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Article Title: “Reverse Lactate Threshold” – A Novel, Single-session Approach to Reliable, High Resolution Estimation of the Anaerobic Threshold

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“Reverse Lactate Threshold” – A novel, single-session approach to reliable, high resolution estimation of the anaerobic threshold

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Abstract

The multi-session Maximal Lactate Steady-State (MLSS) test is the “Gold Standard” for Anaerobic Threshold (AnT) estimation. However, it is highly impractical, requires high fitness level, and suffers additional shortcomings. Existing single-session, AnT-estimating tests are of compromised validity, reliability, and resolution.

The presented “Reverse Lactate-Threshold” test (RLT) is a single-session, AnT-estimating test, aimed at avoiding the pitfalls of existing tests. It is based on the novel concept of identifying blood lactate’s maximal appearance-disappearance equilibrium by approaching the AnT from higher, rather than from lower exercise intensities.

Rowing, cycling, and running case data (4 recreational and competitive athletes, male and female, aged 17-39 yrs) are presented. Subjects performed the RLT test and, on a separate session, a single, 30-min MLSS-type verification test at the RLT-determined intensity. RLT and its MLSS verification exhibited exceptional agreement at 0.5% discrepancy or better. The RLT’s training sensitivity was demonstrated by a case of 2.5-month training regimen following which RLT’s ~15W improvement was fully MLSS-verified. RLT’s test-retest reliability was examined in 10 trained and untrained subjects. Test 2 differed from test 1 by only 0.3% with an Intra-Class correlation of 0.997.

The data suggest RLT to accurately and reliably estimate AnT (as represented by MLSS verification) with high resolution and in distinctly different sports and to be sensitive to training adaptations. Compared with MLSS, the single-session RLT is highly practical and its lower fitness requirements make it applicable to athletes and untrained individuals alike. Further research is needed to establish RLT’s validity and accuracy in larger samples.

Keywords: Endurance, Performance Testing, MLSS, Lactate response
Introduction

The lactate (La) response to steady-rate or graded exercise has been of major significance to physiologists and sports professionals for the last several decades. The anaerobic threshold (AnT), or related estimators, is generally regarded as the foremost indicator of aerobic endurance\textsuperscript{1-6}. However, all existing single-session AnT-estimating tests are variably handicapped by compromised validity, accuracy, resolution, and reliability\textsuperscript{7-12}.

The “Gold Standard” AnT test is the multi-session Maximal Lactate Steady-State (MLSS) test, first introduced by Heck et al.\textsuperscript{13}. The MLSS test’s major shortcoming is its impractical, multi-session nature. It has, however, additional significant weaknesses: a) The MLSS is not determined directly, but rather by a bracketing protocol whose resolution is a function of the number and magnitude of load increments; b) MLSS’s determination criterion of 0.0–1.0mM [La] rise is rather coarse and allows a range of determinations; c) The test’s multi-session protocol presumes no significant physiological changes take place throughout its extended testing period. This has never been validated and is contrary to practical; d) It is only suitable to well-trained, fit individuals. These shortcomings have practically eliminated MLSS testing from general, as well as athletic and scientific use. Indeed, the introduction of alternative indices, such as “Critical power”, “Upper limit for prolonged exercise”, and “Moderate–heavy exercise boundary”\textsuperscript{14}, can be directly attributed to the difficulties associated with AnT estimation, in general, and MLSS testing, in particular.

Most existing single-session tests use either fixed-[La]\textsuperscript{3,4,13,15}, or a transition/inflection-point\textsuperscript{3,16,17,18-19,20} as their determination criteria. They employ the basic La response to incremental exercise, are founded on the unproven threshold concept\textsuperscript{7,9,12}, and their
determination criteria are arbitrary or empirically-derived\(^5,21\). On the other hand, the Lactate Threshold (LT) test is a true physiological test and the only one worthy of the term “threshold”. However, LT is clearly a different entity from and invariably lower than AnT or MLSS\(^8,22\), and is often difficult or impossible to determine\(^6\). The Ventilatory Threshold Test\(^23\), based on gas-exchange rather than La-response criteria, is also a true physiological test. However, it was devised to determine LT, not AnT, and suffers from many validity and reliability issues\(^9,24,25,25\)–\(^27,28\).

