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Camila Peter Hoefelmann\textsuperscript{a}, Fernando Diefenthaeler\textsuperscript{a}, Vitor Pereira Costa\textsuperscript{a}, Ricardo Dantas de Lucas\textsuperscript{a}, Philip Shambrook\textsuperscript{b} & Luiz Guilherme Antonacci Guglielmo\textsuperscript{a}

\textsuperscript{a} Physical Effort Laboratory, Federal University of Santa Catarina, Florianópolis, Brasil
\textsuperscript{b} Sport Science Laboratory, Department of Sport Science, Eastern Institute of Technology, Napier, New Zealand

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Test–retest reliability of second lactate turnpoint using two different criteria in competitive cyclists

CAMILA PETER HOEFELMANN1, FERNANDO DIEFENTHAELER1, VITOR PEREIRA COSTA1, RICARDO DANTAS DE LUCAS1, PHILIP SHAMBROOK2, & LUIZ GUILHERME ANTONACCI GUGLIELMO1

1Physical Effort Laboratory, Federal University of Santa Catarina, Florianópolis, Brasil, 2Sport Science Laboratory, Department of Sport Science, Eastern Institute of Technology, Napier, New Zealand

Abstract
The aim of this study was to determine the relative and absolute reliability of second lactate turnpoint using fixed and individual blood lactate method in competitive cyclists. Twenty-eight male, well-trained cyclists (30.2 ± 10.1 years, 72.0 ± 7.4 kg, 177.3 ± 4.7 cm) were recruited to participate in this study. Cyclists completed two incremental cycling tests to exhaustion over a period of 7 days to determine their peak power output, maximal oxygen uptake, maximal heart rate, maximal blood lactate concentration and two lactate turnpoint criteria. The fixed blood concentration criterion (3.5 mM) and an individual criterion were assessed by a lactate-power curve, considering power output, heart rate and oxygen uptake. The main finding of this study was that both lactate turnpoint criteria showed identical low within-subject variation for power output (2.8% coefficient of variation). High values for test–retest correlations ranging from $r = 0.70$ to $r = 0.94$ were found for all variables in both threshold criteria. In conclusion, the individual and fixed method to determine the second lactate turnpoint showed similar high absolute and relative reliability in competitive cyclists.

Keywords: Reproducibility, cycling, onset of blood lactate accumulation, incremental test

Introduction
The blood lactate ([La]) response during incremental exercise has been studied extensively during the last three decades, with special attention being paid to the identification of two break points (Bentley, Newell, & Bishop, 2007; Bosquet, Léger, & Legros, 2002). The lactate threshold (Ivy, Withers, Van Handel, Helger, & Costill, 1980), onset of blood lactate accumulation (OBLA; Sjödin & Jacobs, 1981), anaerobic threshold (Heck et al., 1985), individual anaerobic threshold (Berg, Jakob, Lehmann, Dickhuth, & Huber, 1990; Stegmann, Kindermann, & Schnabel, 1981) and the lactate turning point (Davis et al., 1983) are terms that have been used to define the exercise intensity at the second break point of the blood lactate exponential curve during an incremental test (Bosquet et al., 2002). The majority of these methods were highlighted in a recent review (Faude, Kindermann, & Meyer, 2009). In fact, this break point attempts to estimate the so-called “maximal lactate steady state” (MLSS) that represents the maximal exercise intensity where the equilibrium between the rate of appearance and disappearance of lactate in the blood is maintained during a constant workload (Heck et al., 1985). The direct determination of MLSS usually requires 3–5 constant workload tests of up to 30 min duration, performed at exercise intensities between 65% and 90% $VO_{2max}$ (Beneke, 2003).

The second lactate turnpoint (LT) provided from a single-graded exercise test is widely used to overcome the difficulties inherent with the direct MLSS determination (Beneke, 2003). However, the second threshold is determined using several different criteria, precisely reviewed by Bosquet et al. (2002). For instance, Sjödin and Jacobs (1981) used a fixed concentration of 4 mmol·l$^{-1}$, while Heck et al. (1985) proposed a 3.5 mmol·l$^{-1}$ for 3-min
stage duration. Using a fixed lactate value as the threshold certainly increases objectivity but rejects individuality since the non-linear increase in [La−] does not always occur at the same level of [La−] (Stegmann et al., 1981). An individual method was introduced by Coyle et al. (1983) and technically extended by Berg et al. (1990), which adds a fixed 1.5 mmol·l−1 [La−] to the minimum ratio between lactate/workload ([La−]·W−1). This method was recently demonstrated to show better validity with MLSS estimation than fixed concentration criteria (De Souza et al., 2012; Grossl, De Lucas, De Souza, & Guglielmo, 2012).

