The midpoint between ventilatory thresholds approaches maximal lactate steady state intensity in amateur cyclists

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ABSTRACT: The aim was to determine whether the midpoint between ventilatory thresholds (MPVT) corresponds to maximal lactate steady state (MLSS). Twelve amateur cyclists (21.0 ± 2.6 years old; 72.2 ± 9.0 kg; 179.8 ± 7.5 cm) performed an incremental test (25 W·min^{-1}) until exhaustion and several constant load tests of 30 minutes to determine MLSS, on different occasions. Using MLSS determination as the reference method, the agreement with five other parameters (MPVT; first and second ventilatory thresholds: VT1 and VT2; respiratory exchange ratio equal to 1: RER = 1.00; and Maximum) was analysed by the Bland-Altman method. The difference between workload at MLSS and VT1, VT2, RER=1.00 and Maximum was 31.1 ± 20.0, -86.0 ± 18.3, -63.6 ± 26.3 and -192.3 ± 48.6 W, respectively. MLSS was underestimated from VT1 and overestimated from VT2, RER = 1.00 and Maximum. The smallest difference (-27.5 ± 15.1 W) between workload at MLSS and MPVT was in better agreement than other analysed parameters of intensity in cycling. The main finding is that MPVT approached the workload at MLSS in amateur cyclists, and can be used to estimate maximal steady state.

INTRODUCTION

The exercise intensity eliciting maximal steady state blood lactate concentration (MLSS; maximal lactate steady state) is a reliable index of endurance capacity [1, 2], i.e. the physiological ability to tolerate long lasting exercises at a higher aerobic rate without intramuscular and blood acid-base perturbations [3-5]. Mean metabolic rate at a workload corresponding to MLSS is about 70-75% of maximum oxygen uptake (VO_{2max}) for cyclists [4], which does not differ from $75 \pm 5\%$ VO_{2max} reported in other sports modalities [2, 6]. Indeed, MLSS relative to workload at VO_{2max} (65-70%) is more independent of motor task performance than blood lactate concentration ([La$^-$]), which ranges from 2 to 8 mmol·L^{-1} at MLSS, and relates to the amount of muscle mass engaged in exercise [2, 7, 8].

Concerning the methodological aspects of MLSS assessment, the gold standard protocol requires three to four 30-minute tests with exercise intensity ranging from 60 to 80% VO_{2max} [2]. By applying this protocol, the velocity or workload at MLSS is defined as the highest exercise intensity attained without blood lactate concentration changes above 1 mmol.L^{-1}, during the final 20 minutes of the bout [9]. However, its usefulness is limited by the need for time-consuming tests [5, 10]. The practical disadvantage of numerous tests for direct MLSS assessment has motivated studies to investigate time saving, less expensive and non-invasive procedures. Many attempts have related protocols of aerobic capacity evaluation, such as critical velocity, to the velocity at MLSS, reporting good relationships between these indexes [11-13]. Even other remarkable indexes of endurance capacity such as critical power (CP) [1, 3], ventilatory (VT) or lactate threshold (LT) have evidenced similarities to the velocity or workload at MLSS [1, 14-20], but none of them confirmed that the physiological responses encompassed by MLSS could be exchanged for these indexes.

Despite CP being recognized as the exercise intensity near to MLSS, the metabolic correspondence between these two points has not been demonstrated [21, 22]. Indeed if, as seems probable, intensity corresponding to MLSS in different sports modalities lies above VT_1 (or LT), and below the point where respiratory compensation for metabolic acidosis starts (RCP, or VT_2) [1, 14, 21, 23-25], it would be expected that an intermediate intensity might be the

nearest to MLSS. For this reason an intensity corresponding to 3.5 mmol$\cdot$L^{-1} [26], referred to as the individual anaerobic threshold (IAT) [17, 20, 27], or the intensity corresponding to a respiratory exchange ratio (RER) equal to 1 [25, 28], has been proposed as an indicator of MLSS.

