TITLE

Lactate Equivalent for Maximal Lactate Steady State Determination in Soccer

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ABSTRACT

Purpose: The association between an overlooked classical Lactate Threshold (LT), named “Minimum Lactate Equivalent” (LE_{min}), with Maximal Lactate Steady State (MLSS) has been recently described with good MLSS prediction results in endurance-trained runners. This study aimed to determine the applicability of LE_{min} to predict MLSS in lower aerobic-conditioned individuals compared to well-established blood lactate-related thresholds (BLTs). **Method:** Fifteen soccer players [velocity at MLSS ($MLSS_V$) 13.2 ± 1.0 $km \cdot h^{-1}$; coefficient of variation (CV) 7.6%] conducted a submaximal discontinuous incremental running test to determine BLTs and 3-6 constant velocity running tests to determine $MLSS_V$. **Results:** LE_{min} did not differ from conventional LTs ($P > 0.05$) and was 24% lower than MLSS ($P < 0.001$; ES: 3.26). Among LTs, LE_{min} best predicted $MLSS_V$ ($r = 0.83$; $P < 0.001$; SEE = 0.59 $km \cdot h^{-1}$). There was no statistical difference between MLSS and estimated MLSS using LE_{min} prediction formula ($P = 0.99$; ES: 0.001). Mean bias and limits of agreement were 0.00 ± 0.58 $km \cdot h^{-1}$ and ±1.13 $km \cdot h^{-1}$, respectively. LE_{min} best predicted $MLSS_V$ ($r = 0.92$; $P < 0.001$; SEE = 0.54 $km \cdot h^{-1}$) in the pooled data of soccer players and endurance-trained runners of the previous study ($n = 28$; $MLSS_V$ range 11.2-16.5 $km \cdot h^{-1}$; CV 9.8%). **Conclusion**: Results support LE_{min} to be one of the best single predictors of MLSS. This study is the sole study providing specific operational regression equations to estimate the impractical *gold standard* $MLSS_V$ in soccer players by means of a BLT measured during a submaximal single-session test.

KEYWORDS: Owles’ point, aerobic-anaerobic threshold, aerobic capacity assessment, OBLA

Competitive soccer is an intermittent team-sport in which the aerobic energy system is heavily challenged contributing with ≈98% of total energy (Bangsbo, 1994; Reilly & Thomas, 1979). During a competitive soccer game elite players perform short-lasting energy-demanding intense actions interspersed with periods of low-intensity jogging or running. Distance covered by elite field-players is commonly around 7-13 km (Bangsbo & Lindquist, 1992; Reilly & Thomas, 1979) and mean heart rate (HR) values of ≈85% maximal HR (HR_{max}) are usually registered (Bangsbo, 1994; Krustrup et al., 2006; Reilly & Thomas, 1979). Albeit soccer is not an endurance sport per se, clearly a minimum level of aerobic fitness is crucial to maintain an elevated intensity work and recover from periods of high-intensity exercise (Krustrup et al., 2006). The aerobic energy system plays a critical role to increase the rate of lactate removal during the phases that are performed at low intensities and to spare muscle glycogen stores during running at different speeds (Bangsbo, 1994; Krustrup et al., 2006). It has been shown that some aerobic performance markers are able to discriminate between players of different performance levels (Wisloff, Helgerud, & Hoff, 1998), and that an improvement of aerobic endurance can promote more ball involvement (Helgerud, Engen, Wisloff, & Hoff, 2001). It is hence considered that there is an aerobic threshold below which an individual player is unlikely to play in top-class soccer (Mohr, Krustrup, & Bangsbo, 2003; Wisloff et al., 1998). The conditioning staff of a soccer team needs therefore to be aware of the aerobic status of each player to design proper trainings and adequately interpret the data registered during the monitoring of soccer training and competition.

Some on-field intermittent tests, such as the Yo-Yo Intermittent Recovery Test, are commonly used to evaluate the aerobic conditioning of soccer players. These intermittent tests, however, do not solely evaluate aerobic conditioning, since the performance exhibited in them is greatly influenced by the anerobic energy system (Bangsbo, Iaia, & Krustrup, 2008). Besides, the prescription of precise aerobic training zones by means of maximal intermittent tests is complicated and hinders proper aerobic training guidance and monitoring in soccer players (Bangsbo et al., 2008). In this sense, the maximal lactate steady state (MLSS), i.e. the maximal constant workload intensity sustainable with no blood lactate concentration (BLC) accumulation, is considered the *gold standard* endurance performance marker among the vast majority of sport scientists (Beneke, 1995). MLSS determination requires 3-6 constant workload tests performed on separate days. On-field utilization of MLSS for endurance performance diagnosis and training guidance in soccer is therefore certainly hampered. Figure 1 (right panel) illustrates the tedious procedure of MLSS determination in an amateur soccer player. Due to the amateur team's training and competition schedule, no more than one constant velocity running test (CVRT) per week was feasible, lengthening MLSS testing to 6 weeks (5 testing sessions) on this particular player.

Decades earlier to the consolidation of the MLSS concept, Owles (1930) first described that during constant workload exercise tests there was also a critical exercise intensity level unique to each individual above which BLC initiates to increase beyond resting values, i.e. the maximal constant workload intensity sustainable with no BLC accumulation above resting values. In the following years, still preceding MLSS consolidation, this critical workload level, which always occurs at lower intensities than MLSS (Aunola & Rusko, 1988; Faude, Kindermann, & Meyer, 2009; Ferguson et al., 2018) and is frequently called "*Lactate Threshold* (LT)" (Garcia-Tabar & Gorostiaga, 2018), was widely considered as the standard criterion measure to determine aerobic capacity, predict endurance performance, and design endurance exercise training programs (Hollmann, 1985; Mezzani et al., 2012; Weltman et al., 1987).

In an attempt to overcome the shortcomings of multiple-day constant-workload testing, simpler methods have unabatedly been proposed to estimate both *gold standard* BLC thresholds (the classical LT and MLSS) from a single-day incremental exercise test involving generally the use of either blood lactate-related thresholds (BLTs) (Denadai, Gomide, & Greco, 2005; Llodio, Garcia-Tabar, Sanchez-Medina, Ibanez, & Gorostiaga, 2015; Loures et al., 2015) or respiratory exchange-based thresholds (Cerezuela-Espejo, Courel-Ibanez, Moran-Navarro, Martinez-Cava, & Pallarés, 2018). Notwithstanding, there are still relevant methodological limitations on the accurate and rigorous estimation of the classical LT and MLSS during a single incremental exercise test (Brooks, 1985; Cerezuela-Espejo et al., 2018). In a recent investigation (Garcia-Tabar & Gorostiaga, 2018) we found very good MLSS prediction results in male endurance-trained runners [velocity at MLSS ($MLSS_V$) 15.0 ± 1.1 $km \cdot h^{-1}$; maximal oxygen uptake 67.6 ± 4.1 $ml \cdot kg^{-1} \cdot min^{-1}$] by the use of an overlooked BLT, named “Minimum Lactate Equivalent” (LE_{min}) and first described by German authors in the early 1980s (Berg et al., 1990; Berg, Stippig, Keul, & Huber, 1980; Lehmann, Berg, Kapp, Wessinghage, & Keul, 1983). LE_{min} should not be confused with the much more popular “Lactate Minimum Test” (LMT) originally described in the 1990s (Tegtbur, Busse, & Braumann, 1993). Velocity at LE_{min} ($_VLE_{min}$), which is measured during a single-session submaximal discontinuous incremental running test (Berg et al., 1980), is suggested (Aunola & Rusko, 1988; Garcia-Tabar & Gorostiaga, 2018) to objectively represent the classical LT (Owles, 1930). The prediction strength and accuracy recently reported (Garcia-Tabar & Gorostiaga, 2018) for $_VLE_{min}$ as a predictor of $MLSS_V$ are among the highest in the literature (Beneke, 1995; Denadai et al., 2005; Figueira, Caputo, Pelarigo, & Denadai, 2008; Grossl, De Lucas, De Souza, & Antonacci Guglielmo, 2012; Philp, Macdonald, Carter, Watt, & Pringle, 2008; Van Schuylenbergh, Vanden Eynde, & Hespel, 2004; Vobejda, Fromme, Samson, & Zimmermann, 2006), thus advocating the use of $_VLE_{min}$ as a major determinant of $MLSS_V$.

