

A 1-day maximal lactate steady-state assessment protocol for trained cyclists

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Abstract

The main aim of this study is to assess the validity of a new cycling protocol to estimate the Maximal Lactate Steady-State workload (MLSS) through a one-day incremental protocol (1day_MLSS). Eleven well-trained male cyclists performed 3 to 4 trials of 30-min constant load test (48-72h in between) to determine their respective MLSS workload. Then, on separate days, each cyclist carried out two identical graded exercise tests, comprised of four 10-minute long stages, with the initial load at 63% of their respective maximal aerobic power, 0.2 W·Kg⁻¹ increments, and blood lactate concentration [La] determinations each 5 min. The results of the 1day_MLSS tests were analysed through three different constructs: i) [La] difference between 5th and 10th min of each stage (DIF_5to10), ii) [La] difference between the 10th min of two consecutive stages (DIF_10to10), and iii) difference in the mean [La] between the 5th and 10th min of two consecutive stages (DIF_mean). For all constructs, the physiological steady state was determined as the highest workload that could be maintained with a [La] rise lower than 1mmol·L⁻¹. No significant differences were detected between the MLSS workload (247 ± 22W) and any of the 1day_MLSS data analysis (250 ± 24W, 245 ± 23W and 243 ± 21W, respectively; p>0.05). When compared to the MLSS workload, strong ICCs and low bias values were found for these three constructs, especially for the DIF_10to10 workload (r=0.960; Bias=2.2 W). High within-subject reliability data were found for the DIF10_10 construct (ICC=0.846; CV=0.4%; Bias=2.2 ± 6.4W). The 1day_MLSS test and DIF_10to10 data analysis is a valid assessment to predict the MLSS workload in cycling, that considerably reduces the dedicated time, effort and human resources that requires the original test. The validity and reliability values reported in this project are higher than those achieved by other previous MLSS estimation tests.

Keywords: Cycling, Habits, Online, Survey, Injury.

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Introduction

The Maximal Lactate Steady State intensity (MLSS) can be used to detect the highest running speed or cycling power output at which blood lactate concentration [La] remains stable during prolonged submaximal constant-workload exercise (Beneke 2003), which has also been considered as the upper limit of the heavy intensity domain (Beneke, Leithaeuser, and Ochentel 2011; Pringle and Jones 2002). The physiological importance of the MLSS lies in the fact that it defines the exercise intensity above which there is a net contribution of energy associated with lactate accumulation due to an increased rate of glycolysis that exceeds the rate of mitochondrial pyruvate utilization (Heck et al. 1985). The Gold Standard method to determine the MLSS intensity requires from two to four 30-minute constant loads, checking the lactate at minute ten and thirty (Beneke 2003). This methodology has been considered quite restrictive since it is a highly time-consuming method, so that, different lactate concepts or even, original single-day tests, have been proposed aiming to

approximate the real MLSS intensity, looking for a fewer resources and time determination protocol.

Due to the multiple exercise sessions undergo in repeated visits to the laboratory, which even implies to restrict the athletes' normal training regime, practitioners and coaches in order to know the MLSS intensity through the Gold Standard tests, alternative proposals have been developed to determine the MLSS speed for running, during a single-session protocol for determination with fewer resources using heart rate, rate of perceived exertion, breath frequency and race pace as predictors (Palmer, Potteiger, Nau, and Tong 1999). This method was latter validated using the single [La] during a sub-maximal running field test to predict the MLSS speed (Kuphal, Potteiger, Frey, and Hise 2004). Garcia-Tabar et al. (2017), used the single [La] during a sub-maximal running field test to predict the MLSS velocity. Billat, Dalmau, Antonini, and Chassain (1994) compared the [La] from two submaximal intensities of 20 minutes tests, carried out on the same day and separated by 40 minutes. A similar method was performed years later by Kilding and Jones (2005). The MLSS speed was also compared with the [La] during field testing (Swensen, Harnish, Beitman, and Keller 1999). Figueira, Caputo, Pelarigo, and Denadai (2008), compared the MLSS with the onset of blood lactate accumulation (OBLA) at 3.5 mmol·L⁻¹ with cyclist and runners, and recently, Llodio, Gorostiaga, Garcia-Tabar, Granados, and Sanchez-Medina (2016) tried to predict



