©Journal of Sports Science and Medicine (2023) **22**, 68-74
http://www.jssm.org DOI: https://doi.org/10.52082/jssm.2023.68

Research article

Easy Prediction of the Maximal Lactate Steady-State in Young and Older Men and Women

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Abstract

Maximal Lactate steady-state (MLSS) demarcates sustainable from unsustainable exercise and is used for evaluation/monitoring of exercise capacity. Still, its determination is physically challenging and time-consuming. This investigation aimed at validating a simple, submaximal approach based on blood lactate accumulation ([Δlactate]) at the third minute of cycling in a large cohort of men and women of different ages. 68 healthy adults (40♂, 28♀, 43 ± 17 years (range 19 - 78), VO_{2max} 45 ± 11 $ml^{-1} \cdot kg^{-1} \cdot min^{-1}$ (25 - 68)) performed 3-5 constant power output (PO) trials with a target duration of 30 minutes to determine the PO corresponding to MLSS. During each trial, [Δlactate] was calculated as the difference between the third minute and baseline. A multiple linear regression was computed to estimate MLSS based on [Δlactate], subjects` gender, age and the trial PO. The estimated MLSS was compared to the measured value by paired t-test, correlation, and Bland-Altman analysis. The group mean value of estimated MLSS was 180 ± 51 W, not significantly different from ($p = 0.98$) and highly correlated with ($R^2 = 0.89$) measured MLSS (180 ± 54 watts). The bias between values was 0.17 watts, and imprecision 18.2 watts. This simple, submaximal, time- and cost-efficient test accurately and precisely predicts MLSS across different samples of healthy individuals (adjusted $R^2 = 0.88$) and offers a practical and valid alternative to the traditional MLSS determination.

Key words: Cycling, maximal metabolic steady state, critical power, blood lactate, aging, functional test.

Introduction

Maximal lactate steady-state (MLSS) is an integrated index of cardiorespiratory and metabolic function that demarcates the boundary between the heavy/sustainable and the severe/unsustainable exercise intensity domains (Hill, 1993; Jones et al., 2010; Poole et al., 2016). Similar to other "threshold" concepts (e.g. critical power, respiratory compensation point, etc.), occurring at a similar metabolic intensity (Keir et al., 2015), it represents the highest strain that is still compatible with prolonged exercise duration and the preservation of metabolic stability (Keir et al., 2015). This feature, together with its high sensitivity to training, makes MLSS a useful marker to monitor human physical fitness, prescribe exercise, manage training loads, predict performance and develop pacing strategies for continuous/intermittent exercises (Jones et al., 2010; Jones and Vanhatalo, 2017). Specifically, in the context of exercise prescription, the use of individualised, threshold-based intensity targets has been proposed to ensure the homogeneity of the training stimulus across different subjects (Pogliaghi et al., 2006; Iannetta et al., 2019), as an alternative to the traditional percentage of the maximum effort approach (American College of Sports Medicine, 2017).

Testing techniques to be used on a large scale should be easy to perform, valid in different populations, minimally invasive and with low risk of injury, and should not rely on expensive or complicated equipment. On the contrary, traditional protocols to determine MLSS are technically complex and require a significant commitment to testing from both subjects and investigators. Typically, a series of two to five 30-min constant load tests in the vicinity of the heavy to severe intensity boundary, as well as frequent capillary blood sampling are necessary to establish the workload compatible with a stable blood lactate concentration, making these protocols time-consuming and physically demanding (Hill, 1993; Jones et al., 2010).

With the aim to simplify the testing approach to establish the boundary between the heavy and severe domains of exercise, a predictive equation was proposed (Fontana et al., 2016) to determine MLSS/Critical intensity based on blood lactate accumulation during a single, non-exhausting sub-maximal 3-min test, performed at a constant power output (PO) over a wide range of relative exercise intensities (i.e. 55 - 95% Peak PO) (Fontana et al., 2016). The study showed, in a small and homogenous sample of young healthy and active adults, very good agreement between the predicted and the actual MLSS/Critical intensity values; however, given the possible differences in the potential of lactate production and clearance between different populations (e.g. older adults, inactive individuals, females), the accuracy of the lactate-based prediction test needs to be further explored, (Seals et al., 1984; Korhonen et al., 2005).

