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Exercise intensity measurement using fractal analysis of heart rate variability: Reliability, agreement and influence of sex and cardiorespiratory fitness

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ABSTRACT

The study aimed to establish the test-retest reliability of detrended fluctuation analysis of heart rate variability (DFA-α1) based exercise intensity thresholds, assess its agreement with ventilatory- and lactate-derived thresholds and the moderating effect of sex and cardiorespiratory fitness (CRF) on the agreement. Intensity thresholds for thirty-seven participants (17 females) based on blood lactate (LT_1/LT_2), gas-exchange (VT_1/VT_2) and DFA-α1 (αTh_1/αTh_2) were assessed. Heart rate (HR) at αTh_1 and αTh_2 showed *good* test-retest reliability (coefficient of variation [CV] < 6%), and *moderate* to *high* agreement with LTs ($r = 0.40 - 0.57$) and VTs ($r = 0.61 - 0.66$) respectively. Mixed effects models indicated bias magnitude depended on CRF, with DFA-α1 overestimating thresholds versus VTs for lower fitness levels (speed at VT_1 <8.5 km·hr^{-1}), while underestimating for higher fitness levels (speed at VT_2 >15 km·hr^{-1}; VO_{2max} >55 mL·kg^{-1}·min^{-1}). Controlling for CRF, sex significantly affected bias magnitude only at first threshold, with males having higher mean bias (+2.41 bpm) than females (−1.26 bpm). DFA-α1 thresholds are practical and reliable intensity measures, however it is unclear if they accurately represent LTs/VTs from the observed limits of agreement and unexplained variance. To optimise DFA-α1 threshold estimation across different populations, bias should be corrected based on sex and CRF.

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Aerobic threshold; anaerobic threshold; DFA-alpha1; HRV; lactate

Introduction

Establishing valid and reliable methods to measure exercise intensity is crucial to understand important outcomes in both general (e.g., cardiorespiratory fitness, all-cause mortality) (Edvardsen et al., 2013; Samitz et al., 2011) and athletic populations (e.g., training-induced adaptation) (Cejuela & Selles-Perez, 2023; Jones & Carter, 2000). Exercise intensity is a complex construct, involving both external (e.g., speed, power output) and internal (e.g., oxygen uptake kinetics, metabolic, ionic alterations) dimensions. However, during exercise, measures should accurately reflect the distinct homeostatic disturbances of recognised exercise intensity domains (Burnley & Jones, 2018; Jamnick et al., 2020). There are a variety of intensity determination methods available, each with their own benefits and limitations. In laboratory settings, direct measurement of physiological thresholds (e.g., first and second lactate [LT_1/LT_2] or ventilatory thresholds [VT_1/VT_2]) is often preferred, but is not easily accessible, nor cost or time effective (Jamnick et al., 2020; Stöggl & Sperlich, 2015). Field-based tests to extrapolate an external intensity threshold such as critical speed (CS) or power (CP) which represent an underlying exercise domain transition are also commonly used (Burnley & Jones, 2018; Jamnick et al., 2020). These are individualised, valid and reliable markers of exercise intensity but require multiple maximal efforts for precise estimation (Burnley & Jones, 2018) and are limited with their sensitivity to monitor and adjust based on changes in acute physiological responses during exercise.

With the growing ease of use and accessibility of wearables such as heart rate (HR) monitors, recording of continuous electrocardiogram (ECG) signals has increased in scale and reach (Cardinale & Varley, 2017). A common method of identifying or prescribing intensity through these signals is based on certain percentages of maximal HR (HR_{max}) or HR reserve (HRR), with the assumption that metabolic responses occurring between individuals will be identical when exercising at fixed HR percentages. However, evidence shows large inaccuracy in estimation of true intensity using %HR_{max} or %HRR in healthy adults, athletes (Iannetta et al., 2020) and cardiac rehabilitation patients (Pymer et al., 2020). As a result, there is a lack of agreement on the most appropriate methods.

Recently, there has been an increasing use of heart rate variability (HRV) based measurements to determine individualised exercise intensity thresholds during a graded incremental test (GXT) (Cassirame et al., 2015; Gronwald et al., 2020; Kaufmann et al., 2023; Zimatore et al., 2021). One of the methods assesses the fractal (self-similarity) properties of ECG-derived RR interval sequence data using alpha-1 of detrended

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fluctuation analysis (DFA-α1) (Gronwald et al., 2020). DFA-α1 is a non-linear dynamics method which represents a scaling exponent and is used to detect long-range temporal correlations in non-stationary time series data such as HRV. Based on the scale-free signal theory, an α of 0.5 within DFA-α1 can be modelled as fractional Gaussian noise (fGn), where the signal is typically uncorrelated and resembles white noise (Hardstone et al., 2012). A $0 < \alpha < 0.5$ indicates signal with anti-correlations, while $0.5 < \alpha < 1$ indicates signal with positive correlations, both characterised by fGn. An $\alpha = 1$ is indicative of a 1/f signal where the amplitude spectrum of frequencies follows a power law, whereas $\alpha > 1$ represents a non-stationary process and can be modelled as fractional Brownian motion (fBm) (Hardstone et al., 2012). With increasing exercise intensity, DFA-α1 generally exhibits a reverse sigmoidal relationship, having a near stable tendency at lower intensity and values around 1.2, indicative of well-correlated fractal-like signal pattern with high complexity and adaptability (Rogers & Gronwald, 2022). This is followed by a linear decrease in DFA-α1 as a result of disintegration of organismic subsystems, before plateauing at higher-end exercise intensities with α values close to under 0.5 representing an anti-correlated signal pattern where interaction of subsystems fails (Rogers & Gronwald, 2022; Rogers et al., 2021, 2021a). Based on the fractal signals theoretical background, Gronwald and colleagues (2020) proposed cut-off values of 0.75 and 0.5 within DFA-α1 signal, which serves as an estimation to aerobic and anaerobic thresholds respectively.

Previous studies have reported mixed findings for agreement between HR at LT_1/VT_1 and DFA-α1-0.75, ranging from *moderate* ($r = 0.31–0.36$) (Fleitas-Paniagua et al., 2024; Sempere Ruiz et al., 2024) to *high* and *nearly perfect* agreement ($r = 0.66–0.96$) (Mateo-March et al., 2023; Rogers et al., 2021; Schaffarczyk et al., 2023), while a *high* to *very-high* agreement between LT_2/VT_2 and DFA-α1-0.5 ($r = 0.62–0.90$) (Fleitas-Paniagua et al., 2024; Mateo-March et al., 2023; Rogers et al., 2021a; Schaffarczyk et al., 2023). Though the mean biases are regularly reported to be < 5 beats per minute (bpm), the limits of agreement (LoA) widths have ranged from 20 to 52 bpm at VT_1 to 22.6 to 40 bpm at VT_2 (Fleitas-Paniagua et al., 2024; Rogers et al., 2021a; Schaffarczyk et al., 2023; Sempere Ruiz et al., 2024; Van Hooren, Mennen, et al., 2023), while 42 to 54 bpm and 28 to 40.7 bpm at LT_1 and LT_2 respectively (Fleitas-Paniagua et al., 2024; Mateo-March et al., 2023; Sempere Ruiz et al., 2024). The wide LoA suggest some caution for using DFA-α1 based thresholds as differences of <10 bpm result in significant differences in accumulated metabolic stress (Azevedo et al., 2022; Black et al., 2017; Ribeiro et al., 1986) and time to exhaustion (de Lucas Rd et al., 2013) during exercise. Therefore, it is critical to understand the underlying factors that might contribute to poor agreement for certain individuals, thus inflating the overall LoA, and ultimately make DFA-α1 feasible to estimate thresholds with more confidence across different populations.

