International Journal of Human Movement and Sports Sciences 11(2): 398-410, 2023 http://www.hrpub.org
DOI: 10.13189/saj.2023.110217

Heart Rate Variability-Established Thresholds to Determine the Ventilatory and Lactate Thresholds of Endurance Athletes

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Received November 30, 2022; Revised February 16, 2023; Accepted March 12, 2023

Cite This Paper in the Following Citation Styles

(a): *[1] Ninette Thiart, Ben Coetzee, Christo Bisschoff , "Heart Rate Variability-Established Thresholds to Determine the Ventilatory and Lactate Thresholds of Endurance Athletes," International Journal of Human Movement and Sports Sciences, Vol. 11, No. 2, pp. 398 - 410, 2023. DOI: 10.13189/saj.2023.110217.*

(b): *Ninette Thiart, Ben Coetzee, Christo Bisschoff (2023). Heart Rate Variability-Established Thresholds to Determine the Ventilatory and Lactate Thresholds of Endurance Athletes. International Journal of Human Movement and Sports Sciences, 11(2), 398 - 410. DOI: 10.13189/saj.2023.110217.*

Abstract This study aimed to determine the use of heart rate variability (HRV)-established thresholds to accurately estimate endurance athletes' ventilatory threshold 1 (VT_1), respiratory compensation point (RCP) and lactate threshold 2 (LT_2). Eleven cyclists (aged: 23.7 ± 3.1 years) from the African Continental Development Cycling team and ten middle- and long-distance athletes (age: 21.2 ± 1.8 years) from a South African university participated in this study. Before the start of an incremental cycling or running maximal oxygen consumption ($\dot{V}O_{2max}$) test each participant was fitted with a Fixed Polar HR Transmitter Belt and Monitor to determine the R-R intervals of the last 60 seconds of each stage. The Kubios HRV Premium software package was used to analyze the R-R-intervals. Blood samples were taken 30 seconds before the end of each stage and analyzed for blood lactate. Ventilatory threshold points were identified using the criteria of an increase in $\dot{V}_E/\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$ (VT_1) and an increase in both $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ (RCP). The LT_2 was identified as the second increase in blood lactate concentration from one increment to the next. Concerning HRV thresholds, VT_1 was determined at a DFAα1 value of 0.75, the first breakpoint in the standard deviation of the instantaneous (SD_1) and continuous long-term RR interval (SD_2) curves, the visual deflection in the squared root of the mean squared differences between successive R-R intervals (RMSSD) curve, and the first abrupt increase in high-frequency (HF) power x HF frequency. The RCP was detected at a DFAα1 value of 0.5 and the final abrupt increase in HF. The Bland-Altman plots revealed that the absolute power outputs at VT_1SD_1 and VT_1RMSSD showed significant agreements with the absolute power outputs at VT_1. Large paired-sample correlations were also found between the absolute power outputs at VT_1SD_1, VT_1RMSSD and VT_1. In conclusion, coaches, sport scientists and other professionals are encouraged to use SD_1 and RMSSD to determine the VT_1-related training program workloads of endurance-trained athletes, especially in areas where laboratories are not available.

Keywords Ventilatory Threshold, Lactate Threshold, Heart Rate Variability, Heart Rate Variability Thresholds

1. Introduction

The estimation of the ventilatory and lactate thresholds is important as it allows sport-related practitioners and scientists to adapt training intensities and monitor and assess endurance performance [1,2]. Last-mentioned thresholds serve as important indicators of individual exercise tolerance which refers to the ability of an individual to perform higher intensities of exercise for longer durations [3]. For this reason, threshold-based