the MLSS velocity through a regression equation using the maximal aerobic speed. Despite the contradictory findings, these results seem to indicate that it is possible to calculate the MLSS workload, reducing substantially the time commitment required by the determination using the Gold Standard method.

Besides, several studies have also tried to guess a methodology to specifically predict the MLSS in cycling. Madrid et al. (2016) estimated the MLSS by using the rate of perceived exertion, where RPE-13 protocol showed a stronger correlation with MLSS ($r = 0.78$). Grossl, De Lucas, De Souza, and Antonacci Guglielmo (2012) found that the minimum equivalent of the blood lactate-power output relationship plus $1.5 \text{ mmol}\cdot\text{L}^{-1}$ (Berg et al. 1990), was the most accurate way to predict the MLSS workload ($r = 0.94$). Finally, and without seeking a specific method to assess the MLSS intensity, Pallares, Moran-Navarro, Fernando Ortega, Emilio Fernandez-Elias, and Mora-Rodriguez (2016) found that the workload at lactate threshold plus $0.5 \text{ mmol}\cdot\text{L}^{-1}$ coincided with MLSS workload (Bias = -4.5 W). None of these methods have obtained completely satisfactory results, due to their questionably validity to predict the MLSS workload in well trained cyclists, and besides that, none of them have either studied the reliability of physiological and/or psychological markers.

With a similar purpose, different studies have previously associated the exercise intensity corresponding to MLSS with a value of respiratory exchange ratio (RER) close to 1.00. Laplaud, Guinot, Favre-Juvin, and Flore (2006) found a strong relationship between $\text{RER} = 1.00$ and MLSS in cyclists ($R^2 = 0.95$). Leti, Mendelson, Laplaud, and Flore (2012), reported a medium correlation between the speed associated with $\text{RER} = 1.00$ and the MLSS ($r = 0.79$; $p = 0.0008$). Peinado et al. (2016) suggested that MLSS could be found between both ventilatory thresholds but they did not find a strong correlation between MLSS and $\text{RER} = 1.00$ ($r = 0.730$; $\text{SEM} = 8.2$). Further, Pallares et al. (2016), neither found a strong correlation between MLSS and $\text{RER} = 1.00$ ($\text{ICC} = 0.17$). Therefore, and given the different correlations reported, there is not strong evidence that it would be a reliably method to predict the MLSS workload.

Therefore, the main purpose of the present study is to validate a new one-day graded exercise test to determine the MLSS workload in cycling. This new assessment must be carry out in a single session, with no need to self-regulate a pacing, and with the lowest possible economic and human resources cost.

Methods

Subjects

Eleven trained male cyclists and triathletes volunteered to participate in this study (age 35.0 ± 9.3 yr, body mass 72.6 ± 10.3 kg, body fat 9.2 ± 1.9 %, $\sum 8$ skinfold 81.9 ± 25.5 mm, height 174.5 ± 6.7 cm, $\text{VO}_{2\text{max}}$ 58.2 ± 6.1 $\text{ml}\cdot\text{kg}\cdot\text{min}^{-1}$), with more than 2 years of endurance training experience. They were recruited from local cycling and triathlon clubs. No physical limitations or