Thus, the present study aimed to extend the use of the 3-min submaximal cycling test and the associated equation for the prediction of MLSS in a heterogeneous group of healthy males and females ranging in age from young to older adults. We hypothesised that the submaximal 3-min test would confirm its validity to accurately and precisely predict MLSS during cycling in this large and heterogeneous population of healthy adults.

Received: 17 October 2022 / Accepted: 15 January 2023 / Published (online): 01 March 2023

Methods

Participants

The study was approved by the University Committee for Approval of Human Research and adhered to the principles of the declaration of Helsinki. 68 healthy adults (40 men, 28 women, 43±17 years (range 19-78), BMI 24±3 (19-31), VO_2max 45±11 $ml^{-1}\cdot kg^{-1}\cdot min^{-1}$ (25-68)) gave written informed consent to participate in the study. Participants were non-smokers, free of any condition that could influence physiological responses during testing.

Protocol

After medical clearance, all participants completed the following cycle-ergometer tests within a maximum of three weeks: i) a preliminary maximal ramp-incremental exercise test to exhaustion; ii) three to five, 30 min constant PO trials.

All exercise tests for a given individual were conducted at a similar time of the day, in an environmentally controlled laboratory, on an electromagnetically braked cycle-ergometer (Sport Excalibur, Lode, Groningen, NL). To minimise variability of glycogen stores and glucose oxidation, participants followed the following standard food intake prescription, 2 hours before all the testing sessions: 2 g per Kg of body weight of low glycaemic index carbohydrates and 500 ml of water. All participants were instructed to avoid caffeine consumption and physical activity respectively for at least 8 h and 24 h before each testing session.

Ramp incremental cycling exercise test: After a 4-min baseline at 20 watts, the workload was increased by 10 to 30 watts·min^{-1}, depending on the subject's anticipated fitness level (Pogliaghi et al., 2014), with the aim to bring the subject to exhaustion within 10-12 minutes. Participants chose a self-selected cadence (range: 60-90 rpm) and were required to maintain it throughout all the tests. Breath-by-breath pulmonary gas exchange and ventilation were continuously measured using a metabolic cart (Quark b2, Cosmed, Italy); the respiratory compensation point was identified as the VO_2 corresponding to a sharp reduction of end-tidal CO_2 and a sharp increase in the ventilatory equivalent for VCO_2 as detailed elsewhere (Colosio et al., 2019); maximal VO_2 (VO_{2max}) was determined as the highest 30-sec average reached upon exhaustion.

Constant load trials: On successive and distinct appointments, participants performed three to five constant, square-wave PO tests (i.e., immediate increase from 4-min baseline at 20 watts to the target PO), with a target duration of 30 min for the identification of MLSS. The first test for MLSS determination was performed at the PO equivalent to the respiratory compensation point, as identified based on the individual VO_2/PO relationship derived from the incremental exercise and a comprehensive translation strategy (strategy 2) that includes a simple mean response time correction below gas exchange threshold and an additional correction above it, up to the respiratory compensation point (Caen et al., 2020). For the above, a population average value (i.e. 14.2 ± 2.4 ml·min^{-1}·$watt^{-1}$) was used for the slope of the VO_2/PO relationship of constant-load trials above the gas exchange threshold (Caen et al., 2020). The PO of the following trials was modified based on the stability/instability of the blood lactate concentration (i.e. lactate accumulation between the 10th and 30th minute of exercise </>1 mmol/L) as previously detailed (Fontana et al., 2016) and suggested by Beneke R. (2003) (Beneke R., 2003); in brief, the PO was increased/decreased by 10 watts, in search of the highest workload still compatible with metabolic stability, i.e. the measured MLSS.

