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Original Research

A Two-test Protocol for the Precise Determination of the Maximal Lactate Steady State

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ABSTRACT

International Journal of Exercise Science 11(4): 681-695, 2018. The purpose of this study was to determine the efficacy of a two-test method for precisely identifying the Maximal Lactate Steady State (MLSS). Eight male competitive cyclists performed two bouts on a cycle ergometer. Following a maximal oxygen consumption ($\dot{V}O_{2max}$) test (66.91 ± 5.29 mL·kg^{-1}·min^{-1}) we identified the lactate deflection point using the visual deflection (T_{Vis}), Log-Log (T_{Log}), D_{max} (T_{Dmax}), RER = 1.00 (T_{RER}), ventilatory threshold (T_{Vent}), and the 1.0 mmol·L^{-1} increase above baseline (T_{+1}) methods. The second incremental test (SIT) consisted of 6-7 stages (5 min each) starting 20-30 W below to 20-30 W above the predetermined deflection point, in 10 W increments. Comparison of the two tests yielded different threshold estimates (range 11-46W) for all methods (P = 0.001-0.019) except the T_{Log} (P = 0.194) and T_{RER} (P = 0.100). The SIT resulted in significantly (P = 0.007) more narrow range of thresholds (27.5 ± 11.01W) compared to the $\dot{V}O_{2max}$ test (70 ± 42.51W). The T_{Vis} from the SIT was identified as the MLSS and was verified using three 45-minute steady-state exercise bouts at 95%, 100%, and 105% of MLSS intensity (average increment 12.8 W). Blood lactate and $\dot{V}O_2$ were recorded every 5 minutes and differed between the three intensities at every time point (P < 0.001). $\dot{V}O_2$ increased from the 5th to the 45th minute by 7.02 mL·kg^{-1}·min^{-1} (100% MLSS), 3.63 mL·kg^{-1}·min^{-1} (95% MLSS) and 7.5 mL·kg^{-1}·min^{-1} (105% MLSS, to the 30th minute). These results indicate that the MLSS was identified correctly by the SIT, the single incremental test overestimated the MLSS intensity, and the T_{Vis} provides a very accurate determination of the lactate breakpoint. The use of a second submaximal test is required for a precise identification of MLSS.

KEY WORDS: Maximal lactate steady state, endurance, exercise, lactate threshold

INTRODUCTION

Evidence accumulated over the last thirty years indicates that an individual's aptitude for success in endurance events is better estimated by the maximal lactate steady state (MLSS) than the $\dot{V}O_{2max}$ (9,26). The workload at MLSS (W_{MLSS}) is defined as the highest workload at which lactate does not progressively accumulate in the blood (5). While the importance of the W_{MLSS} is clear, its determination has proven rather problematic, a fact identified several years ago in the debate between two pioneers in the field (12,17). The abundance of terms and definitions adds to the difficulty of specifically identifying MLSS, as this physiological transition point

sometimes also used synonymously with the lactate threshold (LT) and lactate breakpoint. Furthermore, the experimental protocols used to identify MLSS are often difficult to incorporate into an athlete's training program. Accordingly, the need for an accurate, reliable, and easy to administer test is still evident. These issues have been summarized by Faude et al. (18), who also highlighted the significance of MLSS determination.

Definitions of the LT include, among others, the threshold power identified by visual inspection of the blood lactate curve (T_{Vis})(39); the work rate prior to the stage at which blood lactate increases by 1 mmol·L^{-1} or more (T_{+1})(35); the power output that corresponds to the onset of blood lactate accumulation (OBLA), also known as the point at which blood lactate reaches a concentration of 4 mmol·L^{-1} (T_4)(21); the workload estimated via the log-log method (T_{Log}; 4); the workload at RER = 1.00 (T_{RER})(27); and the workload calculated via the D_{max} method (T_{Dmax})(15). The problems associated with these multiple definitions and the practical applications to exercise training have been elegantly discussed elsewhere (32,40).

In addition to the issues related to terminology, several experimental approaches to the determination of MLSS have been put forth, with varying degrees of accuracy (5,9,14,15,16,20,34). However, subsequent studies have often challenged aspects of these methods or refuted them altogether (1,25,26). The W_{MLSS} is commonly estimated using a hybrid $\dot{V}O_{2max}$ and LT test (1,6,11,15,29,37), despite the fact that this practice negatively impacts the quality of the measurement of either the LT measurement or the VO_{2max} value. Specifically, the W_{MLSS} cannot be estimated using large increments in intensity coupled with short stage durations as in $\dot{V}O_{2max}$ testing because lactate takes longer to stabilize after increases in workload than do $\dot{V}O_2$ and heart rate (30). Therefore, the use of a single test to simultaneously determine $\dot{V}O_{2max}$ and LT is problematic, as test protocol will invariably compromise the accuracy of one variable or the other. Further, the intensity at LT or $MLSS_w$ is commonly reported as percentage of $\dot{V}O_{2max}$ (6), making the accurate determination of $\dot{V}O_{2max}$ essential if valid comparisons for tracking adaptations to training are to be made between different methods. In response, many investigators rely on repeated follow-up exercise bouts lasting at least 30 min to verify the W_{MLSS} (see discussion in 18).

The wide variety of criteria used in estimating the W_{MLSS} has led some authors to conclude that there are multiple thresholds that are all equally valid and useful in predicting endurance performance (36). The use of numerous methods to estimate LT and MLSS leads to widely divergent estimates of W_{MLSS} for the same participant (2). However, for any given set of experimental conditions, there can only be one true physiological W_{MLSS} corresponding to a very narrow range of work rates (10); the LT parameters used to estimate W_{MLSS} from a single incremental exercise test are theoretical constructs or byproducts of testing protocol that may or may not identify the true W_{MLSS}. To complicate matters more, the literature includes single test protocols to estimate W_{MLSS} (27), two-test protocols (9), or more elaborate designs using repeated runs at various intensities above and below MLSS (7,23), a fact previously highlighted (5). For example, the two-test protocol described by Billat et al. (9) required exercise at fixed intensities (67 and 82% of $\dot{V}O_{2max}$) as opposed to a more flexible protocol that gradually increasing intensity that allows for specific determination of MLSS.