Possible factors that could influence agreement include individual cardiorespiratory fitness characteristics (CRF), sex differences or the mode of exercise. Recent studies have investigated the agreement in specific cohorts, such as physically active males (Rogers et al., 2021), females (Schaffarczyk et al., 2023) or elite endurance male athletes (Mateo-March et al., 2023) but it remains uncertain whether agreement differs across CRF levels. As adaptations in the autonomic nervous system are well documented with training status (Plews et al., 2013), it is very likely that the agreement could also be affected due to between-individual differences in fitness characteristics. For example, a recent study using 5-minute DFA-α1 measurement window among recreational runners observed α1 values of 0.68 ± 0.28 at VT_1 and 0.48 ± 0.11 at VT_2, wherein the standard deviation suggests the potential between-individual differences that can exist at these thresholds (Naranjo-Orellana et al., 2021). Insufficient evidence also exists to understand the effect of sex differences, as it is argued that HRV alterations due to hormonal fluctuations within a menstrual cycle can affect the agreement in females (Rogers & Gronwald, 2022). Lastly, most studies have used cycling as the exercise modality for GXT, as running associated impacts can affect the signal quality of ECG and missed beats in the RR sequence which could affect the overall DFA-α1 quality (Åström et al., 2003; Rogers & Gronwald, 2022). A recent study assessed the agreement of DFA-α1 derived first threshold with VT_1 during running (Van Hooren, Mennen, et al., 2023), but the overall evidence is still limited. Therefore, it is equally important to strengthen the evidence of DFA-α1 agreement in a running modality to be able to use the estimated thresholds with confidence in exercise modes like resistance training, swimming, rowing, and field sports, which could have high interference in the ECG signal quality due to upper body activity (Rogers & Gronwald, 2022).

Although many studies have assessed the agreement of DFA-α1 with criterion measures, the test-retest reliability of its estimation is also still unclear. To our knowledge, only one study so far has investigated reliability of DFA-α1 predicted thresholds in a relatively small sample ($n = 15$) and reported *moderate* (ICC = 0.52) to *good* (ICC = 0.85) reliability at first and second threshold respectively (Sempere Ruiz et al., 2024). Other studies either examined the reproducibility of DFA-α1 thresholds after a 4 month period (Fleitas-Paniagua et al., 2024), or have examined the reliability of entire DFA-α1 trace during a 5-min submaximal run before and after an exhaustive ramp test (Van Hooren, Bongers, et al., 2023). Given the natural biological variation present even in lab-derived VTs and LTs (CV = 2.1–3.7%) (Hoefelmann et al., 2015; Pallarés et al., 2016), it is crucial to establish the test-retest reliability of DFA-α1 and determine the smallest worthwhile change at an individual level.

Therefore, this study aims to determine the test-retest reliability of DFA-α1 and extend the current evidence on its agreement with lab-derived lactate and ventilatory thresholds. We also aim to assess the applicability of DFA-α1 estimated intensity thresholds by examining the moderating effect of sex and individual CRF on its agreement with ventilatory thresholds.

Methods

Participants and experimental design

Thirty-seven participants (20 males, 17 females; mean ± standard deviation, age: 32.72 ± 9.26 years; height: 173.95 ± 8.47 cm; body mass: 74.78 ± 17.71 kg; Supplementary Table S1) were recruited following pre-participation health screening. The participants completed three testing visits (1st visit: lactate profiling from a step incremental test; 2nd and 3rd visit: test-retest reliability in a ramp incremental test), at approximately the same time of the day in a 7-day period, with at least 72 hours between each visit. Participants avoided any vigorous physical activity, caffeine, and alcohol consumption 24 hours prior to all visits. During the first visit, participants also provided an informed written consent and anthropometric measurements were taken. The ethical approval of study was granted by Carnegie School of Sport Research Ethics Committee of Leeds Beckett University.

Gas exchange analysis and treadmill protocols

Gas exchange kinetics were recorded continuously using a breath-by-breath online gas analyser system (Metalyzer 3B; Cortex Biophysik GmbH, Leipzig, Germany). Prior to each test, the metabolic cart was two-point calibrated using room air and a standard composition Cranlea gas (15.05% O_2, 4.95% CO_2). Both the O_2 and CO_2 sensors were tested post-calibration against the same concentration Cranlea gas to ensure their standard error was under 0.02%. The flow sensor was calibrated with a manual 3-litre calibration syringe (5530, Hans Rudolph, USA). The Metalyser was connected to a HR monitor (H10, Polar Electro Oy, Kempele, Finland) for concurrent HR recordings.

Step incremental treadmill protocol and determination of lactate thresholds

The step incremental protocol involved 3 minute stages (Bentley et al., 2007) on an indoor treadmill (Ergo ELG 2, Woodway, Germany), interspersed with 1 minute rest for blood sampling and rating of perceived exertion (RPE) assessment using Borg-20 scale (Scherr et al., 2013). The blood sample was collected as soon as the participants stood astride on the treadmill during the rest period following each stage. The treadmill was kept constant at a 1% gradient, and the initial speed was set ~6 km·hr^{-1} below the participants' recent 10 km performance, or at a standard starting speed of 6 km·hr^{-1} if they were uncertain about the pace or were not physically active. Treadmill speed was increased by 1 km·hr^{-1} each stage (Akubat & Abt, 2011; Manzi et al., 2009) with the test terminating after the stage when participants attained the blood lactate (b[La$^-$]) concentration >4 mmol·L^{-1} (Manzi et al., 2009) and RPE ≥ 18 to ensure both lactate thresholds were attained.

The b[La$^-$] concentration was determined using an enzymatic-amperometric based lactate analyser (Biosen C-Line analyser, EKF diagnostics, Germany) calibrated with a standard 12/12 mmol·L^{-1} Glu/b[La$^-$] solution. All blood samples were taken from the right ring fingertip. The finger was first sterilised through a 70% IPA swab, and a puncture was made with a disposable spring-loaded lancet. The first drop of blood was always wiped away to avoid any chances of contamination. A 20 µL blood sample was collected in a capillary tube and then transferred into a haemolysing solution microvette, before placing it in the Biosen analyser.

Lactate thresholds were determined using an online web application (Lactate-OR, https://orreco.io/lactate/) (Newell et al., 2015). The first lactate threshold (LT_1) was calculated by the log-log method, in which a log transformation was first performed for both b[La$^-$] and speed, and a change in linearity "breakpoint" having lowest residuals sum of squares was identified on the log transformed b[La$^-$] versus speed curve through segmented regression (Beaver et al., 1985). All datafiles were reviewed by the investigation leader to confirm the log-log method, and files ($n = 6$) that showed a difference of >5 bpm compared to Lactate-OR derived values were reviewed independently by a second investigator until a consensus to true LT_1 was reached. LT_2 was determined as the intensity at which b[La$^-$] increased 1.5 mmol·L^{-1} above participants' baseline (lowest data point in all stages), as it is observed to be a valid estimate of maximal lactate steady state in a step-incremental GXT of 3-minute stages (Jamnick et al., 2018). The HR and treadmill speeds corresponding to the LT_1 and LT_2 (vLT_1 and vLT_2 respectively) generated through Lactate-OR application were used for further analysis.

Ramp incremental treadmill protocol and determination of ventilatory thresholds

The ramp incremental protocol was a continuous incremental test performed on the indoor treadmill (Ergo ELG 2, Woodway, Germany). The initial speed was set at a standard 4 km·hr^{-1}, and a constant gradient at 1%, and increased by 0.5 km·hr^{-1} every 30 seconds until volitional exhaustion. All participants were provided with verbal encouragement throughout the protocol. Two blood samples were taken, one before the start of the ramp incremental protocol, and the other following 1 minute of passive seated recovery post-cessation.

