $0.50 \leq \text{ICC} < 0.75 = \text{moderate}$ ,  $0.75 \leq \text{ICC} < 0.90 = \text{good}$  and  $\geq 0.90 = \text{excellent}$ .<sup>28</sup> Further, associations between LT1 and MLSS pace were examined using the Pearson product-moment correlation coefficient  $r$  along with 95% CI calculated with the *stats* package. For all tests, statistical significance was accepted at  $p < 0.05$ . All data are presented as mean  $\pm$  SD.

### 3. Results

Determination of RLT was only possible in 17 of the 23 participants due to the lack of the characteristic blood lactate curve. Only marginal differences were observed between the MLSS determination methods. Thus, mean MLSS paces were  $1.225 \pm 0.112$ ,  $1.230 \pm 0.110$  and  $1.231 \pm 0.110 \text{ m} \cdot \text{s}^{-1}$  when determined as the highest speed with an increase

in  $\text{bLa} \leq 1 \text{ mmol} \cdot \text{L}^{-1}$  between the 10th and 30th, 15th and 30th, and 20th and 30th min, respectively. Given the marginal variation between the MLSS determination methods ( $< 0.5\%$ ) and the fact that 14 of the 23 participants could not finish the next 30 min trial, the highest value, determined between the 20th and 30th min, was accepted as MLSS pace according to Iannetta et al.<sup>4</sup>

The average speed, HR and bLa corresponding to each threshold concept are depicted in Table 2. For HR and bLa, statistically significant main effects for threshold concepts were found ( $p < 0.001$ ). HR at OBLA ( $p < 0.05$ ) and LM ( $p < 0.001$ ) and bLa at OBLA ( $p < 0.05$ ), LM ( $p < 0.05$ ) and RLT ( $p < 0.05$ ) differed from those at MLSS as detailed in Table 2. Adding sex as another fixed effect improved the model only for bLa ( $p < 0.05$ ), with male athletes showing higher bLa than females. For speed, a statistically significant main effect for threshold concept was observed ( $p < 0.001$ ), however adding either sex or age as another fixed effect (with and without interaction) did not further improve the model. Across all participants, pairwise comparisons revealed significant differences between speed at MLSS and mDmax ( $p < 0.05$ ) and RLT ( $p < 0.05$ ), albeit with trivial to small effects ( $\text{ES} \leq 0.23$ ) as detailed in Table 2. Excellent measures of agreement ( $\text{ICC} \geq 0.926$ ) demonstrate high conformity of all threshold concepts with MLSS in male and female athletes (Table 2).

Bland-Altman plots confirm the absolute agreement between all threshold concepts and MLSS (Fig. 1). LM showed the smallest bias ( $-0.2\%$ ), but the largest LoA ( $\pm 4.7\%$ ). OBLA ( $+1.0\%$ ), mDmax ( $+1.5\%$ ) and RLT ( $+2.0\%$ ) showed larger biases (overestimation of MLSS pace) but with smaller LoA ( $\pm 4.1\%$ ,  $\pm 3.5\%$  and  $\pm 3.1\%$ ). The coefficients (intercept and slope) for each proportional bias (linear regression between the mean and the difference between the speeds at each threshold concept and MLSS) were  $-0.045$  and  $0.046$  for OBLA,  $0.092$  and  $-0.059$  for mDmax,  $-0.013$  and  $0.009$  for LM and  $-0.085$  and  $0.089$  for RLT. Apart from the slope for RLT ( $p < 0.05$ ), all other coefficients were not statistically significant.

In addition to MLSS estimates, it was found that speed at LT1 ( $1.173 \pm 0.105 \text{ m} \cdot \text{s}^{-1}$ ) was highly associated with speed at MLSS ( $r = 0.97$ , CI:  $0.92$ – $0.99$ ,  $p < 0.001$ ) across all participants.

### 4. Discussion

This study investigated the level of agreement of various threshold concepts (OBLA, mDmax, LM, RLT) with MLSS as a physiological criterion representing MMSS in free-swimming. While previous investigations used speed increments of  $\geq 0.05 \text{ m} \cdot \text{s}^{-1}$  along with fixed step distances (frequently 200 m),<sup>5,9,10,12,13</sup> finer increments of  $0.03 \text{ m} \cdot \text{s}^{-1}$  and fixed step durations (2 or 3 min) were applied in the present study. Likewise, MLSS was determined using additional narrower temporal criteria (i.e., intervals between the 15th and 30th and 20th and 30th min) and a higher resolution ( $0.015 \text{ m} \cdot \text{s}^{-1}$ ) than before ( $\geq 0.03 \text{ m} \cdot \text{s}^{-1}$  and interval between the 10th and 30th min)<sup>1,5,6</sup> to alleviate granularity concerns related to an underestimation of MMSS.<sup>7</sup> Compared to previous studies,<sup>5,9,13,16</sup> a higher agreement was observed between all threshold concepts and MLSS as indicated by small biases and LoAs ( $\leq 2\%$  and  $< 5\%$ , respectively).