Although the midpoint between the ventilatory thresholds (VT$_1$ and VT$_2$) from a progressive ramp protocol would correspond to MLSS, and one maximal aerobic test would be enough to locate MLSS, as far as we have been able to ascertain this has not yet been explored. Therefore, the aim of the present study was to verify whether the intensity corresponding to the midpoint between the ventilatory thresholds (MP$_{VT}$) corresponds to MLSS intensity among an amateur group of cyclists. We hypothesized that the power output corresponding to MP$_{VT}$, determined during a single maximal incremental test, would allow easier calculation of MLSS power output.

MATERIALS AND METHODS

Subjects. Twelve amateur road cyclists (elite-sub23 category) were selected for this investigation (21.0±2.6 years, 179.8±7.5 cm, 72.2±9.0 kg). A physical examination before the start of the study was carried out to ensure that each participant was in good health. The benefits and risks of the protocol were explained, and the subjects signed an informed consent form, following approval from the ethical committee of the Technical University of Madrid.

Procedures

Each subject carried out an incremental test during the first visit. Several constant load tests of 30 minutes were performed thereafter (48 h) in order to determine the intensity corresponding to MLSS. These steady state tests were carried out with a 48 h interval between them. Each cyclist performed all tests at the same time of day under similar environmental conditions (22.8±0.6ºC and 62.4±4.4% relative humidity). Subjects were asked to refrain from hard physical work and consumption of any medication or stimulants for at least 24 h before each experimental session. During the tests, subjects adopted the conventional upright cycling posture. This posture is characterized by a trunk inclination of ~75º and by the subject placing their hands on the handlebars with elbows slightly bent (~10º). Before the tests, each cyclist adjusted the corresponding cycle ergometer and used their own clip-on pedals [29, 30].

Gas exchange data were collected continuously during each test using an automated breath-by-breath system (Jaeger Oxycon Pro gas analyser, Erich Jaeger, Viasys Healthcare, Germany). The following variables were recorded during the tests: oxygen uptake (VO$_2$), carbon dioxide output (VCO$_2$), respiratory exchange ratio (RER), ventilation (V$_E$), respiratory rate (RR), the end tidal partial pressures of O$_2$ (PETO$_2$) and CO$_2$ (PETCO$_2$), and the respiratory equivalents of O$_2$ (VE$\cdot$VO$_2$$^{-1}$) and CO$_2$ (VE$\cdot$VCO$_2$$^{-1}$). A 12-lead electrocardiogram (ECG; Viasys Healthcare, Germany) was continuously recorded during the tests to determine heart rate (HR) [31, 32].

Maximal incremental test

A continuous incremental cycling test was used to determine maximal oxygen uptake (VO$_{2max}$) and ventilatory thresholds (VT). The test was performed on a conventional cycle ergometer (Jaeger ER800, Erich Jaeger, Germany). After a 3-min warm-up at 50 W, the workload was increased by 5 W every 12 s (25 W$\cdot$min^{-1}) until exhaustion. Subjects were allowed to choose their preferred cadence within the 70-90 rpm range. Verbal encouragement was provided to ensure that maximal effort was reached. All the subjects had previous experience with this type of protocol, which has been used for the physiological evaluation of professional cyclists in several previous studies [30, 33-35] and is reliable for the detection of the VT [32]. At least two of the following criteria were required for the attainment of VO$_{2max}$: a plateau in VO$_2$ values despite increasing workload, RER$\geq$1.1, or the attainment of 95% of the age-predicted maximum heart rate (HR$_{max}$) [30, 36].

