Reverse lactate threshold test accurately predicts maximal lactate steady state and 5 km performance in running

AUTHORS: Patrick Wahl^{1,2}, Christian Manunzio², Lukas Zwingmann^{1,2}, Stefan van de Weyer², Wilhelm Bloch^{1,2}

¹ Department of Molecular and Cellular Sport Medicine, Institute of Cardiology and Sports Medicine, German Sport University Cologne, Germany

² The German Research Centre of Elite Sport Cologne, German Sport University Cologne, Germany

ABSTRACT: This study evaluated the accuracy of the reverse lactate threshold (RLT) and the onset of blood lactate accumulation (OBLA; 4 mmol·L⁻¹) to determine the running speed at the maximal lactate steady state (MLSS) and 5 km running performance in a field test approach. Study 1: 16 participants performed an RLT test, and 2 or more constant-speed tests, lasting 30 minutes each, to determine running speed at the MLSS. Study 2: 23 participants performed an RLT test and a 5000 m all-out run as an indicator of performance. The RLT test consisted of an initial lactate-priming segment, in which running speed was increased stepwise up to ~5% above the estimated MLSS, followed by a reverse segment in which speed was decreased by 0.1 m·s⁻¹ every 180 s. RLT was determined using the highest lactate equivalent ([La⁻]/running speed) during the reverse segment. OBLA was determined during the priming segment and was set at a value of 4 mmol·L¹. The mean difference in MLSS was +0.06 ± 0.05 m·s⁻¹ for RLT, and +0.13 ± 0.23 m·s⁻¹ for OBLA. OBLA showed a good concordance with the MLSS (ICC = 0.83), whereas RLT revealed excellent concordance with the MLSS with an ICC = 0.98. RLT showed a very high correlation with 5000 m speed (r = 0.97). The RLT exhibited exceptional agreement to MLSS and 5000 m running performance. Due to this high accuracy, especially concerning the small intra-individual differences, the RLT test may be superior to common threshold concepts. Further research is needed to evaluate its sensitivity during the training process.

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Corresponding author: **Patrick Wahl** The German Research Centre of Elite Sport Cologne German Sport University Cologne Am Sportpark Müngersdorf 6 50933 Cologne, Germany Tel.: -49-221-49828561 Fax: -49-221-49828180 Email: Wahl@dshs-koeln.de

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INTRODUCTION

In competitive sports, and especially in endurance sports, there is a consensus that lactate testing can be used to manage training and to capture indicators that provide insight into an athlete's aerobic endurance capacities. To date, a myriad of lactate-related thresholds have been tested for validity [1, 2]. In general, lactate threshold concepts aim to precisely estimate the so-called maximal lactate steady state (MLSS), which is defined as the highest workload that an athlete can maintain without continual accumulation of blood lactate [3]. Since measuring the MLSS itself is very time-consuming (several 30-min constant-load tests), indirect methods provide an estimation within a single test procedure. Additionally, the most common threshold concepts, such as the onset of blood lactate accumulation (OBLA, 4 mmol·L⁻¹), are arbitrary or empirically derived [4] and therefore do not take sufficient account of the physiological principle of MLSS [2].

The lactate minimum test (LMT) (first introduced by Tegtbur et al. [5]) and the reverse lactate threshold (RLT) test (first introduced by Dotan [6]) are the only single-session tests that are based on the physiologically founded lactate appearance-disappearance equilibrium

concept, which also forms the basis for the MLSS test. The LMT starts with a short, high-lactaemic exercise bout. Subsequently, a graded exercise test, starting well below the estimated MLSS, is performed. Consequently, lactate concentration initially decreases before rising again with increasing exercise intensity, resulting in a U-shaped curve. The minimum point would, therefore, represent a maximal intensity where the blood lactate accumulation and elimination are in balance. In two previous studies, we were able to show that modified LMTs reveal much higher accuracy in the determination of the MLSS in running and cycling compared to step tests using different threshold concepts [7, 8]. However, a problem might arise when using the LMT [9, 10]. As noted by Dotan [6], the range between the first rise in blood lactate concentration above baseline levels (also called the lactate threshold (LT) or aerobic lactate thresholds [1, 6]) and the MLSS predicts a steady-state work intensity and is not necessarily the highest one attainable.

