Reproducibility of lactate markers during 4 and 8 min stage incremental running: a pilot study

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Objective: This study examined the reproducibility of speed corresponding to specific lactate markers during incremental treadmill running of normal and prolonged stage durations. *Design:* Nineteen healthy participants (14 male, 5 female) performed repeated, incremental treadmill running trials of 4 and 8 min stages on separate days to examine the test-retest reproducibility of speed at lactate markers. Two trials were completed for each duration in a randomised order. *Method:* Fingertip blood samples drawn upon stage completion were analysed for plasma lactate, then used to determine running speed at: 2.0, 3.5, and 4.0 mmol·l^{-1} fixed blood lactate accumulations (FBLA), a 1 mmol·l^{-1} rise from baseline, and the markers: the deviation maximum (D_{max}), the D_{max} of the second curve derivative ($D2L_{max}$), the lactate threshold (LT) and log-log LT. *Results:* The 2.0 mmol·l^{-1} FBLA reported the lowest mean bias between 4 min trials (-0.06 km·h^{-1}), with the narrowest limits of agreement (LoA) (-1.78 to 1.66 km·h^{-1}). The D_{max} had the second lowest bias (0.14 km·h^{-1}), $D2L_{max}$ the second narrowest LoA (-1.93 to 2.90 km·h^{-1}). For 8 min stages, the 1 mmol·l^{-1} rise demonstrated, low mean bias (-0.13 km·h^{-1}) and narrowest LoA (-1.22 to 0.97 km·h^{-1}) between trials. *Conclusions:* This preliminary report suggests the reproducibility of running speed at lactate summary markers is influenced by stage duration for incremental treadmill running. Varied marker reproducibility between 4 and 8 min stages indicates different blood lactate response, and therefore workload calculation, according to stage length. Consideration of marker construct is recommended.

Keywords: sports medicine; running; lactate testing; incremental exercise; exercise tolerance; training prescription

i. Introduction

Incremental exercise with blood sampling to characterise the lactate-workload relationship is commonplace to determine exercise intensity and monitor training adaptation.[1] Following endurance training, attenuated lactate response during submaximal exercise results from diminished lactate production at low-, and increased lactate removal at high-workloads.[2] Compared to maximal oxygen uptake ($\dot{V}O_{2max}$), lactate measurement allows greater sensitivity to identify change in training status[3] and can be determined during submaximal incremental exercise.[1] The lactate-workload relationship during incremental exercise can be summarised using lactate markers and the lactate threshold (LT). The LT signifies a metabolic breakpoint, above which, the contribution of anaerobic glycolysis becomes significant to maintain ATP resynthesis.[4] Furthermore, lactate clearance rate appears limited at workloads approaching LT.[5] Lactate response is influenced by the blood sampling site,[6] in particular, venous blood has been seen to overestimate workload during short stage increments.[7] To characterise a curvilinear blood lactate response, various endurance markers and single summary statistics have been proposed.[8] Given the ease with which lactate can be measured, it is important to recognise the associated measurement error for a particular marker and how this influences the lactate-workload relationship for incremental exercise.

Using repeated measurements during incremental treadmill running of 3 min stages, fixed blood lactate accumulations (FBLA) (e.g., 4.0 $mmol \cdot l^{-1}$) have demonstrated good reproducibility ($r = 0.95$).[9] However, comparison of test-retest measures used correlation coefficients. While these describe relationship, they do not indicate agreement and cannot detect systematic error. Applying correlation coefficients, limits of agreement (LoA) and 95% confidence intervals (CI) to treadmill speeds at 4.0 $mmol \cdot l^{-1}$ FBLA and LT for 4 min stages, Grant et al.[10] reported strong relationship, but poor inter-trial reproducibility. In addition to highlighting the importance of supplementing correlation coefficient analysis with LoA, these results also suggest greater reproducibility for individuals of greater aerobic fitness. Reproducibility of the 4.0 $mmol \cdot l^{-1}$ marker has also been reported for repeated incremental running[11] and cycling trials.[12] Recently, the measurement error of several markers was examined for the lactate-power relationship during incremental cycling of 3 min stages.[13] Of several markers only

the deviation maximum (D_{max}) exhibited adequate reproducibility (coefficient of variation (CV) = 3.8%, intraclass correlation coefficient (ICC) r = 0.90, CI width = 31.8 W). Given that blood lactate may not stabilise within 4 min, investigations concerning marker reliability for longer stages remains scant.