The maximum 15 s average value of VO$_2$ attained during the test was reported as VO$_2$max, and the maximum workload achieved during the last stage of the progressive test was identified as the Maximum [25]. The first and the second VT (VT$_1$ and VT$_2$, respectively) were set at the points of maximum agreement of the most common methods of assessment [37]. Briefly, VT$_1$ was calculated 1) according to the V-slope method [31], where VT$_1$ is the break point of the VCO$_2$-VO$_2$ relationship, 2) as the first exponential increment in ventilation [38], and 3) as the first rise in V$_E$$\cdot$VO$_2$$^{-1}$ without increments in V$_E$$\cdot$VCO$_2$$^{-1}$ [39]. VT$_2$ was determined as the second rise in ventilation [38] and as the intensity that accompanied a second rise in V$_E$$\cdot$VO$_2$$^{-1}$ with a concurrent rise in V$_E$$\cdot$VCO$_2$$^{-1}$ [39]. All tests were evaluated by two researchers in a double blind process. The coefficient of variation between the assessments of these two researchers and those of a highly experienced expert was 1.3%.

Determination of MLSS

Constant load tests of 30 min were carried out to determine MLSS. These were performed on a road bicycle fitted with an SRM powermeter (Schoberer Rad Messtechnik SRM, Jülich, Germany). The bicycle was then mounted on a Tacx CycleForce Grand Excel ergometer (Technische Industrie Tacx BV, Netherlands). This ergometer was not used for analysis purposes but only as a platform on which to mount the test rig. Participants were allowed to use their own pedals and saddle. Height and reach were adjusted to match the participant's own bicycle as closely as possible.

The first constant workload trial was performed at an intensity corresponding to MP$_{VT}$, previously calculated in the maximal incremental test [(workload at VT$_1$ + workload at VT$_2$)$\cdot$2^{-1}]. Another 30 min test was performed at a higher intensity with an increase of 5% of maximum load 48 h later if, during the first test, lactate concentration [La$^-$] remained steady or decreased. Subsequent 30 min constant tests were increased by an additional 5% of the previous intensity until no lactate steady state could be maintained. Inversely, if [La$^-$] increased continuously or the exercise was interrupted due to the

subject's fatigue during the first 30 min test, the workload was decreased by 5% of Maximum for each test until a steady state could be maintained. MLSS was defined as the highest workload that could be maintained with an increase in [La$^-$] lower than 1.0 mmol·L^{-1} during the final 20 min of the constant load tests [5, 28, 40-42].

Blood samples

Before each test, an 18G catheter was inserted into a forearm vein for venous blood sampling. Samples were drawn prior to and during exercise at different moments in order to determine [La$^-$] every 2 min and at the moment when maximal effort was deemed to have been reached in the incremental test, every 5 min throughout the steady state tests (0, 5, 10, 15, 20, 25 and 30 min) and at exercise termination if the test could not be maintained. [La$^-$] was analysed by an enzymatic method (YSI 1500, Yellow Springs Instruments Co., Ohio, USA).

Statistical analysis

All data are reported as mean (±SD). One way ANOVA was used to examine the differences between the values obtained at the different points in the incremental test (VT$_1$, MP$_{VT}$, VT$_2$, RER=1.00 and Maximum) with the values at MLSS. Multiple comparisons were made using the Bonferroni post hoc test. The coefficient of variability (CV%), standard error of the mean and Pearson's correlation coefficient were calculated to evaluate the workload differences between MLSS and the different points. Bland–Altman plots [43] were drawn to establish the limits of agreement for the five points of the incremental test plotted against MLSS. Bland-Altman plots were also

used to compare VO$_2$, VE, HR and [La$^-$] assessed using MP$_{VT}$ and MLSS. Linear regression analysis and correlation coefficients were calculated and included in the plots. All analyses were carried out with SPSS version 19 (Chicago, Illinois, USA), and the level of statistical significance was set at p<0.05 for all analyses.