This observation applies to a broad sample including male and female swimmers and triathletes aged from 13 to 33 years. As

**Table 2**

Speed (absolute value and difference [Δ] to MLSS pace), blood lactate concentration (bLa) and heart rate (HR) corresponding to each threshold concept presented as mean ± standard deviation along with the range for the whole group and separated by sex. In addition, effect size (ES) and intraclass correlation coefficient (ICC) indicate the magnitude of difference and agreement, respectively, between each speed estimate and MLSS pace and are presented along with 95% confidence intervals (CI).

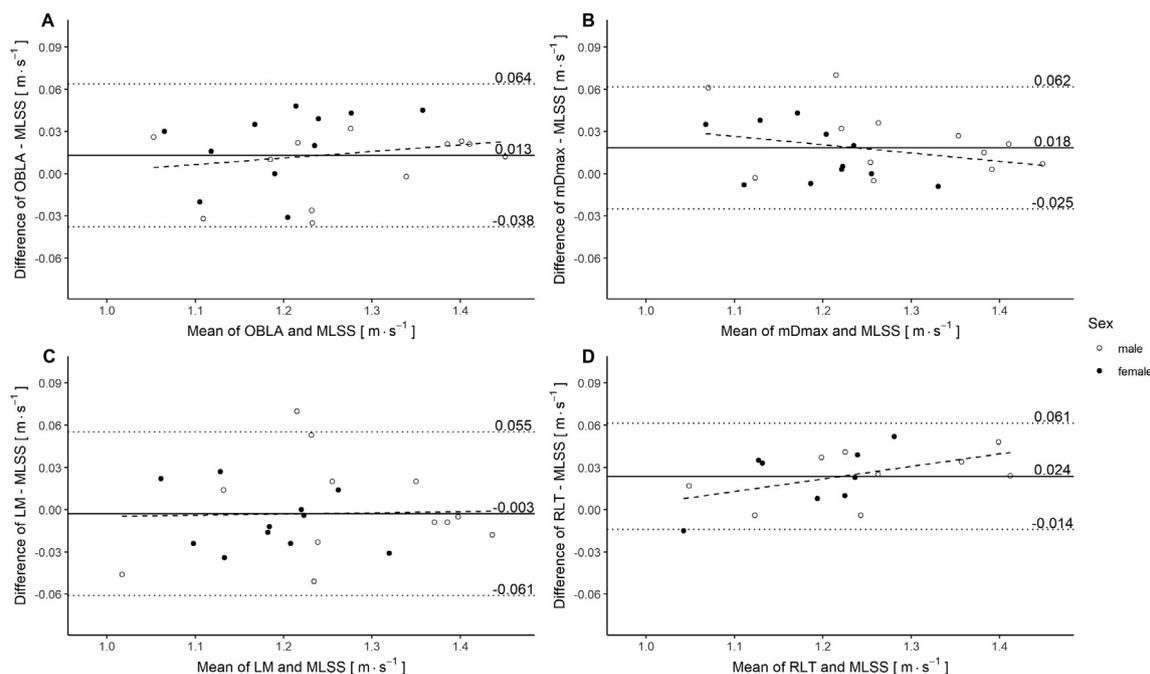
Parameter	Sex	n	Speed [ $m \cdot s^{-1}$ ]	Δ Threshold-MLSS [ $m \cdot s^{-1}$ ]	ES (95% CI)	ICC (95% CI)	bLa [ $mmol \cdot L^{-1}$ ]	HR [ $b \cdot min^{-1}$ ]
MLSS	Male	12	1.271 ± 0.123 (1.040–1.445)				5.28 ± 1.91 (2.83–9.23)	184 ± 10 (162–197)
	Female	11	1.187 ± 0.078 (1.050–1.335)				4.89 ± 1.60 (2.43–7.08)	188 ± 11 (168–205)
	All	23	1.231 ± 0.110 (1.040–1.445)				5.10 ± 1.74 (2.43–9.23)	186 ± 10 (162–205)
OBLA	Male	12	1.277 ± 0.130 (1.066–1.457)	0.006 ± 0.024	0.05 (−0.06–0.15)	0.982*** (0.942–0.995)	4.00 ± 0.00	179 ± 10 (161–193)
	Female	11	1.208 ± 0.089 (1.080–1.380)	0.020 ± 0.027	0.22 (0.04–0.41)	0.926*** (0.644–0.981)	4.00 ± 0.00	186 ± 16* (150–209)
mDmax	All	23	1.244 ± 0.115 (1.066–1.457)	0.013 ± 0.026	0.11 (0.02–0.21)	0.968*** (0.915–0.987)	4.00 ± 0.00*	182 ± 13 (150–209)
	Male	12	1.294 ± 0.113 (1.101–1.452)	0.023 ± 0.024	0.18 (0.06–0.29)	0.963*** (0.716–0.991)	4.48 ± 0.95 (3.13–5.97)	181 ± 10 (163–194)
	Female	11	1.201 ± 0.069 (1.085–1.326)	0.013 ± 0.020	0.16 (0.11–0.31)	0.951*** (0.780–0.987)	3.86 ± 0.86 (2.48–5.00)	185 ± 14 (155–209)
LM	All	23	1.249 ± 0.104* (1.085–1.452)	0.018 ± 0.022	0.16 (0.08–0.25)	0.966*** (0.822–0.989)	4.18 ± 0.94 (2.48–5.97)	183 ± 12 (155–209)
	Male	12	1.273 ± 0.124 (0.994–1.427)	0.001 ± 0.036	0.01 (−0.16–0.19)	0.960*** (0.868–0.988)	4.71 ± 2.12 (1.79–7.75)	178 ± 8 (168–189)
	Female	11	1.180 ± 0.073 (1.072–1.304)	−0.007 ± 0.021	−0.10 (−0.27–0.08)	0.960*** (0.864–0.989)	3.05 ± 0.88 (1.79–4.68)	179 ± 15 (152–202)
RLT	All	23	1.228 ± 0.111 (0.994–1.427)	−0.003 ± 0.030	−0.03 (−0.14–0.09)	0.965*** (0.920–0.985)	3.92 ± 1.82* (1.79–7.75)	178 ± 11*** (152–202)
	Male	9	1.264 ± 0.127 (1.057–1.424)	0.024 ± 0.019	0.17 (0.08–0.27)	0.971** (0.505–0.995)	8.36 ± 2.36 (5.38–11.49)	185 ± 11 (168–196)
	Female	8	1.196 ± 0.085 (1.035–1.307)	0.023 ± 0.021	0.23 (0.07–0.40)	0.928** (0.376–0.987)	5.67 ± 1.70 (3.29–7.86)	186 ± 14 (164–199)
	All	17	1.232 ± 0.112* (1.035–1.424)	0.024 ± 0.019	0.19 (0.11–0.27)	0.961** (0.522–0.991)	7.09 ± 2.44* (3.29–11.49)	186 ± 12 (164–199)