MLSS estimation: During all constant PO trials, capillary blood samples (20 µl) were drawn from the ear lobe at baseline and the 3rd min from exercise onset and five-min intervals from exercise onset thereafter. Samples were immediately analysed using an electro-enzymatic technique (Biosen C-Line, EKF Diagnostics, Barleben, Germany) and lactate accumulation ([Δlactate]) was calculated as the value at the 3rd min of exercise minus the baseline value. Then, the % MLSS corresponding to the test absolute load was predicted based on [Δlactate] using the equation developed by Fontana et al. (Fontana et al., 2016):

$$\% \text{ MLSS} = 76.8 + (10.1 \cdot [\Delta\text{lactate}]\ [\text{mmol/L}]) \qquad \textbf{(Eq. 1)}$$

Finally, the individual's predicted MLSS in watts was calculated by solving the proportion as: MLSS [watts] = (100 · absolute trial intensity [watts]) / %MLSS.

Statistical analysis

Data are presented as means ± SD (range of the measure) throughout. The relationship between exercise intensity (% measured MLSS) and lactate accumulation ([Δlactate]) for trials ≥3 min in duration was modelled by regression analysis. The model was hierarchically structured to overcome the problem of non-independent observations in having multiple participants yield more than one data point in the regression model (Tabachnick and Fidell, 2007; Fontana et al., 2016).

After normality assumption verification (using Shapiro-Wilk test and Q-Q plot), estimated MLSS, as derived using equation 1 (Fontana et al., 2016), and measured MLSS were compared by paired-samples t-test and Pearson's correlation coefficient. The error in the estimated MLSS was evaluated by the standard error of the estimates (SEE) and expressed in percentage with respect to the mean value (Hopkins, 2000). Additionally, the difference between estimated vs. measured values was plotted as a function of the mean of the two measures using a Bland-Altman analysis followed by one-sample t-test. Moreover, the possible effect of the trial intensity (expressed relative to POmax) on the bias of the estimated MLSS values was evaluated by linear regression. Finally, in order to evaluate the possible role of the subject's age, sex and workload as additional predictors of MLSS, in conjunction with lactate accumulation, we run a hierarchical multiple linear regression incorporating these factors. Paired-samples t-test, Pearson's correlation, regression and Bland-Altman analysis were repeated for this new predictive equation and the improvement in prediction resulting from using this new regression model was calculated as the difference between R^2 vs the original equation (Field, 2013).

All statistical analyses were performed using SigmaPlot 11.0 (Systat Software Inc) and $\alpha = 0.05$; statistical significance was accepted when $p < \alpha$.

Results

The total sample of 68 subjects was distributed in 3 age groups (29 subjects ≤ 35 years; 20 subjects 36 - 55 years; 19 subjects ≥ 55 years). A total of 325 tests with a duration of at least 3 min were included in the analysis. The mean [Δlactate] was 3.04 ± 1.65 mmol/L (range 0.3-10.3 mmol/L), while the mean PO was 201 ± 66 watts (range 73 - 385 watts), corresponding to 69 ± 10% (range 51 - 94%) of PO_{max} measured during the ramp incremental test. A full overview of this is presented in Table 1.

In this large and heterogeneous group of subjects, [Δlactate] between baseline and the 3rd min of exercise was confirmed to be linearly related to the intensity of exercise, expressed as % of measured MLSS ($r = 0.63$, $R^2 = 0.39$ $p < 0.001$, SEE = 14%).