To the best of our knowledge, the preceding study (Garcia-Tabar & Gorostiaga, 2018) is the first one in which the association between LE_{min} and MLSS was explored. The generalizability of the results and application of the reported equations are, however, limited to male endurance-trained runners with $MLSS_V$ values ranging from 13.3 to 16.5 $km \cdot h^{-1}$. Therefore, the primary aim of this study was to determine the applicability of the LT, conceptually comprehended as in the old days (Berg et al., 1980; Owles, 1930) (i.e. LE_{min}), to predict MLSS in comparison with well-established BLTs in lower aerobic-conditioned soccer players with $MLSS_V$ values that range somewhat around 11-13.5 $km \cdot h^{-1}$

(Llodio et al., 2015). This study could therefore deliver straightforward practical implications for soccer teams in need of time-efficient non-maximal tests to monitor aerobic capacity.

Methods

Experimental design

A predictive cross-sectional study was conducted to determine $MLSS_V$ from a single-session submaximal discontinuous incremental running test. The study was conducted over 5-8 testing sessions. The first 2 were laboratory-testing sessions. The remaining ones were field-testing sessions. The first session served as a familiarization session to accustom participants to the laboratory testing procedures. This session was also utilized for anthropometric evaluation. During the second laboratory testing-session players completed a submaximal discontinuous incremental running test for BLTs determination, followed by a maximal ramp incremental running test for peak treadmill velocity (PTV) and HR_{max} determination. In the remaining sessions 3-6 CVRTs were conducted for $MLSS_V$ determination.

Participants

Fifteen male amateur outfield soccer players (age 21.9 ± 1.4 yrs, body mass 73.9 ± 7.3 kg, body fat percentage 8.7 ± 2.7%) from a Spanish fourth division soccer team completed the study. Experimental procedures were fully explained to participants, the coach, and the conditioning staff. Participants were free of known cardiorespiratory dysfunction, and were not taking any substances that could have altered the results of the study. Participants acknowledged voluntary participation through written-informed consent. Procedures were approved by the Local Institutional Review Board which conformed to the Declaration of Helsinki.

Procedures

Testing sessions were: (1) integrated into the team's weekly training routine, (2) performed 1-week apart, at the same time of the day, (3) preceded by 2 days of rest or very light exercise and (4) separated by at least 48h from the last competitive game. Subjects were asked to replicate diet and exercise regimens the 2 days preceding each testing session. Testing took place during April-May, i.e. during the final weeks of the competitive season. Laboratory sessions were conducted in ambient (temperature 20.4 ± 0.3ºC; humidity 25 ± 2%; barometric pressure 724 ± 2 mmHg) controlled conditions. During on-field testing-sessions ambient conditions (temperature 22.4 ± 1.4ºC; humidity 33 ± 4%; barometric pressure 722 ± 4 mmHg) were measured (Precision Barometer, Lufft, Germany) and wind velocity (16.7 ± 9.9 km·h^{-1}) obtained from the nearest weather station.

Submaximal discontinuous and maximal ramp incremental running tests.

A submaximal discontinuous incremental running test for BLTs determination, followed by a maximal ramp incremental running test for PTV and HR$_{max}$ determination were conducted on the same running ergometer (Kuntaväline, Hyper Treadmill 2040, Finland) with the gradient set at 1% following procedures previously utilized (Garcia-Tabar & Gorostiaga, 2018). The initial velocity and velocity increments defining the submaximal discontinuous and maximal ramp incremental running tests were lower than in our previous study conducted on endurance-trained runners (Garcia-Tabar & Gorostiaga, 2018) in an attempt to obtain similar trial durations in both studies. The submaximal trial began at 7 km·h^{-1}. Speed was increased by 0.6 km·h^{-1} every 2-min, with 1-min rest pauses between stages for lactate sampling. Two-min stage duration was chosen following previous LE$_{min}$ detection protocols (Aunola & Rusko, 1988; Berg et al., 1990; Lehmann et al., 1983). The submaximal discontinuous incremental running test terminated when a BLC ≥3 mmol·L^{-1} was observed. After a 10-min rest, subjects began the maximal ramp incremental running test. Initial speed was 8 km·h^{-1} and was increased by 0.8 km·h^{-1} every min until volitional exhaustion. HR during both trials was monitored (Polar M400, Polar Electro OY, Finland) and averaged over 30-s. HR_{max} and PTV were determined according to previous procedures (Garcia-Tabar & Gorostiaga, 2018).

Constant velocity running tests (CVRTs).

After the laboratory-sessions, players conducted 3-6 on-field CVRTs on an outdoor artificial-grass soccer pitch (100x50 m). To assure a constant velocity, red pylons were placed every 25-m around the pitch and running pace was set by a customized pre-programmed (MATLAB R2015a, The MathWorks Inc., USA) audio protocol file which was played from an audio-emitting computer (Balise Temporelle, Bauman, Switzerland) and subsequently transferred to portable MP4-players (Sporty II, Sunstech, China). Every player was vigorously encouraged to complete every audio beep. If the subject was ≥ 10 m behind the appropriate pylon at the sound signal, the test was finalized prematurely (Leger & Boucher, 1980). Each CVRT consisted of 30-min running with 1-min interruption every 10-min (Garcia-Tabar & Gorostiaga, 2018). Capillary blood samples were obtained at rest, at min 10 and at the end of exercise. An increase in BLC <1.0 mmol·L^{-1} during the last 20 min of exercise was defined as the criterion for BLC to be considered at a steady state (Beneke, 1995). $MLSS_V$ was defined as the highest running velocity meeting this stability criterion. Velocity of the first CVRT corresponded to ≈70% of the PTV achieved at the maximal ramp incremental running test. Depending on the BLC stability of this first CVRT, the velocity was increased or decreased in the following CVRTs by 0.4 km·h^{-1}, and later by 0.2 km·h^{-1}, until $MLSS_V$ was determined with a precision of 0.2 km·h^{-1} (Figure 1, right panel). HR was monitored and averaged as abovementioned.