These differences in experimental protocols and working definitions present obvious pr oblems for all who rely on a precise determination of the W_{MLSS} for testing or training purposes as do the competitive cyclists in the present study. Thus, the need for an MLSS test that is ac urate, reliable, inexpensive, and practical is still present. The protocol must also allow for flexib ity in testing intensity, in contrast with the rigidity of previous two-stage protocols. The purp ose of this study was to determine whether a two-test protocol determines the W_{MLSS} better th n the traditional $\dot{V}O_{2max}$/LT test procedure and without the need for multiple repeat tests. t was hypothesized that the second, submaximal incremental test (SIT) would yield a narrow r and more accurate range of intensities corresponding to W_{MLSS} than the initial $\dot{V}O_{2max}$ test.

METHODS

Participants

A convenience sample of eight (n = 8) male participants volunteered for this study and pr vided informed consent. Their physical characteristics are provided in Table 1.

Table 1. Physical characteristics of the n = 8 male competitive cyclists who volunteered for this study. D ata are reported as mean ± standard deviations.

Age (Years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	$\dot{V}O_{2max}$ (mL·kg^{-1}·m in^{-1})
22.66 ± 4.82	72.45 ± 8.29	182.22 ± 7.03	21.87 ± 1.60	65.07 ± 2.97

All participants were aerobically trained and competed in regional and national road c cling events. All aspects of this study were approved by the Willamette University Institu tional Review Board. The a-priori power analysis using T_{Vis} as our criterion (comparing the $\dot{V}O_{2max}$ with the SIT using pilot data) indicated a sample of n = 15 participants would be required for this study; however the geographic location of our institution precludes access to a larger population of highly-trained cyclists. Post-facto analysis using the T_{Vis} as criterion yielded effect size d = 0.92 and statistical power P = 0.756.

Protocol

For the main part of the experiment (determination of the MLSS) the participants performed two exercise bouts 2-4 days apart. The first bout was a $\dot{V}O_{2max}$ test, and the second was a submaximal incremental test (SIT) designed to more precisely identify the W_{MLSS}. In the second phase of this project the MLSS and W_{MLSS} estimates from the SIT were subsequently verified by conducting three additional constant-load 45-minute bouts at 95%, 100% and 105% of the W_{MLSS} in counterbalanced order (12.8 W increments on average). Each participant completed the five tests within a 2-week period and always at the same time of the day. Participants were instructed to maintain similar diet and sleep habits for the duration of the study; a brief recall survey administered prior to each test revealed no differences in their daily routine that would influence the results. Participants were asked to maintain their regular training schedule but not engage in strenuous exercise for 24 hours prior to each test to avoid fluctuations in fuel availability or musculoskeletal injury. Finally, they were asked to refrain from eating three hours prior to the tests to avoid gastrointestinal distress during the test, and to drink fluids regularly before and during all tests. Prior to all exercise bouts, participants completed an individualized

warm-up (pace and duration of warm-up were self-selected and varied from person to person) that allowed each to feel comfortable and ready for the testing. The average room temperature during the tests was 23.9 ± 1.1 ^{o}C and the average barometric pressure was 757.1 ± 4.4 mm Hg. All exercise was performed on a calibrated Monark 839E cycle ergometer (Monark Exercise AB, Vansbro, Sweden), which provides a constant given workload independent of the participant's cycling cadence.

Maximal Oxygen Consumption Test ($\dot{V}O_{2max}$): All participants performed a maximal test to volitional exhaustion (initial workload was 160 W, with 40 W increments every 2 min). At the end of every 2-min stage, blood was sampled and analyzed for lactate concentration to determine the threshold. The blood lactate concentrations from the $\dot{V}O_{2max}$ test were plotted against the corresponding workloads, and the deflection point of BL from baseline as determined by visual inspection (T_{Vis}) was independently identified and agreed upon by the investigators.

Second Incremental Test (SIT): For this submaximal test, a new testing protocol was established, with the initial workload set at 20-30 W below each participant's T_{Vis} as determined from the $\dot{V}O_{2max}$ test and progressively increased in 10 W increments every 5 min to 20-30 W above the previously-determined T_{Vis}. The initial intensity for the SIT was below the lowest T value determined by the $\dot{V}O_{2max}$ test, and the final intensity was above the highest T value as determined by the $\dot{V}O_{2max}$ test. The lactate concentrations from the SIT were again plotted against the corresponding power outputs. The new T_{Vis} was again independently identified and agreed upon by the investigators and we hypothesized that the W_{MLSS} would correspond to the workload at this second T_{vis} (see Fig. 1).