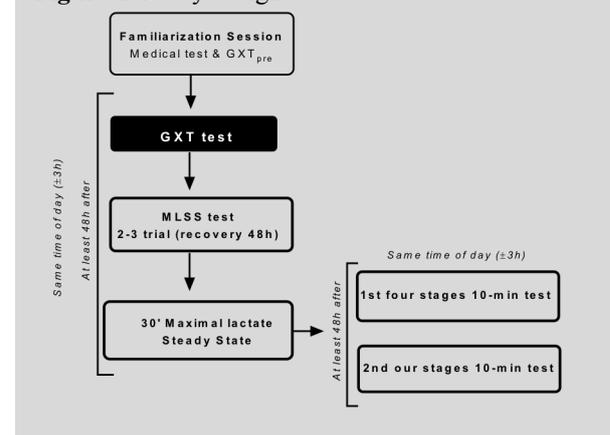
musculoskeletal injuries that could affect training were reported. Cyclist underwent a complete medical examination (including ECG) that showed all were in good health. The study, which was conducted according to the declaration of Helsinki, was approved by the Bioethics Commission of the University of Murcia. Written informed consent was obtained from all subjects prior to participation. Before giving the aforementioned written consent, all subjects were informed of the aim, the possible discomforts and the potential benefits of the experiments.

Study Design

Participants underwent a graded exercise tests which works as either a familiarization and a complete medical examination (including ECG) (GXT_{pre}), fulfilled with three objectives: a) discard cardiac defects or diseases in any of the participants, b) to minimize the bias of progressive learning on test reliability and c) to discard any participant $\text{VO}_{2\text{max}}$ lower than $50.0 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$. Participants visited the lab 7-8 times, within a 3-week period and performed only one test on any given day, separated by at least 48 hours. In the first session, cyclists performed a preliminary GXT to establish the average power output (W) associated to maximal aerobic power, as well as their $\text{VO}_{2\text{max}}$ (Lucia, Hoyos, Perez, and Chicharro 2000; Pallares et al. 2016).

Additionally, participants visited the laboratory 2-3 more times to determine the workload associated with the maximal lactate steady state (MLSS) through 30-minute constant workload test and an additional 30-minute test at the specific MLSS intensity. Finally, subjects performed the one day MLSS test (1day-MLSS) twice, with three to four stages of 10-minute long (Figure 1). The subjects performed the tests on their own bicycles. The bicycles were attached to the Cycleops Hammer ergometer (CycleOps, Madison, USA) (Lillo-Bevia and Pallares 2017) using a hyperbolic mode (the work rate was imposed to the subjects with a constant load independently of the subjects' pedalling rates). Subjects were asked to pedal seated throughout the tests to control the possible differences in the cycling economy (Arkesteijn, Jobson, Hopker, and Passfield 2016), as well as, they were allowed to choose their preferred cadence (Denadai,

Figure 1. Study design



Ruas, and Figueira. 2006). During each test, PO (W) and cadence (rev·min⁻¹) of the direct drive ergometer were transmitted to a unit display fixed on the handlebars, recording at a frequency of 1Hz using a Garmin 1000 cycling computer (Garmin International Inc., Olathe, KS, USA).

All trials were performed in the same time range of day (± 3 h) to control the circadian rhythms effects (Pallares et al. 2014), under similar environmental conditions (22.1 ± 2.5 °C and $39.9 \pm 5.4\%$ relative humidity). In all trials subjects were ventilated at wind velocity of 2.55 m·s⁻¹ with a fan positioned 1.5 meters from the subject's chest. To maintain physical performance during the investigation period (3-4 weeks) participants followed an individual training protocol consisting in cycling sessions up to 150 minutes at individual first ventilatory threshold intensity, interspersed with efforts of 5–7 min at 90–95% of second ventilatory threshold intensity each 20 min. Training sessions were repeated each 48 h with 24 h rest before each evaluation.

All of them were asked to keep their eating habits constant following a similar type of high-carbohydrate diet during the days previous to testing, reaching at least 7 gr·kg⁻¹ during the previous 24 hours (Bussau, Fairchild, Rao, Steele, and Fournier 2002). The last meal was ingested 3 h before the beginning of each testing session. Finally, the intake of any drugs or any other substance that may affect the results of the study were prohibited. During the MLSS and 1day_MLSS tests, subjects were allowed to drink water ad libitum.