Thirty seconds time averaged GXT data files were exported and achievement of $\dot{V}O_{2max}$ was confirmed when two of the following three criteria were fulfilled: no change in $\dot{V}O_2$ (<0.2 L·min^{-1}) despite increasing treadmill speed for at least three stages, respiratory exchange ratio > 1.1 or reaching 95% age-predicted HR_{max} (Akubat & Abt, 2011; Lee & Zhang, 2021). The $\dot{V}O_{2max}$ was identified as the highest observed value in 30-seconds time averaged data during the test. The velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$) was recorded as the minimum speed that elicited $\dot{V}O_{2max}$ over a period of 30 seconds.

VT_1 was identified by a combined three determination approach of using modified v-slope method (intersection of two-line regression between $\dot{V}CO_{2max}$ and $\dot{V}O_2$ graph), ventilatory equivalencies ($\dot{V}E/\dot{V}O_2$ nadir or first rise with no concomitant increase in VE/VCO_2 with increasing HR) and end-tidal pressure ($P_{ET}O_2$ nadir of first increase with increasing HR) (Binder et al., 2008; Schaffarczyk et al., 2023). VT_2 was identified using respiratory compensation point (inflection point in VE and VCO_2 graph), ventilatory equivalencies (VE/VCO_2 nadir or non-linear rise with increasing HR) and end-tidal pressure (deflection point in $P_{ET}CO_2$) methods (Binder et al., 2008). The VTs were first located by the investigation leader and were independently reviewed by another investigator. Datafiles

which had a disagreement in VT occurrence of more than 30 seconds ($n = 17$) were reviewed again prior to reaching a mutual consensus. In cases where VT_2 was indeterminate ($n = 3$) or not achieved ($n = 1$), the test data for those participants was excluded. HR, $\dot{V}O_2$, and treadmill speeds at VT_1 and VT_2 (vVT_1 and vVT_2 respectively) were used for further analyses.

RR-interval measurement and calculation of DFA-alpha1 derived thresholds

Participants wore the chest belt (H10, Polar Electro Oy, Kempele, Finland; sampling rate: 1000 hz) wirelessly paired to a Polar sports watch (Vantage V, Polar Electro Oy) and the metabolic cart. The Polar Vantage V enabled the HRV recordings through H10 to be directly uploaded onto Polar Flow database. The Flow account was linked to Kubios HRV Scientific software Version 4.0.0 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) (Tarvainen et al., 2014), from which RR-interval data were exported in Flexible and Interoperable Data Transfer (FIT) format files. Pre-processing settings were set to the default values, as done previously (Rogers et al., 2021; Schaffarczyk et al., 2023). The RR detrending method was kept at "smoothness priors" (Lambda = 500) and the DFA-α1 window width was set to $4 \leq n \leq 16$ beats (Peng et al., 1995). The Kubios "automatic method" was used for artefacts correction in the RR series data. As DFA-α1 likely shows variable bias at higher artefact levels, datafiles ($n = 4$) were excluded from further analysis when the overall percent artefact exceeded 3% (Rogers et al., 2021b). A time-varying analysis of 2-minute rolling window width and 5 second grid interval for the moving window was adjusted such that DFA-α1 and HR was recalculated for every 5 seconds. The participants stood astride on the treadmill for 2 minutes prior to starting the treadmill protocols to record the initial RR-intervals data.

The DFA-α1 thresholds were calculated using method as previously described (Rogers et al., 2021; Schaffarczyk et al., 2023). The time-series DFA-α1 data was plotted against HR, and a linear regression was performed for values ranging from approximately 1.0 to 0.5. The regression equation was used to identify HR corresponding to DFA-α1 values of 0.75 and 0.5 for first (αTh_1) and second threshold (αTh_2) respectively. Similarly, DFA-α1 over time regression equation was used to identify the timepoints of αTh_1 and αTh_2 for the determination of $\dot{V}O_2$ and treadmill speeds at these thresholds, by use of the $\dot{V}O_2$ and speed over time relations.

Statistical analysis

Agreement and reliability

Normality of lactate – (LT_1, LT_2), ventilatory – (VT_1, VT_2), and DFA-α1 thresholds (αTh_1, αTh_2) was assessed through visual inspection of Q-Q plots, skewness (γ) and confirmed by performing Shapiro-Wilk's test. All variables were normally distributed ($-0.11 < \gamma < 0.40$, $0.11 < p < 0.96$). Due to the nested data structure of test-retest measurements, agreement and reliability of exercise thresholds were assessed using *agree_nest* and *reli_stats* functions respectively in the *SimplyAgree* R package (Caldwell, 2022). Mean biases, Lin's concordance correlation coefficient (CCC) using U-statistics, and 95% limits of agreement (LoA) with 95% confidence intervals (95% CI) were computed to assess agreement. Lin's CCC was interpreted similarly as Pearson's r coefficient (Altman, 1991) with r values < 0.1 *trivial*, 0.1 to 0.29 *small*, 0.3 to 0.49 *moderate*, 0.5 to 0.69 *high*, 0.7 to 0.89 *very high*, 0.9 to 1 *nearly perfect* agreement. For reliability analysis, *reli_stats* function which utilizes a linear mixed model to estimate variance components, was used to produce intraclass correlation coefficients (ICC) with 95% CI, coefficient of variation (CV), and standard error of measurement (SEM) for all variables (Caldwell, 2022). The CV was calculated using the mean squared error from the linear mixed model used to calculate ICC for the specific variable. ICC for reliability was interpreted as values < 0.5 *poor*, 0.5 to 0.75 *moderate*, 0.76 to 0.89 *good*, and ≥ 0.9 *excellent* reliability (Portney & Watkins, 2009).

Effect of sex differences and cardiorespiratory fitness (CRF) on agreement

To identify any potential effect of sex and individuals' CRF on the absolute agreement between HR at DFA-α1 thresholds and VTs, two mixed effects models were built with participants' sex and fitness (linear and quadratic) as predictor variables and random intercept (τ_0) fitted for each participant. The dependent variables were HR differences between DFA-α1 based αTh_1 and gas-VT_1 and the other between αTh_2 and gas-VT_2. Full model specifications are provided in the Supplementary Table S1. The *buildmer* function within the *buildmer* package optimised model fit by performing backward stepwise elimination based on change in log-likelihood (Aikake Information Criterion) (Voeten, 2023). Model assumptions of normality of residuals, homoscedasticity, and multicollinearity (variable inflation factor < 5) were confirmed and found unviolated. The model was visualised using *plotplane* function in *rockchalk* R package, with CRF variables (vVT_1, vVT_2 or $\dot{V}O_{2max}$) as predictors for agreement differences at VT_1/VT_2 and αTh1/αTh2. Significant effect of predictors on agreement differences was determined if $p < 0.05$ and the 95% CI did not enclose "no effect" (i.e., $\beta = 0$).

Results

Table 1 provides the descriptive and test-retest reliability statistics of all physiological variables derived from step and ramp incremental test. All maximal variables showed *excellent* test-retest reliability (ICC = 0.93–0.97, CV < 3%), except $b[La^-]_{max}$ which exhibited *moderate* reliability (ICC = 0.72, CV = 12.9%). Submaximal variables including αTh_1 and αTh_2 showed *good* to *excellent* reliability (ICC = 0.76–0.95, CV = 2.25–5.96%).

Agreement results between LTs, gas-VTs, and DFA-α1 thresholds are presented in Table 2. DFA-α1 thresholds showed a *moderate* to *high* agreement ($r = 0.40$–0.57) with LTs, having mean biases of −4.78 and −3.77 bpm between αTh_1 and LT_1, and −6.03 and −4.08 bpm between αTh_1 and LT_2. A *high* agreement ($r = 0.61$–0.66) between DFA-α1 thresholds and gas-VTs was observed, with bias magnitudes of 0.62 and −7.59 bpm at first and second threshold respectively. Bland Altman plots including mean biases, and LoA with 95% CI for visual

Table 1. Group means ± standard deviation (SD) of all variables during step and ramp incremental tests with test-retest reliability statistics.