Determination of blood lactate-related thresholds (BLTs).

From the data collected during the submaximal discontinuous incremental running test, nine different BLTs were determined: two conventionally-calculated LTs ($LT_{0.2mM}$ and LT_1) (Stratton et al., 2009; Weltman et al., 1987), three lactate equivalent (LE) related thresholds (LE_{min}, $LE_{min+1mM}$ and

$LE_{min+1.5mM}$) (Berg et al., 1990), maximal-deviation threshold (D_{max}) (Cheng et al., 1992), and three fixed blood lactate concentration (FBLC) thresholds ($FBLC_{2mM}$, $FBLC_{2.5mM}$ and $FBLC_{3mM}$) (Garcia-Tabar, Izquierdo, & Gorostiaga, 2017; Seiler, 2010). Determination of BLTs is described in Figure 1 (left panel). For further methodological details of BLTs determination readers are referred to the previous free-access publication (Garcia-Tabar & Gorostiaga, 2018). Blood sampling and BLC measurement procedures during the submaximal discontinuous incremental running test, maximal ramp incremental running test and CVRTs have also been detailed in the mentioned publication. It is worth mentioning that the term LT used in the present investigation refers to thresholds trying to identify the first rise in BLC, as described in the classical literature (Ferguson et al., 2018). The term BLT, however, refers to any threshold determined from the BLC vs. workload curve of an incremental exercise test.

Statistical analysis

Data were analyzed using parametric statistics following confirmation of normality (Kolmogorov–Smirnov test), homoscedasticity (Levene´s test), and when appropriate sphericity (Mauchly's test). Student's paired *t*-tests were used to evaluate differences between each BLT with MLSS and LE_{min}. The magnitudes of the differences were assessed using 90% confidence intervals (CI) and Hedges' *g* effect sizes (ES). Differences in BLC and HR between the sampling time-points during the CVRTs were identified by one-way repeated measures ANOVA with Bonferroni correction for multiple comparisons. Two-factorial ANOVA with the Scheffé post-hoc test was used to identify differences in BLC and HR between the CVRTs at $MLSS_V$ and at 0.2 km·h^{-1} above the $MLSS_V$ ($_VMLSS_{+0.2}$). Linear regression analyses with *Pearson*'s correlation coefficients (*r*) were performed to determine the relationships between the variables of interest. ES and *r* values were interpreted as described elsewhere (Hopkins, Marshall, Batterham, & Hanin, 2009). Agreement with the reference method ($MLSS_V$) was assessed by mean bias and limits of agreement (LOAs) (Krouwer, 2008). Post-hoc power calculation for the linear regressions, assuming type I error of 0.05, indicated a power >99%. Analyses were performed using SPSS Statistics 22 (IBM Corporation, USA). Significance was set at $P < 0.05$ for the analyses that did not require post-hoc adjustment. Descriptive statistics are reported as means ± (standard deviation). Coefficient of variation (CV) is also reported when needed.

Results

The submaximal discontinuous incremental running test lasted 38:48 ± 07:29 min:s. Figure 2 depicts BLC and $\%HR_{max}$ mean pattern response to the submaximal discontinuous incremental running test. Treadmill velocity, BLC and $\%HR_{max}$ at completion of the submaximal discontinuous incremental running test were 14.4 ± 1.5 $km \cdot h^{-1}$ (range 10.6-16.0), 3.3 ± 0.5 $mmol \cdot L^{-1}$ (range 3.0-4.7) and 91 ± 2% (range 87-94), respectively. BLC resting values prior to the maximal ramp incremental running test were 1.1 ± 0.2 $mmol \cdot L^{-1}$ (range 0.8-1.4). The maximal ramp incremental running test lasted 13:11 ± 01:22 min:s. PTV, HR_{max} and BLC attained were 17.8 ± 1.1 $km \cdot h^{-1}$ (range 16.0-19.4), 195 ± 7 $beats \cdot min^{-1}$ (range 183-207) and 8.1 ± 2.7 $mmol \cdot L^{-1}$ (range 5.2-14.5), respectively.

BLC and $\%HR_{max}$ responses to the CVRTs performed at $MLSS_V$ and at ${}_VMLSS_{+0.2}$ are illustrated in Figure 3. BLC during the ${}_VMLSS_{+0.2}$ CVRT increased 1.6 ± 0.5 $mmol \cdot L^{-1}$ ($P < 0.001$; 90% CI: -1.87 to -1.33; ES: 1.23) from min 10 to the end of the trial. During the $MLSS_V$ CVRT, BLC increased 0.5 ± 0.3 $mmol \cdot L^{-1}$ ($P < 0.001$; 90% CI: -0.64 to -0.39; ES: 0.51), but the increment was <1 $mmol \cdot L^{-1}$ in every single case. HR increased ($P < 0.01$) over the course of both $MLSS_V$ and ${}_VMLSS_{+0.2}$ CVRTs. HR ($\%HR_{max}$) at min 10, 21 and 32 of the $MLSS_V$ CVRT were 87 ± 3 (range 83-92), 90 ± 2 (range 84-92) and 91 ± 3% (range 84-94), respectively.

Table 1 reports BLTs and MLSS descriptive values. Among the LTs, ${}_VLE_{min}$ best predicted $MLSS_V$ and PTV (Table 2). Correlation magnitude between ${}_VLE_{min}$ and $MLSS_V$ was 0.83 [$P < 0.001$; standard error of the estimate (SEE) = 0.59; 95% CI: 0.56 to 1.31] (Figure 4A), while the one between $LT_{0.2mM}$ and $MLSS_V$ was 0.69 ($P = 0.005$; SEE = 0.77; 95% CI: 0.19 to 0.84). LT_1 did not correlate with

$MLSS_V$. Correlation magnitudes of $_{V}LE_{min+1mM}$ (Figure 4B) and $_{V}LE_{min+1.5mM}$ (r = 0.84; $P < 0.001$; SEE = 0.58; 95% CI: 0.43 to 0.97) with $MLSS_V$ are identical to the ones previously found in runners (Garcia-Tabar & Gorostiaga, 2018).

There was no statistical difference between $MLSS_V$ and estimated $MLSS_V$ using the $_{V}LE_{min}$ formula reported in Figure 4A ($P = 0.999$; 90% CI: -0.26 to 0.26; ES: 0.001). Mean bias and LOAs were 0.00 ± 0.58 $km \cdot h^{-1}$ and ±1.13 $km \cdot h^{-1}$, respectively, indicating that prediction of $MLSS_V$ from $_{V}LE_{min}$ could be biased up to ≈8.5% above or below actual $MLSS_V$. $_{V}LE_{min+1mM}$ did not differ from $MLSS_V$ ($P = 0.088$; 90% CI: -0.53 to 0.11; ES: 0.27). Mean difference was 0.28 ± 0.6 $km \cdot h^{-1}$ and LOAs were ±1.14 $km \cdot h^{-1}$ (±8.6%).