Abbreviations: MLSS: speed at maximal lactate steady state; OBLA: speed at a blood lactate concentration of 4  $mmol \cdot L^{-1}$ ; mDmax: speed at modified maximal distance method; LM: speed at lactate minimum; RLT: speed at reverse lactate threshold. Statistically significant differences between values (speed, bLa and HR) corresponding to estimates and MLSS: \*  $p < 0.05$ , \*\*\*  $p < 0.001$  and statistically significant ICC values: \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

modeling speed differences between threshold concepts and MLSS was not improved by including sex or age in the model, the agreement between estimates and MLSS pace appears to be valid regardless of both covariates. Apart from sex and age, participants' performance levels were heterogeneous along with wide variation in training routines from 5 to 21 h per week, as indicated by the ranges of  $VO_{2peak}$  and  $V_{400m}$ , i.e., 38.7 to 74.4  $ml \cdot min^{-1} \cdot kg^{-1}$  and 1.14 to 1.59  $m \cdot s^{-1}$ , respectively. However, as evident from the small proportional biases between the speeds corresponding to the

estimates and MLSS shown in Fig. 1 (ranging from −0.059 to 0.089), the accuracy of the threshold concepts was independent of performance level represented by largely different MLSS paces (i.e., 1.040 to 1.445  $m \cdot s^{-1}$ ). Together, linear mixed models and Bland-Altman analyses confirmed the agreement of threshold estimates and MLSS pace in participants with widely varying characteristics including sex, age and performance level.