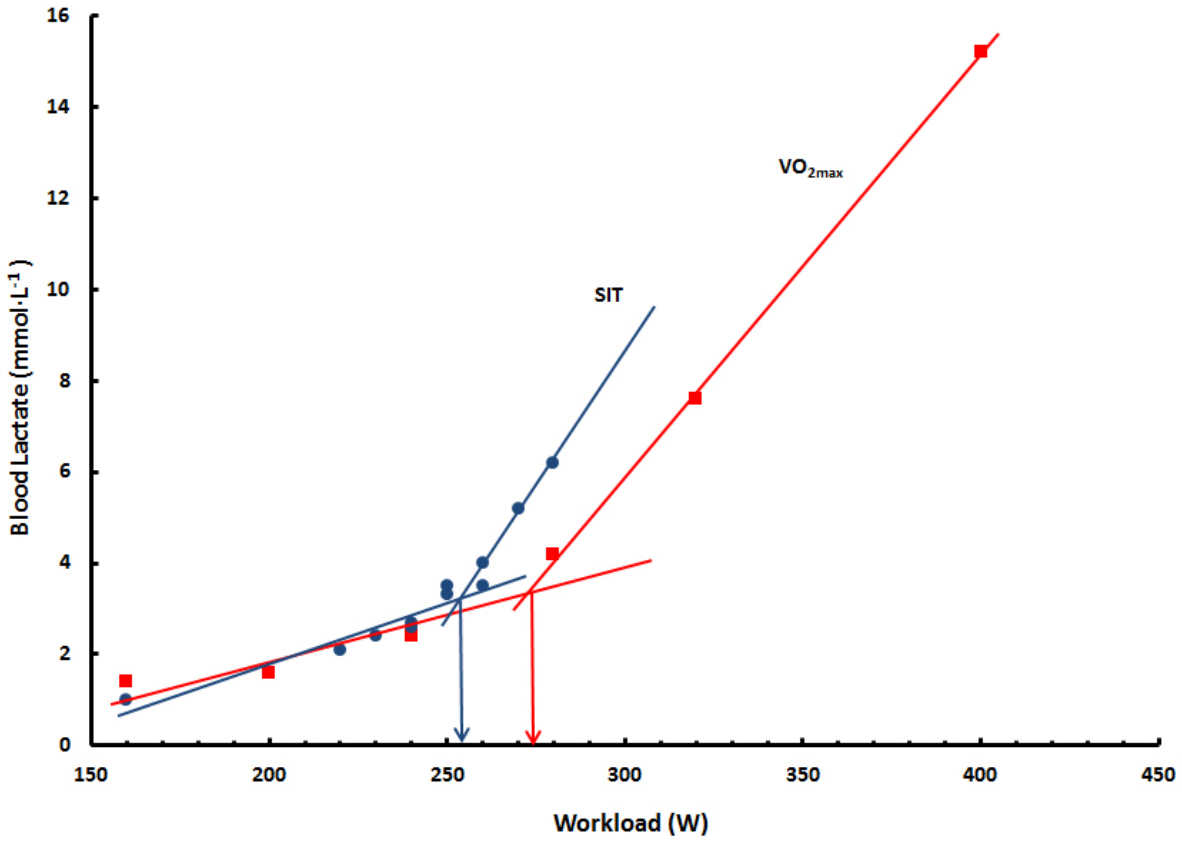

Figure 1. A graphical representation of the blood lactate curves obtained from the two tests for one participant. The first test represents lactate values obtained from the $\dot{V}O_{2max}$ test (red squares) and the second test represents lactate values obtained from the SIT (blue circles). The W_{MLSS} was determined using the T_{Vis} method for both curves. The two tests yielded thresholds that correspond to two different intensities (275 W vs. 255 W respectively).

Constant-Load Bouts: Participants completed three constant-load 45-minute bouts at intensities that corresponded to 95%, 100%, and 105% of the W_{MLSS} in counterbalanced order. Blood lactate, $\dot{V}O_2$, and HR data were recorded every 5 minutes for the duration of the activity or to exhaustion, whichever came first.

Blood Collection: Arterialized blood samples (5-10 µl) were collected from the earlobe at the completion of every intermediate stage during the first two exercise tests and every five minutes during the prolonged bouts. The earlobe was selected because this blood collection site causes minimal discomfort to the participant, it offers easy access during testing, and does not affect the participant's grip on the cycle handlebars. The blood was immediately analyzed using a Lactate Plus analyzer (Nova Biomedical, Waltham, MA) validated daily using the manufacturer's recommendations.

Oxygen Consumption ($\dot{V}O_2$) and Heart Rate (HR): Participants were fitted with a breathing apparatus equipped with two-way breathing valves during all exercise bouts. $\dot{V}O_2$ was measured continuously during exercise bouts using a calibrated ParvoMedics TrueOne Metabolic Measurement System (ParvoMedics, Sandy, Utah), and were averaged every 30 sec.

Participants were also fitted with a Polar HR belt (Polar Electro, Kempele, Finland) during all exercise bouts and the HR was recorded with a Polar HR receiver and recorded in 30-sec intervals.

Threshold Determination: T_{Vis}, T_{+1}, T_4, T_{Log}, T_{Vent}, and $T_{RER=1.00}$ were determined as following established protocols for the both the $\dot{V}O_{2max}$ test and the SIT. T_{Dmax} was calculated using a customized Excel spreadsheet for the two incremental tests as we described previously (33).

Statistical Analysis

The workloads for each of the thresholds were compared for $\dot{V}O_{2max}$ vs. SIT using repeated measures T-test ($\alpha = 0.05$). All the threshold values for the $\dot{V}O_{2max}$ test were compared for differences using repeated measures ANOVA ($\alpha = 0.05$), as were the threshold values for the SIT. The $\dot{V}O_2$, HR, and blood lactate values obtained during the three 45-min constant load bouts were compared at each time point using one-way ANOVA ($\alpha = 0.05$).

RESULTS

The first aim of this study was to identify the different threshold intensities for both the $\dot{V}O_{2max}$ and SIT. The intensities that correspond to the different threshold definitions are summarized in Table 2.

Table 2. The power (W) at each threshold for the $\dot{V}O_{2max}$ and the SIT expressed as mean and standard deviation. The ANOVA revealed significant differences (indicated with the * symbol) between the two tests for all thresholds except the T_{Log} and the T_{RER}.

