Manipulating graded exercise test variables affects the validity of the lactate threshold and VO2peak

Nicholas A. Jamnick
Javier Botella
David B. Pyne
David Bishop
Edith Cowan University

Follow this and additional works at: https://ro.ecu.edu.au/ecuworkspost2013

Part of the Agriculture Commons, Genetics Commons, and the Other Biochemistry, Biophysics, and Structural Biology Commons

10.1371/journal.pone.0199794
Jamnick, N. A., Botella, J., Pyne, D. B., & Bishop, D. J. (2018). Manipulating graded exercise test variables affects the validity of the lactate threshold and VO2peak. PloS one, 13(7), e0199794. Available here.
This Journal Article is posted at Research Online.
https://ro.ecu.edu.au/ecuworkspost2013/4645
Manipulating graded exercise test variables affects the validity of the lactate threshold and \( \dot{V}O_2\text{peak} \)

Nicholas A. Jamnick\(^1\)\(^*\), Javier Botella\(^1\)\(^*\), David B. Pyne\(^2,3\)\(^e\), David J. Bishop\(^1,4\)\(^e\)

1 Institute for Health and Sport, College of Sport and Exercise Science, Victoria University, Melbourne, Australia, 2 Australian Institute of Sport, Canberra, Australia, 3 Research Institute for Sport and Exercise (UCRISE), University of Canberra, Canberra, Australia, 4 School of Medical and Health Sciences, Edith Cowan University, Joondalup, Australia

These authors contributed equally to this work.

* nickolas.jamnick@live.vu.edu.au

Abstract

Background

To determine the validity of the lactate threshold (LT) and maximal oxygen uptake (\( \dot{V}O_2\text{max} \)) determined during graded exercise test (GXT) of different durations and using different LT calculations. Trained male cyclists (n = 17) completed five GXTs of varying stage length (1, 3, 4, 7 and 10 min) to establish the LT, and a series of 30-min constant power bouts to establish the maximal lactate steady state (MLSS). \( \dot{V}O_2 \) was assessed during each GXT and a subsequent verification exhaustive bout (VEB), and 14 different LTs were calculated from four of the GXTs (3, 4, 7 and 10 min)—yielding a total 56 LTs. Agreement was assessed between the highest \( \dot{V}O_2 \) measured during each GXT (\( \dot{V}O_2\text{peak} \)) as well as between each LT and MLSS. \( \dot{V}O_2\text{peak} \) and LT data were analysed using mean difference (MD) and intraclass correlation (ICC).

Results

The \( \dot{V}O_2\text{peak} \) value from GXT\(_1\) was 61.0 ± 5.3 mL kg\(^{-1}\) min\(^{-1}\) and the peak power 420 ± 55 W (mean ± SD). The power at the MLSS was 264 ± 39 W. \( \dot{V}O_2\text{peak} \) from GXT\(_3, 4, 7, 10\) underestimated \( \dot{V}O_2\text{peak} \) by ~1–5 mL kg\(^{-1}\) min\(^{-1}\). Many of the traditional LT methods were not valid and a newly developed Modified D\(_{max}\) method derived from GXT\(_4\) provided the most valid estimate of the MLSS (MD = 1.1 W; ICC = 0.96).

Conclusion

The data highlight how GXT protocol design and data analysis influence the determination of both \( \dot{V}O_2\text{peak} \) and LT. It is also apparent that \( \dot{V}O_2\text{max} \) and LT cannot be determined in a single GXT, even with the inclusion of a VEB.
Abbreviations: LT, lactate threshold; GXT, graded exercise testing; VO_{2max}, maximal oxygen uptake; OBLA, onset of fixed blood lactate accumulation; MLSS, maximal lactate steady state; VEB, verification exhaustive bout; BMI, body mass index; W_{max}, maximum power output; VO_{2peak}, highest measured oxygen uptake value; RCP, respiratory compensation point; RCP_{MLSS}, estimate of the maximal lactate steady state via respiratory compensation point; B + mmol L^{-1}, blood lactate concentration increases above baseline value(s); ModD_{max}, modified D_{max}; Exp-D_{max}, exponential D_{max}; Log-Poly-ModD_{max}, Log-log Modified D_{max}; Log-Exp-ModD_{max}, Log-log Exponential Modified D_{max}; CV, coefficient of the variation; ES, effect size; r, Pearson product moment correlation; ICC, intraclass correlation; SEM, standard error of the measurement.

Introduction

Sampling of expired gas and blood data during a graded exercise test (GXT) to exhaustion permits identification of the gas exchange threshold (GET), the respiratory compensation point (RCP), the lactate threshold (LT), and maximal oxygen uptake (VO_{2max}). These indices can distinguish cardiorespiratory fitness, and demarcate the domains of exercise [1, 2] that can be used to prescribe exercise and to optimize training stimuli [3–6]. However, despite the popularity of these indices, the methods used to determine them can differ substantially and there has been little systematic investigation of their validity [7–9].

The recommended duration of a GXT to assess VO_{2max} is 8 to 12 minutes [10–13]. However, there is little consensus on an appropriate GXT protocol design, including duration, stage length, or number of stages, needed to establish the LT. A stage length of at least 3 minutes has been recommended [13], although an 8-minute stage length has also been suggested for blood lactate concentrations to stabilize [14]. The number of stages and GXT duration will depend on the starting intensity and power increments. Power is typically increased identically [15], regardless of sex or fitness, leading to a heterogenous GXT duration and number of stages completed [16]. A customized approach to LT testing has been recommended to ensure a more homogenous GXT duration [17].

More than 25 methods have been proposed to calculate the LT [18]; these include the power preceding a rise in blood lactate concentration of more than 0.5, 1.0 or 1.5 mmol L^{-1} from baseline [19], the onset of a fixed blood lactate accumulation (OBLA) ranging from 2.0 to 4.0 mmol L^{-1} [20, 21], or the use of curve fitting procedures such as the D_{max} or modified D_{max} methods (ModD_{max}) [22, 23]. However, many of these ‘accepted’ methods are influenced by GXT protocol design [8, 24] and their underlying validity has not been reported.

Assessing the validity of a measurement requires comparison with a criterion measure. The maximal lactate steady state (MLSS) represents the highest intensity where blood lactate appearance and disappearance is in equilibrium and where energy demand is adequately met by oxidative phosphorylation [25]. Exercise performed above the MLSS results in accelerated blood lactate appearance and it has therefore been suggested as an appropriate criterion measure for the LT [25, 26]. The primary advantages of the MLSS test include its independence of participant effort, it’s submaximal and is reliable [27]. However, the disadvantage is the necessity of multiple laboratory visits and that it yields only one index of performance.

VO_{2max} is considered the “gold standard” for assessing cardiorespiratory fitness [28] and the highest recorded VO_{2} from a GXT is often accepted as the VO_{2max} [10]. Establishing the LT requires a GXT that typically exceeds 20 minutes [13]; however, in these instances the highest VO_{2} may underestimate the VO_{2max} [12] and is termed VO_{peak}. Recently, the use of a verification exhaustive bout (VEB) has been recommended to confirm the VO_{2max}. However, it is unknown if a VEB performed after a longer duration GXT provides a valid estimate of VO_{2max}.

The aim of this study was to determine the validity of the LT and VO_{2max} derived from a single visit GXT. We hypothesized that our results would yield one or more GXT stage length and LT calculation method combination that provides a valid estimation of the criterion measure of the LT (i.e., MLSS). We also hypothesized the highest VO_{2} measured during longer duration GXTs would underestimate VO_{2max} and that the highest VO_{2} value measured during each VEB would be similar to the VO_{peak} measured during the 8- to 12-minute GXT.
Materials and methods

Ethical approval

All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Participants/Experimental design

Seventeen trained male cyclists (\(\dot{V}O_2^{max} = 62.1 \pm 5.8 \text{mL kg}^{-1} \text{min}^{-1}\), age 36.2 \(\pm\) 7.4 years, body mass index (BMI): 24.1 \(\pm\) 2.0 kg m\(^{-2}\)) volunteered for this study which required 7 to 10 visits to the laboratory. Informed consent was obtained from all individual participants included in the study.