To avoid the problem that the lactate minimum is not necessarily the highest equilibrium attainable, Dotan [6] suggested that no such ambiguity exists when the load is decreased from intensities higher than the MLSS. Therefore, after a so-called "lactate-priming segment" whose peak intensity was suggested to be ${\sim}5{-}20\%$ higher than the actual MLSS, a "reverse segment" follows where load is decreased by 3-8% of estimated MLSS every 4 min [6]. Therefore, each intensity above the MLSS (even the ones in the "reverse segment") will cause a continuing increase in blood lactate concentrations. Once the intensity declines below the MLSS, blood lactate will be eliminated. According to this concept, the highest lactate appearance-disappearance equilibrium is attained at the highest point of the reverse plot (intensity vs. blood lactate concentration), named the RLT. However, up to now, only one study has investigated the RLT approach, with a rather small number of subjects (4 athletes in different sports disciplines: 1 runner, 1 cyclist and 2 rowers) [6], and another study used a modified version of the RLT test in cycling [7]. Additionally, both studies were conducted under laboratory conditions. Furthermore, the RLT has not been validated against performance so far.

Therefore, the goal of this paper is to address these research gaps by presenting the results from two studies. The first study investigated the accuracy of the RLT test to determine the MLSS in a field test approach, whereas the second study validated the RLT against performance. In addition, this is the first study investigating the RLT approach in running in a larger cohort of subjects.



FIG. 1. Illustration of a blood lactate curve and a lactate equivalent curve (lactate $\cdot v^{-1}$) of one participant during an RLT test and the determination of the RLT and OBLA. RLT: reverse lactate threshold; OBLA: onset of blood lactate accumulation (4 mmol·L¹); eMLSS: estimated maximal lactate steady state (95% of the mean speed of an all-out 5000 m run (v5000m)).

MATERIALS AND METHODS

Study 1

In study 1, 16 healthy, nonsmoking sport students (mean \pm SD, age: 28.1 \pm 5.5 (range: 21–40) years, mass: 69.4 \pm 7.9 (range: 53–80) kg, height: 178.2 \pm 6.7 (range: 160–188) cm, RLT: 4.01 \pm 0.41 (range: 3.4–5.1) m·s⁻¹; 12 male, 4 female) participated. We purposefully recruited a heterogeneous group of participants, i.e. with varied running experience and training background, who were currently running at least 2 hours per week. This was done to investigate whether the tests accurately predict MLSS, even in differently endurance-trained subjects. However, the heterogeneity in the sample can affect the Pearson's r and ICC values [11].

In study 1, running speed at RLT was compared with running speed at MLSS. Prior to all tests, subjects were asked for their latest performance (official race time) over 5000 m on a track (not more than 2 months ago), or, if not available, participants performed a 5000 m all-out run on a track (in a group of at least 3 participants). Afterwards, all subjects performed an RLT test. Finally, participants performed at least two 30-minute constant-load tests to determine running speed at MLSS, which was then compared with running speed at RLT.

Reverse lactate threshold test

The RLT test consisted of an incremental lactate-priming segment and a subsequent reverse segment to determine RLT. The mean speed of the 5000 m run (v5000m) was used as an evaluation of performance and was set as the peak speed during the RLT test. The incremental part started 0.8 m·s⁻¹ below the estimated MLSS (95% of v5000m)

and was increased by 0.4 m·s⁻¹ every 180 s. After running 180 s at the estimated MLSS intensity, speed was further increased by ~5% (~0.2 m·s⁻¹; v5000m). In the following reverse segment, speed was decreased by 0.1 m·s⁻¹ every 180 s. 20 μ l of capillary blood for lactate analysis (Biosen C-line; EKF Diagnostic Sales, Magdeburg, Germany) was taken from the earlobe during a 30 s break after each step of the incremental part and the reverse segment. Running speed at RLT was determined as the highest lactate equivalent ([La]/running speed) during the reverse segment (Figure 1). In addition to determining the RLT, we determined the onset of blood lactate accumulation (OBLA; 4 mmol), which was set at a value of 4 mmol·L¹ during the incremental part of the RLT test (Figure 1).

Maximal lactate steady state (MLSS) tests

MLSS was determined by the means of several constant speed tests. The MLSS was reached when blood lactate was constant for the last 20 min (increase $\leq 1 \text{ mmol}\cdot\text{L}^{-1}$) of a 30-minute constant speed test and rose $> 1 \text{ mmol}\cdot\text{L}^{-1}$ at a speed 0.1 m·s⁻¹ higher. Blood samples were taken under resting conditions after warm-up, and then every 5 min during the 30 min constant load test. Prior to each MLSS test, subjects warmed up for 10 min.

Study 2

In study 2, 23 healthy, nonsmoking sport students (mean \pm SD, age: 25.1 \pm 2.1 (range: 22–30) years, mass: 72.4 \pm 10.9 (range: 55–103) kg, height: 177.7 \pm 7.2 (range: 162–189) cm, RLT: 3.69 \pm 0.43 (range: 2.9–4.3) m·s⁻¹; 19 male, 4 female) participated.