RESULTS

The mean value of workload at MLSS was 284±30 W, within a range from 236 to 323 W. Table 1 shows the results and the differences found between the physiological parameters from the incremental test (VT$_1$, MP$_{VT}$, VT$_2$, RER=1.00 and Maximum) and MLSS. The workload at MLSS was not different from VT$_1$ or MP$_{VT}$ for absolute values (W), those relative to body mass (W.kg^{-1}) or those relative to maximum values, but was located closer to MP$_{VT}$ than VT$_1$. The VO$_2$ at MLSS was not different from VO$_2$ at MP$_{VT}$, VT$_2$ or RER=1.00, comparing the values in absolute terms, relative to body mass, as well as relative to maximum VO$_2$. Otherwise, VO$_2$ values for MLSS and MP$_{VT}$ parameters were the closest. Similarly, HR at MLSS did not differ significantly from HR at MP$_{VT}$, VT$_2$ and RER=1.00. The values for other parameters at MLSS (VE, RR, VE·VO$_2^{-1}$, PETO$_2$ and [La$^-$]) were similar to those at VT$_2$ and RER=1.00.

The Bland-Altman agreement analysis for workload intensity at MLSS with workload at MP$_{VT}$, VT1, VT$_2$, RER=1.00, and Maximum are shown in Figure 1. The mean difference between workload at MLSS and at VT$_1$ (Fig. 1A), VT$_2$ (Fig. 1C), RER=1.00 (Fig. 1D) and Maximum (Fig. 1E) was 31.1±20.0 W (range: 18.3 to 43.8 W), -86.0±18.3 W (range: -74.4 to -97.7 W), -63.6±26.3 W (range: -49.3 to -86.9 W) and -192.3±48.6 W (range: -161.4 to -223.2 W),

TABLE I. Mean ± SD for variables obtained at each point during the incremental test and mean last 20 minute values at MLSS.

	MLSS	VT1	MPVT	VT2	RER=1.00	Maximum
Workload (W)	284 ± 30	253 ± 37	311 ± 32	370 ± 32[a]	347 ± 35[a]	476 ± 62[a]
Workload (W·kg^{-1})	4.0 ± 0.4	3.5 ± 0.5	4.3 ± 0.5	5.2 ± 0.6[a]	4.9 ± 0.6[a]	6.6 ± 0.5[a]
%Workload$_{max}$	60.0 ± 5.6	53.3 ± 6.3	65.8 ± 5.6	78.3 ± 6.3[a]	73.8 ± 8.6[a]	100.0[a]
VO$_2$ (mL·min^{-1})	4225 ± 414	3244 ± 464[a]	4052 ± 362	4588 ± 364	4367 ± 386	5175 ± 474[a]
VO$_2$ (mL·min^{-1}·kg^{-1})	58.8 ± 4.7	45.2 ± 6.5[a]	56.6 ± 6.3	64.2 ± 7.3	61.9 ± 8.4	72.4 ± 8.7[a]
%VO$_{2max}$	81.8 ± 7.0	62.7 ± 7.6[a]	78.5 ± 5.9	88.9 ± 4.8	87.2 ± 4.8	100.0[a]
VCO$_2$ (mL·min^{-1})	3821 ± 404	2802 ± 458[a]	3712 ± 415	4534 ± 416[a]	4384 ± 389	5173 ± 256[a]
VE (L·min^{-1})	121 ± 12	72 ± 15a	99 ± 11a	123 ± 12	119 ± 12	176 ± 12[a]
HR (beats·min^{-1})	175 ± 8	155 ± 14[a]	171 ± 10	183 ± 10	179 ± 8	194 ± 6[a]
RR (breaths·min^{-1})	47 ± 7	31 ± 6a	37 ± 5a	41 ± 5	41 ± 6	59 ± 6[a]
PETO$_2$ (kPa)	13.5 ± 0.5	12.2 ± 0.5[a]	12.8 ± 0.4a	13.2 ± 0.5	13.2 ± 0.4	14.2 ± 0.5[a]
PETCO$_2$ (kPa)	4.7 ± 0.5	5.6 ± 0.5[a]	5.3 ± 0.4	5.3 ± 0.5	5.3 ± 0.4	4.4 ± 0.5
VE·VO$_2^{-1}$	28.5 ± 2.7	21.6 ± 2.3[a]	23.8 ± 2.1a	26.2 ± 2.6	26.5 ± 2.1	34.1 ± 2.9[a]
VE·VCO$_2^{-1}$	31.6 ± 3.8	24.9 ± 2.3[a]	26.0 ± 1.8a	26.5 ± 2.3[a]	26.4 ± 2.1[a]	33.6 ± 2.2
[La-] (mmol·L^{-1})	3.60 ± 0.81	1.32 ± 0.43[a]	1.90 ± 0.69a	3.40 ± 1.27	2.76 ± 1.25	8.10 ± 1.90[a]