training models allow practitioners to set individualized training intensities for endurance sport as it accounts for individual metabolic responses to training [4]. The most recognized and used methods for the determination of these thresholds are indirect calorimetry, open-circuit spirometry, and computerized instrumentation as well as blood lactate sampling during a graded exercise test [5]. The ventilatory threshold 1 or aerobic threshold (VT_1) is regarded to be the point where the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) increases nonlinearly while the ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) remains constant [6]. The VT_1 can also be defined as the point where the first rise in the fraction of expired oxygen ($P_{ET}O_2$) occurs, while the fraction of expired carbon dioxide ($P_{ET}CO_2$) either increases or remains constant [7]. In addition, the intersection between $\dot{V}CO_2$ and $\dot{V}O_2$ (V-slope) graphs where the slope changes from less than one to equal or greater than one, is also used to identify the VT_1 [7]. The ventilatory threshold 2 (VT_2), anaerobic threshold (AT) or respiratory compensation point (RCP) is the point where an increase in both $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ occur and a nonlinear increase in the exhaled carbon dioxide volume ($\dot{V}CO_2$) compared to the inhaled $\dot{V}O_2$ is observed [6,8]. The deflection point of the end-tidal $P_{ET}CO_2$ is also used to identify the VT_2 [7]. The secondary increase in arterial blood lactate concentration (BLa) is known as the second LT point (LT_2) [9].

Despite proof of the reliability and validity of the last-mentioned techniques to determine the thresholds of different populations of athletes, these techniques require expensive equipment which needs to be calibrated frequently, are invasive and need expert personnel to operate [10-15]. Given these difficulties, alternative quick-to-determine, practical, inexpensive-to-obtain, and easy-to-interpret measures are rather needed to determine the thresholds of athletes. In this regard, heart rate variability (HRV) may serve as an easier, non-invasive, and cost-effective measure to determine athletes' thresholds [5,16,17].

Heart rate variability (HRV) serves as a parameter of the autonomic nervous system (ANS) functioning and reflects the sympathetic (SNS) and parasympathetic nervous system (PNS) activity of the ANS [18]. Heart rate variability (HRV) refers to the time between heart beats or the R-R intervals [12,16,18]. The progressive workloads of a graded exercise test will lead to a gradual and linear increase in sympathetic activity in relation to the imposed workload with concurrent and gradual parasympathetic withdrawal [19]. At more or less 60 to 80% of maximal oxygen consumption ($\dot{V}O_{2max}$) total parasympathetic withdrawal occurs followed by a drastic increase in sympathetic activity [20]. Therefore, the workloads at the last-mentioned intensities are characterized by a significant reduction in HRV, which is termed the HRV threshold [21,22]. Consequently, ventilatory thresholds may be under the same nervous system control as HRV, which means that the different thresholds can possibly be determined by examining the changes in HRV during exercise [12,23,24].

Proof that the nervous system control is similar for HRV and the ventilatory thresholds was provided by several researchers who observed strong relationships between HRV and the different threshold points [5,14,23]. For instance, a significant correlation was found for different HRV-threshold indexes (SD_1 and RMSSD) of healthy male participants and the visual inspection of the workload (r = 0.58-0.96) and $\dot{V}O_2$ (ml/kg/min) (r = 0.70-0.98) [25]. The Bland-Altman technique also showed a good agreement between VT_1 and the standard deviation (perpendicular to the line of identity within the Poincaré plot) of correlations between R-R intervals denoted as SD_1 (-0.7 ± 7.98 ml/kg/min), as well as between VT_1 and the squared root of the mean squared differences between successive R-R intervals (RMSSD) (-3.1 ± 7.54 ml/kg/min) [25]. In addition, the relationships between the gas analysis- and HRV-related methods for determining the VT_1 in Spanish professional basketball players indicated significant correlations between VT_1 $\dot{V}O_2$ (r = 0.54, p < 0.005), VT_1 HR (r = 0.57 p < 0.005), VT_1 speed (r = 0.47, p < 0.005) and SD_1 [12]. However, stronger correlations were observed between VT_2 $\dot{V}O_2$ (r = 0.91, p < 0.005), VT_2 HR (r = 0.90, p < 0.005), VT_2 speed (r = 0.93, p < 0.005) and peak frequency in the high-frequency range (fHF) [12]. On the other hand, Cassirame *et al.* [1] found no significant correlations (p > 0.05) between the fHF of elite ski-mountaineers, the HR and the speed at the VT_1, but significant strong correlations between fHF, the HR (r = 0.91, p < 0.001) and the speed at the VT_2 (r = 0.92, p < 0.001).