The mean estimated MLSS using the original equation 1 was 187 ± 55 watts. This value was significantly higher than ($p < 0.001$) yet highly correlated with ($r = 0.93$, $p < 0.01$ SEE = 20 watts and 11%; Figure 1 panel A) the measured MLSS (180 ± 54 watts). A significant bias was detected between measures (d = -7.7 watts, $t < 0.001$; Figure 1 panel B), with a relatively small imprecision (20.5 watts, 11.4% of the mean measure). Furthermore, the bias between estimated and measured MLSS was unaffected by sex (p = 0.27), a negative function of age (difference between measures = -1.22 - (0.168 · age [years]), p = 0.014) and exercise intensity relative to PO_{max} (difference between measures = -19 - (0.38 · PO_{max} [%]), p <0.001).

Table 1. Numerosity, Mean ± SD and range (in parenthesis) of anagraphic, anthropometric and fitness parameters, in the whole group and in the female and male subgroups.

	FEMALES	MALES	ALL
#	28	40	68
Age (years)	45 ± 15 (19-65)	42 ± 18 (22-78)	43 ± 17 (19-78)
Height (m)	1.64 ± 0.05 (1.56-1.74)*	1.76 ± 0.07 (1.62-1.90)	1.72 ± 0.09 (1.56-1.90)
Weight (Kg)	61 ± 8 (49-77)*	76 ± 9 (59-99)	71 ± 11 (49-99)
BMI	22.7 ± 3.1 (18.7-30.6)*	24.5 ± 2.3 (19.4-29.7)	23.9 ± 2.7 (18.7-30.6)
VO_{2max} (ml·kg^{-1}·min^{-1})	37.8 ± 6.6 (24.6-56.6)*	49.4 ± 10.8 (25.2-68.1)	44.6 ± 10.9 (24.6-68.1)
PO_{max} ramp test (watt)	218 ± 53 (137-218)*	342 ± 68 (152-455)	291 ± 87 (137-455)
Measured MLSS (watt)	139 ± 38 (76-230)*	209 ± 44 (93-294)	180 ± 54 (76-294)

* indicates a significant difference between the sex subgroups (t-test, p < 0.05).