Threshold	$\dot{V}O_{2max}$ test	SIT	*P* value
T_{vis}	276.2 ± 36.6	248.7 ± 30.32	0.001*
T_{Dmax}	281.5 ± 35.1	250 ± 28.8	0.001*
T_{+1}	276.8 ± 44.6	252.2 ± 29.1	0.018*
T_{Log}	264.3 ± 44.8	253.1 ± 35.3	0.194
T_4	290.3 ± 41.1	262.3 ± 34.6	0.001*
T_{RER}	293.7 ± 65.2	260 ± 42.4	0.100
T_{Vent}	313.7 ± 61.1	267.5 ± 34.1	0.019*

The average relative $\dot{V}O_2$ at the T_{vis} during the $\dot{V}O_{2max}$ test was 61.29 ± 9.91 mL·kg^{-1}·min^{-1} (91.59% of $\dot{V}O_{2max}$) and 59.43 ± 7.82 mL·kg^{-1}·min^{-1} for the SIT (88.81% of $\dot{V}O_{2max}$, $P = 0.062$). In contrast, the blood lactate concentration at Tvis was 3.18 ± 1.00 mmol · L^{-1} for the $\dot{V}O_{2max}$ test and 3.78 ± 0.45 mmol · L^{-1} for the SIT ($P = 0.008$).

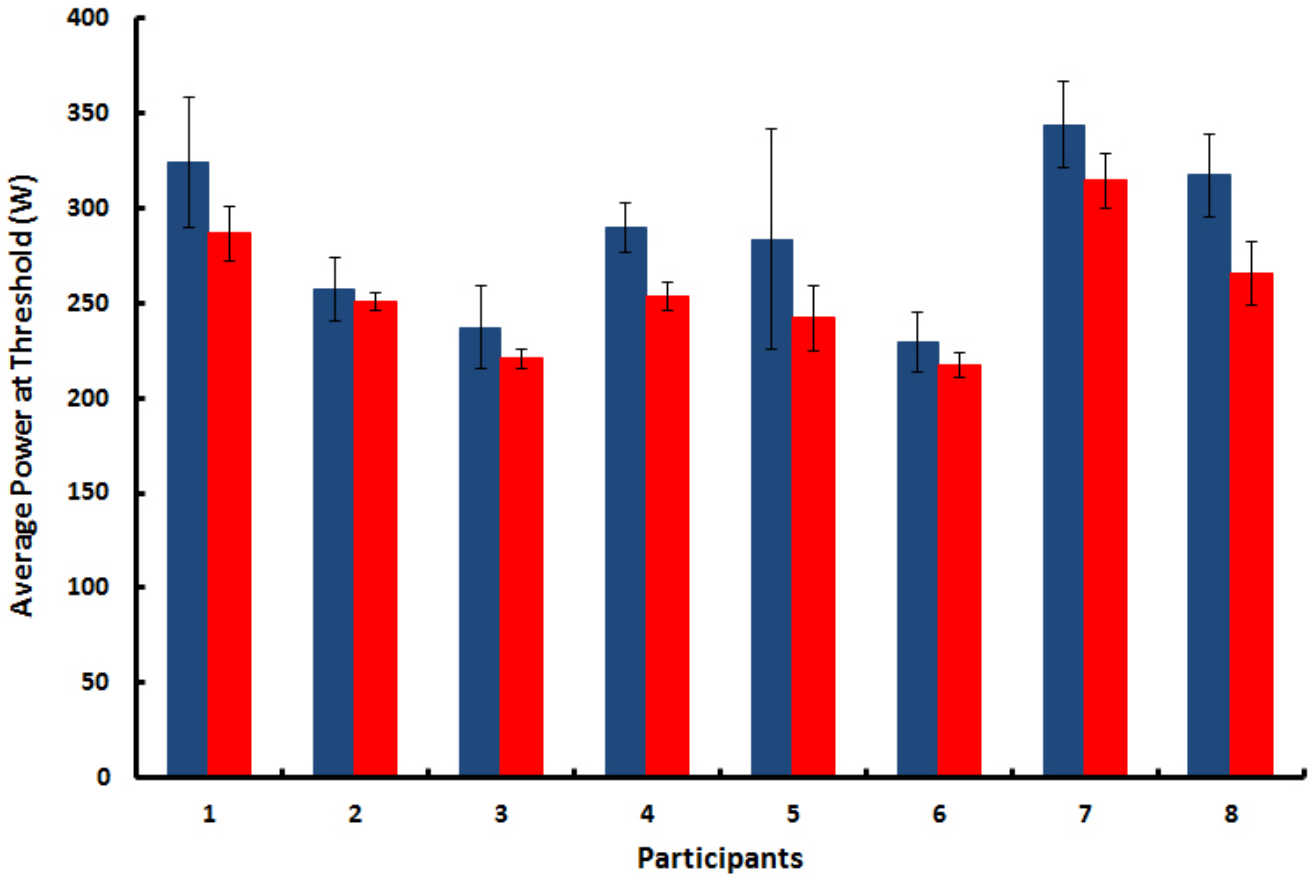

Figure 2. The average power across all threshold methods for each participant for the $\dot{V}O_{2max}$ test (blue bar) and the SIT (red bar). The SIT consistently yielded lower threshold values ($P < 0.001$) and smaller standard deviations than the $\dot{V}O_{2max}$ test.

For each of the participants the $\dot{V}O_{2max}$ tests yielded a greater range of threshold intensities (as calculated by the various LT determination methods) than the SIT (70 ± 42.51 W vs. 27.5 ± 11.01 W respectively, $P = 0.007$). Given the disparity of definitions, the determination of the MLSS can prove very difficult. Overall, intensities identified by the different threshold methods ranged from 30-160 W for the $\dot{V}O_{2max}$ test, and from 15-40 W for the SIT (an example is provided in Figure 3).

