Estimation of the Maximal Lactate Steady State in Endurance Runners

I. Llodio1,2, E. M. Gorostiaga1, I. Garcia-Tabar1, C. Granados1, L. Sánchez-Medina1

1 Studies, Research and Sport Medicine Center, Government of Navarra, Pamplona, Spain
2 Faculty of Physical Activity and Sports Science University of the Basque Country Vitoria-Gasteiz, Spain

Abstract

This study aimed to predict the velocity corresponding to the maximal lactate steady state (MLSSV) from non-invasive variables obtained during a maximal multistage running field test (modified University of Montreal Track Test, UMTT), and to determine whether a single constant velocity test (CVT), performed several days after the UMTT, could estimate the MLSSV. Within 4–5 weeks, 20 male runners performed: 1) a modified UMTT, and 2) several 30 min CVTs to determine MLSSV to a precision of 0.25 km·h⁻¹. Maximal aerobic velocity (MAV) was the best predictor of MLSSV. A regression equation was obtained: MLSSV = 1.425 + (0.756 · MAV); R² = 0.63. Running velocity during the CVT (VCVT) and blood lactate at 6 (La₆) and 30 (La₃₀) min further improved the MLSSV prediction: MLSSV = VCVT + 0.503 – (0.266 · ΔLa₃₀-₆); R² = 0.66. MLSSV can be estimated from MAV during a single maximal multistage running field test among a homogeneous group of trained runners. This estimation can be further improved by performing an additional CVT. In terms of accuracy, simplicity and cost-effectiveness, the reported regression equations can be used for the assessment and training prescription of endurance runners.

Introduction

The exercise intensity corresponding to the maximal lactate steady state (MLSS), a lactate-related threshold, is a consistent physiological phenomenon defined as the highest constant velocity or power output that can be maintained over time without a continual blood lactate accumulation [7,12]. MLSS is regarded by many physiologists as the gold standard for the assessment of endurance capacity [8,22,23,33,34], since it has been proven to be useful in the diagnosis of endurance performance and the prescription of aerobic training [26]. MLSS can predict performance in endurance-type events lasting between 30 and 60 min [12,36], and it itself can be used as a training stimulus [29]. Determination of the MLSS is time-consuming, as it requires the performance of several (3–6) constant workload tests, on separate days and within a 1–2 week period [21]. To avoid such an extensive procedure, simpler methods have been proposed to determine the MLSS from the response to a single incremental test that involves the use of heart rate (HR), blood lactate concentration ([La⁻¹]) or respiratory exchange measurements [8,15,22,29,36].

Several studies have shown that the workload corresponding to the maximal oxygen uptake (VO₂max), or the maximal workload attained at the end of an incremental test to exhaustion, predicts the MLSS with a wide range of correlations (r = 0.67–0.95) [5–9,15,22,29,36]. One of the reasons explaining this disparity in correlation magnitudes might be the fact that the participants under study were not very homogeneous in terms of performance. Thus, the coefficients of variation (CVs) for the MLSS intensity in the aforementioned studies varied between 7% and 17%. These CV values may skew the correlation coefficient (r) because, if the range of values is wide, r tends to be high and vice versa [30]. In a recent study [27], we investigated whether the relationships between MLSS and some variables measured during an incremental running field test to exhaustion were consistent when a very homogeneous sample of soccer players was used (CV for MLSS 4.9%). The results showed that maximal aerobic velocity [10,11] (MAV) and velocity at 80% of the maximum HR, both reached during a maximal incremental running field test, were strong predictors of the running velocity at MLSS (MLSSV), and accounted for 60% of the variance in MLSSV. When, several days after the...
maximal incremental test, the subjects performed an additional 20 min constant velocity field test (CVT) at ~75% of the MAV, which included the measurement of [La−] at the 10th and 20th min of exercise, the prediction of MLSSV was further improved, with explained variance increasing to 66%, and predicted and measured MLSSV values showing a good limit of agreement (~±0.46 km·h−1) [27].