Use of 3 to 5 min stage increments is based upon the attainment of oxygen uptake and heart rate steady state; yet blood lactate likely requires greater duration to achieve stable concentrations. Bentley et al.[14] found similar cycling workloads at lactate markers between 3 and 8 min stage increments. Yet, stages beyond 6 min are recommended to avoid delay in blood lactate response to running increments.[15] Insofar as both studies were limited to measures at a 4.0 $mmol \cdot l^{-1}$ FBLA and the LT, we assessed the reliability of various markers. Extending upon findings of Bentley[14] and Kuipers,[15] we examined the reproducibility of speed at lactate summary markers using incremental treadmill running of traditional, 4 min, and prolonged, 8 min stage durations.

ii. Methods

Nineteen healthy participants (14 male, 5 female; age 22.7 ± 3.8 years; height 174.9 ± 8.0 cm; body mass 69.0 ± 10.0 kg) provided written informed consent. Volunteers completed a medical, and long International Physical Activity Questionnaire;[16] thereby conforming to high levels of habitual physical activity (male, 2855.5 ± 1359.8 $MET \cdot min^{-1}/wk^{-1}$; female, 3224.3 ± 1013.0 $MET \cdot min^{-1}/wk^{-1}$). The investigation was approved by the University of Chichester Ethics Committee.

Five laboratory visits included: one familiarisation, and two repeated incremental running trials of 4 and 8 min stages to volitional exhaustion on separate days. The familiarisation involved two submaximal stages (e.g., 8 and 9 $km \cdot h^{-1}$) of 4 min followed, after a 15 min rest, by two of 8 min. Blind-selection was used to randomly allocate trial order, and testing was completed within a four week period (range of two to eight days between repeated trials). Participants were requested to arrive hydrated and refrain from strenuous exercise 24 h prior. All wore the same footwear for each trial. Testing occurred between 09:00 and 14:00 h, with each participant reporting to laboratory at the same

time of day for each trial. The same pre-calibrated powered treadmill (Woodway Ergo ELG 70, Woodway, Weil am Rhein, Germany) was used for all trials in a temperature controlled laboratory (19–22°C). Participants were instructed to maintain and self-record habitual diet and physical activities 48 h prior to the first trial; arriving 3 h after their last meal and caffeine intake. These records were prescribed in the 48 h preceding all subsequent trials. Resting blood glucose was similar between trials ($p > 0.05$).

Each session began with the explanation of experimental protocol, with anthropometric data collected during the familiarisation. Height was recorded with using a free-standing stadiometer (Holtain Ltd., Crymych, UK) and body mass with digital health scales (Seca Ltd., Birmingham, UK). Fingertip capillary blood was sampled in the final 30 s of a 5 min seated rest; extracted plasma was then measured for lactate. Participants warmed up on a treadmill at a 1% gradient for 5 min at: 7 $km \cdot h^{-1}$ for females, and 8 $km \cdot h^{-1}$ for males. These speeds were selected to commence trials thereafter, as no participant conformed to being endurance trained. All testing began with participants lowering themselves onto a moving treadmill belt set at the required starting speed. Modified from previous,[15] participants completed submaximal stages with 1 $km \cdot h^{-1}$ speed increments every 4 min (first trial, 7 ± 1 stages (range 6 to 10); second trial, 7 ± 1 stages (range 6 to 10)) or 8 min (first trial, 5 ± 1 stages (range 5 to 8); second trial, 5 ± 1 stages (range 5 to 8)). A further two individuals participated, but were excluded from analysis as they failed to complete sufficient trial stages. The investigator provided verbal encouragement and feedback on time remaining during the final minute of each stage. Upon stage completion participants were instructed to straddle the treadmill for ~30 s as speed was increased. Maximal running speed was taken as the last completed stage.