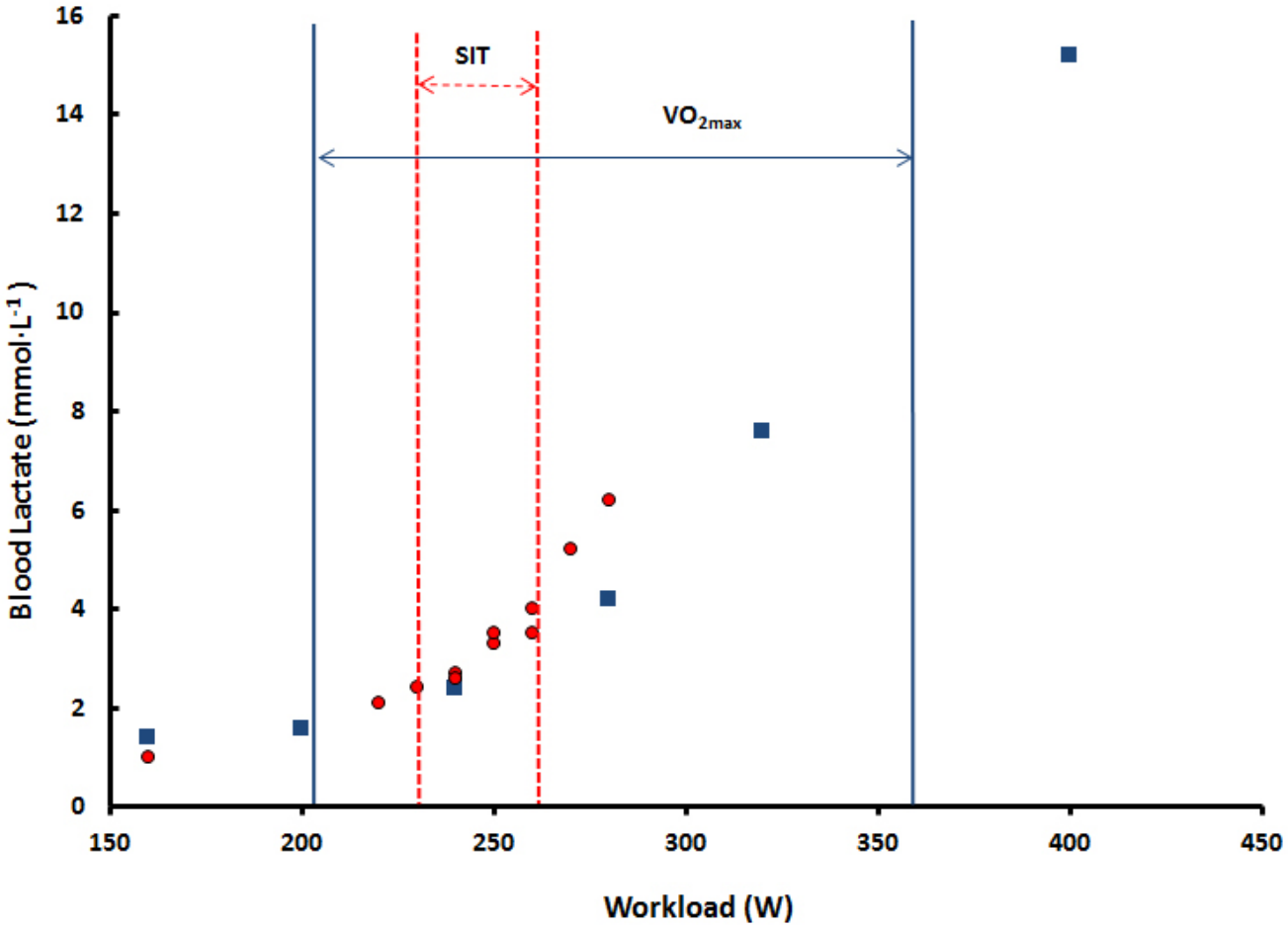

Figure 3. Threshold values for the $\dot{V}O_{2max}$ test (blue squares) and the SIT (red circles) for the same participant as in Fig.1. The perforated lines indicate the range of threshold values for the SIT (35 W) and the solid lines indicate the range of threshold values for the $\dot{V}O_{2max}$ test (160 W). In this participant the wide range for the $\dot{V}O_{2max}$ test was due to the T_{RER} and T_{Vent} occurring at a considerably higher intensity (T_4 occurred at 280 W).

Following the determination of the W_{MLSS} from the SIT the participants conducted three 45-minute bouts at 95%, 100% and 105% of W_{MLSS} , each average intensity differed from the next closest one by 12.8 W (average intensity of 243.4 W, 256.2 W, and 269.1 W respectively).