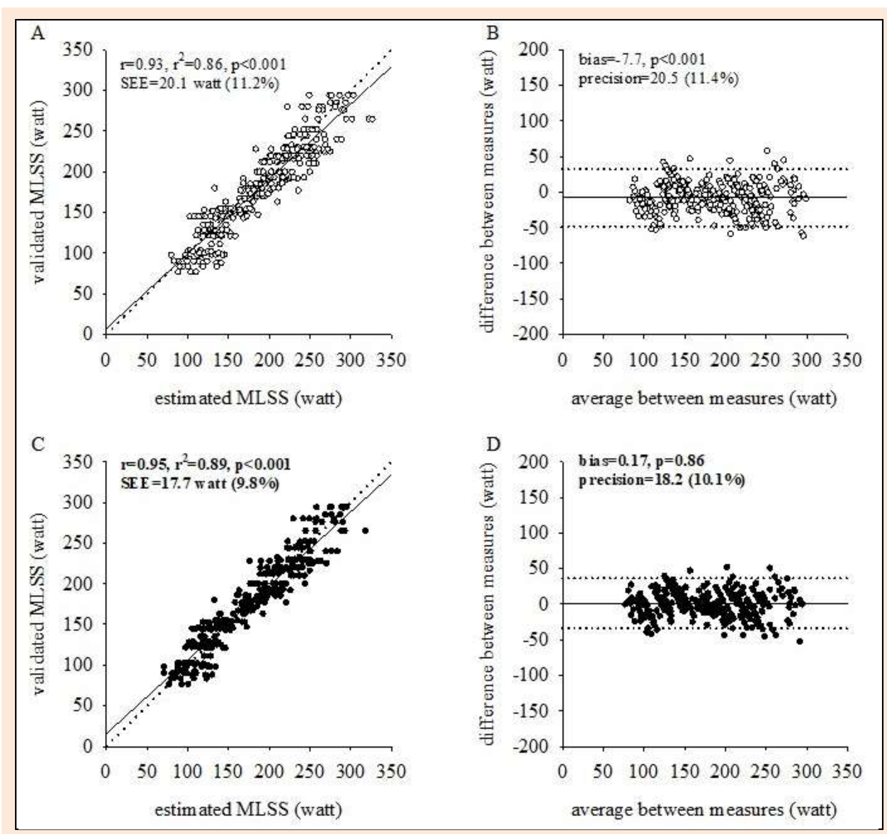

Figure 1. Left panels: Individual validated Maximum Lactate steady-state (MLSS) values are plotted as a function of estimated values based on the original equation proposed by Fontana MLSS (○, panel A) and the new equation that incorporates age, sex and the trial absolute load (●, panel C). The identity (dashed) and the regression (solid) lines are displayed along with the coefficient of determination. Right panels: Individual differences between the values of validated MLSS and estimated values based on the original equation proposed by Fontana MLSS (○, panel B) and the new equation (●, panel D) are plotted as a function of the average of the two measures. The solid lines correspond to the average difference between measures (i.e. bias) while the dashed lines correspond to the limits of agreement.

In the attempt to improve the predictive power of the original equation, we performed a hierarchical multiple linear regression incorporating the subject's age and sex and the trial workload as additional predictors of MLSS, in conjunction with lactate accumulation that indicated all as significant predictors. Then, after assumptions verification, we tested the possible predictive power of the following new multiple linear equation that incorporated all the predictors:

MLSS [watts] = 81.35 - (0.586 · subject's age [years]) - (9.417 · Δlactate [mmol]) + (0.692 · load [watts]) – (17.234 · sex [M = 0, F = 1]) **(Eq. 2)**

The accuracy of the model across different samples was estimated by cross validation (Stein's formula) and expressed as adjusted R^2. The value of adjusted R^2 was equal to 0,88, indicative of a good cross validity (i.e. generalisability of the results outside of our sample) (Field A, 2013). The performance of this new model is presented in Figure 1 C and D: estimated MLSS as calculated from this new equation (Equation 2) was 180 ± 51 watts. This value was not significantly different from ($p = 0.98$), and highly and significantly correlated with the measured MLSS ($r = 0.95$, $p < 0.01$ SEE = 17.6 watts, 9.8%); the bias between values was very small and not significantly different from zero ($d = 0.02$, $p = 0.98$), with a small imprecision (17.5 watts, 9.7% of mean measure). The application of the new equation significantly improved the prediction R^2 compared to the original equation (ΔR^2 0.03, $p < 0.05$) and eliminated the effect of age on the bias between measures ($p = 0.99$). There was a significant effect of trial intensity relative to PO_{max}, on the difference between measures (= -32 - (0.48 · PO_{max} [%]), $p < 0.001$), with intensities >64% PO_{max} being associated with an overestimation and those <64% with an underestimation compared to validated values. However, the average bias between validated and measured MLSS was <5 watts (i.e 2.5%) in the 57 - 76% PO_{max} intensity range.

Discussion

In a large and heterogeneous group of male and female subjects ranging from young to older adults, the present study tested the performance of a predicting equation for determining MLSS based on blood lactate accumulation during a submaximal cycling test lasting 3 minutes. The study confirmed that MLSS can be predicted based on lactate accumulation; however, the inclusion of females and an ample range of ages allowed us to detect a loss of accuracy outside the male population and the narrow age range on which the original equation had been developed. As a result, in the current study, we introduced a new multiple linear model that also incorporates sex and age as predictors. The new equation significantly improved the prediction R^2 compared to the original one and showed a high reproducibility outside our sample (adjusted $R^2 = 0.88$) and a relatively small standard error of estimate of 17.7 watts, across a wide range of relative intensities for testing.

As a demarcation index between the heavy and the severe exercise intensity domains and their distinct physiological responses (Keir et al., 2015; Colosio et al., 2021), MLSS represents a key determinant of endurance performance (Jones et al., 2010) that should be determined at the individual level to ensure appropriate and homogeneous implementation of a desired exercise "dose" towards specific training and health outcomes (Iannetta et al., 2019).