Capillary blood samples (25 µl) were drawn from the right index finger into EDTA-coated microvettes (Sarstedt Aktiengesellschaft & Co., Nümbrecht, Germany) at rest and during the final 30 s of each stage. Blood samples were analysed immediately in duplicate for plasma lactate (2300 STAT Plus™ analyser, YSI Life Sciences, Yellow Springs, USA), with pre-test calibration to standard lactate concentrations of 2.5 and 7.5 $mmol \cdot l^{-1}$. When a test-retest difference less than 0.4 $mmol \cdot l^{-1}$ was

measured, the mean was recorded; when a difference greater than 0.4 mmol·l^{-1} was measured, a third analysis was run with the mean of the two closest samples recorded.[10] Lactate analysis software[8] employing 3rd degree polynomial fitting (r^2 = 0.983 ± 0.030) was used to determine running speed at the fixed markers: 2.0, 3.5, and 4.0 mmol·l^{-1}, the 1 mmol·l^{-1} rise from baseline, and the markers: D_{max}, the D_{max} of the second curve derivative ($D2L_{max}$), LT and log-log LT. In addition, the lactate-speed relationship was constructed for each trial completed by the participants. The D_{max} was the speed at the maximum perpendicular from a line connecting the first and the final lactate-speed points to the polynomial,[17] with the $D2L_{max}$ derived from the maximum acceleration of the lactate curve.[18] The LT method employed a 'broken stick' model,[19] identifying the dividing point between two fitted regression lines as the corresponding speed, with log-log LT applying a log transformation.[20]

Mean speed for repeated trials was compared between 4 and 8 min using Paired t-tests. Speed at lactate markers for incremental running of 4 and 8 min stages were analysed using: Bland and Altman's 95% LoA,[21] and Deming regression.[22] Typical error (mean bias SD divided by the square root; Table 1) and 95% CI of mean bias and LoA (selectively reported) were also calculated. Absolute values are presented as heteroscedasticity was absent in the data. Statistical analyses were calculated using GraphPad Prism 5 for Windows (GraphPad Software, Inc., California, USA), with statistical significance accepted as $p < 0.05$.

iii. Results

There was no effect of duration on speed at fixed markers, but lower speeds were seen for 8 min stages at D_{max} (-1.7 km·h^{-1}), LT (-0.8 km·h^{-1}) and log-log LT (-0.8 km·h^{-1}) markers ($p < 0.05$). The 2.0 mmol·l^{-1} FBLA reported the lowest mean bias between 4 min trials (-0.06 km·h^{-1}) with the narrowest LoA (-1.78 to 1.66 km·h^{-1}; Figure 1). The D_{max} had the second lowest bias (0.14 km·h^{-1}), but widest LoA (-3.77 to 4.04 km·h^{-1}). The $D2L_{max}$ had high mean bias (0.48 km·h^{-1}), with narrow LoA (-1.93 to 2.90 km·h^{-1}). For $D2L_{max}$, 95% CI were wide for lower (-2.96 to -0.90 km·h^{-1}) and upper limits (1.87 to 3.93 km·h^{-1}). Mean bias was 0.31 km·h^{-1} (LoA, -2.25 to 2.87 km·h^{-1}) for LT, and -0.25 km·h^{-1} (LoA,

-3.12 to 2.62 km·h^{-1}; Table 1) for the 1 mmol·l^{-1} baseline rise. The log-log LT had large mean bias (0.44 km·h^{-1}), and wide LoA (-2.23 to 3.12 km·h^{-1}).

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Between 8 min trials, the 1 mmol·l^{-1} baseline rise displayed a -0.13 km·h^{-1} mean bias, with LoA -1.22 (95% CI, -1.71 to -0.73 km·h^{-1}) to 0.97 km·h^{-1} (95% CI, 0.48 to 1.46 km·h^{-1}; Figure 2). The $D2L_{max}$ had a -0.48 km·h^{-1} mean bias, with LoA from -2.63 to 1.67 km·h^{-1}. Mean bias between 8 min trials for the LT were 0.19 km·h^{-1} (LoA, -2.03 to 2.41 km·h^{-1}), and for the log-log LT -0.07 km·h^{-1} (LoA, -2.32 to 2.18 km·h^{-1}). The 4.0 mmol·l^{-1} FBLA showed moderate inter-trial agreement (mean bias, -0.19 km·h^{-1}; LoA, -2.59 to 2.22 km·h^{-1}). The D_{max} reported large mean difference (-0.79 km·h^{-1}) and widest LoA (-3.59 to 2.00 km·h^{-1}).