The average speed corresponding to MLSS observed in the present study was in the range of earlier findings in trained swimmers



**Fig. 1.** Bland-Altman plots: differences between the swimming speeds at a blood lactate concentration of 4  $mmol \cdot L^{-1}$  (OBLA) (A), modified maximal distance method (mDmax) (B), lactate minimum (LM) (C) and reverse lactate threshold (RLT) (D) as well as maximal lactate steady state (MLSS) pace in male (open) and female (filled circles) athletes. The solid line indicates the mean difference (fixed bias), the dotted lines indicate the limits of agreement (fixed bias ± 1.96-fold standard deviation) and the dashed line indicates the linear regression line (proportional bias) of the data.

(e.g.,  $1.22 \pm 0.09 \text{ m}\cdot\text{s}^{-1}$ ,  $1.25 \pm 0.06 \text{ m}\cdot\text{s}^{-1}$ ).<sup>16</sup> In accordance with Iannetta et al.,<sup>4</sup> high sensitivity in MLSS testing was achieved by using narrower temporal criteria and small increments between 30 min trials, which together allowed precise estimation of the physiological criterion. Considering that 14 of the 23 participants could not complete the next trial above MLSS pace, MLSS, defined as the highest speed with an increase in bLa  $\leq 1 \text{ mmol}\cdot\text{L}^{-1}$  between the 20th and 30th min of constant exercise, appears to represent well the 'true' MMSS, above which contrasting physiological responses occur.<sup>4,7</sup> These findings highlight the importance of a high work-rate and temporal resolution for MLSS testing due to the narrow intensity range in swimming, which allows one to remain in a physiological steady state for at least 30 min at a given speed, whereas a speed of only  $0.015 \text{ m}\cdot\text{s}^{-1}$  higher can quickly lead to exhaustion. At the expense of higher temporal resolution, the intermittent MLSS test design, which is required for regular blood sampling, may have yielded a slightly higher speed than the traditional continuous protocol. However, given the low ratio of exercise to resting time of 1:10,<sup>29</sup> the influence of the intermittent protocol appears to be rather small, as also suggested by previous findings in running.<sup>18,19</sup>

Despite the frequent usage of incremental tests in swimming, few studies have validated ST protocols and associated threshold concepts against MLSS.<sup>5,9,10,13</sup> Even fewer studies have reported exact agreement with MLSS pace.<sup>5,9,13</sup> These studies showed a deviation from speed at MLSS (bias  $\pm$  LoA) of  $-0.3 \pm 5.1\%$  (OBLA)<sup>5</sup> and  $-1.3 \pm 6.8\%$  (mDmax),<sup>9</sup> respectively. However, in the study by Espada et al.,<sup>5</sup> MLSS was only determined with a coarse resolution of  $\geq 0.06 \text{ m}\cdot\text{s}^{-1}$ . In contrast, our findings indicate narrower LoA (OBLA:  $+1.0 \pm 4.1\%$  and mDmax:  $+1.5 \pm 3.5\%$ ) in the context of a finely resolved MLSS. Given the excellent agreement between the speeds at OBLA and mDmax with MLSS pace, our findings suggest that constant step duration and high work-rate resolution seem to alleviate MLSS overestimation previously reported by OBLA,<sup>10,15</sup> and hence generally provide a more accurate estimation of MLSS. Despite the high agreement between the speeds at OBLA and MLSS in our sample, the high variability found for bLa at MLSS (range for the whole sample:  $2.43\text{--}9.23 \text{ mmol}\cdot\text{L}^{-1}$ ) generally suggests not to focus on fixed bLa but rather to interpret bLa kinetics such as at mDmax. Since bLa at MLSS already shows a high day-to-day variation (16.6%),<sup>30</sup> it does not seem necessary or possible to approach bLa at MLSS with other threshold concepts.<sup>18</sup> This is further supported by the bLa at LM and RLT in the present study, which deviate even more from that at MLSS compared to OBLA and mDmax, whereas the respective speeds are highly consistent with that at MLSS.

In addition to the MLSS estimates, the ST additionally provides a measure of LT1. Due to the high correlation with MLSS pace ( $r = 0.97$ ), LT1 might be monitored regularly as a submaximal indicator of aerobic performance without the need to perform tests until exhaustion. However, the suitability of LT1 as a submaximal indicator of MLSS in swimming remains to be verified in longitudinal studies. Together, both LT1 and mDmax/OBLA may serve to define training intensity zones, namely high volume  $<$  LT1, threshold  $\leq$  mDmax/OBLA and high intensity training  $>$  mDmax/OBLA, on an individual basis.<sup>3</sup>