One limitation of our previous study [27] was that the generalizability of the results was limited to homogeneous groups of soccer players with MLSSV ranging from 11.0 to 13.5 km·h−1. It was therefore uncertain whether our results could be generalized to other populations with different MLSSV values, and specifically to highly trained endurance runners with MLSSV ≥ 13.5 km·h−1. Strong correlations have been reported between MLSSV and running performance over various distances such as 5 km (r = 0.97) [20] and 8 km (r = 0.92) [22]. The magnitudes of these correlations are similar to those found for the estimated velocity at VO2max (r = 0.93) [22], and higher than the ones found for other variables such as the lactate threshold (r = 0.47) [20], ventilatory threshold (r = 0.37–0.81) [20,22] or the lactate minimum test (r = 0.83) [22]. These studies, among others, underline the importance of the MLSSV assessment or estimation in the physiological profiling of endurance runners [22].

Here, as a follow-up to our previous study conducted with soccer players [27], we aimed to elucidate whether MLSSV could also be predicted in a group of trained endurance runners from a single running field test. Accordingly, the primary purpose of this cross-sectional descriptive study was to determine the relationships existing between the MLSSV and some simple and non-invasive variables measured during a multistage maximal running field test in a homogeneous (CV < 7% for MLSSV) group of endurance runners (MLSSV ≥ 13.5 km·h−1). A secondary purpose of the present study was to determine the extent to which a single CVT, performed several days after the maximal incremental test, could estimate the MLSSV in the same sample of endurance runners. Based on our previous study [27], we hypothesized that MAV and the running velocity corresponding to 80% HRmax would correlate significantly with MLSSV and that an additional CVT performed on subsequent days would improve the estimation of the MLSSV.

Materials & Methods

Subjects

20 well-trained male endurance runners (age 30.7 ± 6.3 years, height 175.3 ± 5.0 cm, body mass 68.2 ± 6.2 kg, body fat 7.9 ± 1.2 %, sum of 6 skinfolds 50.2 ± 11.4 mm) took part in this study. These runners competed in races ranging from 10 km to the half-marathon (their best time in this distance ranged from 71 to 94 min). The subjects and their coaches were informed about the experimental procedures and the possible risks and benefits of their participation. Written informed consent was obtained from all runners before the start of this study, which met the ethical standards of this journal [19] and was conducted in agreement with the guidelines of the local institutional review board. Subjects were not taking any medications or substances known to influence physical performance. Testing was performed in the autumn, when runners had already completed at least 2 months of endurance base training.

Study design

A predictive study was conducted to determine the MLSSV from a maximal multistage running field test. For each subject, testing was conducted over 4–7 sessions, each separated by at least 2 resting days. During the first session, anthropometric measurements and a multistage maximal running field test were performed. Height and body mass were determined using a medical stadiometer and scale (Año Sayol, Barcelona, Spain) to a precision of 0.001 m and 0.01 kg, respectively. 6 skinfold thicknesses (triceps, subscapular, suprailliac, abdominal, front thigh, medial calf) were summed to provide an index of subcutaneous adiposity. Body fat percentage was calculated using the formula 2.585 + 0.1051·sum of 6 skinfolds [14]. Measurements were taken by an experienced investigator in accordance with the guidelines from the International Society for the Advancement in Kineanthropometry. A skinfold caliper accurate to 0.2 mm (Holtain Ltd., Crosswell, UK) was used. In the remaining sessions, several 30 min CVTs were performed to determine the MLSSV. Testing was conducted at the same time of day (±2h) for each subject, on days with no or very light wind (wind speed < 10 km·h−1). All subjects were required not to engage in any vigorous activity during the 2 days before each test. To facilitate the replenishment of carbohydrate stores between the testing sessions, the subjects received specific instructions on how to increase their dietary carbohydrate intake during the period of the study.