	Mean ± SD			SEM	CV (%)	ICC [95%CI]	
Variable	Step	Ramp 1	Ramp 2	Ramp 1 – Ramp 2			N
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	-	48.47 ± 8.87	48.69 ± 8.54	1.24	1.82	0.97 [0.96 to 0.98]	37
HR_{max} (bpm)	-	185.05 ± 10.58	184.67 ± 11.33	2.80	1.52	0.93 [0.88 to 0.96]	37
b[La$^-$] $_{max}$ (mmol·L^{-1})	-	8.52 ± 1.98	8.71 ± 2.34	1.11	12.9	0.72 [0.56 to 0.83]	36
PTV (km·hr^{-1})	-	16.58 ± 2.44	16.79 ± 2.54	0.44	2.67	0.96 [0.94 to 0.97]	37
vLT_1/vVT_1 (km·hr^{-1})	10.28 ± 2.11	10.20 ± 2.25	10.25 ± 2.11	0.45	3.20	0.95 [0.92 to 0.97]	35/37
vLT_2/vVT_2 (km·hr^{-1})	12.15 ± 2.33	13.30 ± 2.22	13.48 ± 2.15	0.61	4.58	0.92 [0.86 to 0.95]	35/35
$\dot{V}O_2$ at LT_1/VT_1 (mL·kg^{-1}·min^{-1})	35.09 ± 6.79	33.48 ± 7.28	33.07 ± 6.28	1.7	3.65	0.94 [0.89 to 0.96]	35/37
$\dot{V}O_2$ at LT_2/VT_2 (mL·kg^{-1}·min^{-1})	41.19 ± 7.43	42.28 ± 7.41	42.36 ± 7.42	2.01	3.42	0.93 [0.87 to 0.96]	35/35
HR at LT_1/VT_1 (bpm)	148.52 ± 12.27	145.02 ± 12.17	143.94 ± 13.67	4.46	2.25	0.88 [0.80 to 0.92]	35/37
HR at LT_2/VT_2 (bpm)	165.60 ± 10.33	169.37 ± 12.94	168.31 ± 13.99	5.35	3.17	0.84 [0.73 to 0.90]	35/35
$\dot{V}O_2$ at αTh_1 (mL·kg^{-1}·min^{-1})	-	32.05 ± 5.49	32.35 ± 5.07	2.00	4.55	0.86 [0.75 to 0.92]	32
$\dot{V}O_2$ at αTh_2 (mL·kg^{-1}·min^{-1})	-	38.37 ± 7.44	39.30 ± 7.39	3.14	5.96	0.82 [0.69 to 0.89]	32
HR at αTh_1 (bpm)	-	146.52 ± 15.99	146.29 ± 15.96	5.88	4.02	0.86 [0.76 to 0.92]	32
HR at αTh_2 (bpm)	-	162.25 ± 15.55	163.51 ± 18.85	8.35	5.13	0.76 [0.61 to 0.86]	32

Test-retest reliability statistics are calculated from Ramp 1 and 2 tests. *SEM = standard error of measurement, CV = coefficient of variation, ICC = Intraclass correlation coefficient, LoA = limits of agreement, VO2max = maximal oxygen uptake, HRmax = maximum heart rate, b[La-]max = maximal blood lactate, PTV = peak treadmill velocity, vLT1 = velocity at first lactate threshold, vVT1 = velocity at first ventilatory threshold, vLT2 = velocity at second lactate threshold, vVT2 = velocity at second ventilatory threshold, VO2 = oxygen consumption, αTh1 = DFA-α1-0.75 threshold, αTh2 = DFA-α1-0.50 threshold

Table 2. Agreement analysis between lactate, ventilatory and detrended fluctuation analysis derived heart rate variability thresholds during incremental treadmill tests. Mean biases, Lin's concordance correlation coefficient and 95% limits of agreement with 95% confidence interval are presented.

		Lin's CCC [95%CI]	Mean Bias [95%CI] (bpm)	Lower LoA [95%CI] (bpm)	Upper LoA [95%CI] (bpm)	N
	VTs versus LTs					
VT_1 vs LT_1	Ramp 1	0.76 [0.59 to 0.87]	−3.72 [−6.25 to −1.18]	−18.20 [−21.07 to −15.33]	10.76 [7.89 to 13.63]	35
	Ramp 2	0.70 [0.49 to 0.83]	−4.46 [−7.52 to −1.40]	−21.93 [−25.39 to −18.47]	13.01 [9.54 to 16.46]	35
VT_2 vs LT_2	Ramp 1	0.67 [0.45 to 0.81]	2.95 [0.06 to 5.97]	−13.99 [−17.40 to −10.58]	19.91 [16.49 to 23.31]	34
	Ramp 2	0.68 [0.46 to 0.82]	2.52 [−0.75 to 5.80]	−15.58 [−19.29 to −11.88]	20.63 [16.93 to 24.34]	34
	αThs versus LTs					
αTh_1 vs LT_1	Ramp 1	0.40 [0.09 to 0.64]	−4.78 [−9.99 to 0.42]	−32.62 [−38.51 to −26.73]	23.06 [17.17 to 28.95]	31
	Ramp 2	0.46 [0.15 to 0.68]	−3.77 [−8.91 to 1.37]	−30.78 [−36.59 to −24.96]	23.23 [17.41 to 29.04]	30
αTh_2 vs LT_2	Ramp 1	0.57 [0.34 to 0.74]	−6.03 [−9.94 to −2.12]	−26.91 [−31.32 to −22.49]	14.83 [10.42 to 19.25]	31
	Ramp 2	0.41 [0.14 to 0.62]	−4.08 [−9.94 to 1.77]	−34.82 [−41.44 to −28.21]	26.65 [20.03 to 33.27]	30
	αThs versus VTs					
αTh_1 vs VT_1		0.61 [0.49 to 0.71]	0.62 [−3.40 to 4.66]	−23.71 [−30.49 to −18.69]	24.96 [19.95 to 31.75]	65
αTh_2 vs VT_2		0.66 [0.55 to 0.75]	−7.63 [−10.88 to −4.38]	−29.14 [−34.48 to −25.24]	13.87 [9.97 to 19.22]	63

Ramp = ramp incremental test, CCC = concordance correlation coefficient, *VT1 = first ventilatory threshold, VT2 = second ventilatory threshold, LT1 = first lactate threshold, LT2 = second lactate threshold, αTh1 = DFA-α1-0.75 threshold, αTh2 = DFA-α1-0.50 threshold.