RLT in a field test approach

We purposefully recruited a heterogeneous group of participants, i.e. with varied running experience and training background, who were currently running at least 2 hours per week. This was done to investigate whether the tests accurately predict performance, even in differently endurance-trained subjects.

In study 2, v5000m was compared with running speed at RLT. All participants performed a 5000 m all-out run and afterwards an RLT test (see above).

Study 1 and 2

All tests were separated by at least 48 hours and were performed outdoor on a 400 m track. Audio signals via MP3 players and marks (cones), at intervals of 25 m each, were used to maintain the individual pace for each participant during the RLT test and the MLSS tests. The athletes had to reach the mark at the time of the audio signal. All tests were carried out at the same time of day to prevent diurnal variations. During testing sessions, ambient conditions were constant (temperature 22.4 \pm 0.8°C; humidity 33 \pm 3%; barometric pressure 744 \pm 4 mmHg; wind 4 \pm 2 km·h⁻¹). Subjects were told to refrain from caffeine intake or any other dietary supplements and intense exercise 24 h before testing days.

The study protocol was approved by the institutional review board and was performed in accordance with the Declaration of Helsinki. Subjects were informed about the benefits and risks of the investigation prior to signing the institutionally approved informed consent document to participate in the study.

Statistical analysis

Statistical analyses of the data were performed using the software IBM SPSS 22 (Chicago, IL, USA). Descriptive statistics of the data are presented as means \pm SD. Data were analysed using parametric statistics following confirmation of normality (Shapiro-Wilk test) and sphericity (Mauchly's test). The Greenhouse-Geisser procedure was used in case of sphericity violation. To assess the differences between MLSS, RLT, and OBLA a one-way repeated-measures ANOVA with Bonferroni post-hoc test was used. Statistical differences were considered to be significant for $p \le 0.05$. Effect size (d) obtained in the analysis was interpreted as proposed by Cohen [12]. Bland-Altman plots were constructed to display agreement of speed at MLSS with running speed at RLT and OBLA, respectively, and of the 5000 m run with running speed at RLT and OBLA, respectively. Mean limits of agreement (\pm 1.96 SD) and Pearson correlations are indicated and interpreted as follows: 0.0–0.3 = negligible, > 0.3–0.5 = low, > 0.5-0.7 = moderate, > 0.7-0.9 = high, > 0.9-1.0 = veryhigh [13]. As a widely used reliability index, the intra-class correlation coefficients (ICC) were calculated based on a single measure 2-way mixed-effects model [14]. For the comparison of RLT and OBLA vs. MLSS, we chose the «absolute agreement» type of analysis (ICC (2,1) according to [15]), and for the comparison of RLT and OBLA vs. v5000m we chose the «consistency» type of analysis (ICC (3,1) according to [15]) [14]. As stated by Koo and Li [14], the agreement of RLT or OBLA and MLSS was interpreted as follows: < 0.50 = poor, between 0.50 and 0.75 = moderate, between 0.75 and 0.90 = goodand > 0.90 = excellent.

RLT	[La] @ v _{peak} [mmol·L ⁻¹]	[La] @ RLT [mmol·L ⁻¹]	No. of steps till RLT after v _{peak} [n]	[La] @ MLSS [mmol·L ⁻¹]
Mean ± SD	5.1 ± 1.5	6.0 ± 1.6	2.3 ± 0.7	4.3 ± 1.1
Min-Max	2.7-7.5	2.9-8.5	1–3	2.9-6.5

TABLE 1. Descriptive values of the RLT test.

v_{peak}: maximal running speed in the incremental part; [La]: lactate concentration; RLT: reverse lactate threshold; MLSS: maximal lactate steady state.

TABLE 2. Mean running speed at MLSS, RLT, and OBLA, mean difference to MLSS, and intraclass correlation coefficient (ICC) between MLSS and the respective test (including lower and upper limits of 95% confidence interval).

	Running speed [m·s ⁻¹]	Mean difference to MLSS [m·s ⁻¹]	ICC (lower and upper limit)
MLSS	3.95 ± 0.41	-	-
RLT	4.01 ± 0.41	0.06 ± 0.05	0.982 (0.704–0.996)
OBLA	4.08 ± 0.48	0.13 ± 0.23	0.834 (0.551–0.941)

Values are shown as mean \pm SD. ICC: intraclass correlation coefficient; MLSS: maximal lactate steady state; RLT: reverse lactate threshold; OBLA: onset of blood lactate accumulation (4 mmol·L¹); OBLA could not be determined in two subjects, as they did not reach 4 mmol·L¹ during the incremental part of the RLT test. Therefore, values were approximated using a 3rd order polynomial function.