However, the validity of a test should not be considered before its reliability has been proven to be acceptable. Reliability data will give an indication of biological and technical noise/error of the protocol and has important practical implications for monitoring an individual during an intervention programme (Hopkins, 2000; Hopkins, Schabort, & Hawley, 2001). According to Hopkins (2000) and Atkinson and Nevill (1998), the majority of sports science studies conducted during the 1980s and 1990s have analysed the relative reliability of measurements. This approach compared only means and showed how measures from test and retest were correlated. These aforementioned authors have shown the importance of analysing the absolute reliability by calculating the intra-individual variability or error.

There is scarce data about absolute reliability of LT, since most of the studies analysed a coefficient of correlation and compared the mean values of fixed concentration criteria (Aunola & Rusko, 1984; Heitkamp, Holdt, & Scheib, 1991; Weltman et al., 1990). To the best of our knowledge, we do not find studies that have investigated the absolute and relative reliability of an individual method that was proposed by Berg et al. (1990) and a 3.5 mmol·l−1 LT in a group of trained athletes. Thus, the aim of the present study was to verify the relative and absolute reliability of LT using the fixed and individual concentrations of [La−] in competitive cyclists.

**Methods**

**Subjects**

Twenty-eight male well-trained cyclists (age 30.2 ± 10.1 years, body mass 72.0 ± 7.4 kg, height 177.3 ± 4.7 cm; values are mean ± SD) were recruited to participate in this study. The cyclists had a minimum experience of 2 years in regular competitions. At the time of the experiment, cyclists were training between 10 ± 2 hours per week and were in a pre-competitive phase of the annual cycling racing programme. The appropriate local ethics committee approved the study. All cyclists were informed of the study purpose and the risks associated with participation before giving their written informed consent.

**Incremental exercise tests**

Cyclists recruited in this study had previously participated in laboratory experiments performed on cycle ergometers and were completely familiar with general exercise testing procedures. Cyclists were instructed to abstain from high-intensity training for 48 h prior to the trials. Cyclists were recommended to replicate their diet as closely as possible before each session and to avoid any caffeine consumption 3 h prior to testing. Subjects reported to the laboratory on two separate occasions over a period of approximately 7 days. During both visits to the laboratory, cyclists completed an incremental exercise test to exhaustion to determine their maximal oxygen uptake (VO2max), peak power output (POO), maximal heart rate (HRmax), maximal blood lactate concentration ([La−]max), individual lactate turnpoint (ILT) and OBLA. Tests were conducted on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode B. V., The Netherlands) in a laboratory under controlled environmental conditions. Air temperature and relative humidity were regulated to 20–22°C and 50–60%, respectively.

The cycle ergometer was initially adjusted to a position which was comfortable and resembled the setup of the subject’s own bicycles; the selected dimensions were recorded and replicated for subsequent tests. Cyclists performed a 10-min warm-up at a self-selected intensity between 70 and 100 W followed by 5 min of rest. Thereafter, a stepwise incremental exercise test was performed starting at 100 W and power output was increased at a rate of 30 W every 3 min until the cyclist reached volitional exhaustion. The use of stepwise protocols with stage duration between 3 and 5 min is necessary for the blood lactate to remain stable in each stage until the second lactate break point is achieved (Heck et al., 1985). The expired respiratory gases were collected and analysed using a Quark PFT ergo metabolic system (Cosmed, Rome, Italy) that was calibrated in accordance with the manufacturers’ instructions. VO2max was defined as the highest oxygen uptake (VO2) recorded over a 30-s period during the test. PPO was defined as follows (Kuipers, Verstappen, Keizer, Geurten, & van Kranenburg, 1985): PPO (W) = Wt + [(a3) × 30], where Wt was the power output of the last complete workload (W), t was the time (min) for the final incomplete workload, 3 was the workload time increment (min) and 30 was the power output increment (W). Heart rate (HR) was continuously recorded during the whole test with a
heart rate monitor (Polar S610, Polar Electro OY, Finland). During the last 30 s of each stage, capillary blood samples were obtained from the right earlobe and analysed using an electromagnetic technique (YSI 2700 STAT, Yellow Springs, OH, USA). The ILT was identified according to Berg’s methodology (Berg et al., 1990) in two steps: (1) identification of minimum value from the ratio Lac•W−1 for each subject; (2) ILT was identified at power output that corresponds to an addition of 1.5 mmol·l−1 at the minimum value reached on Step 1. The OBLA was determined at an intensity on a fixed value 3.5 mmol·l−1 (Heck et al., 1985). Also, power output, oxygen uptake and HR were identified in each threshold by linear interpolation between two segments.