Note: VT1, first ventilatory threshold; MPVT, midpoint between the ventilatory thresholds; VT2, second ventilatory threshold; RER=1.00, respiratory exchange ratio equal to 1; MLSS, maximal lactate steady state; %Workload$_{max}$, percentage of maximal workload; VO$_2$, oxygen uptake; %VO2max, percentage of maximal oxygen uptake; VCO$_2$, carbon dioxide production; VE, ventilation; HR, heart rate; RR, respiratory rate; PETO$_2$, end tidal partial pressure of oxygen; PETCO$_2$, end tidal partial pressure of carbon dioxide. a Significantly different from MLSS.
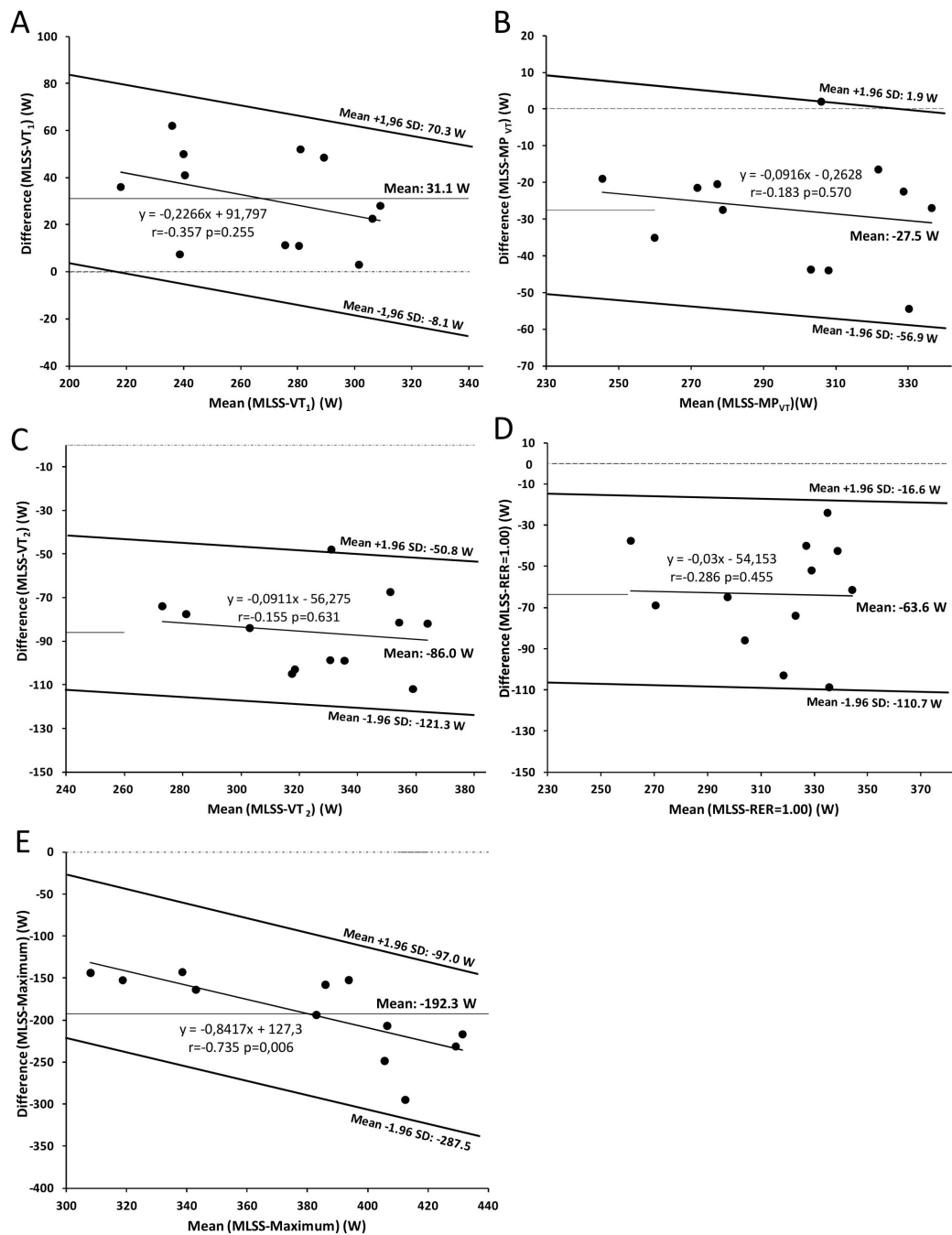
FIG. 1. Bland-Altman plots comparing workload for VT_1-MLSS (A), MP_{VT}-MLSS (B), VT_2-MLSS (C), RER=1.00-MLSS (D) and Maximum-MLSS (E). Linear regression analysis and correlation coefficient (r) are included in the plots.