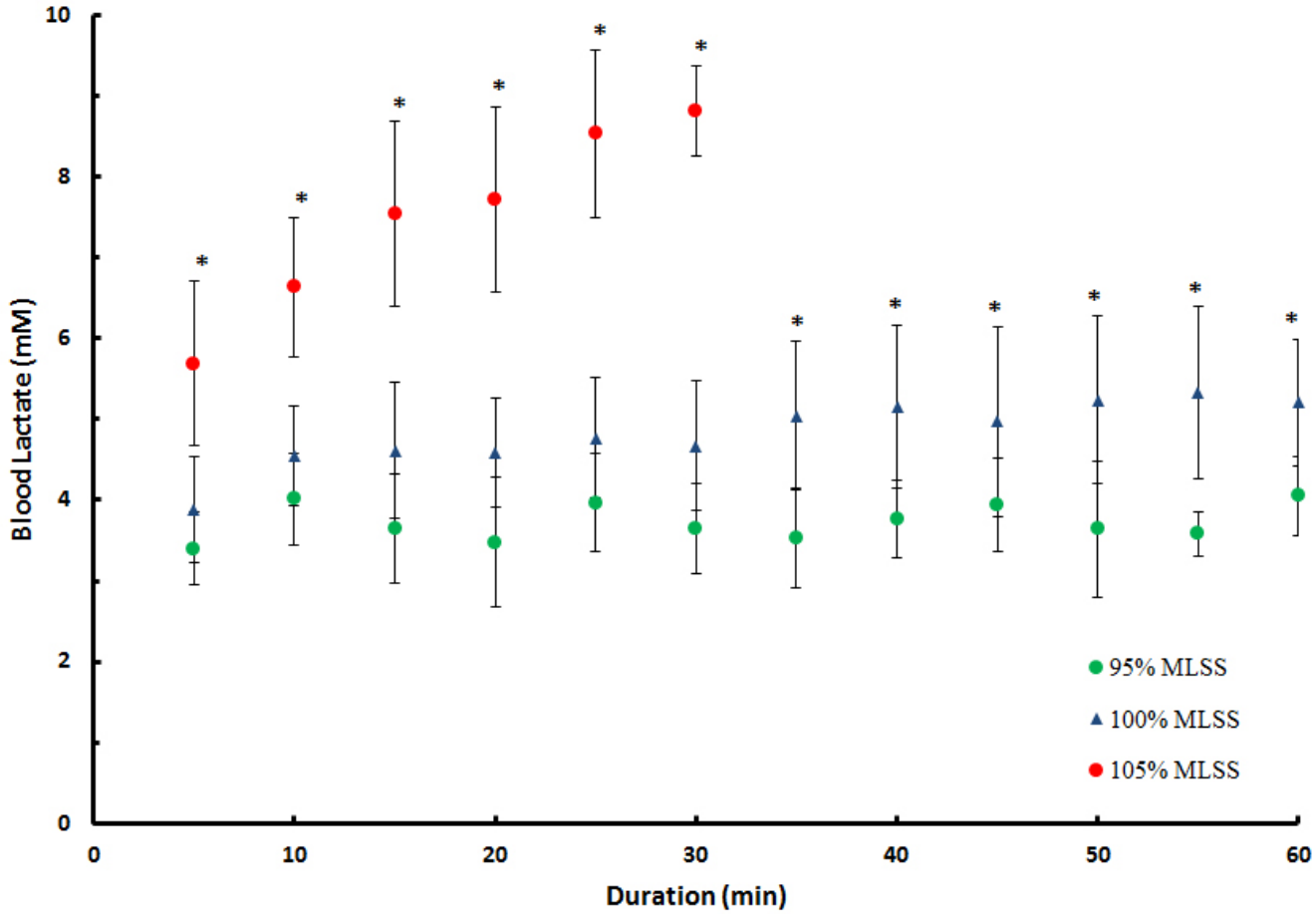

Figure 4. Blood lactate concentration was measured every 5 minutes throughout the long constant-load bouts. As indicated by the * symbol, the ANOVA yielded significant differences ($P < 0.001$) in lactate levels between the three intensities (95% MLSS open circles; 100% MLSS triangles, 105% MLSS closed circles) at every time point.

During the 105% MLSS bout only two participants were able to continue past the 30-min point. There were no differences in the lactate values within the 95% MLSS and 100% MLSS at any point during the 45-min bouts. There were significant differences in blood lactate values at every time point during the 105% MLSS bout except between the 15 min and 20 min time points. There were also significant differences between the HR, $\dot{V}O_2$, and plasma lactate when expressed as percentages of the values corresponding to MLSS ($P < 0.001$ for all variables).