Procedures

Maximal graded exercise tests (GXT)

Participants performed all the experimental trials on their own bicycles attached to a Cycleops Hammer ergometer, with a warm-up of 5 min at 50 W, starting immediately after the ramp protocol with increments of 25 W·min⁻¹ until exhaustion (Pallares et al. 2016). During GXT oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were recorded using breath to breath indirect calorimetry (Cortex Metalyzer 3B, Leipzig, Germany). Before the beginning of the test, each participant ingested 200–250 ml of water to ensure adequate hydration status (1020 usg) (Fernandez-Elias et al. 2014). Heart rate was continuously monitored (Polar Bluetooth H7, Finland). Capillary blood samples were obtained at the beginning (basal values) and three minutes later of the tests ending (Lactate Pro2, Arkray, Japan) (bias ranging from 0.32 to -2.16 mmol·l⁻¹ and coefficient of variation ranging from 0.0 to 1.0 % (Bonaventura et al. 2015). Indirect calorimetry device was calibrated before each test. In order to avoid the local acidosis that could impair the attainment of maximum cardiorespiratory performance, and according to the subjects' maximal peak power output (PPO_{pre}) in the GXT_{pre} (i.e., 350-400W), starting at 50 W, the workload was progressively increased by 25 W·min⁻¹ that ensure that testing duration was not excessively long (i.e., 13.5–15.0 min).

Maximal aerobic power (MAP) was determined as the minimal power output eliciting the maximal oxygen

uptake (VO₂max). At least two of the following criteria were required for the attainment of VO₂max: a plateau in VO₂ values (i.e. an increase in VO₂ between two or more consecutive stages of less than 1.5 ml·kg⁻¹·min⁻¹, a respiratory exchange ratio value ≥ 1.10 , or the attainment of a maximal heart rate value (HR max) above 95% of the age-predicted maximum ($207 - 0.7 \times \text{age}$) (Munoz, Seiler, Alcocer, Carr, and Esteve-Lanao 2015). In case there wasn't a clear VO₂ plateau, or that the subject couldn't end the 60 seconds stage, MAP was computed as follows, "MAP = Wf + [(t/60 x 25)]", where "Wf" is the value of the last completed load (in W), and "t" is the time the last uncompleted workload was maintained (in seconds) (Padilla, Mujika, Cuesta, and Goiriena 1999).

Maximal lactate steady state tests

Several 30-min constant workload pedalling were performed to identify the highest workload (i.e., W) which elicited an increment in lactate blood concentration less than 1 mmol·L⁻¹ between 10th and 30th min of exercise. For all tests, the subjects performed two loads of five minutes at an intensity of 80% and 90% of the VT1 as a warm-up. The first MLSS trial was performed at the 70% of the individual MAP (Pallares et al. 2016). Depending on the result of the first MLSS test, the workload of the second and following MLSS tests increased or decreased 0.2 W·Kg⁻¹ (~ 15W) (Beneke 2003), until criteria was fulfilled. MLSS was identify as the intermediate load between the last two intensities tested (i.e., interpolation). Between 2 and 3 tests were necessary to determine the workload (i.e., W) associated with the MLSS for each cyclist. Finally, and at least 48 hours following the last test, subjects were asked to perform a 30-min test at the intensity corresponding to MLSS previously determined. HR, [La], RPE and cadence were registered every ten minutes.

10-Minute-Stages test (1day-MLSS)

On separate days (48-72 h), each cyclist carried out two identical graded exercise tests, comprised of four 10-minute long stages, with free cadence. During the test, the electromagnetically braked cycle ergometer was in the hyperbolic mode, thus the work rate was independent of cadence. Warm-up consisted of 5 min at 35% and 5 min at 45% of their respective MAP. The initial workload was set at the 63% of the individual MAP previously determined (GXT_{PRE}). The workload of the second and following stages increased 0.2 W·Kg⁻¹ (~ 15W), until either, subjects completed four stages or until volitional exhaustion. Power output, HR, RPE and [La] data were registered at minute 5th and 10th of each stage. To avoid test-retest influence, subjects were only aware of time, although they did not receive any information about the physiological values nor the power output or cadence, what they performed in the first test.