respectively. Thus, VT_1 underestimated MLSS while VT_2, RER=1.00 and Maximum overestimated it. The mean difference of -27.5 ± 15.1 W (range: -17.9 to -37.1 W) between workload at MLSS and at MP_{VT} was the smallest difference among analysed workload intensities (Figure 1B).

Workload corresponding to MLSS was significantly correlated with all points (Table 2). Workload at MP_{VT} was highly correlated with MLSS ($r=0.885$, $p<0.05$), and the standard error of the mean was the lowest between MLSS and the different points of the incremental test (MLSS- MP_{VT}: 4.3 W; Table 2). Moreover, %CV of MLSS-MP_{VT} was 6.6%.

TABLE 2. Correlation coefficient (r), standard error of the mean and coefficient of variability (CV%).

	R	Standard error of the mean (W)	%CV
MLSS-VT1	0.836*	5.8	8.5
MLSS-MPVT	0.885*	4.3	6.6
MLSS-VT2	0.827*	5.3	18.7
MLSS-RER=1.00	0.730*	8.2	14.3
MLSS-Maximum	0.653*	14.0	35.5

Note: VT1, first ventilatory threshold; MPVT, midpoint between the ventilatory thresholds; VT2, second ventilatory threshold; RER=1.00, respiratory exchange ratio equal to 1; MLSS, maximal lactate steady state. * Indicates significant correlation ($p<0.05$).
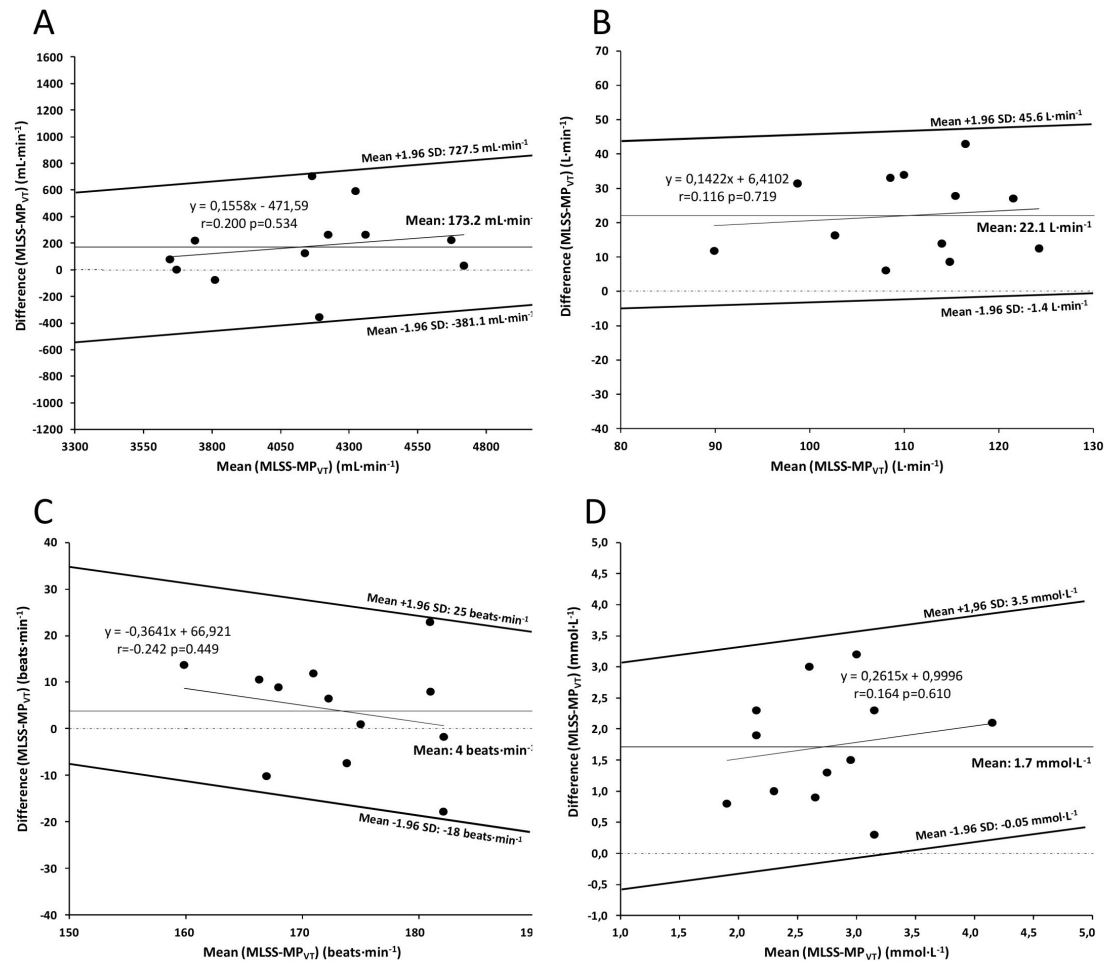
FIG. 2. Bland-Altman analyses between MLSS and MP$_{VT}$: VO$_2$ (A), VE (B), HR (C) and [La$^-$] (D). Linear regression analysis and correlation coefficient (r) are included in the plots.