Visit one included risk stratification using the American College of Sports Medicine Risk Stratification guidelines [29], written informed consent, self-reported physical activity rating (PA-R) [30], measurement of height and body mass, and completion of a cycling GXT with 1-minute stages (GXT\(_1\)) followed by a VEB. The remaining visits consisted of four cycling GXTs with varying stage length (3-, 4-, 7- and 10-min stages) and a series of 30-min constant power bouts to establish the MLSS. The GXTs and constant power bouts were performed in an alternating order and the order of the GXTs was randomised. Prior to each GXT and the constant power bouts a 5-min warm up was administered at a self-selected power followed by 5 min of passive rest. Participants performed each test at their preferred cadence determined during the initial visit. Antecubital venous blood (1.0 mL) was sampled during all visits (excluding GXT\(_1\)) at rest, and at the end of every stage during the GXTs or every 5 min during the constant power exercise bouts. All participants self-reported abstaining from the consumption of alcohol and caffeine or engaging in heavy exercise 24 h prior to each visit. Participants were given at least 48 h between visits and all tests were completed within 6 weeks. The Victoria University Human Research Ethics Committee approved all procedures (HRE 017–035).

Equipment/Instruments

All exercise testing was conducted using an electronically-braked cycle ergometer (Lode Excalibur v2.0, The Netherlands). A metabolic analyser (Quark Cardiopulmonary Exercise Testing, Cosmed, Italy) was used to assess oxygen uptake (\(\dot{V}O_2\)) on a breath-by-breath basis, and heart rate was measured throughout all tests. Antecubital venous blood was analysed using a blood lactate analyser (YSI 2300 STAT Plus, YSI, USA).

GXTs with verification exhaustive bout

Demographic data, PA-R, and measurements of height and body mass were used to estimate \(\dot{V}O_2^{max}\) [31] and maximum power output \(W_{max}\) [30, 32].

\[
\text{Est.} \dot{V}O_2^{max} = 56.363 + (1.921 \times \text{PA - R}) - (0.381 \times \text{AGE}) - (0.754 \times \text{BMI}) + (10.987 \times \text{SEX, } 1 = \text{MALE, } 0 = \text{FEMALE})
\]

\[
W_{max} = \left\{\frac{(\dot{V}O_2^{max} - 7) \times \text{BM}}{1.8}\right\}/6.12
\]

Where \(\dot{V}O_2^{max}\) is expressed in millilitres per kilogram per minute, BMI is in kg m\(^{-2}\), and \(W_{max}\) is in Watts.

A custom GXT protocol with a desired time limit of 10 min was then designed for each participant using: \(W_{max}/10\) min = 1-min intensities (W min\(^{-1}\)). Additional customized protocols
were designed for each of the remaining GXTs based on a percentage of the measured $W_{max}$ from GXT$_1$. The predicted $W_{max}$ was 80%, 77%, 72% and 70% for GXT$_3$, GXT$_4$, GXT$_7$, and GXT$_{10}$, respectively. The target number of stages for each participant was nine; the initial stage and subsequent stages of the remaining GXTs were determined using the following equations:

$$\text{Stage 1 Power} = \text{Predicted } W_{max} \times 0.25$$

$$\text{Subsequent power increments} = \frac{(\text{Predicted } W_{max} - \text{Stage 1})}{8}$$

where stage 1 power and predicted $W_{max}$ subsequent power increments are expressed in Watts.

A 5-min recovery was administered after each GXT, followed by a VEB performed at 90% of $W_{max}$ measured from GXT$_1$ to measure the highest measured $\dot{V}O_2$ peak ([$17$]).

Constant power exercise bouts to establish the maximal lactate steady state

The power associated with the respiratory compensation point (RCP) from GXT$_1$ was used in a regression equation (Eq 5) to estimate the MLSS (RCP$_{MLSS}$) and the first constant power exercise [33]. The RCP was determined as the average of the power output associated with: 1) the break point in ventilation relative to expired carbon dioxide ($\dot{V}_E/\dot{V}CO_2$), 2) second break point in $\dot{V}_E$ and 3) the fall in end-tidal carbon dioxide ($P_{ETCO_2}$) after an apparent steady state [$34$–$36$].

$$\text{Estimated MLSS}(\text{RCP}_{MLSS}) = 23.329 + (0.79127 \times \text{RCP})$$

where the RCP$_{MLSS}$ and RCP are expressed in Watts.

Participants performed 3 min of baseline cycling at 20 W prior to each constant power bout. The MLSS was established as the highest intensity where blood lactate increased $<1.0$ mmol$L^{-1}$ from the 10th to the 30th minute [26]. If the blood lactate concentration increased $>1.0$ mmol$L^{-1}$ the power was decreased by 3%, otherwise the power was increased by 3% [27]. This process continued until the MLSS was obtained.

LT and respiratory compensation point calculations

The LTs were calculated from GXT$_{3,4,7}$ and 10 using 14 methods (4 GXTs $\times$ 14 LTs = 56 LTs in total), and the RCP and the RCP$_{MLSS}$ were also calculated from GXT$_1$ (56 LTs + RCP and RCP$_{MLSS}$ = 58 total estimates) (Fig 1):

1. Log-log: The lactate curve was divided into two segments and the intersection point of the two lines with the lowest residuals sum of squares was taken as the LT [37].
2. OBLA value of 2.0, 2.5, 3.0, 3.5, or 4.0 mmol$L^{-1}$ [1, 24, 38].
3. Baseline + absolute value(s) (B + mmol$L^{-1}$): The intensity at which blood lactate concentration increased 0.5, 1.0 or 1.5 mmol$L^{-1}$ above baseline value(s) [39, 40].
4. $D_{max}$: The point on the third order polynomial regression curve that yielded the maximum perpendicular distance to the straight line formed by the two end points of the curve [23].
5. Modified $D_{max}$ (Mod$D_{max}$): The intensity at the point on the third order polynomial regression curve that yielded the maximal perpendicular distance to the straight line formed by the point preceding the first rise in blood lactate concentration of $>0.4$ mmol$L^{-1}$ lactate and the final lactate point [22].
6. Exponential $D_{\text{max}}$ (Exp-$D_{\text{max}}$): The point on the exponential plus-constant regression curve that yielded the maximum perpendicular distance to the straight line formed by the two end points of the curve [41, 42].

7. Log-log Modified $D_{\text{max}}$ (Log-Poly-Mod$D_{\text{max}}$): The intensity at the point on the third order polynomial regression curve that yielded the maximal perpendicular distance to the straight line formed by the intensity associated with the log-log LT and the final lactate point.

8. Log-log Exponential Modified $D_{\text{max}}$ method (Log-Exp-Mod$D_{\text{max}}$): The intensity at the point on the exponential plus-constant regression curve that yielded the maximal perpendicular distance to the straight line formed by the intensity associated with the log-log LT and the final lactate point.

9. RCP: refer to Constant Power Exercise Bouts to Establish the Maximal Lactate Steady State method section.

10. The estimated MLSS was based on a regression equation based on the RCP from GXT$_1$ ($\text{RCP}_{\text{MLSS}}$) (Eq 5).

**Data analysis**

Breath-by-breath data were edited individually with values greater than three standard deviations from the mean excluded [43]. The data was interpolated on a second-by-second basis and averaged into 5- and 30-s bins [44, 45]. The highest measured $\dot{V}O_2$ value from every GXT and VEB was determined as the highest 20-s rolling average. The $\dot{V}O_{2\text{max}}$ was computed as the
highest \( V_O^2 \) measured from any GXT or VEB. The \( V_O^2_{\text{peak}} \) for each GXT was defined as the highest measured \( V_O^2 \) from either the GXT or the subsequent VEB.