The Lactate-Minimum Test (LMT), introduced by Tegtbur et al.'s\(^29\), begins with a short, high-lactemic exercise bout followed by a typical incremental protocol. Consequently, [La] initially diminishes before rising again with the increasing exercise intensity. The exercise intensity corresponding to the La-minimum point, along the resulting ‘U’-shaped [La]-plot, is LMT’s AnT estimate. Aside from being physiologically based, the LMT is the only single-session test to abandon the unproven threshold premise in favour of the physiologically-founded La appearance-disappearance equilibrium concept that forms the basis for the MLSS test, as well. Despite its conceptual ingenuity, however, the LMT is plagued by methodological or other constraints that make it a consistent under-estimator of the AnT/MLSS\(^10\)–\(^11,28,30\).

The shear multitude of tests and variations thereof, used for estimating AnT, or related criteria, attest to the fact that no single test has proven sufficiently accurate or reliable to become the test of choice. This and MLSS’s noted drawbacks constitute an overwhelming reason for seeking an alternative.

This paper aims to present a novel testing concept and demonstrate its practical application as a single-session, high-resolution, and reliable AnT test. The concept’s validity was demonstrated
Proposed alternative: Reverse Lactate-Threshold

In incremental graded exercise, La equilibrium can exist not only anywhere below the lactate threshold (LT), but also between the LT and AnT exercise intensities. The AnT is defined as the highest sustainable exercise intensity at which aerobic metabolism accounts for the total energy requirements, as previously described\(^5\). The LT is defined as the onset of upward deviation of the [La] response to incremental exercise, as previously described\(^31\). Throughout the LT–AnT transition zone (Figure 1), La equilibrium is maintained despite rising [La] levels, but maximal La equilibrium is, by definition, attained only at the AnT intensity. The ambiguity of where the highest attainable La equilibrium actually takes place is inherent to any incremental La-response test. The LMT is the only test to directly address the La appearance-disappearance equilibrium issue and provide a clear demonstration thereof\(^29\). However, the identified equilibrium (La-minimum) is not necessarily the highest one attainable. This is clearly borne out by consistent findings of LMT’s under-estimation of MLSS\(^{10,28,30,32}\).

No such ambiguity exists when exercise loads are stepped down from intensities higher than the AnT. In this reverse direction, [La] rises as long as exercise intensity exceeds the AnT and declines only once exercise intensity has fallen below AnT levels. The highest point on the reverse [La] plot should therefore closely approximate the highest attainable equilibrium which, by definition, is the AnT or MLSS.

A schematic depiction of this concept’s application as the “Reverse Lactate Threshold” test (RLT) is presented in Figure 2. The initial, “La priming” portion of the test, is incremented to
above MLSS/AnT intensity and then followed by a decrementing, “reverse” segment. The exercise intensity corresponding to the highest attained [La] is RLT’s estimated AnT.

**Methods**

**Subjects**

Full validation testing was carried out on four athletes – two competitive male rowers (#1, age 17; #2, age 20), one male recreational road cyclist (age 39), and one female recreational runner (age 28). All participants volunteered and consented to participate in the study after its purpose, protocol, and possible risks and benefits were fully explained. All testing was approved by the Institution’s Ethics Board.

RLT’s test-retest reliability was examined, in single-blind fashion, on 10 cyclists (8 trained, 2 untrained; 8 male, 2 female; aged 26–51), who performed two cycling RLTS, 2–6 days apart, at similar times-of-day.

**Materials**

Each athlete was tested on an ergometer which best simulates his/her sport. Rowers used the Concept II rowing ergometer (Morrisville, VT, USA; 1W load resolution; 0.1W mean cumulative power resolution). Cyclist used the Lode Excalibur electro-magnetically-braked cycle-ergometer (Lode BV, Groningen, The Netherlands; 1W resolution). Running was tested on a Trackmaster treadmill (Fullvision Inc., Newton, KS, USA; mile•h⁻¹ readings at 0.1 mile•h⁻¹ resolution). All treadmill tests employed a fixed 1% incline to simulate wind resistance in outdoor running. [La]s were determined from fingertip-sampled capillary blood, using the Lactate Pro Test Meter (Arkray Inc., Kyoto, Japan; 5μL blood samples; 0.1mM resolution; 3%
coefficient of variation). Accuracy and reliability of this meter have been found on par with laboratory analyzers.\textsuperscript{33}