Between 4 min, Deming regression slopes (95% CI) were: 1.05 (0.32 to 1.78 km·h^{-1}; 2.0 mmol·l^{-1} FBLA), 0.92 (0.30 to 1.54 km·h^{-1}; 3.5 mmol·l^{-1} FBLA), 0.94 (0.42 to 1.47 km·h^{-1}; 4.0 mmol·l^{-1} FBLA), 1.19 (0.54 to 1.84 km·h^{-1}; 1 mmol·l^{-1} baseline rise), 0.72 (-0.13 to 1.56 km·h^{-1}; D_{max}), 0.82 (0.46 to 1.20 km·h^{-1}; $D2L_{max}$), 1.1 (0.38 to 1.82 km·h^{-1}; LT) and 0.32 (0.27 to 2.21 km·h^{-1}; log-log LT). Between 8 min, slopes (95% CI) were: 0.84 (0.48 to 1.20 km·h^{-1}; 2.0 mmol·l^{-1} FBLA), 1.27 (0.70 to 1.86 km·h^{-1}; 3.5 mmol·l^{-1} FBLA), 1.22 (0.81 to 1.62 km·h^{-1}; 4.0 mmol·l^{-1} FBLA), 0.98 (0.78 to 1.19 km·h^{-1}; 1 mmol·l^{-1} baseline rise), 1.77 (0.30 to 3.24 km·h^{-1}; D_{max}), 1.80 (0.86 to 2.80 km·h^{-1}; $D2L_{max}$), 0.44 (0.15 to 0.73 km·h^{-1}; LT) and 0.60 (-0.05 to 1.24 km·h^{-1}; log-log LT). Typical error ranged from 0.39 to 1.41 km·h^{-1} for both 4 and 8 min trials (Table 1).

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iv. Discussion

This preliminary report suggests the reproducibility of running speed at lactate summary markers is influenced by stage duration for incremental treadmill running. All markers were more reproducible for the prolonged, 8 min stages, when compared to 4 min. For 4 min trials, a 2.0 mmol·l^{-1} FBLA appeared most reproducible, and for 8 min trials, the 1 mmol·l^{-1} baseline rise. Regardless of stage duration, the D_{max} demonstrated poor reproducibility. If a stimulus, such as treadmill speed, fails to elicit reproducible physiological response between test-retest trials, change cannot be reliably quantified. Using LoA, cycling workload at a 4.0 mmol·l^{-1} FBLA has showed agreement with D_{max} (-12.2 ± 29.1 W), but not with single (78.8 ± 44.9 W) or double breakpoint models (-21.1 ± 79.5 W).[23] Elsewhere, Pfitzinger and Freedson[24] reported strong inter-trial relationship between speed and various fixed lactate markers (from r = 0.98 to 0.99), with 5 min stages for endurance trained men. Furthermore, the reliability coefficient of the FBLA 4.0 mmol·l^{-1} was observed as r = 0.92 for trained and r = 0.55 for untrained women.[25] Supporting the assertion that the variance between test-retest trials may be greater among sedentary, than trained individuals. Superior hydrogen ion buffering capacity seen for well-trained individuals (team-sport athletes, but not necessarily endurance-trained athletes)[26] may delay metabolic acidosis at given running speeds; in turn, prolonging maximal exercise duration. Alongside relative biological and motivational homogeneity in trained subjects, this may diminish the inter-test variability herein. Grant et al.[10] considered the fitness levels, classifying participants as unfit or moderately fit according to speed at the first rise from baseline. Narrower LoA were reported for those attaining the LT at speeds greater than 10.5 km·h^{-1}. We did not examine fitness, but our LT LoA for 4 min trials (-2.25 to 2.87 km·h^{-1}) can be interpreted as follows: if LT was reached at 12 km·h^{-1} during the first trial, with the same trial repeated two to eight days later, their LT could range from 9.75 to 14.87 km·h^{-1}. For 8 min protocols, the repeated trial LT could be as low as 9.97 km·h^{-1}, and as high as 14.41 km·h^{-1}.