The \( W_{\text{max}} \) for every GXT was determined as the power from the last completed stage plus the time completed in the subsequent stage multiplied by the slope (Eq 6). The \( V_O^2 \) response at the MLSS was determined by the average \( V_O^2 \) value during the last two minutes of the 30-minute constant power bout.

\[
W_{\text{max}} = \text{Power of Last Stage (W)} + [\text{slope}(W.s - 1) \times \text{time} \text{ (sec.)}] \tag{Eq 6}
\]

Calculated LTs were excluded if the mean difference between the MLSS and calculated LT was greater than the error of the measurement of the MLSS [coefficient of the variation (CV%) = 3%, 7.9 W] [27], the effect size (ES) was greater than 0.2, or the Pearson Product moment correlation coefficient (r) was less than 0.90. Using these criteria, 10 of the 56 LTs and the RCP_{MLSS} (Eq 5) were included in the analysis (Table 1).

Also shown is the mean difference (MD), the Pearson product moment correlation (r) and effect size (ES) of the difference when compared with the MLSS. (log = using the log-log method as the point of the initial data point when calculating the D_{max} or Modified D_{max}; poly = Modified D_{max} method calculated using a third order polynomial regression equation; exp = Modified D_{max} method calculated using a constant plus exponential regression equation; OBLA = onset of blood lactate accumulation, B + = baseline lactate value plus an absolute lactate value). **Bold** represents the LT that met the three criteria for inclusion in our final analysis: mean difference less than 7.9 Watts, Pearson moment product correlation >0.90, and a less than trivial ES difference from the MLSS (ES <0.2)

### Statistical analysis

A one-way analysis of variance with repeated measures was used to assess significant differences between the MLSS and the calculated LTs. Agreement between the MLSS and the calculated LTs was evaluated using a two-way mixed intraclass correlation coefficient (ICC), standard error of the measurement (SEM), Lin’s concordance correlation coefficient (p.c) [46], Bland-Altman plots [47], (r), CV% [48, 49] and a magnitude-based inference approach involving standardised differences (ED) [50, 51]. Differences between \( V_O^2_{\text{peak}} \) values measured during each GXT were assessed using ES, p-values, and the CV%. Agreement between \( V_O^2 \) measured during each GXT and subsequent VEB was evaluated using intraclass calculation coefficient (ICC), SEM, and CV% [49]. Descriptive statistics are reported as the mean ± SD. Alpha was set to \( P \leq 0.05 \).

### Results

#### MLSS

The power associated with the MLSS was 264 ± 39 W, and the blood lactate concentrations at the 10\(^{th}\) and 30\(^{th}\) min were 2.8 ± 0.8 and 3.3 ± 0.8 mmol L\(^{-1}\), respectively. The blood lactate values at 3% above the MLSS (272 ± 41 W) at the 10\(^{th}\) and 30\(^{th}\) min were 3.6 ± 0.8 and 5.0 ± 0.9 mmol L\(^{-1}\), respectively. The \( V_O^2 \) at the MLSS was 81.4 ± 4.7% of \( V_O^2_{\text{max}} \) (3892 ± 441 mL min\(^{-1}\); 50.5 ± 4.0 mL kg\(^{-1}\) min\(^{-1}\)). For each GXT the \( V_O^2 \) at the MLSS and the power at the MLSS are shown in Table 2.

### Validity of LT estimates

Comparisons of the 58 estimations of the MLSS and the calculated MLSS are detailed in Table 1. Fig 2 displays the standardized difference of the 13 LTs calculated for each GXT (52 in
Table 1. The mean ± standard deviation (SD) of the 14 lactate thresholds calculated from the 4 prolonged graded exercise tests (i.e., GXT<sub>3</sub>, GXT<sub>4</sub>, GXT<sub>7</sub> and GXT<sub>10</sub>), and the respiratory compensation point (RCP) and the maximal lactate steady state (MLSS) estimated from the RCP (RCP<sub>MLSS</sub>) calculated from GXT<sub>1</sub>.

|                  | GXT<sub>3</sub> | GXT<sub>4</sub> | GXT<sub>7</sub> | GXT<sub>10</sub> |
|------------------|----------------|----------------|----------------|-----------------|
| Log-log LT       |                |                |                |                 |
| Mean SD (W)      | 211 ± 43       | 202 ± 38       | 200 ± 40       | 196 ± 41        |
| ES               | 1.28           | 1.63           | 1.62           | 1.70            |
| MD (W)           | 53.1           | 62.8           | 64.8           | 68.3            |
| r                | 0.84           | 0.89           | 0.87           | 0.78            |
| OBLA 2.0         |                |                |                |                 |
| Mean SD (W)      | 262 ± 40       | 249 ± 39       | 247 ± 39       | 245 ± 37        |
| ES               | -0.05          | -0.38          | -0.44          | -0.50           |
| MD (W)           | -11.9          | 2.0            | 6.7            | 9.2             |
| r                | 0.89           | 0.95           | 0.94           | 0.93            |
| OBLA 2.5         |                |                |                |                 |
| Mean SD (W)      | 276 ± 42       | 262 ± 40       | 258 ± 40       | 255 ± 38        |
| ES               | 0.30           | -0.05          | -0.17          | -0.23           |
| MD (W)           | -23.2          | -8.8           | -2.2           | 0.4             |
| r                | 0.90           | 0.96           | 0.95           | 0.93            |
| OBLA 3.0         |                |                |                |                 |
| Mean SD (W)      | 288 ± 43       | 273 ± 41       | 267 ± 41       | 264 ± 39        |
| ES               | 0.59           | 0.22           | 0.06           | -0.01           |
| MD (W)           | -32.8          | -18.1          | -10.0          | -7.3            |
| r                | 0.91           | 0.96           | 0.95           | 0.93            |
| OBLA 3.5         |                |                |                |                 |
| Mean SD (W)      | 297 ± 45       | 282 ± 41       | 274 ± 41       | 272 ± 40        |
| ES               | 1.05           | 0.67           | 0.43           | 0.36            |
| MD (W)           | -41.3          | -26.3          | -16.8          | -14.2           |
| r                | 0.91           | 0.97           | 0.95           | 0.93            |
| Baseline + 0.5   |                |                |                |                 |
| Mean SD (W)      | 235 ± 38       | 229 ± 40       | 228 ± 41       | 225 ± 37        |
| ES               | -0.75          | -0.90          | -0.93          | -1.00           |
| MD (W)           | 29.4           | 35.6           | 36.6           | 39.5            |
| r                | 0.74           | 0.81           | 0.83           | 0.82            |
| Baseline + 1.0   |                |                |                |                 |
| Mean SD (W)      | 255 ± 39       | 239 ± 40       | 236 ± 39       | 235 ± 39        |
| ES               | 0.15           | -0.26          | -0.37          | -0.43           |
| MD (W)           | 9.5            | 25.3           | 27.9           | 29.1            |
| r                | 0.88           | 0.92           | 0.93           | 0.91            |
| Baseline + 1.5   |                |                |                |                 |
| Mean SD (W)      | 270 ± 41       | 254 ± 41       | 250 ± 39       | 248 ± 39        |
| ES               | 0.05           | -0.26          | -0.37          | -0.43           |
| MD (W)           | -6.0           | 10.1           | 14.7           | 16.8            |
| r                | 0.90           | 0.94           | 0.94           | 0.92            |
| Dmax             |                |                |                |                 |
| Mean SD (W)      | 246 ± 34       | 232 ± 36       | 223 ± 31       | 216 ± 33        |
| ES               | -0.47          | -0.81          | -1.06          | -1.24           |
| MD (W)           | 18.6           | 31.9           | 41.6           | 48.8            |
| r                | 0.94           | 0.97           | 0.96           | 0.95            |
| Modified Dmax    |                |                |                |                 |
| Mean SD (W)      | 278 ± 37       | 267 ± 39       | 255 ± 40       | 248 ± 37        |
| ES               | 0.33           | 0.07           | -0.25          | -0.40           |
| MD (W)           | -15.5          | -1.1           | 9.5            | 16.5            |

(Continued)
total) and the MLSS (all log-log methods were excluded given an ES > 1.0). Ten of the calculated LTs and the RCPMLSS met our inclusion criteria for final analysis—detailed comparisons with the MLSS are provided in Table 3 and Fig 3. Figs 3–7 shows Bland-Altman plots of the 11 estimations included in our analysis; the newly developed ModD<sub>max</sub> LT calculations (Fig 5 Panel C and D; Fig 6 Panel C) had the lowest limits of agreement with the MLSS. The log-log polynomial modified D<sub>max</sub> (Log-Poly-ModD<sub>max</sub>) method derived from GXT<sub>4</sub> provided the best estimation of the MLSS (Fig 5 Panel C). There was an inverse relationship between the power calculated for each of the 14 LTs and stage length (Tables 1 and 4).