Three new 1day-MLSS constructs were defined for this project as follows: i) 1day-MLSS was considered the workload of the last stage were [La] was ≤ 1 mmol·L⁻¹ between minute five and ten of each stage (DIF_5to10); ii) 1day-MLSS was considered the workload of the last

Table 1. Comparison of power output values attained for the MLSS and 1day-MLSS tests.

	MLSS (W)		1day-MLSS (W)	
		DIF_5to10	DIF_10to10	DIF_mean
Mean \pm SD	247 \pm 22	250 \pm 25	245 \pm 23	243 \pm 21
ICC (r value)	--	0.850	0.960	0.925
Bland Altman (W)				
Bias	--	-3.4 W	2.2 W	3.6 W
SD Bias	--	12.5 W	6.4 W	8.4 W
LoA	--	-28.4 to 21.6	-10.6 to 15.1	-13.2 to 20.4
Effect Size (d)	--	0.14	-0.10	-0.17

ICC = Intraclass correlation coefficient; SD = Standard Deviation; LoA = Limits of Agreement;

stage were [La] was ≤ 1 mmol·L⁻¹ comparing [La] of the 10th-minute of the stage compared with the 10th-minute of the previous stage (DIF_{10to10}); iii) 1day-MLSS was considered the workload of the last stage were the mean [La] of the minutes 5 and 10 of each stage was ≤ 1 mmol·L⁻¹ comparing the mean [La] of the minutes 5 and 10 of the previous stage (DIF_{mean}). If some subject was unable to performed 10 minutes of any stage, 1day-MLSS was considered the workload of the previous stage.

Body composition

On the first day of testing, baseline measures of height, body mass, and sum of eight skinfolds were taken (bicep, tricep, subscapula, supraspinale, suprailiac, abdomen, front thigh and calf), six perimeters (arm relaxed and tensed, gluteal, waist, calf and mid-thigh) and three breadths (bicipicondylar humerus, bicipicondylar femur and wrist), always in duplicate by the same researcher using Harpenden skinfold calipers (British Indicators, West Sussex, UK). The body lean mass was calculated for each athlete as described (Lee et al. 2000).

Statistical analysis

Standard statistical methods were used for the calculation of means, standard deviations (SD) and 95% confidence interval. Data were screened for normality of distribution and homogeneity of variances using a Shapiro-Wilk normality test and a Levene test respectively. Some data were deemed in violation of normality; therefore, a log-transformation was done to ensure the normal distribution. The validity between the Gold Standard MLSS and the three constructs (i.e., DIF_{5to10}, DIF_{10to10} and DIF_{mean}) was assessed using one way repeated measures ANOVA followed by pairwise comparisons (Bonferroni's adjustment), intraclass correlation coefficient (ICC) and Bland-Altman plots (Bland and Altman 1999). The reliability of these three constructs was assessed using coefficients of variation (CV), ICC and Bland-Altman plots. The size of the correlations was evaluated as follows; $r < 0.7$ low; > 0.7 to $r < 0.9$ moderate and $r > 0.9$ high (Vincent 2005). Effect sizes (d) were also calculated for each construct as the mean 30-min MLSS test power output minus the mean 1day-MLSS power output, divided by the pooled standard deviation (SD). Analyses were

performed using commercially available software GraphPad Prism 6.0 (GraphPad Software, Inc., CA, USA) and (IBM SPSS version 21.0, SPSS Inc., Chicago, IL). Significance was set at an alpha level ≤ 0.05 .