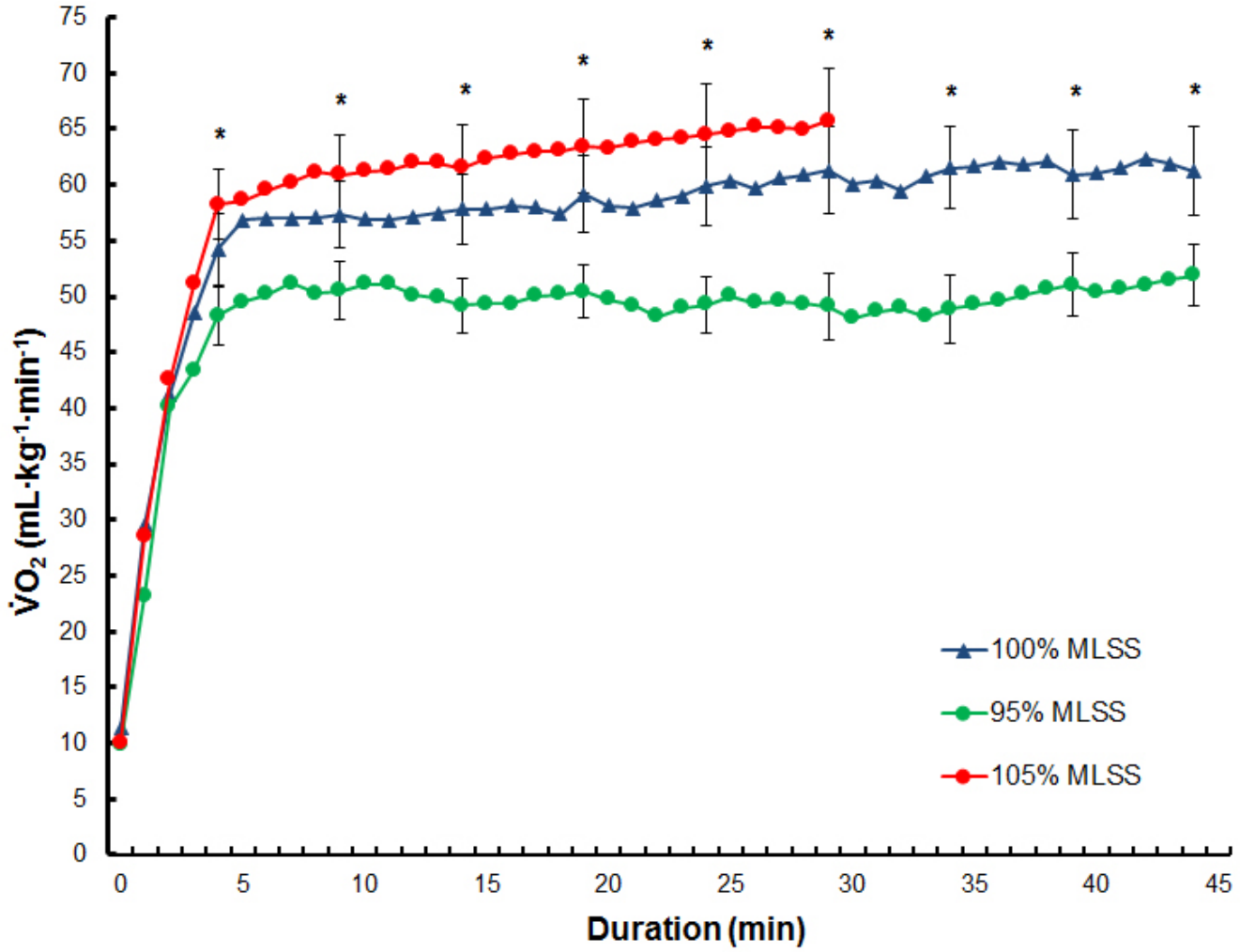

Figure 5. Oxygen consumption was measured continuously throughout the long constant-load bouts, but data were analyzed at 5-min intervals. As indicated with the * symbol, the ANOVA yielded significant differences ($P < 0.001$) in $\dot{V}O_2$ between the three intensities (95% MLSS open circles; 100% MLSS triangles, 105% MLSS closed circles) at every time point.

The average increase of $\dot{V}O_2$ from the 5th to the 45th minute of exercise was 7.02 mL·kg^{-1}·min^{-1} for the 100% W_{MLSS} bout, 3.63 mL·kg^{-1}·min^{-1} for the 95% W_{MLSS} bout, and 7.5 mL·kg^{-1}·min^{-1} for the 105% W_{MLSS} bout (until the 30th minute). A single-factor ANOVA revealed significant differences in the $\dot{V}O_2$ increase between the 95% MLSS and 100% MLSS bouts ($P = 0.001$) and the 95% MLSS and 105% MLSS bouts ($P = 0.032$), but not between the 100% MLSS and 105% MLSS bouts ($P = 0.728$).

DISCUSSION

This study examined whether a second incremental test could improve the accuracy of the MLSS estimate beyond the $\dot{V}O_{2max}$ test in competitive cyclists. It was hypothesized that the SIT would provide a more accurate determination of the work intensity at MLSS as compared to the $\dot{V}O_{2max}$ test alone. The results confirm that the combination of a traditional $\dot{V}O_{2max}$ test followed by a SIT

yields a very accurate estimate of the MLSS, a finding validated through repeated prolonged constant-load bouts of exercise.

The recent renewed emphasis on a protocol that identifies the MLSS is indicative of the importance of this workload intensity for training and physiological adaptations. This is certainly not the first study to establish a protocol for precise determination of MLSS intensity, and others have provided good reviews of the available literature (32). With regard to the precise determination of the MLSS, the minimum stage duration in incremental exercise for LT detection is the time necessary to reach a quasi-steady-state in blood lactate, which represents 95% of the steady-state level. For example, during work increments of 10 W, the minimum stage duration is two minutes, while for work increments of 50 W, the minimum stage duration is five minutes (33). However, many studies utilize work increments of 50 W with one to three minute stage durations (1,7,15,37). This choice of protocol is justified when the main independent variable is the $\dot{V}O_2$, but such tests are not sensitive enough for precise determination of the LT or W_{MLSS}. Furthermore, large work increments (< 40 W) are further contraindicated as the detection of W_{MLSS} is only improved when the lactate curves are constructed with more data points (such as smaller work increments around the lactate curve) particularly by the T_{Vis} method (31,38). The problem, of course, is that while determination of the $MLSS_w$ requires small power increments, prolonged test duration compromises the $\dot{V}O_{2max}$ value. Buchfuhrer et al. determined that $\dot{V}O_{2max}$ values were significantly higher when the incremental test protocol resulted in volitional fatigue in 8–17 minutes than when the incremental test duration was more prolonged (13). For these reasons, we elected for smaller intensity increments (10 W) and longer duration per stage (5 min) to ensure that participants reached true steady state at each interval.

Many researchers have followed along the thinking of Beneke (5) whose protocol requires several submaximal 30-minute tests of constant intensity with 1.0 mmol · L^{-1}increase in blood lactate after the 10^{th} minute to pinpoint the MLSS. For example, Billat et al. (1) presented a model that closely estimates MLSS from two submaximal bouts of constant intensity at 67% and 82% of $\dot{V}O_{2max}$ conducted during a single visit. While certainly faster, this approach was shown by Kilding and Jones (26) to substantially underestimate the true MLSS. In an attempt to make the determination of MLSS even more manageable Laplaud et al. (27) presented evidence that a single bout of exercise of increasing intensity can identify the workload at RER = 1.00. They then used repeat bouts of constant load at increments that corresponded to5% of the intensity of the workload at RER = 1.00 to show a very close relationship (R^2=0.95) between workload at MLSS and RER = 1.00. Our own data (see Table 2) indicate at best a moderate correlation (R^2 = 0.41) between the T_{Vis} and T_{RER} (R^2 = 0.54 for the $\dot{V}O_{2max}$ test and R^2 = 0.25 for the more accurate SIT). In our population of nationally competitive cyclists the two estimates differed by up to 100W (Figure 3), obviously an unacceptably wide range of values.