Maximal multistage running test

A modified version of the University of Montreal Track Test (UMTT) [25], a maximal incremental multistage running field test, was used. The test started at 8 km·h−1 and velocity was increased by 1 km·h−1 every 2 min [10, 11]. The UMTT was conducted around an athletic track where 16 red cones were placed at every 25 m on the 400 m inside lane [10, 11]. Running pace was determined by audio beeps emitted from a pre-recorded digital audio file. Each time a beep sounded, the runner had to be reaching one of the cones. When the runner could not keep the imposed running pace, and failed to reach the cone in time for the beep on 2 consecutive occasions, the test was terminated. This track configuration and digital audio system allowed running pace to be set easily and accurately. Subjects were encouraged to give a maximal effort. According to previous research [1,24], MAV was estimated as follows:

\[ MAV = \text{Velocity of last completed stage} \left( \text{km} \cdot \text{h}^{-1} \right) + \left[ t \left( \text{s} \right) / 120 \right] \cdot \text{stage increment} \left( \text{km} \cdot \text{h}^{-1} \right) \]

where ‘t’ is the time sustained during the incomplete stage. HR was registered at 5 s intervals using a HR monitor (Sporttester, Polar, Kempele, Finland) and maximum HR (HRmax) was considered as the highest recorded value. HR was plotted against running velocity, and a second-degree polynomial regression fit was calculated. The resulting formula was used to determine the running velocities corresponding to 70, 80 and 90% of HRmax.

Constant velocity tests for the determination of MLSSV

Subjects completed three to six 30 min constant velocity tests (CVTs). Track configuration was the same as that used for the UMTT. Running pace for the CVT tests was imposed with sound (beep) protocols that were pre-recorded in MP3 audio format and the appropriate file was selected for each CVT. Each CVT consisted of 5 stages of 6 min running (5×6 min) at constant
pace, with 30 s interruptions after every 6 min for blood sampling; thus, total test duration was 32 min. The reason for choosing 6 min running stages, instead of the more usual 5 min stages, was that an increment of 0.25 km·h\(^{-1}\) in running velocity (our precision in the MLSS\(_V\) determination) corresponded to completing exactly 25 m more distance during each 6 min stage. Thus, from a practical standpoint, this information enabled us to know where exactly on the track the subjects were going to finish each stage, facilitating blood sampling and field test development. An increase \(>0.9\) mmol·L\(^{-1}\) in [La\(^-\)] during the last 18 min of exercise (0.05 mmol·L\(^{-1}\)·min\(^{-1}\)), i.e., the difference in [La\(^-\)] at the end of the 5\(^{th}\) and 2\(^{nd}\) stages, was defined as the criterion for lactate to be considered at a steady state (R. Beneke, personal communication, October 15, 2004). The MLSS\(_V\) was defined as the highest running velocity meeting this stability criterion. Running velocity of the first CVT corresponded to \(-83\%\) of the MAV reached during the UMTT. Depending on the result of this test, the velocity was increased or decreased in the following tests. If during the first CVT a steady state or decrease in [La\(^-\)] was found, the velocity for the next CVT was increased by 0.50 km·h\(^{-1}\). Conversely, if an increase in [La\(^-\)] superior to the stability criterion was observed, running velocity for the next CVT was decreased by 0.50 km·h\(^{-1}\). This process of increasing or decreasing running velocity by 0.50 km·h\(^{-1}\) or later by 0.25 km·h\(^{-1}\), in subsequent tests was further repeated until MLSS\(_V\) could be determined to a precision of 0.25 km·h\(^{-1}\). HR was averaged every min of exercise.

**Blood sampling**

A 5 μL sample of whole blood was aspirated from a hyperemized earlobe into an enzyme-coated electrode test strip. [La\(^-\)] was determined via amperometric measurement using a portable analyzer (Lactate Pro LT-1710; Arkray, Japan) calibrated before every test. This analyzer has been shown to have high accuracy, reliability and linearity and deemed suitable for research [4]. Manufacturers report a CV of 3.2% and 2.6% for lactate standards of 2 and 11 mmol·L\(^{-1}\), respectively.