Statistical analysis

Descriptive statistics are shown as mean ± standard deviation. Mean differences and their 95% confidence limits were estimated with a spreadsheet (Hopkins, 2000) via the unequal-variance t-statistic computed for change scores. Heteroscedasticity was examined by plotting the absolute differences against the individual means and calculating the correlation coefficient, in order to assess the significance of the relationship. Intraclass correlation coefficients (ICC), typical error of measurement (TE) and coefficient of variation (CV) were calculated according to Hopkins (2000) to determine the test–retest reliability. Statistical significance was set at p ≤ 0.05.

Results

There was no systematic bias (i.e. heteroscedasticity) in the data from test–retest for any variable measured (i.e. maximal and sub-maximal). Table I shows the maximal parameters from incremental exercise tests and reliability scores. There were no significant differences in the means for PPO, VO2max, HRmax and [La]max. High scores were found for ICC and low values of CV in most part of variables except in [La]max (Table I).

Table II shows the sub-maximal parameters from incremental exercise tests and reliability scores at ILT. There were no significant differences in the means for power output, VO2 and HR. However, the [La] was significantly (p < 0.05) higher during Test 2 compared to Test 1. We also found high scores for ICC in all measures. Furthermore, low values of CV were found for power output and HR (Table II).

Table III shows the sub-maximal parameters from incremental exercise tests and reliability scores at OBLA. There was no significant difference in the means in all measures. High scores were found for ICC in all variables. Moreover, we found low values of CV in power output and HR.

Discussion

The main finding of this study was that both criteria to determine the power output and HR at LT showed low within-subject variation. Furthermore, high values for retest correlations and non-significant difference in the means were also found in all physiological measures at LT. Collectively, the individual and fixed criterion to determine the LT showed similar high absolute and relative reliability in competitive cyclists.

The studies that have analysed the test–retest of LT have been using the relative reliability as criterion in repeated trials on the same individuals. Aunola and Rusko (1984) used a fixed 4.0 mmol·l−1 lactate method (OBLA) in 33 men aged 20–50 years using a cycle ergometer. The authors found high relative reliability of power, HR and VO2 throughout with a regression analysis all above r = 0.90, although this analysis is recommended to verify the relationship between a variable and one or more predictive factors (Atkinson & Nevill, 1998). Thereafter, Weltman et al. (1990) and Heitkamp et al. (1991) investigated the reliability of OBLA in trained male runners and endurance-trained females, respectively. They also found high correlations for the velocity at OBLA (above r = 0.90); however, the physiological variables showed lower correlations (r = 0.61 to r = 0.88) compared to Aunola and Rusko (1984). In addition, Heitkamp et al. (1991) reported low correlation coefficients of r = 0.65 for VO2, r = 0.55 for running speed and r = 0.42 for HR in a group of 27 untrained women. A likely reason for the lower correlations found at LT in untrained women in the latter study would be the level of fitness compared to the trained women.

Pfitzinger and Freedson (1998) established the reliability of several lactate markers using a treadmill. The authors used analysis of variance (ANOVA) to calculate a range of high ICC at different LT methods for velocity (r = 0.98 to r = 0.99), VO2 (r = 0.91 to r = 0.96), HR (r = 0.75 to r = 0.96). Frequently, the ANOVA is used to detect large systematic errors in results; however, it has the same limitation as the paired t-test; the detection of systematic bias is affected by large random variations (Atkinson & Nevill, 1998). Therefore, the reliability results of Aunola and Rusko (1984) and Pfitzinger and Freedson (1998) may provide non-realistic information about the reliability of LTs as the statistical analyses have been characterised by shortcomings (Grant et al., 2002).