<math>W_{\text{max}} \text{ and } \dot{V}O_2_{\text{peak}}</math>

There was an inverse relationship between GXT duration and both <math>W_{\text{max}}</math> and <math>\dot{V}O_2_{\text{peak}}</math> (Table 5). The <math>\dot{V}O_2_{\text{peak}}</math> values derived from GXT<sub>3</sub> and GXT<sub>4</sub> were similar to the <math>\dot{V}O_2_{\text{peak}}</math> measured during GXT<sub>1</sub> (Table 6); however, the values were outside the variability of the measurement (CV > 3%) [27]. <math>\dot{V}O_2_{\text{peak}}</math> values from GXT<sub>1</sub> and the corresponding VEB had the highest agreement (MD = 0.5 mL·kg<sup>-1</sup>·min<sup>-1</sup>, ICC = 0.96, SEM = 1.1 mL·kg<sup>-1</sup>·min<sup>-1</sup> and CV = 2.0%) compared with any GXT and corresponding VEB. The remaining GXTs and corresponding

---

Table 1. (Continued)

|       | GXT<sub>3</sub> | GXT<sub>4</sub> | GXT<sub>7</sub> | GXT<sub>10</sub> |
|-------|---------------|---------------|---------------|---------------|
| r     | 0.94          | 0.96          | 0.96          | 0.92          |
| ES    | 0.39          | 0.03          | -0.24         | -0.42         |
| Exp-D<sub>max</sub> | Mean SD (W) | 256 ± 35 | 243 ± 36 | 234 ± 34 | 228 ± 35 |
|       | MD (W) | 8.0 | 21.8 | 30.8 | 36.8 |
| r     | 0.92 | 0.97 | 0.96 | 0.94 |
| ES    | -0.20 | -0.55 | -0.78 | -0.93 |
| Log-Exp-MD<sub>max</sub> | Mean SD (W) | 286 ± 42 | 271 ± 42 | 260 ± 39 | 253 ± 40 |
|       | MD (W) | -21.7 | -7.0 | 4.3 | 11.1 |
| r     | 0.94 | 0.97 | 0.96 | 0.93 |
| ES    | 0.55 | 0.18 | -0.11 | -0.28 |
| RCP<sub>MLSS</sub> | Mean SD (W) | 271 ± 39 |
|       | MD (W) | -6.71 |
| r     | 0.92 |
| ES    | -0.17 |
| RCP   | Mean SD (W) | 315 ± 40 |
|       | MD (W) | -50.4 |
| r     | 0.91 |
| ES    | 1.27 |

Table 2. Mean, standard deviation, and range of the \(\dot{V}O_2\) and power associated with the maximal lactate steady state (MLSS) expressed as a percentage of the maximal power (\(W_{\text{max}}\)) and \(\dot{V}O_2_{\text{peak}}\) measured during each GXT. Note: The \(\dot{V}O_2\) at the MLSS was 81.4 ± 4.7% of the \(\dot{V}O_2_{\text{max}}\) (Defined as the highest measured \(\dot{V}O_2\) during any GXT).

|       | GXT<sub>3</sub> | GXT<sub>4</sub> | GXT<sub>7</sub> | GXT<sub>10</sub> |
|-------|---------------|---------------|---------------|---------------|
| \(\dot{V}O_2\) at MLSS | 83.0 ± 4.5 | 84.7 ± 4.7 | 86.1 ± 5.9 | 88.4 ± 6.0 |
| (\% of \(\dot{V}O_2_{\text{peak}}\)) | [75.5–90.7] | [76.6–91.9] | [73.9–94.2] | [77.4–103.2] |
| Power at MLSS | 62.9 ± 3.9 | 78.4 ± 4.3 | 82.4 ± 3.6 | 87.3 ± 4.4 |
| (\% of \(W_{\text{max}}\)) | [56.8–71.7] | [69.8–84.4] | [73.7–88.8] | [79.8–96.0] |

https://doi.org/10.1371/journal.pone.0199794.t001

https://doi.org/10.1371/journal.pone.0199794.t002
Table 3. Mean ± standard deviation, mean difference (MD), intraclass correlation coefficient (ICC), Lin’s concordance correlation coefficient (ρc), standard error of the measurement (SEM), effect size (ES) with 95% confidence limits, and coefficient of the variation (%CV) between the maximal lactate steady state (MLSS) and the eleven thresholds included in our analysis. The main findings of the present study are as follows. Only 11 of the 58 threshold values met our inclusion criteria as valid estimates of the MLSS. Of the 11 methods included in our analysis, three of the ModD<sub>max</sub> methods compared with the OBLA and baseline plus absolute lactate value methods. The V<sub>O</sub><sub>2peak</sub> values measured during the longer duration GXTs (GXT<sub>3-10</sub>) underestimated the V<sub>O</sub><sub>2peak</sub> values derived from GXT<sub>1</sub> (Table 6).

### Discussion

The main findings of the present study are as follows. Only 11 of the 58 threshold values met our inclusion criteria as valid estimates of the MLSS. Of the 11 methods included in our analysis, three of the ModD<sub>max</sub> methods yielded the most favourable estimations of the MLSS, and the Log-Poly-ModD<sub>max</sub> derived from GXT<sub>1</sub> provided the best estimation of the MLSS. There was an inverse relationship between stage length and LT, and this effect was larger in all D<sub>max</sub> methods compared with the OBLA and baseline plus absolute lactate value methods. The V<sub>O</sub><sub>2peak</sub> values measured during the longer duration GXTs (GXT<sub>3-10</sub>) underestimated the V<sub>O</sub><sub>2max</sub> and the V<sub>O</sub><sub>2peak</sub> values obtained from GXT<sub>1</sub> (MD = 1.2 to 4.8 mL kg<sup>-1</sup> min<sup>-1</sup>). Finally, contrary to our hypothesis, the VEB after the longer duration GXTs did not yield V<sub>O</sub><sub>2peak</sub> values comparable to the V<sub>O</sub><sub>2peak</sub> derived from GXT<sub>1</sub>.

The use of five GXT protocols, 14 common LT methods, the RCP and RCP<sub>MLSS</sub> resulted in 58 unique thresholds. However, despite their common use, we observed that only 11 of these values met our criteria for inclusion (MD < 7.9 W; ES < 0.2; r < 0.90). Of the four D<sub>max</sub> methods included in our analysis, one consisted of the traditional ModD<sub>max</sub> method [22]. This had the poorest agreement relative to the other ModD<sub>max</sub> methods included in our analysis. The remaining three D<sub>max</sub> methods are new variations of the ModD<sub>max</sub> method, and the Log-Poly-
ModD_{max} derived from GXT_{2} had the highest correlation and lowest mean difference with the MLSS. These variations of the ModD_{max} method use the power at the log-log LT as the initial intensity to calculate the ModD_{max} and then either the traditional third-order polynomial or exponential plus-constant regression curve to fit the lactate curve [23, 41]. Although the validity of these three methods has not previously been assessed, the favourable estimations of the MLSS may be related to the greater objectivity with which they determine the intensity that corresponds with the initial rise in blood lactate concentration [37].
Fig 5. (A-D) Bland-Altman plots displaying agreement between measures of the power associated with the (A) OBLA of 2.5 mmol L\(^{-1}\), (B) Modified D\(_{max}\), (C) Log-Poly-Modified D\(_{max}\), (D) Log-Exp-Modified D\(_{max}\). The differences between measures (y-axis) are plotted as a function of the mean of the two measures (x-axis) in power (Watts). The horizontal solid line represents the mean difference between the two measures (i.e., bias). The two horizontal dashed lines represent the limits of agreement (1.96 x standard deviation of the mean difference between the lactate threshold and the maximal lactate steady state). The dotted diagonal lines represent the boundaries of the 95% CI for MLSS reliability (CV = 3.0%; 95% CI = 3.8%) calculated from Hauser et al., 2014) (log = Modified D\(_{max}\) method using the log-log method as the point of the initial lactate point; poly = Modified D\(_{max}\) method calculated using a third order polynomial regression equation; exp = Modified D\(_{max}\) method calculated using a constant plus exponential regression equation; OBLA = onset of blood lactate accumulation).