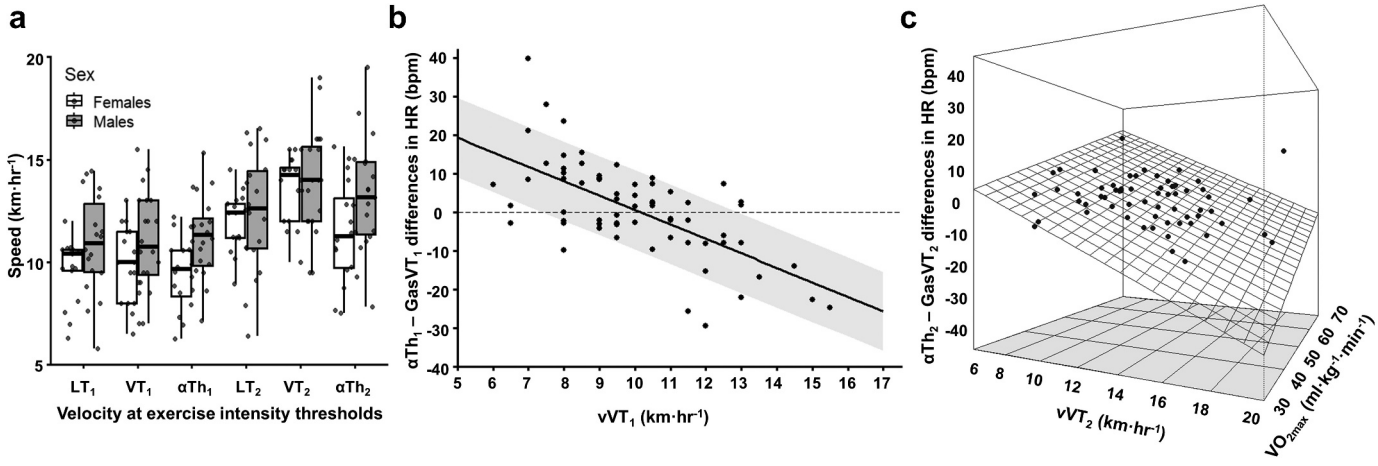

Figure 1. Plots representing (A) treadmill speeds at lactate, ventilatory and dfa-α1 thresholds as measures of cardiorespiratory fitness (CRF), (B and C) changes in the HR differences between dfa-α1 and gas-VTs across CRF levels. Abbreviations: LT_1, first lactate threshold (log-log); VT_1, first ventilatory threshold; αTh_1, dfa-α1-0.75 threshold; αTh_2, dfa-α1-0.5 threshold; LT_2, second lactate threshold (baseline +1.5 mmol·L^{-})[1]; VT_2, second ventilatory threshold; vVT_1/vVT_2, treadmill speed at VT_1/VT_2.

representation of agreement between different intensity thresholds are provided in Supplementary Figure S1.

CRF varied considerably among participants, with VO_{2max} (48.78 ± 8.54 mL·min^{-1}·kg^{-1}) ranging from 30.6 to 70.4 mL·min^{-1}·kg^{-1}, and similar heterogeneity evident at speed at first threshold (Figure 1(a); mean ± SD [range], vLT_1 = 10.28 ± 2.11 km·hr^{-1} [5.8 to 14.4 km·hr^{-1}], vVT_1 = 10.47 ± 2.22 km·hr^{-1} [6.5 to 15.5 km·hr^{-1}]) and second threshold (mean ± SD [range], vLT_2 = 12.15 ± 2.33 km·hr^{-1} [6.4 to 16.5 km·hr^{-1}], vVT_2 = 13.81 ± 2.19 km·hr^{-1} [9.5 to 19 km·hr^{-1}]).

Supplementary Table S2 describes the effect of CRF on differences in agreement between DFA-α1 and gas-derived thresholds, with the grand intercept and standardised (beta) coefficients (β) presented for significant predictors. The magnitude of mean bias between αTh_1 and gas-VT_1 was significantly affected by vVT_1 (β_{vVT1} = −5.80 [95%CI −7.86 to −3.74]) and sex (β_{sex} = 9.26 [95% CI 3.44 to 15.08]). Similarly, bias magnitude between αTh_2 and gas-VT_2 was significantly affected by vVT_2 (β_{vVT2} = −3.94 [95% CI −6.19 to −1.68]) and VO_{2max} (β_{VO2max} = 0.74 [95%CI 0.18 to 1.30]). The visual representation of influence of individuals' CRF on the difference in bias magnitude at first and second thresholds is presented in Figure 1(b,c).

Discussion

This study examined the test-retest reliability of DFA-α1 thresholds and their agreement with ventilatory and lactate derived exercise thresholds in males and females of varied cardiorespiratory fitness (CRF) levels. DFA-α1 derived thresholds showed *good* test-retest reliability with similar variation as those observed in gas-VTs. Secondly, *moderate* to *high* agreement between DFA-α1 and lactate thresholds (LTs), and *high* agreement with ventilatory thresholds (VTs) was observed. The magnitude of disagreement between DFA-α1 thresholds and gas derived VTs depended on sex and individual fitness characteristics, with higher mean bias in males and overestimated thresholds by DFA-α1 at lower CRF and underestimated thresholds at higher CRF levels.

Reliability of DFA-α1 derived thresholds

DFA-α1 based thresholds showed *good* test-retest reliability (ICC = 0.76–0.86, CV = 4–5.13%) with an approximate typical error of 6 and 8 bpm in HR at αTh_1 and αTh_2 respectively. The results agree with the recent findings by Sempere-Ruiz et al. (2024) which reported *moderate* to *good* test-retest reliability (ICC = 0.52 and 0.85, typical error = 8.83 and 4.08 bpm) at DFA-α1 first and second threshold respectively, in a relatively smaller sample size (n = 15) than the present study (n = 32). Moreover, gas-VTs as a criterion measure in the study showed similar levels of reliability (ICC = 0.84–0.88) at VT_1 and VT_2, with slightly lower CV ~ 3% and typical error of ~5 bpm at both thresholds. These agree with studies that have reported between-day variation in gas-VTs, LTs (Pallarés et al., 2016) and various other physiological markers (Zinner et al., 2023) as a result of within-individual biological day-to-day variability. Taken together, DFA-α1 like any other physiological threshold indicates potential as a reliable measure of exercise intensity, keeping into account the typical variation that exists in its estimated thresholds.

Agreement between ventilatory, lactate and DFA-α1 derived thresholds

In the present study, gas-derived VTs and LTs showed *high* agreement at both first and second threshold (r = 0.67–0.76) with minimal mean biases (<5 bpm). This agrees with the suggested co-occurrence of VTs and LTs (Loat & Rhodes, 1993; Pallarés et al., 2016), explored in the early works by Wasserman and colleagues (Wasserman et al., 1973). DFA-α1 based αTh_1 showed *moderate* agreement with LT_1 (r = 0.40–0.46), and a *high* agreement with VT_1 (r = 0.61) with mean biases of approximately −4 and 1 bpm respectively. Similar recent observations of *low* to *moderate* agreement (r = 0.23–0.32) between αTh_1 and LT_1 (Fleitas-Paniagua et al., 2024), while a *high* agreement with VT_1 (Schaffarczyk et al., 2023; Van Hooren, Mennen, et al., 2023) have been reported. Comparative to the first threshold, αTh_2 also showed *moderate* to *high* agreement with LT_2 (r = 0.41–0.57), and VT_2 (r = 0.66). The lower agreement of DFA-α1 with LTs than gas-VTs can be explained by a multitude of reasons including different treadmill protocols (i.e., step versus ramp) used for LTs/VTs determination, various LT concepts and quantification methods (Faude et al., 2009), or the underpinning mechanisms that constitute these exercise thresholds. Factors like lactic acidosis during exercise explain the changes in ventilatory parameters such as VE to some extent, however other factors including parasympathetic withdrawal and sympathoadrenal activity via autonomic nervous system (ANS) have been argued to directly modulate ventilation with increasing intensity (Cottin et al., 2007; Yamamoto et al., 1992). Similarly, lactate kinetics with increasing exercise intensity have been observed to be primarily dictated by peripheral bioenergetic mechanisms within the skeletal muscles (Hargreaves & Spriet, 2020; van Hall, 2010). On the other hand, the DFA-α1 response with incremental exercise intensity can be understood as a whole-system organismic regulation during exercise represented through the correlation properties of HRV, based on the concept of "network physiology" (Gronwald et al., 2020; Rogers & Gronwald, 2022). The paired innervation and regulation of parasympathetic and sympathetic branches of ANS, along with other neuromuscular, biochemical, peripheral and central nervous system inputs forms the overall concept of "organismic demand" reflected in DFA-α1. The likely dissociated mechanisms may explain lower absolute agreement between DFA-α1 thresholds and other intensity thresholds estimates such as LTs/VTs which are estimated from specific subsystem physiological responses.