Relative analyses by using correlation coefficients have been considered only as part of reliability...
(Weir, 2005), since it does not give any information on systematic bias. Moreover, the results of the study can be greatly influenced by a range of values in the sample (Atkinson & Nevill, 1998; Hopkins, 2000). Thus, the novelty of the present study was to investigate the absolute reliability using two methods to determine the LT. This included the individual criteria that has been reported to be more accurate to identify MLSS than the fixed concentration method (De Souza et al., 2012; Grossl et al., 2012). Our results showed similar and low within-subject variability for power output in both methods, although it is worthy of note that the mean values presented a difference of 20 W between methods (Tables II and III).

Jensen and Johansen (1998) tested seven well-trained cyclists to report absolute reliability of OBLA using a "limits of agreement approach". This study found a CV associated to limits of agreement of 5.9% for power output and 2.4% for HR. Absolute \( \dot{V}O_2 \) at LT showed worse reliability with 7.7% limits of agreement. These different reliabilities considering different variables are in line with the present study (Tables II and III). These values could be transformed to typical error in order to compare this study with others. As an example, Hopkins et al. (2001) recalculated the data from Jensen and Johansen (1998), and the power output at OBLA was with a CV of 4.2%. This value is higher than the power output at OBLA (3.5 mmol·l\(^{-1}\)) and ILT found in the present study (both 2.8%). There are a number of possible theories that could explain the large within-subject variability in the latter results. Jensen and Johansen (1998) tested a very small sample of well-trained cyclists using a stationary magnetic braked wheel that replaced the rear wheel of the bicycle. Hopkins et al. (2001) suggested that researchers should investigate the stability of the load with a dynamic calibration rig, or perform a reliability study on the ergometer, before using to assess individuals.

From a practical point of view, Hopkins (2000) pointed out that about 1.5–2.0 times the typical error could be used as a threshold above which any individual change would be interpreted as “a real change” following an intervention. In the present study, the typical error for the power output expressed as CV was 2.8% in both lactate break point criteria. This suggests that any individual change following an intervention needs to be greater than 4.2–5.6% for it to be “detectable” and the intervention to be considered effective. Interestingly, the meaningful change is not different between lactate break points investigated in our study, so the criterion does not influence the sensibility of the LT to detect a meaningful change in performance. Additionally, the PPO presented a similar typical error (CV = 2.6%), suggesting that this maximal parameter has the same sensibility (i.e. based on noise) to detect changes in performance.

### Table I. Maximal parameters from incremental exercise test and reliability scores

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test 1</th>
<th>Test 2</th>
<th>TE</th>
<th>CV (%)</th>
<th>ICC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO (w)</td>
<td>333 ± 30</td>
<td>333 ± 33</td>
<td>8.5</td>
<td>2.6</td>
<td>0.93 (0.85–0.97)</td>
</tr>
<tr>
<td>( \dot{V}O_2 )max (ml·kg(^{-1})·min(^{-1}))</td>
<td>63.5 ± 7.1</td>
<td>64.0 ± 6.5</td>
<td>2.7</td>
<td>4.2</td>
<td>0.85 (0.70–0.93)</td>
</tr>
<tr>
<td>( \dot{V}O_2 )max (l·min(^{-1}))</td>
<td>4.5 ± 0.4</td>
<td>4.5 ± 0.3</td>
<td>0.2</td>
<td>4.2</td>
<td>0.76 (0.54–0.88)</td>
</tr>
<tr>
<td>HRmax (bpm)</td>
<td>186 ± 9</td>
<td>185 ± 9</td>
<td>2.9</td>
<td>1.6</td>
<td>0.90 (0.80–0.95)</td>
</tr>
<tr>
<td>[La]max (mmol·l(^{-1}))</td>
<td>9.5 ± 2.2</td>
<td>9.6 ± 2.4</td>
<td>1.5</td>
<td>16.1</td>
<td>0.55 (0.23–0.76)</td>
</tr>
</tbody>
</table>

PPO, peak power output; \( \dot{V}O_2 \)max, maximal oxygen uptake; HRmax, maximal heart rate; [La]max, maximal blood lactate concentration; ICC, intra-class correlation; TE, typical error; CV, coefficient of variation; CI, 95% confidence interval.