Results

Validity of the 1day-MLSS test

The mean power output at which the MLSS intensity was found in these well-trained athletes was 247 ± 22 W, while the mean power output calculated with the two trails of the 1day-MLSS were 250 ± 25 W, 245 ± 23 W and 243 ± 21 W for the DIF_{5to10}, DIF_{10to10} and DIF_{mean} constructs, respectively. No significant differences were detected between de MLSS results (Gold Standard) and any of the 1day-MLSS constructs ($p > 0.05$) (Table 1). Strong correlations coefficients between MLSS workload and the DIF_{10to10} and DIF_{mean} constructs were found (ICC = 0.960 and 0.925 respectively), while only a moderate correlation was found with the DIF_{5to10} (ICC = 0.850) (Table 1). The Bland-Altman plots revealed low Bias, SD of Bias and limits of agreement for the three 1day-MLSS data analysis or constructs (i.e., DIF_{5to10}, DIF_{10to10} and DIF_{mean}) (Figure 1), specifically for the comparison of the DIF_{10to10} approach (Bias = 2.2 ± 6.4 W; Table 1; Figure 1B).

Significantly lower mean values were found between the rate of perceived exertion reported by participants at the 10th minute of the MLSS (12.7 ± 1.1) and the three constructs of the 1day-MLSS analysed (15.5 ± 1.5 , 14.3 ± 1.3 and 14.1 ± 1.6 for DIF_{5to10}, DIF_{10to10}, and DIF_{mean} respectively; $p < 0.05$). Additionally, heart rate values detected in the three 1day-MLSS constructs (160 ± 8 , 157 ± 8 , 156 ± 7 bpm for DIF_{5to10}, DIF_{10to10}, DIF_{mean} and MLSS respectively) were significantly higher than the mean heart rate values achieved at 10th minute of the MLSS tests (154 ± 8 bpm; $p < 0.05$).

Within-subject reliability of the 1day-MLSS test

Within-subject reliability (Trial 1 vs. Trial 2) revealed low CV (ranging from 0.4 ± 7.4 to 2.4 ± 5.0), low Bias (specifically the DIF_{10to10} construct (2.2 ± 6.4 W) and moderate ICC, especially the DIF_{10to10} and DIF_{mean} constructs (0.846 and 0.841). Table 2

displays the mean results and data analysis of the power output obtained in both 1day-MLSS trials.