RESULTS

Study 1

The descriptive values of the RLT test and MLSS test are presented in Table 1.

Repeated measures analysis revealed no significant differences between MLSS, RLT, and OBLA (p = 0.074). Effect sizes were small for RLT (d = 0.152) and OBLA (d = 0.289) compared to MLSS. OBLA showed a good concordance with the MLSS. RLT demonstrated excellent concordance with the MLSS (Table 2).

RLT showed a very high correlation with MLSS (r = 0.99). The Bland-Altman plot for RLT showed good agreement with MLSS with a very small mean difference and small limits of agreement (Figure 2). The largest discrepancy was $+0.1 \text{ m} \cdot \text{s}^{-1}$ (Figure 2).

OBLA showed a high correlation with MLSS (r = 0.88). The Bland-Altman plot for OBLA showed a mean difference to MLSS twice as high compared to RLT. Additionally, the limits of agreement were much larger (Figure 2). The largest discrepancies were 0.37 and +0.50 m·s⁻¹ (Figure 2).



data points — mean difference …… limits of agreement (1.96 · SD) – regression line

FIG. 2. Bland-Altman plots: difference in running velocity between the maximal lactate steady state (MLSS) and the reverse lactate threshold (RLT) (A) or the onset of blood lactate accumulation (OBLA; 4 mmol·L¹) (B), respectively.



FIG. 3. Bland-Altman plots: difference in running velocity between the mean velocity of an all-out 5000 m run (v5000) and the reverse lactate threshold (RLT) (A) or the onset of blood lactate accumulation (OBLA; 4 mmol·L¹) (B), respectively.

RLT in a field test approach

Study 2

RLT showed a very high correlation with v5000m (r = 0.97). The Bland-Altman plot for RLT showed an underestimation of v5000m with a mean difference of -0.18 m·s⁻¹ but very narrow limits of agreement (-0.38 to 0.03 m·s⁻¹) (Figure 3A). Concordance between the two measures is excellent, with ICC = 0.97

OBLA showed a high correlation with v5000m (r = 0.85). The Bland-Altman plot for OBLA showed an underestimation of v5000m with a mean difference of -0.26 m·s⁻¹ and much larger limits of agreement (-0.88 to 0.36 m·s⁻¹) (Figure 3B). The intraclass correlation revealed good concordance between OBLA and v5000m (ICC = 0.80).

DISCUSSION

The main objective of this study was to evaluate the RLT in the field concerning its validity as an indicator of the MLSS and to analyse whether it is related to 5 km running performance. The RLT showed very high accuracy in determining the MLSS and very high correlations with performance, and can easily be implemented in a field test setting. The mean difference and the limits of agreement $(0.06 \pm 0.1 \text{ m}\cdot\text{s}^{-1})$ (Fig. 2 A) for the comparison of RLT and MLSS speed indicate that the RLT is an excellent indicator for aerobic endurance. For OBLA, determined in the same test during the priming segment, the mean difference, and especially the limits of agreement $(0.13 \pm 0.45 \text{ m}\cdot\text{s}^{-1})$ (Fig. 2 B), were much larger.

Our study supports the recommendation of previous research to use the physiologically founded RLT test to estimate MLSS [6, 7, 16] because of its high accuracy. In his pilot study with four subjects from different sports disciplines (rowing, cycling, running), Dotan [6] found exceptional agreement at 0.5% discrepancy or better between the RLT and the MLSS. Our study is the first to investigate this approach in a field test setting and in a larger cohort of subjects in running. Our results also showed good agreement with the MLSS $(1.6 \pm 1.3\%)$. However, in our setting the RLT seems to systematically slightly overestimate the MLSS in 10 out of 16 subjects by 0.1 m·s⁻¹ (Fig. 2 A). Dotan [6] chose 4-min stages (compared to 3-min in our study) and very similar decrements of 0.09 m·s⁻¹ (compared to 0.1 m·s⁻¹) in the reverse segment. This shorter duration might be one explanation for the slight overestimation in our study, as longer stage durations are generally considered to allow for a better equilibration between muscle and blood lactate. The main reason why we chose 3-min stages, instead of 4-min stages, is the high risk of overstraining the athlete in the reverse segment with increasing duration. Therefore, a reduction in time per step will decrease this risk. Moreover, it must be emphasized that very small decrements in the load, like in the present study, need less time to achieve a muscle to blood equilibrium compared to larger decreases [17]. A main determinant for the slight overestimation might be the necessary 30 s rest intervals for blood sampling in our field test approach. Dotan [6] showed that rest intervals (15-20 s and 60 s) during the reverse segment of the RLT test result in an earlier [La] decline,