**Statistical analyses**

Standard statistics were used for the calculation of means and standard deviations (SD). Normal data distribution was confirmed with the Shapiro-Wilk test. A repeated measures ANOVA with Bonferroni post-hoc tests was used to compare [La\(^-\)] and HR at different time points during the CVTs. Pearson product-moment correlation coefficients (\(r\)) were used to determine associations between variables. The adjusted \(R^2\) was used to assess the proportion of variance explained by the independent variables. Validity of the MLSS\(_V\) predictions was investigated by the standard error of the estimate (SEE) and by the 95% limits of agreement method (mean difference±1.96SD) originally reported by Bland and Altman [13]. A regression analysis between mean MLSS\(_V\) and MLSS\(_V\) difference was applied to explore whether the degree of systematic error was uniform over the range of MLSS\(_V\) values studied [3]. A stepwise regression analysis to predict MLSS\(_V\) (dependent variable) from variables derived from the UMTT (independent variables) was performed. [La\(^-\)] at minutes 6, 12, 18, 24 and 30 of the CVT, as well as the difference of [La\(^-\)] and HR between the 30\(^{th}\), 24\(^{th}\), 18\(^{th}\), 12\(^{th}\) and 6\(^{th}\) min of exercise during the CVT, were used as independent variables to predict MLSS\(_V\) from the CVT. The difference between the running velocity of the CVT (\(V_{CVT}\)) and MLSS\(_V\) was employed as a dependent variable. Statistical significance was set at \(P \leq 0.05\). Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, USA).

**Results**

### UMTT and CVT tests

Test results are presented in Table 1. Fig. 1a shows the [La\(^-\)] at the MLSS\(_V\) and at a 0.25 km·h\(^{-1}\) faster velocity than MLSS\(_V\) (MLSS\(_V+0.25\)). All runners completed the CVT at MLSS\(_V\) whereas 3 runners could not finish the CVT at MLSS\(_V+0.25\) due to exhaustion. From the 12\(^{th}\) min to the end of the exercise trial at MLSS\(_V+0.25\), [La\(^-\)] increased > 0.9 mmol·L\(^{-1}\) (1.6 ± 1.4 mmol·L\(^{-1}\); range: 1.0–6.6), [La\(^-\)] at minutes 12, 18 and at the end of the CVT was higher at MLSS\(_V+0.25\) than at MLSS\(_V\) (\(P < 0.05\)). HR at MLSS\(_V\) and at MLSS\(_V+0.25\) is shown in Fig. 1b. Average HR values at MLSS\(_V+0.25\) were 3–4 beats·min\(^{-1}\) higher than at MLSS\(_V\), although the differences were not statistically significant (\(P = 0.07\)). When expressed as a percentage of the HR\(_{max}\) attained during the UMTT, HR at the MLSS\(_V\) corresponded to 88 ± 4% HR\(_{max}\) after 6 min of exercise, increased to 90 ± 3% HR\(_{max}\) at 12 min, and reached 91 ± 2% HR\(_{max}\) at the end (30 min of running) of the CVT (Fig. 2).

Fig. 3a shows that MLSS\(_V\) correlated with MLSS\(_V\) expressed as a percentage of MAV in this sample of endurance runners, yielding the following equation:

\[
\text{MLSS} \% = 60.112 + 2.957 \times \text{MLSS}_V
\]