The mean differences in VO$_2$ (173.2±282.8 mL·min^{-1}), V$_E$ (22.1±12.0 L·min^{-1}), HR (4±11 beats·min^{-1}) and [La$^-$] (1.7±0.9 mmol·L^{-1}) between MLSS and MP$_{VT}$ are shown in Figure 2. No significant correlations were found between MLSS and MP$_{VT}$ in VO$_2$, V$_E$, HR and [La$^-$].

DISCUSSION

The main finding of this study was to locate the workload corresponding to MLSS in well-trained amateur cyclists close to the midpoint between the ventilatory thresholds. This intensity is the closest to MLSS, as the average mean difference was -27.5±15.1 W, whereas the intensities at VT$_1$, VT$_2$, RER=1.00 and Maximum are further away and these points cannot be taken as indicators of intensity at MLSS.

The role of MLSS as an index of aerobic endurance [4, 18, 44-46] and as a training stimulus to improve this ability [10, 47, 48] has motivated the search for a single assessment protocol [45, 49-51], since the gold standard protocol comprises the performance of an incremental test followed by successive constant intensity tests [5, 9, 52]. Although some of the defined points during an incremental

test have been proposed as intensities that indicate MLSS, which would permit its determination with one single test [1, 15, 19, 25, 27, 28, 52-55], none of these studies is definitive, and the challenge remains to be able to determine this intensity with just one test.