leading in turn to a rightward shift (higher intensity) of the RLT compared to "on-the-fly" blood sampling. Therefore, he suggested that the need for "on-the-fly" blood sampling excludes the RLT test from being used in exercise conditions where this sampling method is technically impossible. However, the same problem might appear during a normal graded exercise test on a treadmill in a laboratory setting as well. Nonetheless, the overestimation of $1.6 \pm 1.3\%$ in the present study is still a very good result which justifies the use of this concept also in a field test setting compared to OBLA vs. MLSS $(3.4 \pm 5.5\%)$ and other threshold concepts (Table 2). The RLT test produced even better results in terms of the limits of agreement (mean difference and the limits of agreement: 0.06 \pm 0.1 m·s⁻¹) (Fig. 2A) than our recently published modified LMT (0.01 \pm 0.28 m·s⁻¹) [8]. This might underline the previously mentioned problem that, when using the LMT [6, 10], the lactate equilibrium can exist anywhere in the transition zone between LT1 and the MLSS, and is not necessarily the highest one attainable.

Due to the field test conditions and the problem of accurate pacing, we determined running speed at MLSS and RLT with a resolution of "only" 0.1 m·s⁻¹. Accordingly, for RLT determination, we decided to use the highest lactate equivalent ([La⁻]/running speed) stepwise, rather than a smoothed line function to trace the reverse plot and to determine the apex, or, in the case of missing data points, a best-fit nth-order polynomial trend line like in the study of Dotan [6]. The more standardized laboratory conditions, including a higher resolution of pacing, might also explain the slightly higher agreement with MLSS in the study of Dotan compared to our field test approach.

Even though the MLSS may have relevance for athletic performance, this concept has recently and justifiably been criticized. Some have argued that "the rationale for the very specific, but apparently arbitrary, definition of MLSS, including the 10-30 min timeframe and the acceptable magnitude of change in blood [lactate], is not clear" [18]. Therefore, the MLSS determination might also only be an approximation. Unlike the MLSS's multi-session testing protocol, Dotan [6] claims that the RLT provides a true "snapshot" of the subject's fitness at the time of testing, manifested by its exceptional test-retest reliability (ICC = 0.997). This test-retest reliability is very similar to one of the MLSS tests (ICC = 0.98) [19]. However, Hauser et al. [19] did not mention which ICC calculation they used. The statement that the RLT is a true "snapshot" of the subject's fitness at the time of testing is confirmed by our second study, showing very high correlations of RLT with v5000m (Fig. 3A). The relationship between running performance and the anaerobic threshold has already been proven in previous studies [1], although concordance analyses have very rarely been reported so far. Besides correlation coefficients of 0.92 and 0.88 for D_{max} and OBLA, Forsyth et al. [20] also investigated the limits of agreement in relation to v5000m. D_{max} showed the narrowest limits with \pm 0.42 m·s⁻¹ and OBLA the highest variability with $\pm 1.61 \text{ m}\cdot\text{s}^{-1}$, which we confirmed in our study (Fig. 3B). It is worth mentioning that the RLT in our study showed limits of agreement (\pm 0.21 m·s⁻¹) (Fig. 3A) that are only the half of the D_{max}

reported in the study of Forsyth et al. [20] and thus illustrates its relevance as a predictor of endurance performance. Additionally, the fact that the RLT showed a very high correlation with MLSS and v5000m respectively indirectly confirms the MLSS as an important indicator of endurance performance.

CONCLUSIONS

The RLT and its MLSS verification exhibited exceptional agreement of $1.6 \pm 1.3\%$ discrepancy. The mean deviation for OBLA, determined during the incremental part, was $3.4 \pm 5.5\%$. The present findings suggest that the estimation of the MLSS using the RLT concept in a field test setting is worthwhile. Especially with regard to intra-individual differences between the methods, it was found that the RLT

did not deviate from the MLSS in any subject by more than +0.1 m·s⁻¹. Due to these results, its physiological basis, and its very high correlation with performance, the RLT test might also be a promising approach for elite athletes, where high precision of performance evaluation and MLSS determination is desired to identify training intensity zones. This needs to be proven by future studies involving elite athletes. Additionally, the RLT test requires previous knowledge of the athletes' performance level to assess the peak speed of the protocol. Furthermore, if maximal aerobic performance is also of interest, a common graded exercise test or a modified lactate minimum test might be more appropriate, even though one has to accept a lower precision of MLSS determination.

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