\textbf{Test protocol}

\textit{Test-intensity Determination.} The RLT’s peak intensity (final “priming load”; see below and Figure 2) should, ideally, be ~5–20\% (preferably, ~10–15\%) higher than the subject’s actual MLSS intensity. Higher intensities would lead to pre-mature exhaustion, while lower ones would prevent AnT determination by coinciding with the [La] peak. For athletes, final priming load can typically be determined from recent racing/training data. Whenever estimates are too broad or unreliable, real-time estimates can normally be inferred from the rate of [La] rise in the test’s Priming segment.

\textit{Blood-sampling regimen.} Due to the nature of La-kinetics during exercise breaks, a continuous protocol was found essential for RLT’s AnT estimation in both cycling and running. In rowing, due to the infeasibility of “on-the-fly” sampling, 15–20s sampling intervals were successfully employed (for further elaboration on this issue, see Discussion/Blood-sampling regimen, below).

\textit{Stage duration.} Stage durations in most existing AnT/La-response tests are 3–5 min. The shorter durations typically produce [La] values that under-represent exercise intensity, while the longer stages allow for better muscle–blood La equilibration, but lengthen the test, allow for more La accumulation, and make it increasingly more difficult to complete. Full 4-min exercise duration at the prescribed intensities was opted for as a practical resolution of the conflicting considerations. Extra 10-20s were typically taken by blood sampling and load adjustment for the subsequent stage. Thus, overall stage duration was typically ~4.25 min.
Lactate-priming segment. The RLT’s first part serves as a controlled warm-up and for gradual, homogeneous [La] elevation to above MLSS/AnT levels (see under “Gradual vs. Acute La Priming”, below). Typically, this should be done in 3–4 graded stages. For example, with 240W estimated MLSS intensity, priming loads might be: 180, 210, 240 and 270W. For a runner with 16.0 km•h\(^{-1}\) (~10.0 mile•h\(^{-1}\)) estimated MLSS intensity, appropriate choices might be: 13.0, 14.5, 16.0, and 17.5 km•h\(^{-1}\) (~8.2, 9.1, 10.0, 10.9 mile•h\(^{-1}\)). Recommended increments are ~10–15% of estimated MLSS.

Reverse segment. Following the lactate-priming segment, intensity is retrograted for AnT estimation. For good resolution, decrements should be substantially smaller than the priming segment’s increments. Ten-watt decrements (~3–8% of estimated MLSS) were successfully used throughout this study. However, since intensity can be regulated at 1W increments in most professional ergometers, different decrements (e.g., 5–20W) can be used, depending on the magnitude of the subject’s power output. Treadmill speeds are typically controlled by coarser, 0.1 km•h\(^{-1}\) or mile•h\(^{-1}\) increments, leaving a more limited adjustment latitude. Convenient treadmill decrements would normally be 0.2–0.3 km•h\(^{-1}\) (~0.2 mile•h\(^{-1}\)). Large decrements shorten and make the test easier at the expense of resolution. Small decrements, however, while improving resolution, increase the number of necessary stages and thus, the risk of premature termination.

AnT estimation. The exercise intensity corresponding to the apex of the reverse [La] plot represents maximal La appearance-disappearance equilibrium and is the RLT’s AnT estimate (Figure 2). [La]-apex determination is in essence not unlike [La]-minimum determination which has previously been shown highly reliable in LMT testing\(^{10-11,28}\).
using a “smoothed line” function. With reliable data, this provides the best apex determination. However, when this procedure was compromised by 1-2 missing, or discarded (invalid) data points, a best-fit $n^{th}$-order polynomial trend-line was substituted (see Figure 3 for examples).

**MLSS verification.** As MLSS is presently the Gold Standard of AnT estimation; it served as the test-of-choice for RLT validation. A 30-min MLSS-type testing session was conducted two, but no more than five days, following the RLT test being verified. The RLT’s AnT estimate was considered validated if [La] rose by $\leq 1.0$ mM between the 10$^{th}$ and 30$^{th}$ min$^{13,28,34-35}$. The high resolution of rowing and cycling ergometry ($\leq 1$ W) facilitated MLSS testing at the precise RLT-determined value. The treadmill’s resolution of 0.1 mile$\cdot$h$^{-1}$ dictated the MLSS verification increments (e.g., if the RLT estimate was 7.84 mile$\cdot$h$^{-1}$, it could only be tested at 7.8 and/or 7.9 mile$\cdot$h$^{-1}$).