**Table 1** Variables obtained from the UMTT and CVT tests (\(n = 20\)).

<table>
<thead>
<tr>
<th>Test</th>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMTT</td>
<td>MAV (km·h(^{-1}))</td>
<td>18.2 ± 1.1</td>
<td>16.6–19.7</td>
</tr>
<tr>
<td></td>
<td>HR(_{max}) (beats·min(^{-1}))</td>
<td>159 ± 6</td>
<td>179–205</td>
</tr>
<tr>
<td></td>
<td>Running velocity at 70% HR(_{max}) (km·h(^{-1}))</td>
<td>9.3 ± 1.3</td>
<td>7.3–11.4</td>
</tr>
<tr>
<td></td>
<td>Running velocity at 80% HR(_{max}) (km·h(^{-1}))</td>
<td>11.3 ± 1.7</td>
<td>8.4–13.8</td>
</tr>
<tr>
<td></td>
<td>Running velocity at 90% HR(_{max}) (km·h(^{-1}))</td>
<td>14.2 ± 1.5</td>
<td>12.0–16.5</td>
</tr>
<tr>
<td>CVT</td>
<td>MLSS(_V) (km·h(^{-1}))</td>
<td>15.2 ± 1.0</td>
<td>13.3–17.0</td>
</tr>
<tr>
<td></td>
<td>MLSS(_V) ( % MAV)</td>
<td>83.5 ± 3.4</td>
<td>77.5–89.8</td>
</tr>
<tr>
<td></td>
<td>[La(^-)] at MLSS (mmol·L(^{-1}))</td>
<td>4.4 ± 1.1</td>
<td>2.5–6.7</td>
</tr>
<tr>
<td></td>
<td>HR at MLSS(_V) during UMTT (beats·min(^{-1}))</td>
<td>173 ± 8</td>
<td>163–188</td>
</tr>
<tr>
<td></td>
<td>HR at MLSS(<em>V) during CVT ( % HR(</em>{max}))</td>
<td>92.6 ± 2.7</td>
<td>86.3–97.3</td>
</tr>
</tbody>
</table>

UMTT: Modified University of Montreal Track Test; MAV: maximal aerobic velocity; HR\(_{max}\): maximum heart rate; [La\(^-\)] at MLSS: blood lactate concentration at the maximal lactate steady state (average concentration measured at minutes 12, 18, 24 and 30); CVT: 30 min constant velocity running test; MLSS\(_V\): running velocity at the maximal lactate steady state; HR at MLSS\(_V\): heart rate observed during the UMTT when running at MLSS\(_V\).
Fig. 3b shows the relationship between the individual MLSSV values and the percentages of \( HR_{\text{max}} \) observed after 18 min (\( HR_{18} \)) of constant running at the MLSSV in the present sample of endurance runners, the previous sample of soccer players [27], and both groups combined. No significant correlation was observed in the endurance runners between both variables, whereas a significant but moderate correlation had been observed in soccer players [27]. When the data from both groups of endurance runners and soccer players were pooled together, the magnitude of the correlation increased noticeably and reached statistical significance, albeit the correlation magnitude was moderate (\( r = 0.42; P < 0.01 \)). This relationship yielded the following equation:

\[
HR_{18}(\%HR_{\text{max}}) = 80.454 + 0.678 \times MLSSV
\]  

[Equation 3]
Analysis of the variance, and yielding the equation:

\[ \text{MLSS}_V = 0.756 \times \text{MAV} \]  

[Equation 4]

Stepwise linear regression analyses did not identify any other significant determinants of MLSS\(_V\) derived from the UMTT. The Bland-Altman plot of \(\Delta\text{[La-]}_{30-6}\) shows good agreement between the predicted and actual MLSS\(_V\) based on the low bias and relatively narrow limits of agreement (Bias: 95% confidence interval; \(-0.009\) to 1.17 km·h\(^{-1}\)). The gradient of the regression line was not different from zero (P = 0.14).

**Prediction of the MLSS from a single CVT**

Stepwise linear regression analysis identified the following key determinants for the prediction of the MLSS\(_V\) from the running velocity of the first CVT (\(V_{\text{CVT}}\):

\[ \text{MLSS}_V = V_{\text{CVT}} + 0.503 - (0.266 \times \Delta\text{[La-]}_{30-6}) \]  

[Equation 5]

\(\Delta\text{[La-]}_{30-6}\) accounted for 66% of the variance (P < 0.001; SEE = 0.30 km·h\(^{-1}\)).