On the other hand, as shown in Table 2, even by using the same method the threshold intensity determined by the $\dot{V}O_{2max}$ test differed from the one from the SIT in most cases. Accordingly, and given the fact that there can only be one true MLSS intensity, it is clear that the various threshold methods cannot all represent the MLSS. This point has been previously raised by others (6) who reported inconsistent correlations between the D_{max}, T_{Log} and OBLA, thus

proving a weak connection of these methods to the real MLSS. Billat et al. (9) made a very strong case as to why the 4.0 mmol · L^{-1} OBLA threshold (T_4) does not correspond to individual MLSS intensity, as did Figueira et al. (19) for the fixed OBLA value of 3.5 mmol · L^{-1}.

It is perhaps purely coincidental that the data presented here revealed consistently that the $\dot{V}O_{2max}$ test overestimated the MLSS compared to the SIT. Theoretically it is equally possible that the MLSS could be higher or lower from the estimate from the $\dot{V}O_{2max}$ test. On the other hand, given the large workload increments, it is rather unlikely that the shorter stages of the $\dot{V}O_{2max}$ test would ever underestimate the W_{MLSS}, a fact pointed out by others (3). Accordingly, it must be noted that when summarizing group data the average difference in W_{MLSS} between the $\dot{V}O_{2max}$ and the SIT may not be a good indication of the ability of the tests to estimate W_{MLSS}. In that case, the test may accurately identify the average W_{MLSS} for a population but would be a poor predictor of an individual's W_{MLSS}. In other words, the negative and positive differences from W_{MLSS} may average out, obscuring any true differences between the two W_{MLSS} estimates. For example, the T_4, now considered a poor indicator of MLSS or W_{MLSS} because of the great inter-individual variation in the $MLSS_c$ (10), was once thought a valid measure because the T_4 corresponds to the average MLSS for a population (21). Thus, for less homogeneous populations the most valid indication of the ability of a test to estimate W_{MLSS} is the absolute value of the difference of the W_{MLSS} obtained from the $\dot{V}O_{2max}$ and the SIT.

One of the biggest advantages of this two-test approach is the very narrow range of thresholds obtained with the SIT compared to the $\dot{V}O_{2max}$ test (Fig. 3). Narrowing the wide range of threshold values inherent to the one-incremental test method might reduce the current controversy over which threshold most accurately estimates W_{MLSS}.

From the data presented in this study it is clear that the SIT provides a much more narrow range of values wherein the threshold is located. Yet, there was some uncertainty as to whether the 100% MLSS prolonged bout accurately represent the W_{MLSS} intensity. After the initial incremental test, subjects in other studies typically perform constant-load prolonged bouts at a T_w, and then the workload is adjusted up or down in subsequent constant-load bouts until the highest workload is found at which lactate increases no more than 1 mmol ·L^{-1} in the last twenty minutes of exercise (i.e. 5). In the present study participants performed constant-load bouts at 95%, 100%, and 105% of the T_{Vis} from the SIT in random order, with the assumption that the 100% MLSS bout would correspond to W_{MLSS}. Based on the different lactate kinetics observed at the three different exercise intensities (Fig. 4) we are confident that we accurately identified W_{MLSS} with the SIT method.

Given the differences in $\dot{V}O_2$ and blood lactate concentration observed with just small deviations from W_{MLSS} (± 12.8 W, on average) in the three long bouts (see Figures 4 and 5), the importance of precise identification of the $MLSS_w$ is clear. Furthermore, the W_{MLSS} is considered an important training intensity for endurance athletes (9). Small errors in estimation of the W_{MLSS} will lead to workloads that do not represent the appropriate amount of physiological stress. Based on these findings it is concluded that, if an athlete's training regime calls for training at the W_{MLSS}, and the W_{MLSS} is determined with a $\dot{V}O_{2max}$ test, then it is likely that the athlete will

be overtraining, and the result will be diminished performance (28). Accordingly, the present study is in agreement with the results of others in calling into question the assumption that a single incremental test can be used to identify the W_{MLSS}.

The data indicate that even a small (~13 W) overestimation of the MLSS intensity will considerably tax the energy production mechanisms of the body. The increase in $\dot{V}O_2$ during the 105% MLSS bout probably corresponds to the slow component of $\dot{V}O_2$ at intensities above threshold, first described by Jones et al. (24). The significant increase in lactate production, oxygen consumption, and heart rate (data not shown) at the 105% MLSS intensity should concern all athletes who rely on MLSS intensity for performance. On the other hand, it is obvious that even such a small underestimation of equal magnitude would not provide an adequate training stimulus. We have previously shown this effect to hold true in recreational athletes, where the 5% load difference between the long bouts is as little as 6-8 W (41).

Future work should address some of the limitations of this study. The participants were all competitive cyclists, used to maintaining specific intensities for extended periods of time. This is an obvious result of training, and an effect that may not be present in less trained participants. Accordingly, the SIT should be validated with a more heterogeneous group of participants. While the results of study yielded significant effect size (d = 0.92) and high statistical power (0.756), a larger number of participants would strengthen the validity of the SIT for determination of the MLSS.

In conclusion, the SIT significantly improves the detection of the W_{MLSS} over the single $\dot{V}O_{2max}$ test method. The visual deflection from baseline (T_{Vis}) method proved to be the most accurate method in determining MLSS. Furthermore, we identified the W_{MLSS} within ± 12.8 W, which, to our knowledge, is the narrowest range reported in the literature. This two-test approach to the determination of MLSS offers flexibility, accuracy, and is easy to administer.

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