Note: Although the RLT is a demanding test, it is easier than a 30-min MLSS-testing session at MLSS intensity, which can be properly performed only by trained individuals. Therefore, only trained athletes were recruited for the RLT’s MLSS verification.

**Results**

**RLT and its verification.** Figures 3 & 4 show the RLT results of the four athletes, along with their corresponding MLSS verifications. In three athletes (cyclist, and rowers 1 & 2; Figure 3), MLSS verification precisely matched the RLT-estimated MLSS. In the runner (Figure 4), the RLT-MLSS discrepancy was $\leq 0.5\%$. It is worth noting that the 1.0mM-wide MLSS determination “window” did not add uncertainty to RLT determination. In all 5 verifications reported in this study, $\Delta$[La] (last 20 min) was also very consistent, ranging in only a 0.25mM span (0.75 to 1.0mM).
Test-retest reliability. The range of RLT-determined AnT values of the 10 reliability-group subjects was 117.5–343.5W. The mean test-1-vs.-2 values were 238.5±69.4 and 237.7±70.0W, respectively (Δ=-0.3%; p>0.05). The Pearson test-retest correlation coefficient was 0.998, and the intra-class correlation: 0.997 (Figure 5). Repeat, randomised and blinded test 1 and 2 determinations in 9 subjects (18 data pairs) indicated intra-reviewer reliability of 0.996.

Discussion

Technical considerations

Blood-sampling regimen. During incremental exercise, La concentrations are higher in La-producing, active muscles than in non-active, or lightly-active muscles and other organs. Consequently, as the intermediary between La-producing and La-absorbing compartments, the blood does not display stable [La]s, unless exercise is sufficiently long and of sub-AnT intensity. Further instability ensues upon cessation of exercise, as La flux from the previously-active muscles falls precipitously, but La continues being drawn out by the La-absorbing organs. Thus, exercise interruptions provide additional time for equilibration and result in [La] values that are inversely related to interval durations. Thus, rest (sampling) intervals during RLT’s Reverse segment result in an earlier [La] decline, leading in turn to a right-shift (higher intensity) of the observed [La] peak and to overestimation of AnT. This is clearly shown in Figure 6. Thus, the need for “on-the-fly” blood sampling excludes RLT from being used in exercise modes where this sampling mode is technically impossible (e.g., swimming).

Rowing does not readily lend itself to the uninterrupted regimen. Although rowers’ toes remain stationary during exercise, pilot toe-tip sampling produced inconsistent results while a
previous report\textsuperscript{37} showed 3–9\% lower toe-vs.-finger [La]s. Nevertheless, unlike running (Figure 6\textsubscript{B}) and presumably cycling (not tested), 15–20-s sampling intervals in rowing (Figure 6\textsubscript{A}) still provided complete MLSS agreement (Figure 3). A likely explanation is the rowers’ larger active-to-non-active compartment-size ratio compared with runners or cyclists. That is, while runners and cyclists heavily engage only their lower-body muscles, rowers rely on both their upper- and lower-body musculature. The larger active-to-non-active muscle ratio implies that a larger muscle mass is responsible for a given level of La production and [La]. This, in turn, means that the active muscles’ mean La concentration is smaller and, consequently, so is the muscle-blood La gradient. It thus appears that rowing’s smaller gradients sufficiently slow down [La] changes to allow brief sampling intervals.

\textit{Gradual vs. acute La priming.} Possible differential effects on AnT estimation of gradual \textit{vs.} abrupt [La] increases were not investigated. Nevertheless, two arguments support incremental, stage-wise approach to the RLT’s priming phase: \textbf{a)} Incremental priming attenuates La-concentration gradients between active and non-active body compartments. This presumably stabilizes [La]s and makes them more reliable in reflecting given exercise loads, particularly at the critical transition from the Priming to Reverse segment; \textbf{b)} The RLT’s Priming segment can serve as a graded La-response test and provide useful information in addition to AnT estimation (see “Lactate profile”, below).