To overcome the limitations of the reference method for MLSS determination and the detection of other indexes of the heavy to the severe boundary, such as the Critical Power (Moritani et al., 1981; Hill, 1993), previous studies in athletes have used delta blood lactate during short, constant load trials of fixed absolute intensity to predict different indexes of the heavy to the severe boundary (Jacobs, 1981; Jacobs et al., 1983; Sirtori et al., 1993). The physiological rationale behind such a testing approach is that delta lactate over a given time reflects the early lactate and the extent of the mismatch between lactate production and removal which in turn is a function of relative intensity (i.e., % of MLSS) (Sirtori et al., 1993; Fontana et al., 2016). In agreement with the above idea, and with previous work from our group (Fontana et al., 2016), the current study confirmed, in healthy male and female participants ranging in age from ~20 - 80 years old, the existence of a linear relationship between lactate accumulation and exercise intensity relative to MLSS. This confirmation is important as tests are frequently developed on male individuals only and because the submaximal approach proposed in our study is particularly useful for the evaluation of unfit participants and older individuals; in these populations, the lower exercise tolerance may reduce the feasibility and accuracy of the heavy to severe boundary determination based on either the power-duration relationship or ventilatory thresholds, while the maximal nature of these tests may involve undesired health risks (Wasserman et al., 1973; American College of Sports Medicine, 2017).

Importantly, our current study documented a low accuracy and an overestimation of MLSS as a function of age when the original equation (Fontana et al., 2016) was applied. The above finding led to the identification of a new predictive equation that, thanks to the introduction of age, sex and test PO as predictors, improved the prediction of the PO associated with MLSS. In the updated prediction equation, age and sex were negative terms. This is compatible with the notion that older adults and women present lower levels of lactate accumulation at a given relative intensity. This might be explained by factors such as sarcopenia (Bruseghini et al., 2015) and selective atrophy of type II muscle fibres, which may characterise ageing (Lexell, 1995; Korhonen et al., 2005) and the different strength levels, the prevalence of type I muscle fibres, and the possible lower absolute lactate production that characterises females (Isacco et al., 2012). Moreover, the increased sympathetic tone (Seals et al., 1994), chronic dehydration and reduced muscle mass (Bruseghini et al., 2015) that accompany ageing as well as the lower overall muscle mass and higher potential for fat oxidation that characterises women (Beaudry and Devries, 2019; Ferrari et al., 2020), may affect the blood lactate removal/exercise intensity relationship in these populations compared to young, healthy males.

A last aspect to consider as potentially impacting lactate-based estimates is the behaviour of lactate accumulation above MLSS. In fact, in the severe domain of exercise lactate accumulation displays an exponential increase as a function of workload, while in our study a linear increase was detected. This difference, probably due to the relatively short time window investigated (i.e., 3 min) could be responsible for a reduction of accuracy of our predictive equation above the 76% peak power output (~0.4 watt every 1% increase in % peak power output).

In agreement with our previous work, the present study confirmed that MLSS can be accurately and precisely predicted, within but also outside of the tested population, with the proposed 3-min submaximal test over a wide range of relative exercise intensities (45 - 95% peak PO). Importantly, when workloads between 57 and 76 % of the individual's PO_{max} were used, the bias was below the minimum detectable difference (i.e. ±2.5% of the MLSS). However, tests performed outside this optimal intensity range were associated with an increasing overestimation or underestimation respectively if > or < 64 % PO_{max}. As an example, a test performed at 32 and 100% PO_{max} would be on average associated with an under/overestimation of ±16 watts (or 9%). To investigate the applicability of the present method outside our sample, we tested the model on a different group using MLSS data collected in our laboratory with the same procedure as this study. In this external group of 23 individuals (8 women, 27 ± 5 years (range 20 - 40), 52 ± 10 $ml^{-1}\cdot kg^{-1}\cdot min^{-1}$), the newly developed equation showed a mean estimated MLSS of 166 ± 43 W versus a measured MLSS of 169 ± 53 W. These values were non-statistically different (t-test, $p = 0.17$) highly correlated ($R^2 = 0.83$) and showed a small, non-significant bias (-3.1 ± 22.4 W). Although these values support the applicability of our test, we acknowledge the need to further verify the performance of this test in people presenting different characteristics (elderly and athletic populations).