https://doi.org/10.1371/journal.pone.0199794.g005

Although the original D\(_{max}\) method is a commonly cited method for determining the LT [23], we observed large mean differences (19 to 49 W) between the D\(_{max}\) and MLSS. Three previous studies have purported to investigate the validity of this method to estimate the MLSS in trained male cyclists [15, 52, 53]. One concluded that the D\(_{max}\) method derived from GXT\(_3\) was a valid estimation of the MLSS (r = 0.97) [54]. We also observed a high correlation between D\(_{max}\) and the MLSS (r = 0.94 to 0.97) (Table 1), but, as indicated by the MD and other measures, a high correlation is not sufficient to establish validity [55]. Another study examined D\(_{max}\) derived from two GXTs with similar durations (36 vs. 39 min), but with different stage lengths (30-s vs. 6-min) [15]. The D\(_{max}\) derived from GXT\(_{30s}\) was not correlated (r = 0.51) with the MLSS, even though the MD was 5 W, whilst the D\(_{max}\) derived from GXT\(_6\) was correlated (r = 0.85); however, it underestimated the MLSS (MD = 22 W). The third study concluded the D\(_{max}\) derived from GXT\(_1\) yielded poor estimates of the MLSS (r = 0.56; bias = -1.8 ± 38.1 W) [53]. Thus, although some studies [15, 54] have used correlation analysis to suggest the D\(_{max}\) provides a valid estimate of the MLSS, this is not supported by the more comprehensive assessment of validity performed in the present and other studies [53].

There were five fixed blood LT methods and one baseline plus an absolute value that met our inclusion criteria, and, as previously reported [15, 24], these varied with the GXT protocol used. The baseline + 1.5 mmol L\(^{-1}\) was the only LT derived from GXT\(_3\) included in our analysis (bias = -6 ± 35 W). This is consistent with the results of one previous study (bias = 0.5 ± 24 W), which also recruited trained male cyclists and had a similar GXT protocol design [56]. Consistent with our findings, this study also reported that an OBLA of 3.5 mmol L\(^{-1}\) derived from GXT\(_1\) did not provide a valid estimation of the MLSS. In contrast, another study confirmed the validity of the OBLA of 3.5 mmol L\(^{-1}\) [52], despite recruiting trained cyclists and using an identical GXT protocol. These conflicting results are likely attributable to the low reproducibility of the OBLA methods [16].

While none of the OBLAs from GXT\(_3\) met our inclusion criteria, the OBLA methods of 2.5 mmol L\(^{-1}\) derived from GXT\(_4\) and GXT\(_7\) provided valid estimations of the MLSS, as did the OBLA of 3.0 mmol L\(^{-1}\) derived from GXT\(_7\) and GXT\(_{10}\). The OBLA of 3.5 mmol L\(^{-1}\) from GXT\(_{10}\) was the highest fixed blood LT that identified the MLSS. There is no previous data investigating the validity of these OBLA methods. However, it is worth noting that these five methods provided superior estimations of the MLSS compared with the original ModD\(_{max}\), but were less favourable than the newly-developed ModD\(_{max}\) methods.

An OBLA of 4.0 mmol L\(^{-1}\) is the most commonly-accepted fixed blood lactate value for estimating the LT or MLSS. Three previous studies have attempted to validate use of an OBLA of 4.0 mmol L\(^{-1}\) with cycle ergometry [15, 53, 57]. One study found that it overestimated the MLSS (MD = 49 W) when derived from GXT\(_4\) [53]. The other study reported poor agreement (bias 7 ± 49 W) when OBLA of 4.0 mmol L\(^{-1}\) was derived from GXT\(_4\) [57]. The final study observed a poor correlation between an OBLA of 4.0 mmol L\(^{-1}\) and the MLSS (r = 0.71) [15]. Our results indicated the OBLA of 4.0 mmol L\(^{-1}\) overestimated the MLSS across all GXTs.
Fig 6. (A-C) Bland-Altman plots displaying agreement between measures of the power associated with the (A) OBLA 2.5 mmol L\(^{-1}\) (GXT\(_{7}\)), (B) OBLA 3.0 mmol L\(^{-1}\) (GXT\(_{7}\)), (C) Log-Exp-Modified \(D_{\text{max}}\) calculated from GXT\(_{7}\) and the MLSS. The differences between measures (y-axis) are plotted as a function of the mean of the two measures (x-axis) in power (Watts). The horizontal solid line represents the mean difference between the two measures (i.e., bias). The two horizontal dashed lines represent the limits of agreement (1.96 x standard deviation of the mean difference between the lactate threshold and the maximal lactate steady state). The dotted diagonal lines represent the boundaries of the 95%
Thus, in agreement with previous research, our results indicate; the OBLA of 4.0 mmol L\(^{-1}\) does not accurately estimate the MLSS. It is also worth noting that the original authors cautioned the use of this OBLA method, given the lack of a significant correlation when comparing OBLA methods from a GXT and the MLSS [24].

The RCP derived from an 8- to 12-minute GXT consistently overestimates the MLSS [44, 53], and this was confirmed in our study (Table 1). Therefore, we used a regression equation based on the RCP (RCP\(_{MLSS}\) (Eq 5) to estimate the starting intensity for establishing the MLSS [33]. Our results indicate there was good agreement between the MLSS and RCP\(_{MLSS}\).
Nonetheless, for many participants the difference between MLSS and RCP exceeded the CV% for the MLSS (Fig 3). Therefore, although the RCP can be used as a convenient ‘starting point’ when establishing the MLSS, we recommend methods based on blood sampling from the current study and assessing blood lactate kinetics in real time as recommended by Hering et al. [58] for a more accurate estimation of the MLSS.

(Table 3). Nonetheless, for many participants the difference between MLSS and RCP exceeded the CV% for the MLSS (Fig 3). Therefore, although the RCP can be used as a convenient ‘starting point’ when establishing the MLSS, we recommend methods based on blood sampling from the current study and assessing blood lactate kinetics in real time as recommended by Hering et al. [58] for a more accurate estimation of the MLSS.
Validation of a single visit graded exercise test

Table 5. Mean and standard deviation of \(\dot{V}O_2\text{peak}\)—highest measured \(\dot{V}O_2\) during any graded exercise test (GXT); GXT \(\dot{V}O_2\)—highest measured \(\dot{V}O_2\) during each GXT; VEB \(\dot{V}O_2\)—highest measured \(\dot{V}O_2\) during each verification exhaustive bout (VEB); \(\dot{V}O_2\text{peak}\), highest measured \(\dot{V}O_2\) during either the GXT or corresponding VEB. Mean and standard deviation of GXT duration, max power (Watts) from each GXT, percentage of maximum power from the prolonged GXT expressed as a percentage (% of the maximal power measured during the GXT. The subscript (i.e., 1, 3, 4, 7 or 10) refers to the stage duration (minutes) for each test.