Discussion

To the best of our knowledge, the present study is the first to describe the associations between LTs (not BLTs) and MLSS in soccer players. It is also the first to provide an equation for the prediction of MLSS from an LT. The reported equation (Figure 4A) is the equation for the prediction of MLSS from LE_{min}, which has turned out to be the best MLSS predictor among the LTs. The high sustained variance by $_{V}LE_{min}$ in $MLSS_V$ prediction (70%) compares favorably with the rest of the previous cross-sectional $MLSS_V$ predictive studies in soccer (Denadai et al., 2005; Llodio et al., 2015; Loures et al., 2015). These studies proposed the velocity associated with a FBLC of 3.5 $mmol \cdot L^{-1}$ (Denadai et al., 2005), the velocity associated with a FBLC of 4 $mmol \cdot L^{-1}$ (Loures et al., 2015), PTV (Llodio et al., 2015) and delta BLC during a CVRT (Llodio et al., 2015) as functional alternatives to $MLSS_V$ determination in soccer with $MLSS_V$ prediction variances reported ranging from 52 to 66%. Homogeneity of the sample, test protocol, precision in MLSS determination, as well as the choice of variables derived from the exercise tests all constitute potential factors affecting the observed differences between studies in the magnitude of correlations. Participants of the present study and our previous investigation in soccer (Llodio et al., 2015) were quite homogeneous in terms of $MLSS_V$ (CV 7.6% and

4.9%, respectively), and determination of $MLSS_V$ were very accurate (±1.5% and ±2.9% mean $MLSS_V$, respectively). Study samples in the rest of $MLSS_V$ prediction publications in soccer (Denadai et al., 2005; Loures et al., 2015) were more heterogeneous (CVs ≈10-12%) and precision in $MLSS_V$ determination was lower (≈4-5%), which are factors that may bias comparisons between studies but further support our results. It is well-established that the greater the heterogeneity of a group, the greater the magnitude of the correlation coefficient. $_{V}LE_{min}$ can be therefore considered as a major $MLSS_V$ determinant in soccer players.

With regard to the prediction accuracy, a relatively low SEE (4.5% mean $MLSS_V$, Figure 4A) was found in the prediction of $MLSS_V$ from $_{V}LE_{min}$. The obtained SEE value 1) is similar to the ≈4% SEE found in our other $MLSS_V$ predictive study performed in a different soccer population (Llodio et al., 2015), 2) is lower than the accuracy in MLSS determination frequently utilized (Beneke, 1995; Loures et al., 2015), and 3) compares favorably with SEE values of ≈6-21% reported by other authors (Figueira et al., 2008; Vobejda et al., 2006). The observed LOAs in this study are also generally narrower compared to those of other studies predicting MLSS from the LMT (±7-16%) (Wahl et al., 2017; Wahl, Zwingmann, Manunzio, Wolf, & Bloch, 2018), FBLC thresholds (±9-18%) (Grossl et al., 2012; Wahl et al., 2017; Wahl et al., 2018), D_{max} (±11-14%) (Jamnick, Botella, Pyne, & Bishop, 2018; Wahl et al., 2017; Wahl et al., 2018) or other BLTs (± 10-17%) (Grossl et al., 2012; Jamnick et al., 2018). Strength and accuracy of $_{V}LE_{min}$ for the prediction of $MLSS_V$ reported in this study lend further support to our previous findings (Garcia-Tabar & Gorostiaga, 2018) and suggest that $_{V}LE_{min}$ can be considered one of the best single predictors of $MLSS_V$.

During the 80s, before the appearance of the MLSS concept and based mainly on the early work of Owles (1930), the classical LT (Berg et al., 1980; Hollmann, 1985; Owles, 1930) became the *gold standard* endurance performance marker (Brooks, 1985; Hollmann, 1985; Mezzani et al., 2012). However, there still exist some relevant methodological limitations that make it difficult to accurately determine the LT from a single incremental test using conventional approaches. These limitations are mainly due to the utilization of subjective and/or imprecise LT identification procedures and unsuitable exercise protocols (Brooks, 1985; Hollmann, 1985). In this sense, LE_{min} (Berg et al., 1980) is suggested (Aunola & Rusko, 1988; Garcia-Tabar & Gorostiaga, 2018) to objectively represent the classical *gold standard* LT (Owles, 1930). Using the appropriate exercise protocol the BLC/workload vs. workload curve displays an idiosyncratic "U-shaped" curve fitting profile allowing mathematical impartial location of the transition at the LT (i.e. $_{V}LE_{min}$) with a very fine resolution (Figure 1, left panel). The reason why LE_{min} would offer significant advantages over FBLC thresholds, conventionally-calculated LTs, or other BLTs (e.g. D_{max} or LMT) can be related to different factors. 1) The resolution in the determination of the LE_{min} is finer than that observed in other BLTs [e.g. conventionally-calculated LTs (Philp et al., 2008) and FBLC thresholds (Denadai et al., 2005; Loures et al., 2015)] because all the data points before and after the transition are used to project the LE_{min} value. 2) Undesired error effects due to statistical scatter of the data points are minimized by the least squares curve-fitting procedure. 3) LE_{min} could essentially take on an infinite number of values using the least squares curve-fitting procedure, whereas LT_1 and $LT_{0.2mM}$ could only be based on the discrete values of the specific velocity-rate stages. 4) The troublesome identification of the first BLC elevation above baseline values (LT) due to initial BLC fluctuations associated with the error of the analyzer (Weltman et al., 1987) is resolved by the "U"-shape of the LE curve used for the identification of LE_{min} without the need of a previous high level of exertion phase to induce hyperlactatemia, as it is required for LMT identification (Tegtbur et al., 1993) which hampers HR data interpretation, and therefore, its on-field application. 5) Relative changes in BLC based on the shape and slope of the BLC/workload vs. workload curve (i.e. LE_{min}) during incremental exercise may be more advantageous, sensitive and robust compared with the use of absolute BLC values (i.e. FBLC thresholds). It is known that BLC absolute values are influenced by substrate availability, exercise protocol, pre-testing physical and hydration status, dietary or pharmacological manipulations, environmental conditions (Dickhuth et al., 1999; Halson, 2014) and subjects' aerobic endurance-related characteristics. The comparison of the present results with those of our previous study (Garcia-Tabar & Gorostiaga, 2018) reveals that the FBLC threshold approximating MLSS differed among the study samples (soccer players vs. runners) used (i.e. $FBLC_{2.5mM}$ vs. $FBLC_{2mM}$). The relevance of LE_{min} is underpinned by the fact that the other two LE_{min}-related thresholds better correlated with $MLSS_V$ in comparison with the conventionally calculated LTs and D_{max}, whereas average $_VLE_{min+1mM}$ (12.9 km·h^{-1}) was similar to average $MLSS_V$ (13.2 km·h^{-1}).. These results, therefore, support LE/running-velocity to be a very good predictor of the individual and group average $MLSS_V$ in soccer players.