The present study has identified MP$_{VT}$ as the nearest intensity to MLSS, as the rest of the points determined were further away. The difference between MP$_{VT}$ and MLSS could be taken as a reference for determining MLSS. Bearing in mind that the maximal test was performed on a different ergometer from the one used in the constant load tests, the difference in the load between MP$_{VT}$ and MLSS could be attributed to this circumstance, decreasing the internal validity of the study. However, external validity increases, as the data obtained in the laboratory can be transferred to training sessions, using a portable ergometer such as the SRM system. We suggest that a difference of 27 W in training intensity is probably realistic for amateur cyclists and easy to adjust with a field test, such as a 40 km time trial. In any case, the variability of MLSS-MP$_{VT}$ is low and in line with the results of Hauser et al. [56], who reported variability values of 3% for MLSS power. Furthermore, our results suggest an easy approach to determine MLSS, since the mean difference between

workload at MLSS and at MP_{VT} is reduced to -3.2±12.4 W by subtracting 27 W from MP_{VT}. Only one subject shows a difference greater than 20 W, while the variability is 2.4%.

The difference between RER=1.00 and MLSS reported by Laplaud et al. [28] was 3.6±8.1 W, which is less than the difference observed in the present study, although they did not report the intensity at RER=1.00 as MP_{VT}, despite this point coinciding with the mean value between ventilatory thresholds. The estimation of MLSS from the intensity at RER=1.00, VT_1 and VT_2, determined during a single maximal incremental field test in well-trained long and middle distance male runners, showed a better agreement between velocity at MLSS and RER=1.00, than with velocity at VT_1 and VT_2 [25]. Again, RER=1.00 coincided with the velocity at MP_{VT}. The level of training could affect exercise intensity where RER=1.00 during a maximal test. In highly trained athletes this is near to the VT_2, as shown in our results, due to greater energy production from lipid metabolism [28, 57, 58]. Thus, in well-trained athletes it is not advisable to consider that the intensity at RER=1.00 represents MLSS. Workload at VT_1 seems to underestimate the intensity at MLSS [1, 28, 59], as even though at this intensity lactate concentration is steady [53], it does not match the maximum steady state level. Our results showed that MLSS was also above VT_1. Conversely, VT_2 overestimates MLSS [1, 28], although both are physiologically related [1]. By definition, MLSS should be between the two ventilatory thresholds, as reported by Benito et al. [60], observing a steady state [La⁻] for exercise intensity at MP_{VT}.

Different lactate threshold methods have been proposed for estimating MLSS, such as the IAT, or anaerobic thresholds at fixed [La⁻] of 3.5 and 4 mmol·L⁻¹ [16, 19, 61, 62], but none of these methodologies have presented conclusive results. The differences in test protocols used in the original threshold investigations, the large individual differences shown by the lactate thresholds, and [La⁻] applied as references for the anaerobic threshold explain the discrepancies [26, 63, 64]. Probably, lactate threshold methods do not ac-curately estimate the intensity corresponding to MLSS, as the correspondence found between the fixed lactate concentrations and the intensity corresponding to MLSS may be due to a mere coincidence, and an overall interpretation of the result neglecting the individual differences [48, 54, 64]. The range of [La⁻] at MLSS [2, 7] and high day-to-day variability for lactate at MLSS [56] support the coincidental similarity between a given lactate value from incremental and constant intensity exercise. Therefore a comparison of lactate "intensities" should be avoided, being more adequate using power or workload parameters. Indeed, differences in physiological profile during exercise at constant intensity (and steady metabolic rate) from a non-constant and increasing exercise rate have been well documented [1, 65] and likewise shown by our results.

CONCLUSIONS

The main conclusions of the study were: (a) the workload corresponding to MLSS in amateur cyclists is located at a point which is near to the MP_{VT}, being the nearest intensity, while VT_1, VT_2, RER=1.00 and Maximum cannot be taken as indexes of MLSS; and (b) MLSS could be determined with a single maximum incremental test, as it is located at a workload fairly close to the MP_{VT}, or even just below it. Further information is required to confirm that the MP_{VT} is a good estimator of MLSS, focusing on a broad sample of elite cyclists from different specialties, and non-elite and elite endurance athletes from other cyclic sports.

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