\textit{Lactate profile.} While gradual La priming for the RLT can be accomplished in various ways, the stage-by-stage Priming-segment protocol was chosen to be similar to (albeit shorter than) typical La-response tests. When a repeatable, consistent protocol is used, the resulting La plot
can characterize the subject’s submaximal La-response, or “lactate profile”. This can be used to follow-up and compare training effects at sub- and circum-AnT intensities (see\textsuperscript{21} for examples).

**Findings and conclusions**

This pilot study presents a novel concept in aerobic-endurance testing. The RLT test demonstrated a high level of reliability in both athletes and non-athletes. Although limited in scope, the presented data suggest the RLT to be a highly valid AnT test among trained athletes. This was similarly shown in three distinct sports that widely differ in the involved muscle-mass as well as the nature of its activity. It should be noted that since MLSS testing can only be administered to trained individuals, untrained subjects could not be used for RLT’s validation (MLSS verification).

Unlike the MLSS’s multi-session testing protocol, the RLT provides a true “snapshot” of the subject’s fitness at the time of testing. RLT’s robustness is manifested by its exceptional reliability (ICC=0.997; Figure 5), not approached by any other lactic or ventilatory tests. This is particularly significant in light of the fact that the reliability of the “Gold Standard” MLSS test has never been established.

Using high-resolution cycling and rowing ergometers, full RLT–MLSS agreement could be shown in the rowers and cyclist (Figure 3). Hampered by the treadmill’s limited velocity resolution, running’s RLT–MLSS agreement was still excellent at ~0.5% (Figure 4). An accuracy level of better than 1% is unprecedented in any existing test and should be of major consequence to sports physiologists, coaches, and athletes, who require sensitive, high-resolution monitoring of endurance capacity.
Illustrating RLT’s sensitivity and the validity of its verification process is the testing of rower #1. His RLT was determined at 222W (Figure 3A) and MLSS-verified (Δ[La]=0.75mM; Figure 3B). A day later, in a repeat MLSS test at 227W, just 5W/2.3% above the established MLSS intensity, Δ[La] jumped nearly 1mM, to 1.7mM (data not presented), reaffirming the validity of the earlier determination.

Various investigators have argued against AnT being a threshold phenomenon⁷-⁹,¹² (i.e., an acute physiologic/metabolic change or transition). Indeed, as an unequivocal threshold has never been demonstrated, it may be argued that the development of a valid, single-session AnT test has been hampered by preoccupation with the unfounded threshold concept. By relying on the physiologically-sound criterion of highest attainable La equilibrium, the RLT does away with the threshold controversy. The RLT’s “threshold” only refers to the intensity above which aerobic sufficiency and [La] equilibrium are no longer maintained. By approaching the “threshold” from higher rather than from lower exercise intensities, the RLT avoids the ambiguity of the LT–AnT transition zone that has apparently confounded other La-based tests and particularly, the LMT. Like the LMT, the RLT provides clear visual representation of the threshold intensity.

As has been previously demonstrated by Beneke & Duvillard⁴, the results of the present study expose the fallacy of fixed-[La] criteria (e.g., 4.0mM) used in various existing tests⁴,¹³,¹⁵. Amongst the four subjects, only two had mean MLSS [La] (last 20min) near 4mM (Figure 3B), while one had MLSS [La] clearly below 4mM (Figure 4B), and another was much higher (~7.4mM; Figure 3B).
To examine RLT’s sensitivity to training and fitness changes, rower #1 was first tested (Figure 3) prior to his season’s preparatory phase (mainly aerobic power and endurance training) and at its conclusion, 2.5 months later. The RLT-estimated AnT improved by 15W (222 to 237W; Figure 7A), with corresponding changes in heart-rate responses (Figure 7B). The post-training MLSS verification exhibited full agreement with the new RLT determination (Δ[La]=1.0mM; Figure 7C). Thus, contrary to the training insensitivity reported for LMT by Carter et al., the RLT appears quite sensitive. Confirmation on a larger athlete sample is required.

Fixed-[La] tests have been shown to be affected by diet and other factors. Both LMT and RLT determinations, on the other hand, rely on La-response features (La-minimum and La-apex, respectively) rather than on absolute or fixed [La] values. Since LMT has been shown to be independent of preceding dietary variations, RLT can likewise be expected to be fully or partially immune to dietary variations and possibly other [La]-affecting factors. However, research to directly address these issues is clearly warranted.