The prediction strength of BLTs for the estimation of $MLSS_V$ found in this study are similar to those observed in runners (Garcia-Tabar & Gorostiaga, 2018). Concerning the $_VLE_{min}$ vs. $MLSS_V$ relationship, the correlation magnitude (r = 0.834 vs. 0.912) and prediction accuracy (4.5% vs. 3.1% of mean $MLSS_V$) are slightly lower. The main difference among studies resides in the specific conditions in which the $MLSS_V$ was determined. Thus, while in runners $MLSS_V$ was determined in well-controlled laboratory conditions, field conditions were used for soccer players. Atmospheric conditions (e.g. wind velocity 16.7 ± 9.9 km·h^{-1}, range: 3.7-27.8) and/or other on-field testing limitations such as the feasibility of carrying out the CVRT within a reasonably short period of time (i.e. in runners ≥2 CVRTs per week were feasible while in soccer players a maximum of 1 CVRT session could be scheduled per week) could have influenced the determination of $MLSS_V$ and its relationship with $_VLE_{min}$. Nonetheless, when data from runners (Garcia-Tabar & Gorostiaga, 2018) and soccer players are taken together, evidence supporting the greater predictive capacity of LE-related BLTs over the rest of BLTs becomes clearer. Thus, LE-related BLTs were *extremely largely* correlated (r = 0.90 to 0.92) with $MLSS_V$, whilst the rest of the BLTs correlated *very largely* (r = 0.75 to 0.89) with $MLSS_V$. The sustained variance (85%) by $_VLE_{min}$ (Figure 5A) and $_VLE_{min+1mM}$ (Figure 5B) in $MLSS_V$ prediction in the combined population of runners and soccer players ($MLSS_V$ range 11.2-16.5 km·h^{-1}; CV 9.8%) is among the highest reported in the literature. In addition, the pooling of data from the runners and soccer players showed ${}_{V}LE_{min+1mM}$ to be the only BLT not differing from $MLSS_V$ with average values being nearly identical (14.0 vs. 14.1 km·h^{-1}). Taken together, these results seem to indicate that LE/running-velocity is a superior predictor of the individual and group average $MLSS_V$ when compared to well-established BLTs. Thus, LE is advocated as a major $MLSS_V$ determinant in individuals with $MLSS_V$ values ranging from 11.2 to 16.5 km·h^{-1}.

Finally, we must acknowledge that the present study is not limitation-free. First, the on-field MLSS testing might have induced higher day-to-day variability due to external conditions (wind, ambient temperature, relative humidity, floor surface characteristics, body aerodynamics, etc.) and could have slightly altered the relationships between variables derived from the submaximal discontinuous incremental running test vs. CVRTs, as well as the determination of $MLSS_V$. Nevertheless the present investigation was a field-based study conducted during regular in-season soccer competition, and hence, the study design might have enhanced the applicability of the results. Second, the applicability of the results is limited to male individuals with $MLSS_V$ values ranging from 11.2 to 16.5 km·h^{-1}. Even though the vast majority of $MLSS_V$ values of male athletes from most sports fall within this range (Garcia-Tabar et al., 2017), caution should be taken when generalizing these results to other populations with higher or lower levels of aerobic conditioning. Third, validation of the prediction equations presented in this study would be needed in different and larger populations and in different gender and age specific samples before ${}_{V}LE_{min}$ can be established for mass field testing. Fourth, a test-retest analysis of LE_{min} was beyond the scope of this study, and therefore, the extent to which LE_{min} is a reliable measure was not assessed, although a good test-retest reproducibility of LE_{min} determined during a submaximal discontinuous incremental running test in males has been previously reported (Dickhuth et al., 1999). Finally, the submaximal discontinuous incremental running test protocol characteristics such as initial running speed and subsequent speed increments might influence the resolution in the determination of the velocity corresponding to LE_{min}. Thus, the obtained prediction equations (Figures 4 and 5) are recommended to be used only when identical testing protocols and procedures to those employed in this study are followed.

Conclusion

In summary, the results of the present study reinforce our previous results (Garcia-Tabar & Gorostiaga, 2018) and add novel and prominent practical information with the reporting of operational regression equations to estimate the impractical *gold standard* MLSS in the specific populations studied. The accuracy in the prediction of MLSS from LE_{min} is among the highest reported in the literature and presents a reasonable alternative to classical MLSS assessment by means of a single-session submaximal test. The relationships between LE vs. MLSS observed in this study deserve further examination. Validity and longitudinal research exploring the possible physiological mechanisms underpinning their close relationship is warranted.

What does this article add?

LE_{min} is an objective variable that is easy to measure by means of a submaximal running test and it is strongly associated with the *gold standard* reference for endurance performance (i.e. MLSS). Operational prediction equations are provided for its use in a sample of soccer players with $MLSS_V$ values ranging from 11.2 to 14.4 km·h^{-1} (Figure 4) and individuals with $MLSS_V$ values ranging from 11.2 to 16.5 km·h^{-1} (Figure 5). The use of these equations could provide a reasonable alternative to reduce costs and alleviate the burden associated with the classical assessment of MLSS. LE_{min} and $LE_{min+1mM}$ absolute values could also serve for the assessment of endurance capacity and training prescription and monitoring in soccer. Other team-sport athletes possessing similar lactate/velocity characteristics (such as those in futsal, basketball or handball) could also benefit from the use of these prediction equations.

Abbreviations

BLC	blood lactate concentration
BLT	blood lactate-related thresholds
CI	confidence intervals
CV	coefficient of variation
CVRT	constant velocity running test
D_{max}	maximal-deviation method
ES	effect size
FBLC	fixed blood lactate concentration
$FBLC_{2mM}$	fixed blood lactate concentration of 2 mmol·L^{-1}
$FBLC_{2.5mM}$	fixed blood lactate concentration of 2.5 mmol·L^{-1}
$FBLC_{3mM}$	fixed blood lactate concentration of 3 mmol·L^{-1}
HR	heart rate
HR_{max}	maximal heart rate
LE	lactate equivalent
LE_{min}	minimum lactate equivalent
$LE_{min+1mM}$	minimum lactate equivalent plus 1 mmol·L^{-1}
$LE_{min+1.5mM}$	minimum lactate equivalent plus 1.5 mmol·L^{-1}
LMT	lactate minimum test
LOA	limits of agreement
LT	lactate threshold
$LT_{0.2mM}$	the stage prior to a ≥0.2 mmol·L^{-1} blood lactate concentration elevation above baseline values
LT_1	the highest stage above which blood lactate concentrationincreased by ≥0.1 mmol·L^{-1} in the following stage and ≥0.2 mmol·L^{-1} in the subsequent stage
MLSS	maximal lactate steady state
PTV	peak treadmill velocity
SEE	standard error of the estimate
SD	standard deviation
$_{V}LE_{min}$	velocity at the minimum lactate equivalent
$_{V}LE_{min+1mM}$	velocity at the minimum lactate equivalent plus 1 mmol·L^{-1}

${}_{V}LE_{min+1.5mM}$	velocity at the minimum lactate equivalent plus 1.5 $mmol \cdot L^{-1}$
$MLSS_{V}$	velocity at the maximal lactate steady state