Prolonging stage duration has the effect of reducing both running speed at the FBLA 4.0 mmol·l^{-1}, and the number of stages performed above the anaerobic threshold.[11] Longer stages may decrease variance in speed at particular markers between test-retest trials. It is likely that prolonged stages allow stabilisation between arterial and muscle lactate concentrations, facilitating lactate steady state, thus

limiting differences in lactate diffusion capacity for the sprint and endurance trained. Fixed blood lactate markers appeared consistently agreeable across 4 and 8 min stages, whereas the D_{max} and $D2L_{max}$ were prone to stage length. Unlike D_{max}, estimated from first and final lactate concentrations; $D2L_{max}$ is the mathematically-derived, second maximal derivative accounting for final lactate. This, in part, may explain the greater 8 min trial reproducibility seen for $D2L_{max}$ in comparison to D_{max}. Prolonged stages may cause insufficient increase in lactate between first and final workloads to fit polynomial regression, but not at the point of maximum acceleration. Longer stages may reduce the number of lactate samples, presenting unsatisfactory model-fitting using linear regression.[20] Based on five samples, each regression line would consist of two points; resulting in inaccurate and poor threshold reproducibility. Outliers would induce even greater variability in workload estimation. Criterion measures are often validated during maximal lactate steady state (MLSS) tests, with individuals sustaining marker speed for extended periods. The MLSS was not our experimental aim, but should be considered for marker validation studies. Even using the 1 $mmol \cdot l^{-1}$ baseline rise, a 2.19 $km \cdot h^{-1}$ change in running speed would be required to be deemed training-induced.

Fixed blood lactate markers do not account for the individual variation in lactate response to exercise. However, workloads derived from the D_{max}, $D2L_{max}$, LT and log-log LT are dependent upon the nature of the underlying lactate curve.[8] Of these markers, the $D2L_{max}$ was more reproducible for 4 min stages, and similar reproducibility was seen between $D2L_{max}$, the LT and log-log LT for 8 min stages. Elsewhere, the D_{max} has been found to correlate favourably with endurance performance in trained runners[27] and cyclists,[28] representing good reproducibility for incremental cycling exercise.[13] In contrast, we found speed at D_{max} to be unstable between trials. Mean bias was low (0.14 $km \cdot h^{-1}$), but 95% of trial differences resided within a -3.77 to 4.04 $km \cdot h^{-1}$ range for 4 min trials. Of seven markers, Morton et al.[13] found the D_{max} to have the highest ICC (0.903), and the narrowest CI width (31.8 W) for cycling power output. However, as all subsequent trials were terminated at the maximum workload achieved in trial one; the reliability of markers dependent upon peak power may have been inflated.

The LT appeared more stable across 4 and 8 min stages, in comparison to D_{max}, $D2L_{max}$ and log-log LT. Deming regression revealed no proportional or systematic bias between repeated trials of 4 or 8 min. The LT at 8 min stages exhibited the lowest regression slope (0.44), and a narrow 95% CI range defined by 0.15 to 0.73 $km \cdot h^{-1}$. This may indicate less susceptibility to blood lactate outliers sampled for 8 min stages. Higher intensity markers, such as the LT, may better reflect the aerobic-anaerobic transition, assuming lactate responds exponentially to increasing workload. Earlier studies by Foxdal and colleagues[29] examined the effect of incremental stage duration on blood lactate concentration and the corresponding validity to predict endurance performance.[30] Stages of 4 and 6 min tended to overestimate MLSS when using the FBLA 4.0 $mmol \cdot l^{-1}$, in comparison to 8 min stages. Of methods examined, 0.9 $km \cdot h^{-1}$ increments every 8 min with haemolysed capillary sampling was proposed the most accurate predictor of maximal endurance running velocity. However, longer stages may demotivate, evoke premature fatigue and compromise assessment of peak measures. Equally, the reduced laboratory times, and swift analyses for fixed markers, make shorter tests appealing to coaches and athletes. That venous blood lactate may not achieve steady state within 8 min has been contested.[15] Measuring FBLA 4.0 $mmol \cdot l^{-1}$ for incremental treadmill running of 1, 3 and 6 min stages, these authors argued for the use of stages exceeding 6 min. Faster mean running speed at FBLA 4.0 $mmol \cdot l^{-1}$ was reported for 3 min (14.4 $km \cdot h^{-1}$), in comparison to 6 min (12.9 $km \cdot h^{-1}$) stages. However, a delayed venous blood lactate response was observed for the respective running speed that was absent for 6 min stages.