Past studies that have examined other HRV-based methods for quantification of thresholds such as spectral frequency peak, power density in high frequency band or time-varying spectral analysis, have either reported similar or better agreement levels with gas-VTs (r = 0.91–0.98), as compared to DFA-α1 (Cassirame et al., 2015; Cottin et al., 2006; Mourot et al., 2012). An important feature in virtually all former HRV

methods was the identification of an inflection point to estimate thresholds. Given the introduction of DFA-α1 method has been to practically demarcate training boundaries, exploring different approaches that provide valid and individualised intensity distribution using the entire DFA-α1 response without necessarily matching it with discrete physiological thresholds, gives future direction for method development to improve its accuracy.

Effect of sex differences and CRF on DFA-α1 and gas-VTs agreement

The present study is the first to explore the influence of sex and CRF on the bias magnitude which can likely explain the inflated LoA observed between DFA-α1 thresholds and gas-VTs in recent literature. A novel finding was the overestimation by DFA-α1 at VT_1 for individuals with lower fitness (vVT_1 <8.5 $km{\cdot}hr^{-1}$) and underestimation for highly-fit individuals (vVT_1 >12 $km{\cdot}hr^{-1}$). Similarly, DFA-α1 underestimated VT_2 for individuals with higher fitness (vVT_2 >15 $km{\cdot}hr^{-1}$, VO_{2max} >55 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$). Although direct comparison is difficult, these observations are similar to studies that investigated DFA-α1 agreement in relatively homogeneous samples. For example, with respect to CRF, DFA-α1 thresholds underestimated LT_1 and LT_2 by approximately 11 and 8 bpm among elite cyclists ($VO_{2max} = 70.5 \pm 4.6$ $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) (Mateo-March et al., 2023), but overestimated by over 20 bpm at VT_1 in a group of untrained individuals with lower VO_{2max} (49.7 ± 8.0 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) (Sempere Ruiz et al., 2024). Equally, Schaffarczyk et al. (2023) also observed an overestimation by DFA-α1 at VT_1 by 4.7 bpm in females ($VO_{2max} = 36.4 \pm 4.5$ $mL{\cdot}kg^{-1}{\cdot}min^{-1}$). According to the network physiology standpoint (Rogers & Gronwald, 2022), wherein the changes in the correlation properties of HRV are reflected from interaction of multiple subsystems, it is likely that in untrained individuals the DFA-α1 responses appear later than the intensity estimates based on homeostatic disturbances occurring at a particular subsystem level, and therefore the overestimated thresholds. On the other side, training responses such as decrease in vagally-related HRV even with increasing fitness has been observed with extensive training status (e.g., in elite athletes) (Plews et al., 2013). As a result of lower overall HRV, its correlation properties may tend to white noise (α1 ~ 0.5) earlier with increasing intensity and thereby leading to underestimated DFA-α1 thresholds in these populations. Besides fitness characteristics, effect of sex on agreement were also observed, with males having a mean bias of approximately 9 bpm higher than females at VT_1, when controlling for CRF variables within the mixed-effects model. An ongoing discussion still exists around the likely sex differences in agreement (Rogers & Gronwald, 2022), though emerging evidence showed a similar higher mean bias for males than females at first threshold (Fleitas-Paniagua et al., 2024). The higher mean bias in males can be attributed to greater variability in fitness within this cohort (Figure 1(a)) which could have raised the overall bias in comparison to females. These results imply that individual fitness characteristics may have a higher weightage on affecting the bias magnitude of DFA-α1 predicted thresholds.

From a practical application perspective, a bias correction factor based on CRF can be applied to DFA-α1 thresholds to improve the accuracy of estimation across different population. Given that CRF variables like vVTs and VO_{2max} would require access to GXT, a similar model and standardised correction factor could be built based on field-based measures of CRF. However, future work is needed to determine this. The study findings should however be interpreted keeping into account certain limitations. As running was used for the testing modality during GXT, concurrent recordings of lactate, gas-exchange and HRV data was unobtainable, and therefore may have affected the agreement levels of LTs with the VTs and DFA-α1 thresholds. It should be noted that different treadmill protocols (step versus ramp) were performed on separate days that may have influenced the agreement levels between thresholds. In addition, various concepts exist for lactate profiling and threshold determination (Faude et al., 2009; Jamnick et al., 2018), which are also dependent on specific protocol designs and manipulating GXT variables, and therefore could affect the accuracy of the lactate threshold estimates with the protocol used in present study. The other consideration is the use of predefined methodology for quantification of DFA-α1 (Rogers & Gronwald, 2022), including set window width, grid interval and discrete points for thresholds estimation, which might be a limiting factor affecting the agreement.

Overall, DFA-α1 predicted first and second thresholds proved to be reliable estimates of individualised exercise intensity, however its validity to represent aerobic or anaerobic thresholds is questionable. Though DFA-α1 can be seen to represent a certain underlying physiological response as a function of increasing exercise intensity, considering the unexplained variance in agreement and wide LoA observed in the previous literature and in the present study, it is arguable whether it is perfectly representative of lab-based intensity thresholds. Nevertheless, as DFA-α1 showed high agreement with VTs from a statistical threshold perspective, it may potentially be used a practical measure of classifying individualised intensity zones. However, due to variability in CRF which affects the bias magnitude between individuals, it is recommended to perform necessary corrections based on fitness measures to increase accuracy of DFA-α1 thresholds across different population. Future research should aim to look at DFA-α1 in context of longitudinal changes in fitness and chronic training adaptations to better understand the validity of its thresholds. Although the current use of absolute values of 0.75/0.5 for threshold estimation is based on signal theory, reflecting distinct systemic regulation with increasing intensity, future studies may explore identifying other transition points in correlation properties of HRV that may demarcate individualised exercise intensity zones.

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Data availability statement

The datasets generated and analysed during the current study are not publicly available as part of Leeds Beckett University Data Management Plan but will be made available by the corresponding author upon request.

References

Akubat, I., & Abt, G. (2011, May 1). Intermittent exercise alters the heart rate–blood lactate relationship used for calculating the training impulse (TRIMP) in team sport players. *Journal of Science & Medicine in Sport*, *14* (3), 249–253. https://doi.org/10.1016/j.jsams.2010.12.003

Altman, D. G. (1991). *Practical statistics for medical research*. CRC Press.

Åström, M., García, J., Laguna, P., Pahlm, O., & Sörnmo, L. (2003, March 1). Detection of body position changes using the surface electrocardiogram. *Medical & Biological Engineering & Computing*, *41*(2), 164–171. https://doi.org/10.1007/BF02344884

Azevedo, R. D. A., Forot, J., Gy, M., & Murias, J. M. (2022, March). Comparing muscle V̇o2 from near-infrared spectroscopy desaturation rate to pulmonary V̇o2 during cycling below, at, and above the maximal lactate steady state. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *132*(3), 641–652. https://doi.org/10.1152/japplphysiol.00754.2021

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1985, December). Improved detection of lactate threshold during exercise using a log-log transformation. *Journal of Applied Physiology*, *59*(6), 1936–1940. https://doi.org/10.1152/jappl.1985.59.6.1936

Bentley, D. J., Newell, J., & Bishop, D. (2007, Jul 1). Incremental exercise test design and analysis. *Sports Medicine*, *37*(7), 575–586. https://doi.org/10.2165/00007256-200737070-00002

Binder, R. K., Wonisch, M., Corra, U., Cohen-Solal, A., Vanhees, L., Saner, H., & Schmid, J.-P. (2008, December 1). Methodological approach to the first and second lactate threshold in incremental cardiopulmonary exercise testing. *European Journal of Cardiovascular Prevention & Rehabilitation*, *15*(6), 726–734. https://doi.org/10.1097/HJR.0b013e328304fed4