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Figure Legends

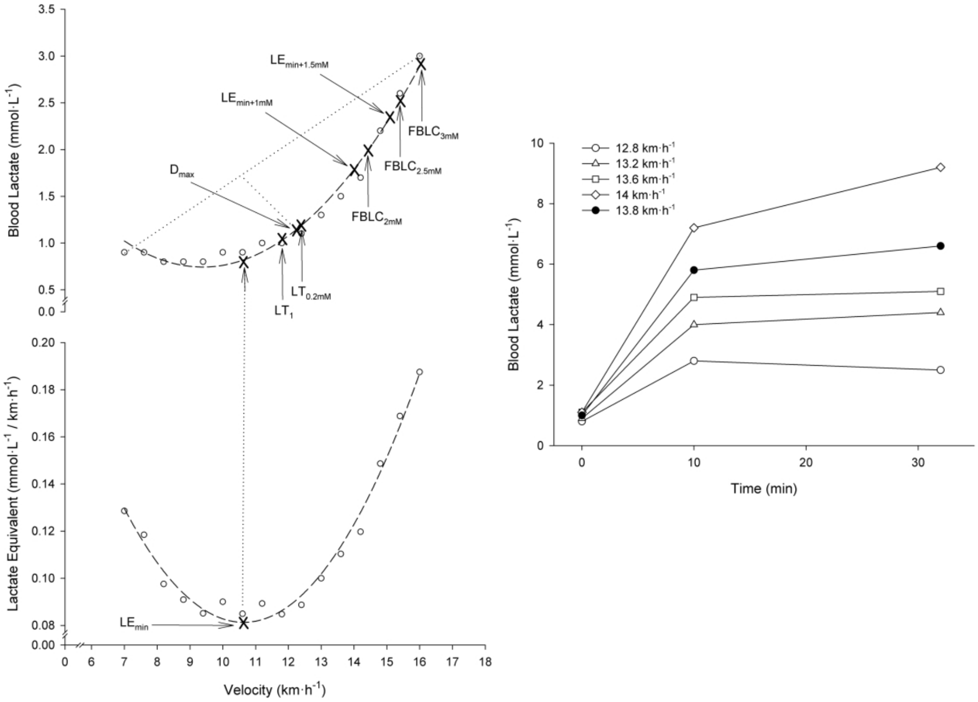
Figure 1 Left panel: Illustration of blood lactate-related thresholds determination in a representative participant. Dashed lines: second-order polynomial curve fits. Dotted lines: the greatest perpendicular distance from the third-order polynomial BLC-velocity curve fit to the generated straight line by the two end data-points of this curve. To improve figure clarity, D_{max} determination is illustrated together with the rest of BLTs on a second-order polynomial curve fit, although actually it was determined on third-order curvilinear fits as originally described (Cheng et al., 1992). Right panel: Determination procedure of the velocity associated with the maximal lactate steady state in the same representative participant. The highest velocity of an increase in blood lactate concentration of <1.0 mmol·L^{-1} during the last 20 min of exercise (i.e. $MLSS_V$), determined with a precision of 0.2 km·h^{-1}, was the 13.8 km·h^{-1} velocity (filled symbol).

Figure 2 Mean (SD) blood lactate and heart rate responses to the submaximal discontinuous incremental running exercise test. All subjects completed the 10.6 km·h^{-1} exercise stage. Mean (SD) values at completion of the test for subjects achieving ≥11.2 km·h^{-1} are indicated by dashed lines.

Figure 3 Mean (SD) blood lactate (triangles) and heart rate (circles) responses to the constant velocity running tests (CVRTs) at the maximal lactate steady state velocity ($MLSS_V$) (open symbols) and at 0.2 km·h^{-1} faster velocity ($_VMLSS_{0.2}$) (filled symbols). Dashed lines indicated that four players did not terminate the CVRT at $_VMLSS_{+0.2}$ due to premature exhaustion. * Significantly different from the rest of the time-points within the same CVRT ($P < 0.0125$). # Significantly higher in comparison with the corresponding time-points at the maximal lactate steady state velocity CVRT ($P < 0.0125$)

Figure 4 Linear relationships between the velocity at the Minimum Lactate Equivalent ($_{V}LE_{min}$) (A) and velocity at $_{V}LE_{min}$ plus 1 mmol·L^{-1} ($_{V}LE_{min+1mM}$) (B) with the velocity at the Maximal Lactate Steady State ($MLSS_{V}$). Solid lines: linear regressions. Dashed lines: 95% confidence intervals.

Figure 5 Linear relationships between the velocity at the Minimum Lactate Equivalent ($_{V}LE_{min}$) (A) and velocity at $_{V}LE_{min}$ plus 1 mmol·L^{-1} ($_{V}LE_{min+1mM}$) (B) with the velocity at the Maximal Lactate Steady State ($MLSS_{V}$) in the combined population of soccer players (open symbols) and endurance-trained runners (filled symbols) of the preceding study (Garcia-Tabar & Gorostiaga, 2018).



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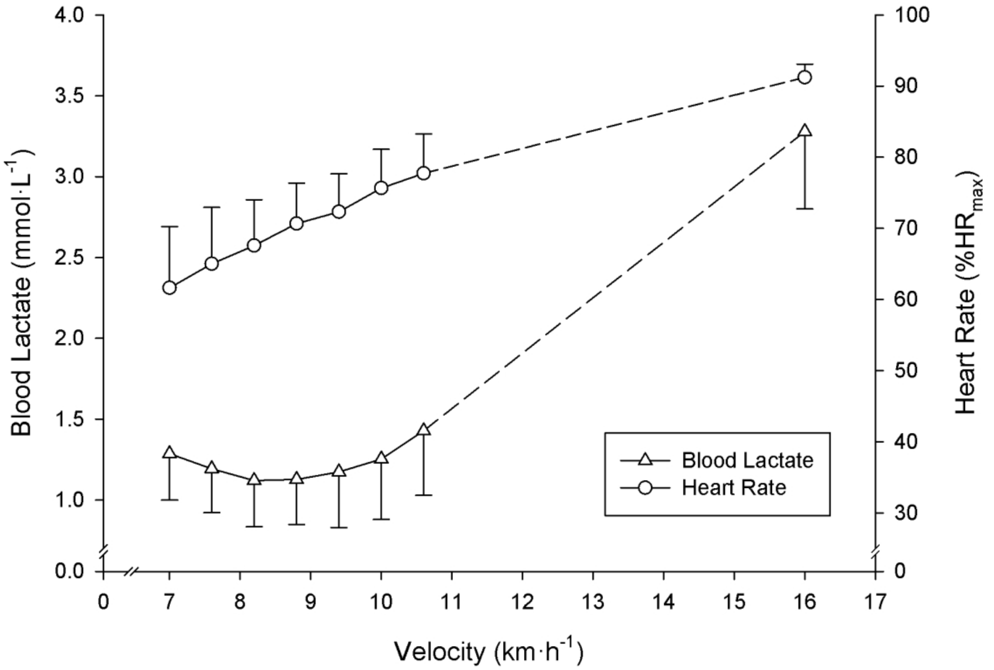

Figure 2 Mean (SD) blood lactate and heart rate responses to the submaximal discontinuous incremental running exercise test. All subjects terminated the 10.6 km•h-1 exercise stage. Mean (SD) values at completion of the test of subjects achieving ≥11.2 km•h-1 are indicated by dashed lines.