### Table II. Sub-maximal parameters from incremental exercise test and reliability scores at ILT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test 1</th>
<th>Test 2</th>
<th>TE</th>
<th>CV (%)</th>
<th>ICC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (W)</td>
<td>251 ± 30</td>
<td>251 ± 29</td>
<td>7.1</td>
<td>2.8</td>
<td>0.94 (0.87–0.97)</td>
</tr>
<tr>
<td>( \dot{V}O_2 ) (ml·kg(^{-1})·min(^{-1}))</td>
<td>52.7 ± 6.8</td>
<td>53.3 ± 4.6</td>
<td>2.7</td>
<td>5.0</td>
<td>0.78 (0.58–0.89)</td>
</tr>
<tr>
<td>( \dot{V}O_2 ) (l·min)</td>
<td>3.8 ± 0.5</td>
<td>3.8 ± 0.3</td>
<td>0.2</td>
<td>5.4</td>
<td>0.72 (0.48–0.86)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>162 ± 11</td>
<td>161 ± 11</td>
<td>4.7</td>
<td>2.9</td>
<td>0.82 (0.65–0.91)</td>
</tr>
<tr>
<td>[La] (mmol·l(^{-1}))</td>
<td>2.5 ± 0.3</td>
<td>2.6 ± 0.3*</td>
<td>0.2</td>
<td>6.5</td>
<td>0.70 (0.45–0.85)</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \).

P, power output; \( \dot{V}O_2 \), oxygen uptake; HR, heart rate; [La], blood lactate concentration; ICC, intra-class correlation; TE, typical error; CV, coefficient of variation; 95% CI, 95% confidence interval.
Several papers have featured the reproducibility of physical performance tests and associated measures (Aunola & Rusko, 1984; Jensen & Johansen, 1998). However, few of them have specifically considered the number of repeated measurements as a factor affecting reproducibility (Morton, Stannard, & Kay, 2012). Morton et al. (2012) reported the total error in power output at several differing markers of LT measured during an incremental cycling exercise protocol repeated six–seven times per subject. The results showed high variability within-subject for the power output at OBLA (CV = 8.2%) and ICC of $r = 0.81$. The authors highlighted that the magnitude of the variation observed is such that the change in power output required to detect a change in an individual physiological profile with 95% confidence is apparently quite unrealistic for trained athletes. In agreement with this result, Grant et al. (2002) analysed the absolute reliability of OBLA during running incremental tests using a sample of 36 active men and women. The within-subject variability was analysed by 95% limits of agreement of Bland and Altman (1986). They found high variability of certain parameters (i.e. velocity and HR) at OBLA, indicating poor reliability in untrained subjects. Unfortunately, the authors did not provide the mean values in order to calculate the limits of agreement of the CV. Although, these previous studies have shown low reliability of power output at a fixed 4.0 mmol·l$^{-1}$ method, Hopkins et al. (2001) in a review of reliability of power in physical performance tests suggested that OBLA is the most reliable lactate break point method with a CV of approximately 1.5% at best. In accordance with this, the results of our study showed similar high reliability with low CV for the individual and fixed lactate turnpoint criteria in a sample of competitive cyclists (Tables II and III).

### Conclusion

The study represents the first assessment of absolute and relative reliability of a fixed and an individual lactate break point in a large group of competitive cyclists with previous exercise test experience. The low within-subject variability of power output and HR at both LT methods combining with high ICC, characterises high reliability in cyclists. From a practical point of view, we found the same low CV for the power output using fixed and individual LT methods; consequently, an option could be the use of any of these lactate threshold criteria in a controlled intervention. The intervention needs to promote individual changes greater than 4.2–5.6% for the intervention to be considered effective in a large group of competitive cyclists.

### Acknowledgements

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### Funding

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### References


### Table III. Sub-maximal parameters from incremental exercise test and reliability scores at OBLA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test 1</th>
<th>Test 2</th>
<th>TE</th>
<th>CV (%)</th>
<th>ICC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P$ (w)</td>
<td>273 ± 32</td>
<td>272 ± 33</td>
<td>7.7</td>
<td>2.8</td>
<td>0.94 (0.87–0.97)</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>56.2 ± 6.9</td>
<td>56.3 ± 5.0</td>
<td>2.6</td>
<td>4.6</td>
<td>0.81 (0.63–0.91)</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (l·min$^{-1}$)</td>
<td>4.0 ± 0.4</td>
<td>4.1 ± 0.4</td>
<td>0.2</td>
<td>5.2</td>
<td>0.73 (0.50–0.87)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>169 ± 9</td>
<td>167 ± 10</td>
<td>4.2</td>
<td>2.5</td>
<td>0.82 (0.65–0.91)</td>
</tr>
</tbody>
</table>

$P$, power output; $\dot{V}O_2$, oxygen uptake; HR, heart rate; $[La]$, blood lactate concentration; ICC, intra-class correlation; TE, typical error; CV, coefficient of variation.