Black, M. I., Jones, A. M., Blackwell, J. R., Bailey, S. J., Wylie, L. J., McDonagh, S. T. J., Thompson, C., Kelly, J., Sumners, P., Mileva, K. N., Bowtell, J. L., & Vanhatalo, A. (2017, March). Muscle metabolic and neuromuscular determinants of fatigue during cycling in different exercise intensity domains. *Journal of Applied Physiology*, *122*(3), 446–459. https://doi.org/10.1152/japplphysiol.00942.2016

Burnley, M., & Jones, A. M. 2018, January 2. Power–duration relationship: Physiology, fatigue, and the limits of human performance. *European Journal of Sport Science*, *18*(1), 1–12. https://doi.org/10.1080/17461391.2016.1249524

Caldwell, A. R. 2022, March 22. SimplyAgree: An R package and jamovi module forSimplifying agreement and reliability analyses. *Journal of Open Source Software*, *7*(71), 4148. https://doi.org/10.21105/joss.04148

Cardinale, M., & Varley, M. C. (2017, April 1). Wearable training-monitoring technology: Applications, challenges, and opportunities. *International Journal of Sports Physiology & Performance*, *12*(s2), S2–62. https://doi.org/10.1123/ijspp.2016-0423

Cassirame, J., Tordi, N., Fabre, N., Duc, S., Durand, F., & Mourot, L. (2015, October 3). Heart rate variability to assess ventilatory threshold in ski-mountaineering. *European Journal of Sport Science*, *15*(7), 615–622. https://doi.org/10.1080/17461391.2014.957729

Cejuela, R., & Selles-Perez, S. (2023). Training characteristics and performance of two male elite short-distance triathletes: From junior to "world-class". *Scandinavian Journal of Medicine & Science in Sports*, *33* (12), 2444–2456. https://doi.org/10.1111/sms.14474

Cottin, F., Leprêtre, P. M., Lopes, P., Papelier, Y., Médigue, C., & Billat, V. (2006, December). Assessment of ventilatory thresholds from heart rate variability in well-trained subjects during cycling. *International Journal of Sports Medicine*, *27*(12), 959–967. https://doi.org/10.1055/s-2006-923849

Cottin, F., Médigue, C., Lopes, P., Leprêtre, P. M., Heubert, R., & Billat, V. (2007, April). Ventilatory thresholds assessment from heart rate variability during an incremental exhaustive running test. *International Journal of Sports Medicine*, *28*(4), 287–294. https://doi.org/10.1055/s-2006-924355

de Lucas Rd, de Souza Km, Costa, V. P., Grossl, T., Guglielmo, L. G. A., de Lucas, R. D., & de Souza, K. M. (2013, February 1). Time to exhaustion at and above critical power in trained cyclists: The relationship between heavy and severe intensity domains. *Science & Sports*, *28*(1), e9–14. https://doi.org/10.1016/j.scispo.2012.04.004

Edvardsen, E., Hansen, B. H., Holme, I. M., Dyrstad, S. M., & Anderssen, S. A. (2013 Jul 1). Reference values for cardiorespiratory response and fitness on the treadmill in a 20- to 85-year-old population. *Chest*, *144*(1), 241–248. https://doi.org/10.1378/chest.12-1458

Faude, O., Kindermann, W., & Meyer, T. 2009 Jun 1. Lactate threshold concepts. *Sports Medicine*, *39*(6), 469–490. https://doi.org/10.2165/00007256-200939060-00003

Fleitas-Paniagua, P. R., Marinari, G., Rasica, L., Rogers, B., & Murias, J. M. 2024, February 12. Heart rate variability thresholds: Agreement with established approaches and reproducibility in trained females and males. *Medicine & Science in Sports and Exercise*. https://doi.org/10.1249/MSS.0000000000003412

Gronwald, T., Rogers, B., & Hoos, O. (2020). Fractal correlation properties of heart rate variability: A new biomarker for intensity distribution in endurance exercise and training prescription? *Frontiers in Physiology* [Internet]. *11*. Retrieved January 30, 2024. https://doi.org/10.3389/fphys.2020.550572

Hardstone, R., Ss, P., Schiavone, G., Jansen, R., Vv, N., & Mansvelder, H. D. (2012, November 30). Detrended fluctuation analysis: A scale-free view on neuronal oscillations. *Frontiers in Physiology* [Internet]. *3*. https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2012.00450/full

Hargreaves, M., & Spriet, L. L. (2020, September). Skeletal muscle energy metabolism during exercise. *Nature Metabolism*, *2*(9), 817–828. https://doi.org/10.1038/s42255-020-0251-4

Hoefelmann, C. P., Diefenthaeler, F., Costa, V. P., de Lucas Rd, Shambrook, P., Guglielmo, L. G. A., & de Lucas, R. D. (2015, May 19). Test–retest reliability of second lactate turnpoint using two different criteria in competitive cyclists. *European Journal of Sport Science*, *15*(4), 265–270. https://doi.org/10.1080/17461391.2014.944874

Iannetta, D., Inglis, E. C., Mattu, A. T., Fontana, F. Y., Pogliaghi, S., Keir, D. A., & Murias, J. M. (2020, February). A critical evaluation of current methods for exercise prescription in women and men. *Medicine & Science in Sports and Exercise*, *52*(2), 466–473. https://doi.org/10.1249/MSS.0000000000002147

Jamnick, N. A., Botella, J., Pyne, D. B., Bishop, D. J., & Sandbakk, Ø. (2018, Jul 30). Manipulating graded exercise test variables affects the validity of the lactate threshold and V̇O2peak. *PLOS ONE*, *13*(7), e0199794. https://doi.org/10.1371/journal.pone.0199794

Jamnick, N. A., Pettitt, R. W., Granata, C., Pyne, D. B., & Bishop, D. J. (2020, October 1). An examination and critique of current methods to determine exercise intensity. *Sports Medicine*, *50*(10), 1729–1756. https://doi.org/10.1007/s40279-020-01322-8

Jones, A. M., & Carter, H. (2000, Jun 1). The effect of endurance training on parameters of aerobic fitness. *Sports Medicine*, *29*(6), 373–386. https://doi.org/10.2165/00007256-200029060-00001

Kaufmann, S., Gronwald, T., Herold, F., & Hoos, O. (2023, Jul 18). Heart rate variability-derived thresholds for exercise intensity prescription in endurance sports: A systematic review of interrelations and agreement

with different ventilatory and blood lactate thresholds. *Sports Medicine - Open*, *9*(1), 59. https://doi.org/10.1186/s40798-023-00607-2

Lee, J., & Zhang, X. L. (2021, September 1). Physiological determinants of VO2max and the methods to evaluate it: A critical review. *Science & Sports*, *36*(4), 259–271. https://doi.org/10.1016/j.scispo.2020.11.006

Loat, C. E. R., & Rhodes, E. C. (1993 February 1). Relationship between the lactate and ventilatory thresholds during prolonged exercise. *Sports Medicine*, *15*(2), 104–115. https://doi.org/10.2165/00007256-199315020-00004

Manzi, V., Iellamo, F., Impellizzeri, F., Dottavio, S., & Castagna, C. (2009, October 1). Relation between individualized training impulses and performance in distance runners. *Medicine & Science in Sports and Exercise*, *41*(11), 2090–2096. https://doi.org/10.1249/MSS.0b013e3181a6a959

Mateo-March, M., Moya-Ramón, M., Javaloyes, A., Sánchez-Muñoz, C., & Clemente-Suárez, V. J. (2023, April 3). Validity of detrended fluctuation analysis of heart rate variability to determine intensity thresholds in elite cyclists. *European Journal of Sport Science*, *23*(4), 580–587. https://doi.org/10.1080/17461391.2022.2047228

Mourot, L., Tordi, N., Bouhaddi, M., Teffaha, D., Monpere, C., & Regnard, J. (2012, December 1). Heart rate variability to assess ventilatory thresholds: Reliable in cardiac disease? *European Journal of Preventive Cardiology*, *19*(6), 1272–1280. https://doi.org/10.1177/1741826711423115