Optimal stage duration was not investigated in the present study. However, the level of accuracy attained with the employment of ~4.25-min stage-durations, likely attests to its suitability in most cases. Nevertheless, further investigation of this issue is clearly warranted, particularly in relation to different exercise modes (e.g., kayaking vs. rowing) and subject populations (e.g., trained vs. untrained, children vs. adults).

As in all other tests, ultimate accuracy can be affected by many factors, such as timing, pacing, and sampling errors; spurious values, etc. Thus, RLT’s actual accuracy in routine testing
cannot be expected to always fall within 0.5%. RLT’s inherent robustness however is strongly suggested by the consistency of results and the test’s high degree of reliability.

As noted, RLT testing offers several distinct advantages over MLSS. Foremost is its practicality as a single-session test. Nearly as important is RLT’s applicability to both untrained individuals and endurance-trained athletes, making the RLT applicable to considerably larger populations.

Compared with other single-session tests, the RLT’s main disadvantage is its inapplicability to sports in which sampling intervals are unavoidable and expected to affect results. Future research could investigate the effects of interval-duration and other factors in an attempt to devise a correction method that would facilitate valid testing in sports where a continuous protocol cannot be implemented. However, the primary objectives of future research should be confirmation of the present study’s finding in larger and more diverse subject populations and in diverse sports.
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Conflict of Interest

No conflicting interests were involved in this study. The funding body (Brock University) had no involvement whatsoever in any aspect or decision concerning this study.
References


Figure 1 – Schematic representation of the relationship between LMT (dashed line) and a conventional La-response curve (solid line). Typically, LMT’s La-minimum point is considerably lower than actual MLSS/AnT, sometimes as low as LT. Data based on actual testing.
Figure 2 – Schematics of RLT testing. The first, Priming segment (lower portion), is a graded La-response protocol, used to elevate [La] in all body compartments, as evenly as possible. It is carried out to a workload and [La] clearly above AnT levels. The second part, the Reverse segment (upper portion), is a retro-graded protocol, carried out in small decrements, to workloads well below AnT intensity (see text for more details).
Figure 3  – A. RLT [La] plots of three athletes whose performance was measured by power output (watt). Note polynomial trend-line use, in the Reverse segments of the cyclist and rower #1, due to missing [La] values. B. MLSS-verification plots for the same athletes. In all three, [La] rose by <1.0mM (0.75–0.9mM) between the test’s 10th minute and its completion (≥30min), verifying the RLT-determined AnT estimates. Note the large MLSS [La] variance, ranging from ~4.0 to ~7.4mM.
Figure 4 –  A. RLT plot of the runner (treadmill; mile•h⁻¹). B. Two MLSS verifications of the RLT. Note that the 7.2 mile•h⁻¹ RLT is a slight over-estimate (Δ=1.3mM) while the 7.1 mile•h⁻¹ value is likely an under-estimate (only 0.2mM rise). Since the treadmill’s speed resolution could be set no lower than 0.1 mile•hr⁻¹, the true MLSS (at Δ=0.9-1.0 mM) was estimated to lie at ~7.17 mile•h⁻¹. Notably, the ~0.03 mile•h⁻¹ difference from the 7.2 mile•h⁻¹ RLT determination, constitutes <0.5% error – considerably smaller than that of existing tests.
Figure 5 – RLT test-retest plot of 10 trained and untrained subjects (cycling).
Figure 6 – Effect of sampling-interval duration on the RLT. **A.** In rowing: 60- vs. 15–20-s intervals (rower #2). **B.** In running: 15–20 vs. 0-s (“on-the-fly” sampling) intervals. Note the shift to higher intensities with longer interval durations. While intervals of 15–20s were still appropriate for correct RLT determinations in both rowers (Figure 2), 60-s intervals produced ~9% RLT over-estimate in the rower (#2). In running, on the other hand, 15–20-s intervals resulted in a ~1.8% RLT over-estimate.
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Figure 7 – RLT response to a 2.5-month aerobic training regimen (rower #1). A. La response; B. Heart-rate response. C. MLSS validation of the post-training RLT determination.