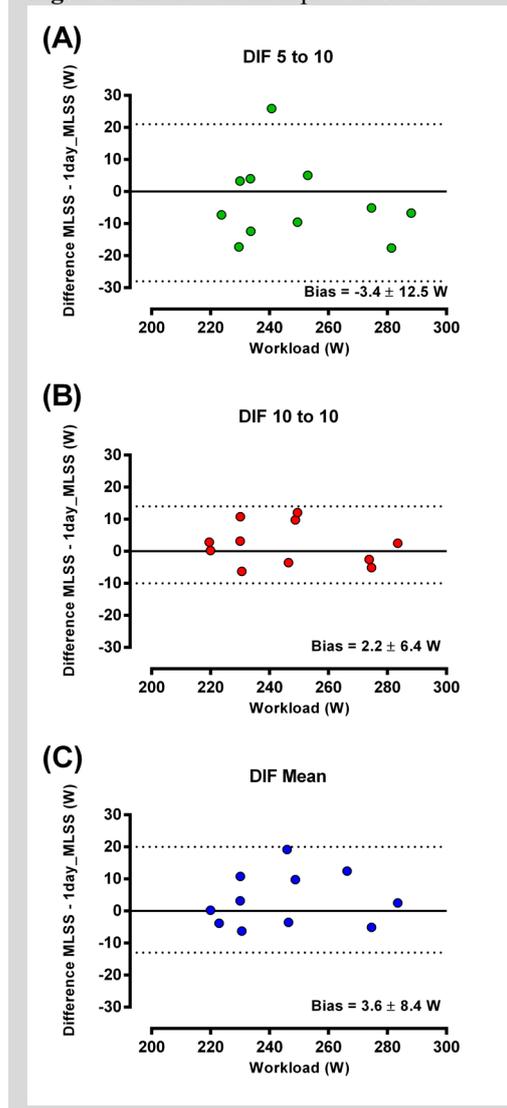
Discussion

The first aim of this study was to confirm if the 1day-MLSS test provides a valid and reliable surrogate of the directly determined MLSS intensity in cycling. The main finding of this study is that the DIF_10to10 construct of the 1day-MLSS is a valid and reliable method to estimate the aforementioned MLSS workload, demanding substantially lower resources and time.

Detection of MLSS intensity is particularly important since a substantial portion of aerobic training in athletes is carried out at MLSS intensities (Pallares and Moran-Navarro 2012; Ronnestad et al. 2014). Different studies have tried to estimate the MLSS in cycling and running by using the power output associated with RER = 1.00 with contradictory results. Leti, Mendelson, Laplaud, and Flore (2012) reported strong correlation in runners (VO_{2max} 60.8 ± 5.7 ml·kg⁻¹·min⁻¹) between the MLSS intensity with the speed at RER = 1.00 ($r = 0.79$; $p = 0.0008$). Laplaud, Guinot, Favre-Juvin, and Flore (2006) also reported a stronger relation between RER = 1.00 and MLSS ($r^2 = 0.95$, $p < 0.0001$) in cyclists (VO_{2max} 62.1 ± 4.6 ml·kg⁻¹·min⁻¹). Finally, Pallares et al. (2016) show very close values in well trained cyclists (VO_{2max} 62.1 ± 4.6 ml·kg⁻¹·min⁻¹) between RER = 1.00 and MLSS (259 ± 36 vs 255 ± 32 W), but conversely, the correlation coefficient between both results was very low ($r = 0.17$; $p = 0.397$). Despite the fact that all these publications seem to indicate that RER = 1.00 might be a good predictor of the MLSS workload or speed, even sometimes better than the ventilatory thresholds (Pallares et al. 2016), this methodology is very demanding due to the fact that indirect calorimetry is required, so becoming inaccessible to most coaches, practitioners and sport scientists.

Another method to predict in a single day an intensity similar to MLSS was performed by Billat, Bernard, Pinoteau, Petit, and Koralsztein (1994). These authors performed two constant-speed treadmill runs of 20-minute duration at approximately 65% and 90% of VO_{2peak} , separated by 40-minute rest. A validation protocol was developed by Kilding and Jones (2005), comparing the results previously mentioned, with the traditional and Gold Standard protocol (3 to 4 stages of 30-minute long), but they founded poor correlations between each other ($r = 0.29$, $p = 0.49$). They stated that the two-stages of 20-minute long substantially

Figure 2. Bland–Altman plots results.



underestimated the speed, blood lactate concentration and % VO_{2max} utilized from the actual MLSS.

MLSS was also predicted through the rate of perceived exertion (RPE), where a value of 13 correlated strongly with MLSS ($r = 0.78$) (Madrid et al. 2016). The validation protocol consisted in three ten-minute stages corresponding to each RPE identified during and GXT session as RPE-10, RPE-13 and finally RPE-16. The one which best fitted was RPE 13 ($r = 0.78$; $p < 0.01$), but high between-subject variability was found (bias = -4.7 W; 95% LoA -27.0 to 17.6 W), whereas no within-

Table 2. Test-retest data of the 1day-MLSS test.