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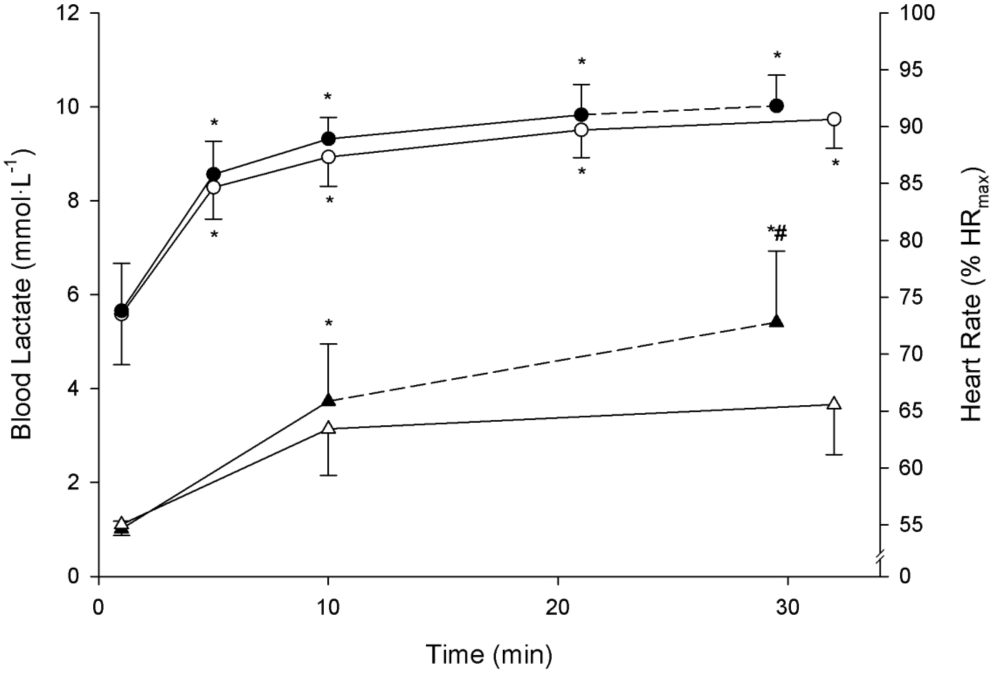

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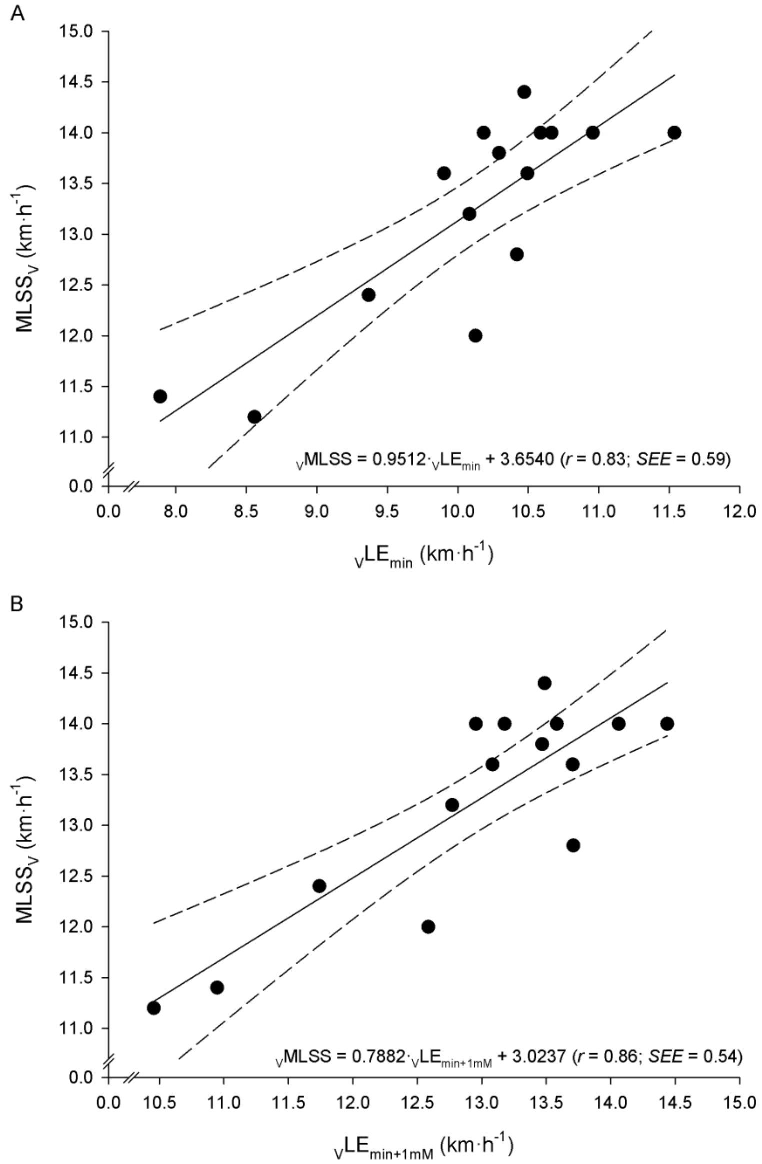

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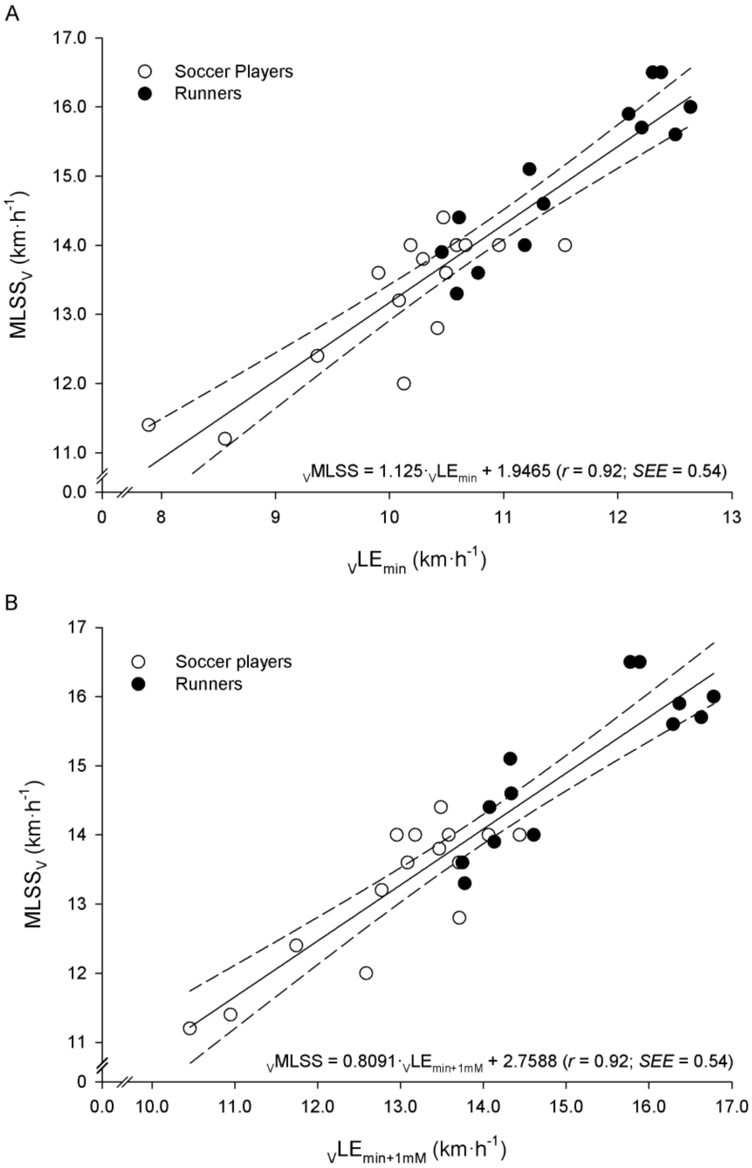