|                      | GXT1 | GXT3 | GXT4 | GXT7 | GXT10 |
|----------------------|------|------|------|------|-------|
| \(\dot{V}O_{2\text{max}}\) (mL.kg\(^{-1}\).min\(^{-1}\)) | 60.6 ± 5.4 | 58.2 ± 5.3 | 57.3 ± 5.7 | 56.4 ± 5.2 | 54.9 ± 4.9 |
| GXT \(\dot{V}O_2\) (mL.kg\(^{-1}\).min\(^{-1}\)) | 60.1 ± 5.8 | 58.9 ± 5.9 | 58.8 ± 6.1 | 56.4 ± 5.9 | 54.7 ± 6.6 |
| VEB \(\dot{V}O_2\) (mL.kg\(^{-1}\).min\(^{-1}\)) | 61.0 ± 5.3 | 59.7 ± 5.4 | 58.9 ± 6.0 | 57.3 ± 5.4 | 56.2 ± 5.5 |
| GXT Duration (min)   | 11.3 ± 0.9 | 26.8 ± 1.4 | 34.9 ± 1.9 | 59.2 ± 3.3 | 81.6 ± 4.6 |
| Maximum Power (Watts) | 420 ± 55 | 337 ± 46 | 321 ± 47 | 303 ± 43 | 295 ± 43 |
| Percent \(\dot{W}_{\text{max}}\) of GXT1 (%) | 100 | 80.3 ± 2.9 | 76.4 ± 3.1 | 72.1 ± 3.6 | 70.3 ± 4.0 |
| VEB (Watts)          | 378 ± 50 |          |          |          |       |
| VEB (% of GXT \(\dot{W}_{\text{max}}\)) | 90 | 109.7 ± 3.8 | 118.4 ± 18.7 | 125.4 ± 19.3 | 128.8 ± 20.4 |

Although a single GXT can be used to estimate both \(\dot{V}O_{2\text{max}}\) and LT, the optimal test duration for each measure is different [11, 13]. To address this challenge, we added a supramaximal VEB after each GXT, equivalent to that performed following GXT1, expecting all VEBs would yield similar \(\dot{V}O_2\) values. However, the \(\dot{V}O_{2\text{peak}}\) values from the VEB after the longer duration GXTs underestimated the \(\dot{V}O_{2\text{peak}}\) from GXT1. Although the \(\dot{V}O_{2\text{peak}}\) values from GXT1 and GXT4 were similar to GXT1, the differences were larger than the typical coefficient of variability for \(\dot{V}O_{2\text{peak}}\) (CV < 3%) [59]. Our results are consistent with previous recommendations that longer duration GXTs are not optimal for establishing \(\dot{V}O_{2\text{peak}}\) [10, 60]. Furthermore, while a VEB can be used to verify that \(\dot{V}O_{2\text{peak}}\) was achieved, it appears that a VEB following a prolonged GXT cannot be used to establish \(\dot{V}O_{2\text{max}}\).

Extending the duration of the GXT stages results in a lower \(\dot{W}_{\text{max}}\) [61]. This has implications for exercise prescription, as it is common in sport and exercise science research to prescribe exercise intensity as a percentage of \(\dot{W}_{\text{max}}\). For example, in the present study the MLSS ranged from 63 ± 4% (range = 52 to 72%) of \(\dot{W}_{\text{max}}\) from GXT1 to 82 ± 4% (range = 74 to 88%)

Table 6. Mean difference (MD) and standard deviation, effect size (ES), coefficient of the variation (CV) and p-value (p) for the measured \(\dot{V}O_{2\text{peak}}\) values from GXT1 compared with the \(\dot{V}O_{2\text{peak}}\) values from GXT3, GXT4, GXT7, and GXT10 and for the \(\dot{V}O_{2\text{peak}}\) values from GXT4 compared with the \(\dot{V}O_{2\text{peak}}\) values from the VEB following GXT3, GXT6, GXT7, and GXT10. The subscript (i.e., 1, 3, 4, 7 or 10) refers to the stage duration (minutes) for each test.

|                      | GXT1 vs. GXT3 | GXT1 vs. GXT4 | GXT1 vs. GXT7 | GXT1 vs. GXT10 |
|----------------------|---------------|---------------|---------------|---------------|
| MD (mL.kg\(^{-1}\).min\(^{-1}\)) | -1.2 ± 3.3 | -2.1 ± 4.2 | -3.7 ± 4.7 | -4.8 ± 3.7 |
| ES                   | 0.23          | 0.36          | 0.69          | 0.88          |
| CV (%)               | 3.8           | 4.9           | 5.6           | 4.6           |
| p                    | 0.13          | 0.06          | < 0.01        | < 0.01        |
|                      | GXT1 vs. VEB GXT3 | GXT1 vs. VEB GXT4 | GXT1 vs. VEB GXT7 | GXT1 vs. VEB GXT10 |
| MD (mL.kg\(^{-1}\).min\(^{-1}\)) | -2.1 ± 5.9 | -2.1 ± 6.1 | -4.6 ± 5.9 | -6.2 ± 6.6 |
| ES                   | 0.37          | 0.37          | 0.81          | 1.04          |
| CV (%)               | 4.2           | 4.9           | 6.1           | 5.9           |
| p                    | 0.02          | 0.98          | 0.03          | 0.03          |

https://doi.org/10.1371/journal.pone.0199794.0105

https://doi.org/10.1371/journal.pone.0199794.0106
of \( W_{\text{max}} \) from GXT\(_4\). Prescribing exercise in the current study cohort at a fixed percentage of \( W_{\text{max}} \) (e.g., 73% of \( W_{\text{max}} \)), would result in all participants exercising above or below the MLSS, GXT\(_1\) and GXT\(_4\), respectively. This is important as it has previously been reported that prescribing exercise relative to LT results in a more homogenous physiological response than when exercise performed relative to \( W_{\text{max}} \) [62]. This also highlights why it is important to consider the GXT protocol and the method used to determine relative exercise intensity when comparing results between studies.

The wide range of \( W_{\text{max}} \) for each GXT is also noteworthy, the \( W_{\text{max}} \) range for GXT\(_1\) was 320 to 517 W and the duration ranged from 9 to 12 minutes. Had we employed a standardized GXT (e.g., 35 W increments), and assuming \( W_{\text{max}} \) stayed constant, the range would have been 9- to 15 min. Applying this to our longer duration GXTs resulted in a homogenous duration (GXT\(_4\): 32- to 39 min), whereas a standardised approach (e.g., 35 W increments) would have resulted in a range of 27- to 46 min [57]. Thus, individualizing GXT protocol design is a useful approach to ensure homogenous test duration [17].

**Conclusion**

In conclusion, the traditional \( D_{\text{max}} \) and OBLA of 4.0 mmol L\(^{-1}\) did not provide valid estimates of the MLSS. The best estimation of the MLSS was the Log-Poly-Mod\( D_{\text{max}} \), derived from GXT\(_4\). The validity of our newly-developed Mod\( D_{\text{max}} \) model may relate to the objectivity for determining the initial rise in blood lactate concentration. However, we must advise caution with the use of our newly-developed method until future research investigates the reliability and reproducibility. It is apparent that both \( \dot{V}O_{2\text{max}} \) and LT cannot be determined in a single GXT, even if the GXT is followed by a VEB. Therefore, to appropriately determine \( \dot{V}O_{2\text{max}} \) the optimum duration of a GXT is 8–12 minutes and the \( \dot{V}O_{2} \) values measured during the GXT and VEB be within 3% = CV [63]. Our data also highlight how differences in GXT protocol design and methods used to calculate the relative exercise intensity may contribute to the conflicting findings reported in the literature.

**Author Contributions**

**Conceptualization:** Nicholas A. Jamnick, Javier Botella, David B. Pyne, David J. Bishop.

**Data curation:** Nicholas A. Jamnick, Javier Botella, David B. Pyne, David J. Bishop.

**Formal analysis:** Nicholas A. Jamnick, Javier Botella, David B. Pyne, David J. Bishop.

**Investigation:** Nicholas A. Jamnick, Javier Botella, David J. Bishop.

**Methodology:** Nicholas A. Jamnick, Javier Botella, David B. Pyne, David J. Bishop.

**Project administration:** Nicholas A. Jamnick, David J. Bishop.

**Resources:** Nicholas A. Jamnick, Javier Botella, David B. Pyne, David J. Bishop.

**Software:** Nicholas A. Jamnick.

**Supervision:** David B. Pyne.

**Validation:** Nicholas A. Jamnick, Javier Botella, David J. Bishop.

**Visualization:** Javier Botella.

**Writing – original draft:** Nicholas A. Jamnick, Javier Botella, David B. Pyne, David J. Bishop.
Writing – review & editing: Nicholas A. Jamnick, Javier Botella, David B. Pyne, David J. Bishop.