**Discussion**

One of the main findings of this study was that the MAV attained during a UMTT was a strong predictor of the MLSS\(_V\) in a homogeneous group of endurance trained runners, accounting for 63% of the variance. No other variable under study provided a better estimation of MLSS\(_V\) than that obtained with the MAV alone. In our previous study [27], performed with soccer players who showed 25% lower average MLSS\(_V\) values (12.2 km·h\(^{-1}\)), the prediction of MLSS\(_V\) from MAV presented a lower correlation magnitude \((R^2 = 52\%)\) compared to that of the present group of endurance runners. However, in that case, when the running velocity corresponding to 80% HR\(_{\text{max}}\) was added to the prediction model, the combination of this variable and MAV accounted for 60% of the explained variance, a value which is similar to the explained variance \((R^2 = 63\%)\) observed for the endurance runners sample of the present study. These findings are in line with previous research showing that maximal workload or the workload/velocity at VO\(_{2\text{max}}\) obtained during a maximal incremental test in cycling [5–7, 9, 15, 36], rowing [6–8], running [22, 29] and...
speed-skating [7] are significant determinants of the MLSS. The explained variance by MAV in this study is, however, among the lowest values reported in the literature (44–90%). Differences such as homogeneity of the sample, test protocol characteristics and specificity, precision and stability criterion in the MLSS determination, as well as the exact variables derived from the incremental test chosen for each study might explain these differences. For instance, endurance trained runners in the present study were homogeneous in terms of MLSSV (CV 6.6%) and the determination of the MLSSV was very accurate (±0.25 km·h⁻¹; ±1.6% of mean MLSSV). In contrast, most of the abovementioned studies used more heterogeneous samples (CV 7–17%) and lower precision (3–10%) in their MLSS determinations, which are factors that can bias the comparisons of the explained variances between studies. The present findings, and those of our previous study [27] confirm that the running velocity attained at the end of a maximal incremental test is a good predictor of the MLSSV in subjects with MLSSV values ranging between 11 and 17 km·h⁻¹. Previous studies have shown that the maximal workload attained during an incremental test to exhaustion is as good or better predictor of the MLSS than other invasive, more expensive or difficult-to-measure lactate or ventilatory-related methods, such as the onset of blood lactate accumulation [8,22,36], individual anaerobic threshold [8], Dmax [36], lactate minimum test [22], lactate turn-point [22], lactate threshold [29], or the first and second ventilatory thresholds [15,22]. Therefore, in terms of accuracy, simplicity and cost-effectiveness, the maximal workload or velocity attained during a multistage maximal test can be considered the best single predictor of the MLSS.

The prediction of MLSSV from MAV in this study resulted in a SEE of 0.61 km·h⁻¹, which is only 4% of the mean MLSSV. This SEE is very similar to the one previously found in soccer players (0.43 km·h⁻¹; 3.5% of the mean MLSSV) [27] and compares favorably with other studies predicting MLSSV from the velocity corresponding to a 4 mmol·L⁻¹ [La⁻] and velocity at the maximal constant HR maintainable for 30 min, where SEE values of 0.67 km·h⁻¹ (5.5% of the mean MLSSV) have been reported [37]. The *Bland-Altman* limits of agreement (−1.17 to 1.17 km·h⁻¹ or ±7.7% of mean MLSSV) of equation 4 are narrower or only slightly higher compared to those of other studies predicting MLSSV from a 1600 m time trial (−0.8 to 0.7 km·h⁻¹ or ±6.0% of the mean) [31], lactate minimum test (−0.9 to 0.7 km·h⁻¹ or ±6.6% of the mean) [22,34], power output at a fixed blood lactate concentration (±10.3% of the mean) [17], power output from the minimum equivalent of the blood lactate-power relationship plus 1.5 mmol·L⁻¹ (±9.5% of the mean) [17], velocity associated with a respiratory exchange ratio equal to 1.00 (−1.2 to 1.6 km·h⁻¹ or ±9.0% of the mean) [26] or ventilatory threshold (−1.3 to 2.5 km·h⁻¹ or ±12.0% of the mean) [26], in cyclists [17], runners [22,26] and physically active men [33,34]. This evidence lends further support to the finding that MAV, a non-invasive, cheap and objective variable, may provide a similar or better estimation of the MLSS intensity than other more sophisticated, expensive and time consuming lactate or ventilatory-related thresholds.