Another HRV parameter that can be used as an indicator of VT_1 or LT_1 is the short-term scaling exponent alpha 1 of the nonlinear detrended fluctuation analysis method (DFA↦1) with a value of ~0.75 [21]. In this regard, results from a group of elite endurance cyclists showed significant correlations between power output/HR at LT_1 and power output/HR at DFA ↦1 (~0.75) (r = 0.85, p < 0.001) as well as between power outputs/HRs at LT_2 and DFA↦1 (~0.5) (r = 0.93, p < 0.001) but no significant correlations were found between HRs at LT_1 and DFA↦1 (~0.75) [22]. Although researchers also use ~ 0.5 as the cut-off value for the identification of RCP, the accuracy of this value is still being debated [21].

Other researchers did not only report significant strong correlations between the VT_1 and the heart rate ventilatory threshold (HRVT) of healthy participants (r = 0.92, p < 0.001) but also between LT_2 and HRVT (r = 0.85, p < 0.001) [14]. These results are very similar to the significant strong correlation that was found between the LT_2 (182.0 ± 8.1 b min^{-1}) and power HF (Hz) (181.1 ± 8.2 b min^{-1}) of high-level swimmers when researchers assessed HR (r = 0.93, p < 0.05) and velocity (r = 0.98, p < 0.05) [16]. In another study of recreational long-distance male runners, a significant correlation was observed between the second HRV threshold ($HRVT_2$) and

the LT_2 ($r = 0.86$, $p < 0.01$) [13]. These results gave rise to the conclusion that good agreements exist between the LT and HRV threshold points and that HRV has the potential to be used as a non-invasive measure to determine LT during a maximal incremental running test [13].

The above-mentioned findings suggest that HRV-related parameters, which serve as indicators of cardiovascular ANS function, may serve as indicators of the VT and LT points. However, up until now, researchers narrowed their focus on the use of very specific HRV-related variables when investigating the usefulness and accuracy of HRV-related parameters to determine different thresholds in a wide range of non- and sport-participating populations [1,5,11,13,14,16,22,24,25]. No researchers used a variety of available HRV-related measures to examine their accuracy in determining different thresholds in high-level endurance athletes. Therefore, the purpose of this study was to determine whether the HRV-established threshold absolute power outputs can be used to accurately estimate the absolute power outputs at VT_1, RCP and LT_2 of endurance athletes.

We hypothesized that the HRV-established thresholds of endurance athletes as determined through changes in DFAα1, SD_1, RMSSD, and HF power can be used to accurately estimate VT_1, RCP, and LT_2. Study results may allow coaches, sport scientists and sport-related professionals to determine the precise threshold-related training program workloads of athletes for the continuous monitoring of training adaptations in places where expensive and sophisticated testing equipment and laboratories are not available.

2. Materials and Methods

2.1. Study Design

The hypothesis of the study was tested by making use of a selected group, quasi-experimental research design.

2.2. Participants

Ten middle- and long-distance athletes from a university in South Africa (M ± SD = age: 23.7 ± 3.1 years; height: 175.8 ± 6.8 cm; mass: 66.3 ± 8.8 kg), as well as eleven cyclists from the African Continental Development Cycling team (M ± SD = age: 21.2 ± 1.8 years; height: 182.1 ± 7.8 cm; mass: 73.4 ± 5.3 kg) volunteered to participate in this study. According to the classification of Swann *et al.* [26], cyclists could be classified as elite/expert cyclists whereas athletes could be classified into three categories, namely: competitive, successful, and world-class elite athletes. The study followed the ethical principles of the Declaration of Helsinki as well as the ethical guidelines of the National Health Research Ethics Council of South Africa. The Health Research Ethics Committee of the institution where the research was conducted approved the study with the following number: NWU-00005-17-A1. Participants gave written informed consent which thoroughly explained the benefits, risks as well as expectations of study participation.