	Mean \pm SD (W)		ICC (r value)	Bias \pm SD	95% LoA	CV
	Trial 1	Trial 2				
DIF_5to10	252 \pm 25	249 \pm 27	0.739	3.9 \pm 18.2 W	-32.4 to 40.3	1.4 \pm 7.4%
DIF_10to10	248 \pm 27	242 \pm 22	0.846	8.7 \pm 14.8 W	-23.4 to 35.9	0.4 \pm 5.5%
DIF_mean	245 \pm 23	242 \pm 22	0.841	6.4 \pm 12.6 W	-21.7 to 28.7	2.4 \pm 5.0%

ICC = Intraclass correlation coefficient; SD = Standard deviation; CV = Coefficient of variation; LoA = Limits of agreement

subject variability was assessed. On the other hand, Pallares et al. (2016) assess that the RPE associated to the MLSS workload, and also found a RPE of 13 out of 20 during a GXT with stages of one-minute long. The results achieved at the present study show that the mean RPE of the three different constructs of the 1day_MLSS were significantly higher than those achieved at the MLSS determination tests. Such a difference may be explained by the fact that as the time goes by, the physiological and psychological fatigue accumulated consequently increases the subjective rate of perception. A large number of authors have tried to validate other tests to estimate the MLSS workload using incremental graded exercises test and [La] analysis. Hauser, Adam, and Schulz (2014) reported significant correlations between MLSS and the “onset of blood lactate accumulation (OBLA_{4mmol})” (Sjodin and Jacobs 1981), “the individual anaerobic threshold (IAT)” (Jones and Doust 1998), and the “+ 1.5 mmol·L⁻¹ lactate model” (Dickhuth et al. 1999) ($r = 0.89$; $r = 0.83$ and $r = 0.88$, respectively), but with large individual differences based on the Bland-Altman model. Pallares et al. (2016) also found high coefficient of correlation between Lactate threshold+0.5 and OBLA_{4mmol} and MLSS ($r > 0.78$, $p < 0.05$ in all cases). Again, large individual differences based on the Bland-Altman analysis were found between MLSS and Lactate Threshold OBLA_{4mmol}, but surprisingly a low bias was found between MLSS and Lactate threshold + 0.5 (-4.5 ± 23.2 W). In the present study, mean values of 4.7 ± 0.7 , 3.8 ± 1.0 and 3.7 ± 1.0 mmol·L⁻¹ were found at the different constructs tested. A key factor to assess the validity of a method is to know how likely it predicts the true value. Palmer et al. (1999) reported that their method (which consisted in two stages of 27-minute runs on a treadmill, collecting blood samples every 3 min of each 9-min stage), was successful in predicting the MLSS in 9 out of 12 subjects. Leti et al. (2012) reported that 5 out of 14 subjects showed some disagreement between intensities of MLSS and RER = 1.00. Paton and Hopkins (2001) suggested that in elite athletes, a magnitude lower than 2% is required to detect changes in performance from an ergogenic or training intervention. Applying this very demanding as an acceptable error of the real MLSS power output value, this method was successful for 4 out of 11, 7 out of 11 and 6 out of 11 for the DIF_5to10, DIF_10to10 and DIF_mean respectively. In conclusion, the main findings of the present study were that the DIF_10to0 method of the 1day-MLSS is a valid and reliable method that could allow to estimate the MLSS intensity in well trained cyclists with lower resources and time. The small bias and CV as well as the consistent correlations and small differences found, lead to use this protocol for assessing the MLSS workload in a single testing session. There are some limitations in the current study which may be possible to overcome in future studies. Since the tests were performed at laboratory, additional research must be done at field, either at flat or hilly conditions, to confirm these results. Furthermore, it must be mentioned that our results are limited to male cyclists and triathletes

with a similar performance status and physiological level (~ 55 ml.kg.min⁻¹). A transfer to other populations (women, inactive people or even elite cyclists), or exercise modes (running, swimming or paddling) must be done carefully.

Practical applications

This study confirms that the 1day-MLSS test is a highly valid and reliable test for coaches and practitioners to estimate the MLSS intensity by comparing the lactate values achieved at 10th minutes of each stage of a 10-minute-stages test, providing an alternative to the Gold Standard determination test in cycling with lower resources and time.

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Conflict of interest

The investigators in the present study have no conflicts of interest.

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