A significant relationship was observed between the MLSSV and the percentage of MAV at which MLSSV occurred, as we previously observed in male soccer players [27]. When the groups of endurance runners and soccer players were combined (Fig. 3a), the correlation improved noticeably and reached a large magnitude (r = 0.89). This finding seems to indicate that, within MLSSV values ranging from 11 to 17 km·h⁻¹, those subjects with higher values of MLSSV are more likely to possess their MLSSV at a higher percentage of MAV than those with lower MLSSV values, regardless of the type of sport practiced. This finding is in agreement with previous studies showing that experienced endurance trained athletes attain their lactate or ventilatory related thresholds at a higher %VO₂max or %MAV compared to lower level or less experienced athletes [28,38]. This relationship allows the prediction of MAV from MLSSV when MLSSV values are known and a maximal test is not feasible or desirable. This may be useful for coaches and athletes who prescribe training intensities as percentages of MAV.

During exercise at MLSSV, absolute HR differed markedly between subjects but relative HR (%HRmax) was maintained within a reasonably narrow range over time. Between 18 and 24 min of exercise at MLSSV, average HR was −90–91% HRmax. This finding is consistent with previous research carried out with samples of well trained endurance runners (average MLSSV between 14.5 and 15.8 km·h⁻¹), which showed that average HR after 20 min of exercise at the MLSSV ranged from 89 to 94% of HRmax [2,16,26,32]. Furthermore, no significant relationship (r = 0.31; P = 0.187) was observed in our sample of endurance trained runners between their MLSSV and their percentage of HRmax at 18 min of constant exercise at MLSSV (Fig. 3b). However, when the present sample of runners was combined with the group of soccer players of our previous study [27], the magnitude of the relationship increased noticeably and reached a significant, albeit moderate, correlation (r = 0.42; P < 0.01; Fig. 3b). Similar to that observed for the MLSSV vs. MLSSV (% MAV) relationship (Fig. 3a), this finding seems to indicate that those subjects with higher MLSSV values may be able to reach HR values closer to their HRmax while exercising at MLSSV when compared to those with lower MLSSV values. In agreement with others [32], this finding could lead to suggest that the MLSS could be estimated non-invasively, and with acceptable precision, during constant velocity running, based solely on the basis of a percentage of HRmax. However, Fig. 3b also shows that the individual values of %HRmax after 18 min of exercise at the MLSSV varied considerably between subjects (84–96% HRmax), which indicates that the HR zone corresponding to MLSS should be estimated on an individual basis [18]. An interesting and practical observation derived from Fig. 3b is that when HR values at 18 min of exercise at MLSSV were lower than 85% of HRmax, none of the soccer players [27] or the endurance runners was exercising above his MLSSV. We therefore may conclude that, at least in this sample of subjects, when the individual assessment of MLSS is not possible, exercising below 85% HRmax may prevent individuals from exceeding their MLSS. This has practical relevance since it has been suggested that training at or below the MLSS intensity may optimize training adaptations [31], may constitute the most time-efficient tradeoff between the volume and intensity of endurance training, and may contribute to reduce the risk of overreaching and overtraining [32,35].