Different approaches, alternative to direct MLSS determination, have been proposed for the identification of the heavy to severe intensity boundary, with the aim to facilitate the use of this variable for exercise testing and prescription. Among them, an equation-based estimate (Iannetta et al., 2018) or the direct measurement of Critical Power and other "thresholds" such as the respiratory compensation point and the deoxygenated haemoglobin deflection point (Bellotti et al., 2013; Fontana et al., 2015). While the equivalence of the metabolic intensity at the above-mentioned indexes and the interchangeability/translation among them and MLSS is the object of an unsettled debate (e.g., Broxterman et al., 2018; Keir et al., 2018), none of the above methods is superior to the others from a practical standpoint. All tests require one or more maximal exercise sessions and, except for critical power, expensive and sophisticated equipment. Additionally, the values derived from the above tests remain estimations as they all have measurement errors (i.e. ~20 watts) (Mattioni Maturana et al., 2016) and/or the necessity for a "smart" translation (Iannetta et al., 2018; Caen et al., 2020), calling for a verification procedure to confirm the actual highest external power still compatible with metabolic stability (Keir et al., 2015).

Compared to the above methods for MLSS identification, the sub-maximal test proposed in the current study has practical advantages and good statistical prediction qualities. The main practical advantages of this new 3-min approach are that: (1) MLSS can be established in a single 3-min trial, which makes the test time-effective; (2) exercise tests are performed at a sub-maximal intensity and, hence, do not require exhaustive efforts; (3) only two lactate samples are required, reducing the overall cost and increasing the "field" applicability of the procedure; and (4) a wide range of exercise intensities (45 - 95% PO_{max}, with best performance between 57 and 76 %) can be used to estimate MLSS, allowing a versatile application and customisation of this test. Regarding the prediction qualities, our estimate of MLSS predicts validated MLSS with a linear function close to the line of identity, with a small standard error of the estimate, a high degree of explanatory power (Field, 2013) along with a systematic error (i.e., accuracy; 0.02 vs. -5.0 W) and a random error (i.e., precision; 18 vs 20 watts) that are comparable to those observed in other prediction approaches (Mattioni Maturana et al., 2016; Iannetta et al., 2018) or other indirect methods to estimate metabolic intensity (Colosio and Pogliaghi, 2018; Colosio et al., 2020), yet with the valuable advantages of time efficiency and a submaximal intensity.

Conclusion

In conclusion, data from a fairly large and heterogeneous group of young to older men and women indicated that the heavy to severe boundary (i.e. MLSS) can be accurately and precisely predicted, based on blood lactate accumulation measured at the third minute of a single sub-maximal, non-exhaustive exercise trial performed at a constant power output over a wide range of relative exercise intensities, on the subject's age and sex and on the absolute PO of the trial. Based on these observations, this new 3-min sub-maximal test could offer an economical, practical, and valid alternative to the traditional determination of MLSS.

Acknowledgements

We would like to express our gratitude to the subjects in this study. The experiments comply with the current laws of the country in which they were performed. The authors have no conflict of interest to declare. The datasets generated and analyzed during the current study are not publicly available, but are available from the corresponding author who was an organizer of the study.

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Key points

- Lactate accumulation from a submaximal test can be used to predict the maximal lactate steady state (MLSS).
- This approach was previously proposed in young men, and it is here extended to women and elderly people.
- Within the limitations of lactate measurements, this 3-min submaximal test offers an economical and practical approach to obtain a first estimate of the MLSS.

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