2.3. Procedures

Participants completed the demographic questionnaire and were familiarized with the HRV equipment and the maximal aerobic capacity ($\dot{V}O_{2max}$) test one week before testing commenced. A week later, participants completed the maximal incremental $\dot{V}O_{2max}$ tests. Before the start of the tests, participants completed a recovery and hydration status questionnaire, followed by body mass and stature measurements. Next, each participant was fitted with a Fixed Polar HR Transmitter Belt and Monitor (Polar Team^2 Pro, Polar Electro, Kempele, Finland) to record HRs and HRV where after a fifteen-minute dynamic warm-up was performed followed by a $\dot{V}O_{2max}$ test.

2.4. Instruments and Tests

2.4.1. General Recovery and Hydration Status Questionnaire

The general recovery and hydration status questionnaire indicated participants' quantity as well as the quality of sleep during the previous night, their hydration status, and their degree of muscle soreness. The sleep quantity was indicated by noting the sleep duration and the sleep quality and hydration status were indicated through a 5-point Likert scale, and muscle soreness through a 3-point Likert scale [27,28,29]. Results of the general recovery and hydration status questionnaire aided in evaluating the overall recovery status of participants to ensure that they were in the best possible physiological state to successfully execute the $\dot{V}O_{2max}$ test.

2.4.2. Standard incremental maximal oxygen uptake ($\dot{V}O_{2max}$) tests

To measure the metabolic parameters during the running and cycling $\dot{V}O_{2max}$ tests the Oxycon Pro METALYZER@ 3B high-resolution spiroergometry system (Cortex Biophysik GmbH, Leipzig, Germany) with breath-by-breath technology was used. The MetaSoft@ Studio application software analysed the results of the spiroergometry system. The Oxycon Pro is regarded to be a valid, reliable, and accurate system for the measurement of athletes' and healthy participants' metabolic parameters during low- and maximal-intensity exercise [30-32]. Before the test commenced, the gas analyzer was calibrated with standard gases. The sampled $\dot{V}O_2$ and $\dot{V}CO_2$ from the Oxygen Pro gas analyzer apparatus were used to calculate the respiratory exchange ratio (RER). Before the start of the test, each participant was fitted with a face mask and a soft nose clip.

Before the start of the tests, cyclists performed a ten-minute warm-up at a resistance of 100 W on the

Wattbike Pro Air and Magnetic Resistance Trainer (Wattbike Limited, Nottingham, England), followed by high-intensity cycling at 250 W for 30 s, stretching and mobility exercises. The incremental cycling test was completed on the Wattbike Pro according to the adapted maximal ramp test protocol of Wattbike Ltd (2015:33-41) exactly two minutes after the warm-up. Cyclists were instructed to start at 150 W using an air resistance setting of one and to increase the power output with 35 W every two minutes. The test was terminated when the following criteria were met: when a minimum cadence of 80 rpm could not be maintained, when the criteria for $\dot{V}O_{2max}$ was met (e.g. a RER-value higher than 1.15, oxygen consumption ceased to rise and reached a plateau or began to fall with an increase in work rate, and the maximum age-specific HR as calculated through the formula: Maximum HR (208–0.7*age) was reached), or they wanted to stop [33,34]. The Wattbike Pro automatically determined the absolute (W) and relative power outputs (W kg^{-1}) at the end of each increment.