References
1. Skinner JS, McLellan TH. The transition from aerobic to anaerobic metabolism. Research Quarterly for Exercise and Sport. 1980; 51(1):234–48. https://doi.org/10.1080/02701367.1980.10609285 PMID: 7394286
2. Londeree BR. Effect of training on lactate/ventilatory thresholds: a meta-analysis. Medicine and Science in Sport and Exercise. 1997; 29(6):837–43. PMID: 9219214
3. Wenger HA, Bell GJ. The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness. Sports Medicine. 1986; 3(5):346–56. PMID: 3529283
4. Mann T, Lamberts RP, Lambert MI. Methods of prescribing relative exercise intensity: physiological and practical considerations. Sports Medicine. 2013; 43(7):613–25. https://doi.org/10.1007/s40279-013-0045-x PMID: 23620244
5. Coen B, Schwarz L, Urhausen A, Kindermann W. Control of training in middle-and long-distance running by means of the individual anaerobic threshold. International Journal of Sports Medicine. 1991; 12(06):519–24.
6. Granata C, Jamnick NA, Bishop DJ. Principles of Exercise Prescription, and How They Influence Exercise-Induced Changes of Transcription Factors and Other Regulators of Mitochondrial Biogenesis. Sports Medicine. 2018:1–19.
7. González-Haro C. Differences in Physiological Responses Between Short-vs. Long-Graded Laboratory Tests in Road Cyclists. Journal of Strength and Conditioning Research. 2015; 29(4):1040–8. https://doi.org/10.1152/jsm.00163.2015 PMID: 26153008
8. McNaughton LR, Roberts S, Bentley DJ. The relationship among peak power output, lactate threshold, and short-distance cycling performance: effects of incremental exercise test design. Journal of Strength and Conditioning Research. 2006; 20(1):157. https://doi.org/10.1519/R-15914.1 PMID: 16506862
9. Bentley D, McNaughton L, Batterham A. Prolonged stage duration during incremental cycle exercise: effects on the lactate threshold and onset of blood lactate accumulation. European Journal of Applied Physiology. 2001; 85(3–4):351–7. https://doi.org/10.1007/s004210010452 PMID: 11560091
10. Poole DC, Jones AM. Measurement of the maximum oxygen uptake \( \dot{V}O_2 \text{max} \). \( \dot{V}O_2 \text{peak} \) is no longer acceptable. Journal of Applied Physiology. 2017; 122(4):997–1002. https://doi.org/10.1152/japplphysiol.01063.2016 PMID: 28153947
11. Buchfuhrer MJ, Hansen JE, Robinson TE, Sue DY, Wasserman K, Whipp BJ. Optimizing the exercise protocol for cardiopulmonary assessment. Journal of Applied Physiology. 1983; 55(5):1558–64. https://doi.org/10.1152/jappl.1983.55.5.1558 PMID: 6643191
12. Yoon B-K, Kravitz L, Robergs R. VO2max, Protocol Duration, and the VO2 Plateau. Medicine and Science in Sport and Exercise. 2007; 39(7):1186–92. https://doi.org/10.1249/mss.0b13e3188054e304 PMID: 17596788
13. Bentley DJ, Newell J, Bishop D. Incremental exercise test design and analysis. Sports Medicine. 2007; 37(7):575–86. PMID: 17595153
14. Foxdal P, Sjödin A, Sjödin B. Comparison of blood lactate concentrations obtained during incremental and constant intensity exercise. International Journal of Sports Medicine. 1996; 17(05):360–5.
15. Van Schuylenbergh R, Vanden EB, Hess P. Correlations between lactate and ventilatory thresholds and the maximal lactate steady state in elite cyclists. International Journal of Sports Medicine. 2004; 25(6):403–8. https://doi.org/10.1055/s-2004-819942 PMID: 15346226
16. Morton RH, Stannard SR, Kay B. Low reproducibility of many lactate markers during incremental cycle exercise. British Journal of Sports Medicine. 2012; 46(1):64–9. https://doi.org/10.1136/bjsm.2010.076380 PMID: 21343140
17. Pettitt R, Clark I, Ebner S, Sedgeman D, Murray S. Gas Exchange Threshold and VO2max Testing for Athletes: An Update. Journal of Strength and Conditioning Research. 2013; 27(2):549–55. https://doi.org/10.1519/JSC.0b013e31825770d7 PMID: 22531615
18. Faude O, Kindermann W, Meyer T. Lactate threshold concepts. Sports Medicine. 2009; 39(6):469–90. https://doi.org/10.2165/00007256-200939060-00003 PMID: 19453206
19. Ivy JL, Withers RT, Vanhanel PJ, Elger DH, Costill DL. Muscle respiratory capacity and fiber type as determinants of the lactate threshold. Journal of Applied Physiology. 1980; 48(3):523–7. PubMed PMID: WOS:A1980JJ95100018. https://doi.org/10.1152/jappl.1980.48.3.523 PMID: 7372524
20. Kindermann W, Simon G, Keul J. Significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. European Journal of Applied Physiology and Occupational Physiology. 1979; 42(1):25–34. https://doi.org/10.1007/bf00421101 PubMed PMID: WOS: A1979HN19000003. PMID: 499194

21. Heck H, Mader A, Hess G, Mucke S, Muller R, Hollmann W. Justification of the 4-mmol/L lactate threshold. International Journal of Sports Medicine. 1985; 6(3):117–30. https://doi.org/10.1055/s-2008-1025824 PubMed PMID: WOS:A1985AL51800002. PMID: 4030186

22. Bishop D, Jenkins DG, Mackinnon LT. The relationship between plasma lactate parameters, Wpeak and 1-h cycling performance in women. Medicine and Science in Sports and Exercise. 1998; 30(8):1270–5. PMID: 9710868

23. Cheng B, Kuipers H, Snyder A, Keizer H, Hesselink M. A new approach for the determination of ventilatory and lactate thresholds. International Journal of Sports Medicine. 1992; 13(7):518–22. https://doi.org/10.1055/s-2007-1021309 PMID: 1459746

24. Billat VL, Sirvent P, Py G, Koralsztein J-P, Mercier J. The concept of maximal lactate steady state. Sports Medicine. 2003; 33(6):407–26. PMID: 12744715

25. Beneke R. Methodological aspects of maximal lactate steady state—implications for performance testing. European Journal of Applied Physiology. 2003; 89(1):95–9. https://doi.org/10.1007/s00421-002-0783-1 PMID: 12627312

26. Hauser T, Bartsch D, Baumgärtel L, Schulz H. Reliability of maximal lactate- steady-state. International Journal of Sports Medicine. 2013; 34(3):196–9. https://doi.org/10.1055/s-0032-1321719 PMID: 22972242

27. Hill AV, Long C, Lupton H. Muscular exercise, lactic acid, and the supply and utilisation of oxygen. Proceedings of the Royal Society of London Series B, Containing Papers of a Biological Character. 1924; 97(681):84–138.

28. Riebe D, Franklin BA, Thompson PD, Garber CE, Whitfield GP, Magal M, et al. Updating ACSM’s recommendations for exercise preparticipation health screening. Medicine and Science in Sport and Exercise. 2015; 47(11):2473–9. https://doi.org/10.1249/MSS.0000000000000664 PMID: 26473759

29. Jamnick NA, By S, Pettitt CD, Pettitt RW. Comparison of the YMCA and a Custom Submaximal Exercise Test for Determining VO2max. Medicine and Science in Sports and Exercise. 2016; 48(2):254–9. https://doi.org/10.1249/MSS.0000000000000763 PMID: 26339726

30. Jackson AS, Blair SN, Mahar MT, Wier LT, Ross RM, Stuteville JE. Prediction of functional aerobic capacity without exercise testing. Medicine and Science in Sports and Exercise. 1990; 22(6):863–70. Epub 1990/12/01. PMID: 22872267.