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TABLE 1: Descriptive features of blood lactate-related thresholds and maximal lactate steady state (MLSS) (*n* = 15)

	km·h^{-1}		***%$_V$MLSS***		***%PTV***		***%HR$_{max}$***	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
LT$_1$	9.9 ± 1.2**	8.2 – 12.4	75 ± 9**	59 - 89	56 ± 6**	42 - 69	75 ± 6**	68 - 86
LE$_{min}$	10.1 ± 0.9**	7.9 – 11.5	76 ± 4**	69 - 84	57 ± 4 **	49 - 64	76 ± 4**	70 - 85
LT$_{0.2mM}$	10.7 ± 1.4**	8.8 – 13.0	81 ± 7**	63 - 93	60 ± 6**	51 - 77	78 ± 6**††	66 - 86
D$_{max}$	11.4 ± 1.1**††	8.8 – 12.9	85 ± 6**††	73 - 95	64 ± 5**††	54 - 73	79 ± 3**††	73 - 83
FBLC$_{2mM}$	12.7 ± 1.4*††	9.3 – 14.4	96 ± 6*††	83 - 106	71 ± 6*††	55 - 77	86 ± 2*††	83 - 90
LE$_{min+1mM}$	12.9 ± 1.1††	10.5 – 14.4	98 ± 4††	93 - 107	73 ± 4††	62 - 81	87 ± 3††	83 - 94
MLSS	13.2 ± 1.0††	11.2 – 14.4	100 ± N/A††	N/A	74 ± 4††	66 - 81	90 ± 2††	84 - 92
FBLC$_{2.5mM}$	13.6 ± 1.4††	10.1 – 15.4	103 ± 6††	90 - 114	76 ± 6††	60 - 82	90 ± 2††	87 - 94
LE$_{min+1.5mM}$	13.7 ± 1.2*††	10.9 – 15.3	104 ± 5*††	97 - 115	77 ± 5*††	64 - 86	91 ± 3**††	86 - 96
FBLC$_{3mM}$	14.3 ± 1.5**††	10.6 – 16.2	108 ± 7**††	95 - 121	80 ± 6**††	63 - 87	93 ± 2**††	90 - 97

LT$_1$, the highest stage above which blood lactate concentration increased by ≥0.1 mmol·L^{-1} in the following stage and ≥0.2 mmol·L^{-1} in the subsequent stage; *LE$_{min}$,* Minimum Lactate Equivalent; *LT$_{0.2mM}$,* the stage prior to a ≥0.2 mmol·L^{-1} blood lactate concentration elevation above baseline values; *D$_{max}$,* Maximal-Deviation method; FBLC$_{2mM}$, Fixed blood lactate concentration (FBLC) threshold of 2 mmol·L^{-1}; *LE$_{min+1mM}$,* LE$_{min}$ plus 1 mmol·L^{-1}; FBLC$_{2.5mM}$, FBLC threshold of 2.5 mmol·L^{-1}; *LE$_{min+1.5mM}$,* LE$_{min}$ plus 1.5 mmol·L^{-1}; FBLC$_{3mM}$, FBLC threshold of 3 mmol·L^{-1}

Significantly different from MLSS at $P < 0.05$ (*) and at $P < 0.001$ (**)

Significantly different from LE$_{min}$ at $P < 0.05$ (†) and at $P < 0.001$ (††)

TABLE 2: *Pearson*'s correlation magnitudes between the selected (MLSS, BLTs and PTV) endurance performance variables (n = 15)

	FBLC$_{2mM}$	**LE$_{min+1mM}$**	**FBLC$_{2.5mM}$**	**FBLC$_{3mM}$**	**LE$_{min+1.5mM}$**	**LE$_{min}$**	**PTV**	**LT$_{0.2mM}$**	**D$_{max}$**	**LT$_1$**	**MLSS**
FBLC$_{2mM}$		0.966***	0.997***	0.992***	0.972***	0.847***	0.773***	0.679**	0.833***	0.369	0.865***
LE$_{min+1mM}$			0.971***	0.969***	0.993***	0.933***	0.747**	0.706**	0.859***	0.472	0.861***
FBLC$_{2.5mM}$				0.998***	0.982***	0.838***	0.771***	0.698**	0.834***	0.386	0.852***
FBLC$_{3mM}$					0.984***	0.824***	0.763***	0.714**	0.814***	0.395	0.840***
LE$_{min+1.5mM}$						0.889***	0.737**	0.724**	0.847***	0.466	0.839***
LE$_{min}$							0.645**	0.566*	0.817***	0.485	0.834***
PTV								0.621*	0.477	0.294	0.723**
LT$_{0.2mM}$									0.357	0.357	0.689**
D$_{max}$										0.229	0.673*
LT$_1$											0.377

MLSS, maximal lactate steady state, *BLTs,* blood lactate-related thresholds; *FBLC$_{2mM}$*, fixed blood lactate concentration (FBLC) threshold of 2 mmol·L^{-1}; *LE$_{min+1mM}$,* Minimum Lactate Equivalent (LE$_{min}$) plus 1 mmol·L^{-1}; *FBLC$_{2.5mM}$*, FBLC threshold of 2.5 mmol·L^{-1}; *FBLC$_{3mM}$*, FBLC threshold of 3 mmol·L^{-1}; *LE$_{min+1.5mM}$,* LE$_{min}$ plus 1.5 mmol·L^{-1}; *LE$_{min}$,* Minimum Lactate Equivalent; *PTV*, peak treadmill velocity; *LT$_{0.2mM}$,* the stage prior to a ≥0.2 mmol·L^{-1} blood lactate concentration elevation above baseline values; *D$_{max}$,* Maximal-Deviation method; *LT$_1$,* the highest stage above which blood lactate concentration increased by ≥0.1 mmol·L^{-1} in the following stage and ≥0.2 mmol·L^{-1} in the subsequent stage

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$