Naranjo-Orellana, J., Nieto-Jiménez, C., & Ruso-Álvarez, J. F. (2021 January 12). *Non-linear heart rate dynamics during and after three controlled exercise intensities in healthy men*. Retrived September 9, 2024, from https://akjournals.com/view/journals/2060/107/4/article-p501.xml

Newell, J., Korir, P., Moore, B., & Pedlar, C. App for the calculation of blood lactate markers. *Journal of Sports Sciences*, *33*(6), 568–569. 2015 April 3. https://doi.org/10.1080/02640414.2014.953982

Pallarés, J. G., Morán-Navarro, R., Ortega, J. F., Fernández-Elías, V. E., Mora-Rodriguez, R., & Sandbakk, Ø. Validity and reliability of ventilatory and blood lactate thresholds in well-trained cyclists. *PLOS ONE*, *11*(9), e0163389. 2016 September 22. https://doi.org/10.1371/journal.pone.0163389

Peng, C. K., Havlin, S., He, S., & Goldberger, A. L. Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. *Chaos Interdiscip J Nonlinear Sci*, *5*(1), 82–87. 1995 March 1. https://doi.org/10.1063/1.166141

Plews, D. J., Laursen, P. B., Stanley, J., Kilding, A. E., & Buchheit, M. Training adaptation and heart rate variability in elite endurance athletes: Opening the door to effective monitoring. *Sports Medicine*, *43*(9), 773–781. 2013 September 1. https://doi.org/10.1007/s40279-013-0071-8

Portney, L. G., & Watkins, M. P. (2009). *Foundations of clinical research: Applications to practice* (3rd ed. p. 892). Pearson/Prentice Hall.

Pymer, S., Nichols, S., Prosser, J., Birkett, S., Carroll, S., & Ingle, L. Does exercise prescription based on estimated heart rate training zones exceed the ventilatory anaerobic threshold in patients with coronary heart disease undergoing usual-care cardiovascular rehabilitation? A United Kingdom perspective. *European Journal of Preventive Cardiology*, *27*(6), 579–589. 2020 April 1. https://doi.org/10.1177/2047487319852711

Ribeiro, J. P., Hughes, V., Fielding, R. A., Holden, W., Evans, W., & Knuttgen, H. G. Metabolic and ventilatory responses to steady state exercise relative to lactate thresholds. *European Journal of Applied Physiology and Occupational Physiology*, *55*(2), 215–221. 1986 March 1. https://doi.org/10.1007/BF00715008

Rogers, B., Giles, D., Draper, N., Hoos, O., & Gronwald, T. (2021). A new detection method defining the aerobic threshold for endurance exercise and training prescription based on fractal correlation properties of heart rate variability. *Frontiers in Physiology* [Internet]. *11*. https://doi.org/10.3389/fphys.2020.596567

Rogers, B., Giles, D., Draper, N., Mourot, L., & Gronwald, T. (2021a, June). Detection of the anaerobic threshold in endurance sports: Validation of a new method using correlation properties of heart rate variability. *Journal of Functional Morphology and Kinesiology*, *6*(2), 38. https://doi.org/10.3390/jfmk6020038

Rogers, B., Giles, D., Draper, N., Mourot, L., & Gronwald, T. (2021b, January). Influence of artefact correction and recording device type on the practical application of a non-linear heart rate variability biomarker for aerobic threshold determination. *Sensors*, *21*(3), 821. https://doi.org/10.3390/s21030821

Rogers, B., & Gronwald, T. (2022). Fractal correlation properties of heart rate variability as a biomarker for intensity distribution and training prescription in endurance exercise: An update. *Frontiers in Physiology* [Internet]. *13*. Retrieved January 30, 2024. https://doi.org/10.3389/fphys.2022.879071

Samitz, G., Egger, M., & Zwahlen, M. Domains of physical activity and all-cause mortality: Systematic review and dose–response meta-analysis of cohort studies. *International Journal of Epidemiology*, *40*(5), 1382–1400. https://doi.org/10.1093/ije/dyr112

Schaffarczyk, M., Rogers, B., Reer, R., & Gronwald, T. Validation of a non-linear index of heart rate variability to determine aerobic and anaerobic thresholds during incremental cycling exercise in women. *European Journal of Applied Physiology*, *123*(2), 299–309. 2023 February 1. https://doi.org/10.1007/s00421-022-05050-x

Scherr, J., Wolfarth, B., Christle, J. W., Pressler, A., Wagenpfeil, S., & Halle, M. Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity. *European Journal of Applied Physiology*, *113*(1), 147–155. 2013 January 1. https://doi.org/10.1007/s00421-012-2421-x

Sempere Ruiz, N., Sarabia Marín, J. M., Baladzhaeva, S., & Moya, M. Reliability and validity of a non-linear index of heart rate variability to determine intensity thresholds. *Frontiers in Physiology*, *15*. 2024 February 515; 515. https://doi.org/10.3389/fphys.2024.1329360

Stöggl, T. L., & Sperlich, B. (2015). The training intensity distribution among well-trained and elite endurance athletes. *Frontiers in Physiology* [Internet]. *6*. https://doi.org/10.3389/fphys.2015.00295

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Po, R.-A., & Karjalainen, P. K. H. Kubios HRV – Heart rate variability analysis software. *Computer Methods and Programs in Biomedicine*, *113*(1), 210–220. 2014 January 1. https://doi.org/10.1016/j.cmpb.2013.07.024

van Hall, G. (2010). Lactate kinetics in human tissues at rest and during exercise. *Acta Physiologica*, *199*(4), 499–508. https://doi.org/10.1111/j.1748-1716.2010.02122.x

Van Hooren, B., Bongers, B. C., Rogers, B., & Gronwald, T. The between-day reliability of correlation properties of heart rate variability during running. *Applied Psychophysiology and Biofeedback*, *48*(4), 453–460. 2023 December 1. https://doi.org/10.1007/s10484-023-09599-x

Van Hooren, B., Mennen, B., Gronwald, T., Bongers, B. C., & Rogers, B. (2023). Correlation properties of heart rate variability to assess the first ventilatory threshold and fatigue in runners. *Journal of Sports Sciences*, *0*(), 1–10. https://doi.org/10.1080/02640414.2023.2277034

Voeten, C. (2023). *Stepwise elimination and term reordering for mixed-effects regression [internet]*. CRAN. https://cran.r-project.org/web/packages/buildmer/buildmer.pdf

Wasserman, K., Whipp, B. J., Koyl, S. N., & Beaver, W. L. (1973, August). Anaerobic threshold and respiratory gas exchange during exercise. *Journal of Applied Physiology*, *35*(2), 236–243. https://doi.org/10.1152/jappl.1973.35.2.236

Yamamoto, Y., Hughson, R. L., & Nakamura, Y. Autonomic nervous system responses to exercise in relation to ventilatory threshold. *Chest*, *101*(5, Supplement), 206S–210S. 1992 May 1. https://doi.org/10.1378/chest.101.5_Supplement.206S

Zimatore, G., Falcioni, L., Gallotta, M. C., Bonavolontà, V., Campanella, M., Spirito, M. D., Guidetti, L., & Baldari, C. Recurrence quantification analysis of heart rate variability to detect both ventilatory thresholds. *PLOS ONE*, *16*(10), e0249504. 2021 October 7. https://doi.org/10.1371/journal.pone.0249504

Zinner, C., Gerspitzer, A., Düking, P., Boone, J., Schiffer, T., Holmberg, H. C., & Sperlich, B. (2023, March). The magnitude and time-course of physiological responses to 9 weeks of incremental ramp testing. *Scandinavian Journal of Medicine & Science in Sports*, *33*(7), 1146–1156. https://doi.org/10.1111/sms.14347