v. Conclusions

To be deemed reliable, a marker must demonstrate adequate reproducibility across test-retest sessions. In this pilot study, the reproducibility of running speed at various lactate summary markers may be influenced by stage duration during incremental treadmill running. Greater reproducibility for 8 min, as opposed to 4 min stage trials, is likely attributable to, a) reduced workloads at and beyond the anaerobic threshold, and b) greater duration to attain lactate steady state in capillary blood. However, moderate stages allow a) more increments for sampling, therefore b) greater exercise intensities, and c) sufficient time for muscle-to-blood lactate equilibrium.

vi. Practical Applications

This pilot study suggests for future studies to examine lactate marker reproducibility in trained individuals and/or larger cohorts:

- 8 min stages offer more reliable measures of lactate markers, in comparison to 4 min stages during treadmill running.
- Varied marker reproducibility between 4 and 8 min stages indicates different blood lactate response, and therefore workload calculation, according to stage length. Consideration of marker construct is recommended.
- Future research is required to advance these findings to larger cohort of endurance trained athletes enabling a more refined analysis of lactate marker reliability.

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viii. References

1. Bentley DJ, Newell J, Bishop D. Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes. *Sports Med* 2007; 37(7):575-586.
2. MacRae HS, Dennis SC, Bosch AN et al. Effects of training on lactate production and removal during progressive exercise in humans. *J Appl Physiol* 1992; 72(5):1649-1656.
3. Yoshida T, Suda Y, Takeuchi N. Endurance training regimen based upon arterial blood lactate: effects on anaerobic threshold. *Eur J Appl Physiol Occup Physiol* 1982; 49(2):223-230.
4. Wasserman K, Whipp BJ, Koyl SN et al. Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol* 1973; 35(2):236-243.

5. Messonnier LA, Emhoff CAW, Fattor JA et al. Lactate kinetics at the lactate threshold in trained and untrained men. *J Appl Physiol* 2013; 114(11):1593-1602.
6. Yeh MP, Gardner RM, Adams TD et al. "Anaerobic threshold": problems of determination and validation. *J Appl Physiol* 1983; 55(4):1178-1186.
7. Yoshida T. Effect of exercise duration during incremental exercise on the determination of anaerobic threshold and the onset of blood lactate accumulation. *Eur J Appl Physiol* 1984; 53:196-199.
8. Newell J, Higgins D, Madden N et al. Software for calculating blood lactate endurance markers. *J Sports Sci* 2007; 25(12):1403-1409.
9. Weltman A, Snead D, Stein P et al. Reliability and validity of a continuous incremental treadmill protocol for the determination of lactate threshold, fixed blood lactate concentrations, and $\dot{V}O_{2max}$. *Int J Sports Med* 1990; 11(1):26-32.
10. Grant S, McMillan K, Newell J et al. Reproducibility of the blood lactate threshold, 4 $mmol \cdot l^{-1}$ marker, heart rate and ratings of perceived exertion during incremental treadmill exercise in humans. *Eur J Appl Physiol* 2002; 87(2):159-166.
11. Coen B, Urhausen A, Kindermann W. Individual anaerobic threshold: methodological aspects of its assessment in running. *Int J Sports Med* 2001; 22(1):8-16.
12. Aunola S, Rusko H. Reproducibility of aerobic and anaerobic thresholds in 20-50 year old men. *Eur J Appl Physiol Occup Physiol* 1984; 53(3):260-266.
13. Morton RH, Stannard SR, Kay B. Low reproducibility of many lactate markers during incremental cycle exercise. *Br J Sports Med* 2012; 46:64-69.
14. Bentley DJ, McNaughton LR, Batterham AM. Prolonged stage duration during incremental cycling exercise: effects on the lactate threshold and onset of blood lactate accumulation. *Eur J Appl Physiol* 2001; 85:351-357.
15. Kuipers H, Rietjens G, Verstappen F et al. Effects of stage duration in incremental running tests on physiological variables. *Int J Sports Med* 2003; 24(7):486-491.
16. Craig CL, Marshall AL, Sjöström M et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; 35(8):1381-1395.