While high agreement between RLT and MLSS has been shown in cycling and running,<sup>17,19</sup> this is the first investigation to apply the RLT test in swimming. RLT showed a similar overestimation of MLSS pace as OBLA and mDmax, which may be explained by the short resting periods inevitable for blood sampling, leading to an earlier decline in bLa and thus a shift in RLT towards higher speeds.<sup>8,19</sup> However, the major challenge in RLT testing was the prior selection of individual intensities that exceed MMSS while avoiding premature test termination due to fatigue. In contrast to running and cycling, the narrow speed range due to the aquatic environment made it difficult to accurately determine swimming speeds. Thus, in accordance with Greco et al.<sup>3</sup> reporting a narrow range of submaximal speeds (i.e., between LT1 and MLSS), we observed a similarly narrow range between the speed associated with MLSS and  $v_{\text{peak}}$  obtained during the ST ( $0.090 \pm 0.028 \text{ m}\cdot\text{s}^{-1}$ ). Given the increments of

$0.03 \text{ m}\cdot\text{s}^{-1}$ , a maximum of two stages remains to obtain the characteristic blood lactate curve of the RLT test. Despite detailed prior knowledge of athlete performance based on the ST results, no 'valid' RLT (i.e., no further increase in bLa during the reverse segment) could be recorded in six out of 23 participants. Therefore, since the RLT test did not show higher accuracy compared to the ST, it may be less practicable because of the need for accurate prior knowledge of athlete performance.

Based on the experience from previous investigations with the LMT in cycling and running,<sup>17,18</sup> we chose a short rest interval between both segments (5 min, because pilot testing revealed that peak bLa was often reached after this time and athletes should restart with the highest possible bLa) and targeted a similar duration below MMSS ( $\sim 8$  min) by using 2 min stages to avoid underestimation of MLSS pace. In addition, we applied fixed step durations in combination with finer increments ( $0.03$  instead of  $0.05 \text{ m}\cdot\text{s}^{-1}$ ) compared to previous studies in swimming.<sup>16,20</sup> Indeed, LM was highly consistent with MLSS pace, albeit unlike previous investigations in running and cycling,<sup>17,18</sup> it did not show higher agreement with MLSS pace compared to the other threshold concepts. Nevertheless, compared to the only study presenting the absolute agreement between the speeds at LM and MLSS in free-swimming, our protocol design allowed for a halving of the observed variation (i.e.,  $2.7 \pm 10.0\%$ ).<sup>16</sup>

When interpreting the results, some limitations need to be considered and addressed in future research. It should be considered that the tests were not conducted in randomized order since preliminary testing showed that detailed prior knowledge (from the ST) was necessary to select appropriate initial intensities to obtain valid results in the LMT and RLT test. Therefore, learning effects cannot be completely excluded, although we estimate them to be small, if any, since no novice swimmers or triathletes were studied. Still, no valid RLT could be determined for six participants, which limits the comparability with the other tests and highlights the difficulty of this test design in swimming. Furthermore, unlike the ST and RLT test, we chose a step duration of 2 instead of 3 min for the LMT as detailed above underlining the impact of step duration on threshold determination. However, since stage duration should always be considered together with the increment per step,<sup>18,29</sup> 2 min steps might still be appropriate for an increment of  $0.03 \text{ m}\cdot\text{s}^{-1}$  during the LMT, especially compared with previous studies that used increments of  $\geq 0.05 \text{ m}\cdot\text{s}^{-1}$  for a step duration of  $< 3$  min (i.e., 200 m steps).<sup>16,20</sup> Since our study was the first to use tests with such a high resolution of  $0.015$  and  $0.03 \text{ m}\cdot\text{s}^{-1}$ , the reliability of the presented test protocols and associated MLSS estimates needs to be verified in future studies. In particular, athletes' compliance with the required speeds needs to be quantified (e.g., by video analysis), to ensure that, given the narrow intensity range in swimming, even small changes in speed are detected. Although we cannot yet quantify whether athletes met the required speeds, the fact that 14 of 23 participants were able to complete a 30 min test at a given pace but not the test that was only  $0.015 \text{ m}\cdot\text{s}^{-1}$  faster clearly indicates that athletes can follow such small adjustments.

## 5. Conclusion

Test protocols with a fixed step duration and fine increments allowed high accuracy in estimating speed at MLSS, which was likewise determined with high temporal and speed resolution, in swimmers or triathletes of different levels, ages and sexes. As LMT and RLT test protocols did not provide higher accuracy than STs, the latter appear more practicable because little prior knowledge is required and individual training intensity zones can be derived from LT1 and mDmax.

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## Declaration of Interest Statement

The authors declare no conflict of interest.

## Confirmation of Ethical Compliance

The study was conducted according to the Declaration of Helsinki and was approved by the ethical committee of the German Sport University Cologne (approval number: 124/2020).

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