31. Medicine ACoS. ACSM’s guidelines for exercise testing and prescription: Lippincott Williams & Wilkins; 2013.

32. Smekal G, von Duvillard SP, Pokan R, Hofmann P, Braun WA, Arciero PJ, et al. Blood lactate concentration at the maximal lactate steady state is not dependent on endurance capacity in healthy recreationally trained individuals. European Journal of Applied Physiology. 2012; 112(8):3079–86. https://doi.org/10.1007/s00421-011-2283-7 PMID: 22194004

33. Beaver W, Wasserman K, Whipp B. A new method for detecting anaerobic threshold by gas exchange. Journal of Applied Physiology. 1986; 60(6):2020–7. https://doi.org/10.1152/jappl.1986.60.6.2020 PMID: 3087938

34. Whipp BJ, Davis JA, Wasserman K. Ventilatory control of the ‘isocapnic buffering’region in rapidly-incremental exercise. Respiration Physiology. 1989; 76(3):357–67. PMID: 2501844

35. Caiozzo VJ, Davis JA, Ellis JF, Azus JL, Vandagriff R, Pietto C, et al. A comparison of gas exchange indices used to detect the anaerobic threshold. Journal of Applied Physiology. 1982; 53(5):1184–9. https://doi.org/10.1152/jappl.1982.53.5.1184 PMID: 7174412

36. Beaver W, Wasserman K, Whipp B. Improved detection of lactate threshold during exercise using a log-log transformation. Journal of Applied Physiology. 1985; 59(6):1936–40. https://doi.org/10.1152/jappl.1985.59.6.1936 PMID: 4077801

37. Kindermann W, Simon G, Keul J. The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. European Journal of Applied Physiology and Occupational Physiology. 1979; 42(1):25–34. PMID: 499194

38. Zoladz JA, Rademaker A, Sargeant AJ. Non-linear relationship between O2 uptake and power output at high intensities of exercise in humans. Journal of Physiology. 1995; 488(Pt 1):211.

39. Berg A, Jakob E, Lehmann M, Dickhuth H, Huber G, Keul J. Current aspects of modern ergometry. Pneumologie (Stuttgart, Germany). 1990; 44(1):2.
41. Machado FA, Nakamura FY, Moraes SMFD. Influence of regression model and incremental test protocol on the relationship between lactate threshold using the maximal-deviation method and performance in female runners. Journal of Sports Sciences. 2012; 30(12):1267–74. https://doi.org/10.1080/02640414.2012.702424 PMID:22775431
42. Hughson RL, Weisiger KH, Swanson GD. Blood lactate concentration increases as a continuous function in progressive exercise. Journal of Applied Physiology. 1987; 62(5):1975–81. https://doi.org/10.1152/jappl.1987.62.5.1975 PMID:3597269
43. Lamarrà N, Whipp BJ, Ward SA, Wasserman K. Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. Journal of Applied Physiology. 1987; 62(5):2003–12. https://doi.org/10.1152/jappl.1987.62.5.2003 PMID:3110126
44. Keir DA, Fontana FY, Robertson TC, Murias JM, Paterson DH, Kowalchuk JM, et al. Exercise Intensity Thresholds: Identifying the Boundaries of Sustainable Performance. Medicine and Science in Sport and Exercise. 2015; 47(9):1932–40. https://doi.org/10.1249/MSS.0000000000000613 PMID:25606817
45. Keir DA, Murias JM, Paterson DH, Kowalchuk JM. Breath-by-breath pulmonary O2 uptake kinetics: effect of data processing on confidence in estimating model parameters. Experimental Physiology. 2014; 99(11):1511–22. https://doi.org/10.1113/expphysiol.2014.080812 PMID:25063837
46. Lawrence I, Lin K. A concordance correlation coefficient to evaluate reproducibility. Biometrics. 1989;255–68. PMID:2720055
47. Bland JM, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. The Lancet. 1986; 327(8476):307–10.
48. Hopkins W. Analysis of validity by linear regression (Excel spreadsheet). 2015; 19:36–44. https://doi.org/10.1249/MSS.0b013e31818cb278 PMID:19092709
49. Hopkins W. Measures of reliability in sports medicine and science. Sports Medicine. 2000; 30(1):1–15. PMID:10907753
50. Hopkins W, Marshall S, Batterham A, Hanin J. Progressive statistics for studies in sports medicine and exercise science. Medicine and Science in Sports and Exercise. 2009; 41(1):3. https://doi.org/10.1249/MSS.0b013e3181318cb278 PMID:19092709
51. Cohen J. A power primer. Psychological Bulletin. 1992; 112(1):155. PMID:19565683
52. Denadai B, Figueira T, Favaro O, Gonçalves M. Effect of the aerobic capacity on the validity of the anaerobic threshold for determination of the maximal lactate steady state in cycling. Brazilian Journal of Medical and Biological Research. 2004; 37(10):1551–6. https://doi.org/10.1590/S0100-879X2004001000015 PMID:15448877
53. Pallarés JG, Morán-Navarro R, Ortega JF, Fernández-Elias VE, Mora-Rodríguez R. Validity and Reliability of Ventilatory and Blood Lactate Thresholds in Well-Trained Cyclists. PloS one. 2016; 11(9):e0163389. https://doi.org/10.1371/journal.pone.0163389 PMID:27657502
54. Czuba M, Zając A, Cholewa J, Poprzęcki S, Waśkiewicz Z, Mikolajek K. Lactate threshold (D-max method) and maximal lactate steady state in cyclists. Journal of Human Kinetics. 2009; 21:49–56.
55. Aldrich J. Correlations genuine and spurious in Pearson and Yule. Statistical Science. 1995:364–76.
56. Grossi T, De Lucas RD, De Souza KM, Antonacci Guglielmo LG. Maximal lactate steady-state and anaerobic thresholds from different methods in cyclists. European Journal of Sport Science. 2012; 12 (2):161–7. https://doi.org/10.1007/s00221-011-1286-2
57. Hauser T, Adam J, Schulz H. Comparison of selected lactate parameters with maximum lactate steady state in cycling. International Journal of Sports Medicine. 2014; 35(6):517–21. https://doi.org/10.1055/s-0033-1353176 PMID:24227122
58. Hering GO, Hennig EM, Riehle HJ, Stepan J. A Lactate Kinetics Method for Assessing the Maximal Anaerobic Thresholds: Identifying the Boundaries of Sustainable Performance. Medicine and Science in Sport and Exercise. 1986; 327(8476):307–10. PMID:2720055
59. Keir DA, Fontana FY, Robertson TC, Murias JM, Paterson DH, Kowalchuk JM, et al. Exercise Intensity Thresholds: Identifying the Boundaries of Sustainable Performance. Medicine and Science in Sport and Exercise. 2015; 47(9):1932–40. https://doi.org/10.1249/MSS.0000000000000613 PMID:25606817
60. Keir DA, Murias JM, Paterson DH, Kowalchuk JM. Breath-by-breath pulmonary O2 uptake kinetics: effect of data processing on confidence in estimating model parameters. Experimental Physiology. 2014; 99(11):1511–22. https://doi.org/10.1113/expphysiol.2014.080812 PMID:25063837
61. Aldrich J. Correlations genuine and spurious in Pearson and Yule. Statistical Science. 1995:364–76.
62. Grossi T, De Lucas RD, De Souza KM, Antonacci Guglielmo LG. Maximal lactate steady-state and anaerobic thresholds from different methods in cyclists. European Journal of Sport Science. 2012; 12 (2):161–7. https://doi.org/10.1007/s00221-011-1286-2
63. Pettitt RW, Jamnick NA. Commentary on “Measurement of the maximum oxygen uptake $\dot{V}o_2$max: $\dot{V}o_2$peak is no longer acceptable”. Journal of Applied Physiology. 2017; 123(3):696–. https://doi.org/10.1152/japplphysiol.00338.2017 PMID:28947630