17. Cheng B, Kuipers H, Snyder AC et al. A new approach for the determination of ventilatory and lactate thresholds. *Int J Sports Med* 1992; 13(7):518-522.
18. Newell J, McMillan K, Grant S et al. Using functional data analysis to summarise and interpret lactate curves. *Comput Biol Med* 2006; 36(3):262-275.
19. Lundberg MA, Hughson RL, Weisiger KH et al. Computerized estimation of lactate threshold. *Comput Biomed Res* 1986; 19(5):481-486.
20. Beaver WL, Wasserman K, Whipp BJ. Improved detection of lactate threshold during exercise using a log-log transformation. *J Appl Physiol* 1985; 59(6):1936-1940.
21. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1(8476):307-310.
22. Cornbleet PJ, Gochman N. Incorrect least-squares regression coefficients in method-comparison analysis. *Clin Chem* 1979; 25(3):432-438.
23. Thomas V, Costes F, Chatagnon M et al. A comparison of lactate indices during ramp exercise using modelling techniques and conventional methods. *J Sports Sci* 2008; 26(13):1387-1395.
24. Pfitzinger P, Freedson PS. The reliability of lactate measurements during exercise. *Int J Sports Med* 1998; 19(5):349-357.
25. Heitkamp HC, Holdt M, Scheib K. The reproducibility of the 4 $mmol \cdot l^{-1}$ lactate threshold in trained and untrained women. *Int J Sports Med* 1991; 12(4):363-368.
26. Edge J, Bishop D, Hill-Haas S et al. Comparison of muscle buffer capacity and repeated-sprint ability of untrained, endurance-trained and team-sport athletes. *Eur J Appl Physiol* 2006; 96:225-234.
27. Nicholson RM, Sleivert GG. Indices of lactate threshold and their relationship with 10-km running velocity. *Med Sci Sport Exerc* 2001; 33(2):339-342.
28. Bishop D, Jenkins DG, Mackinnon LT. The relationship between plasma lactate parameters, Wpeak and 1-h cycling performance in women. *Med Sci Sport Exerc* 1998; 30(8):1270-1275.
29. Foxdal P, Sjödin A, Sjödin B. Comparison of blood lactate concentrations obtained during incremental and constant intensity exercise. *Int J Sports Med* 1996; 17(5):360-365.

30. Foxdal P, Sjödin B, Sjödin A et al. The validity and accuracy of blood lactate measurements for prediction of maximal endurance running capacity. Dependency of analyzed blood media in combination with different designs of the exercise test. *Int J Sports Med* 1994; 15(2):89-95.

ix. Table legend

Table 1 Mean bias and absolute limits of agreement for blood lactate markers during repeated incremental exercise trials of 4 and 8 min stages (values presented as $km \cdot h^{-1}$).

x. Figure legends

Figure 1 Bland-Altman plot of mean bias and absolute limits of agreement of speed at the 2.0 $mmol \cdot l^{-1}$ fixed blood lactate accumulation between first and second trials of incremental treadmill running of 4 min stages (dotted lines: 95% limits of agreement).

Figure 2 Bland-Altman plot of mean bias and absolute limits of agreement of speed at the 1 $mmol \cdot l^{-1}$ rise from baseline between first and second trials of incremental treadmill running of 8 min stages (dotted lines: 95% limits of agreement) (n = 18; data point excluded where blood lactate response did not satisfy marker criteria for speed calculation).