The second purpose of the present study was to investigate whether the MLSS could be predicted from a single CVT after having performed a maximal multistage test several days before. The results show that Δ[La⁻] between 6 and 24 min (R² = 62%) or between 12 and 30 min (R² = 55%) of exercise.
Prediction of MLSS\textsubscript{V} using equation 5 (between 6 and 30 min) resulted in a SEE of 0.30 km·h\textsuperscript{−1}, which is only 2.0\% of the mean MLSS\textsubscript{V}, whereas the Bland-Altman plot's limits of agreement clearly demonstrated a good precision (0.57 km·h\textsuperscript{−1} or ±3.8\% of the mean). These values are similar to those observed with soccer players [27], and compare favorably with the prediction of MLSS\textsubscript{V} from MAV (equation 4), as well as with those of other studies estimating MLSS from blood lactate or ventilatory-related measurements obtained during a maximal incremental test [8,15,22,29,36]. The better precision in the MLSS\textsubscript{V} prediction from the CVT compared to that of other studies could result in a better estimation of MLSS for individual subjects. It is therefore suggested that, in situations in which blood lactate assessment is available, and only 2 testing sessions are allowed (one maximal incremental test and one CVT), the change in [La\textsubscript{\text{\textsuperscript{−}}} from the 6\textsuperscript{th} to the 30\textsuperscript{th} min of a single CVT (at a suggested velocity of ±83\% MAV) could be considered the best predictor of MLSS\textsubscript{V} in endurance runners.

The present investigation is limited in some aspects. First, with regards to the experimental protocol, the exercise field tests were performed in an outdoor running track (400 m). Although more ecologically valid, when compared with a controlled laboratory environment, field testing external conditions (wind, ambient temperature, relative humidity, floor surface characteristics and gradients, body aerodynamics, etc.) may induce higher day-to-day variability and influence the performance variables analyzed. Furthermore, the extent to which testing in other indoor or outdoor settings may alter the relation between the variables obtained from a maximal incremental test and a CVT, together with its impact on MLSS\textsubscript{V} determination, is uncertain.

Second, the applicability of the results of the present study and those of our previous one with soccer players [27] is limited to subjects with MLSS\textsubscript{V} values ranging from 11 to 17 km·h\textsuperscript{−1}. Even though the vast majority of MLSS\textsubscript{V} values of elite male athletes from most sports fall within that range, caution should be taken when generalizing these results to other populations, especially to those with significantly different MLSS\textsubscript{V} values, such as to world-class endurance athletes with higher MLSS\textsubscript{V} values or to people with chronic diseases and disabilities showing much lower MLSS\textsubscript{V} values. Despite these limitations, the results of the present study provide important and novel information about the prediction of the MLSS, which is considered the gold standard for the assessment of endurance capacity.

In conclusion, the present results further support those of our previous study [27] and indicate that when direct blood lactate assessment is undesirable or unfeasible, and only one testing session can be carried out in endurance runners, MAV attained during an incremental test to exhaustion is the strongest predictor of the MLSS\textsubscript{V}, accounting for 63\% of the variance. If direct blood lactate measurement is available and only 2 testing sessions can be arranged: one maximal incremental test and, several days later, one CVT at ~83\% of the MAV, the prediction of MLSS\textsubscript{V} is improved when taking into account the [La\textsubscript{\text{\textsuperscript{−}}} at the 6\textsuperscript{th} and 30\textsuperscript{th} min of the CVT, since they account for 66\% of the variance and show a good limit of agreement (±0.57 km·h\textsuperscript{−1}). A practical guideline for endurance trained runners consisting in exercising below 85\% \text{HR}_{\text{max}} between the 10\textsuperscript{th} and the 20\textsuperscript{th} min during constant velocity exercise can be established to prevent individuals from exceeding their MLSS. The prediction equations reported in this study and in the previous one with young soccer players [27] can be used for the physiological assessment and training prescription of male athletes for most sports in which the subjects’ MLSS\textsubscript{V} values range from 11 to 17 km·h\textsuperscript{−1}. Being able to estimate the MLSS with acceptable precision from one or 2 relatively simple and inexpensive field tests, is a reasonable alternative to reduce costs and considerably alleviate the burden associated with classical MLSS assessment.

**Conflict of interest:** The authors have no conflicts of interest to declare.

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