Before the start of the test, athletes completed a ten-minute warm-up at a running speed of 13 km h^{-1} on a Woodway Pro XL Treadmill (Woodway, W229 N591, Foster Ct, Waukesha, WI), followed by a speed increase to 16 km/h for 30 s after which stretching and mobility exercises were executed. The incremental test was performed according to the adapted method of Peserico *et al.* [35] exactly two minutes after the warm-up. The test commenced at an incline of 1˚ and a speed of 12 km·h^{-1}, increasing with 1 km h^{-1} every two minutes until volitional exhaustion. The test was terminated when the participants indicated they wanted to stop, when they failed to maintain the speed of the incremental level for at least 30 s, or when the $\dot{V}O_{2max}$ criteria were reached. The studio application software automatically determined the absolute (W) and relative power outputs (W kg^{-1}) at the end of each incremental stage.

Two physiological gas exchange points were also identified from the standard incremental $\dot{V}O_{2max}$ test data. The ventilatory threshold 1 (VT_1) was determined using the criteria of an increase in $\dot{V}_E/\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$ and departure from the linearity of $\dot{V}_E$ [5]. The ventilatory threshold 2 (VT_2) or RCP was taken as the point which corresponds to an increase in both $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ [8]. VT_1 and RCP were visually detected by two independent researchers, with no discrepancies between the visually detected values of the two researchers.

Throughout the $\dot{V}O_{2max}$ tests, blood samples were taken 30 seconds before the end of each stage and analysed with a Simplified Lactate Pro LT-1710 Blood Lactate Test Meter (Arkray Factory Inc., KDK Corporation, Shiga, Japan). The LT_2 was determined by the identification of the secondary increase in arterial blood lactate concentration from one increment level to the next [9,36].

To record the variations in beat-to-beat intervals (R-R intervals) during the standard incremental $\dot{V}O_{2max}$ test a Fix Polar Heart Rate Monitor and Transmitter Belt were used. These monitors are valid devices for obtaining R-R intervals [37]. The Polar device-obtained R-R-intervals were analysed via the Kubios HRV Premium software package (Version 3.4.2, Biosignal Analysis and Medical Imaging Group at the Department of Applied Physics, University of Kuopio, Kuopio, Finland). The Polar Team software package was used to export the R-R-related data sets from the Polar Monitors and the "automatic artefact correction" function was selected to automatically filter out the erroneous values before it was exported as a raw R-R data set [38].

The R-R intervals of the last 60 seconds of each stage were used to measure HRV. A one-minute window from the R-R data was used to measure DFAα1 [5]. The window width of the DFAα1 was set to $4 < n < 16$ beats. To detect the VT_1 a DFA α1 value of 0.75 was selected as this is also the midpoint between a fractal behavior of the HR time series of 1.0 (for low-intensity exercise) and an uncorrelated value of 0.5 (for high-intensity exercise) representing random behavior of the HR time series [21]. The first breakpoint in the SD_1 and SD_2 curves of the Poincar éplot was used to detect VT_1 [11,13]. To determine VT_1 through RMSSD a visual deflection in the curve of intensity versus RMSSD indexes was identified [25]. For power HF, VT_1 was calculated from the R-R interval series using a time-varying short-term Fourier transform with a 64-second moving window [1,23]. The first abrupt increase in Power HF x HF frequency after it has reached a minimum was used to determine VT_1 [1,23]. A DFAα1 value of 0.5 was used to detect the second threshold (RCP) [21]. Thereafter, the breakpoints of DFAα1 were matched to the power output values obtained during the incremental test. RCP was determined from HF power by identifying the final abrupt increase in the HF band [11,23].

2.5. Data Analysis

The descriptive statistics of each variable were first calculated. Next, the Shapiro-Wilk test for data normality was performed with a p-value of less than 0.05 determined as the cut-off point for the rejection of the null hypothesis that the data are normally distributed. Thereafter, paired-sample T-tests and correlation analyses were performed to determine whether significant differences or associations exist between the different HRV-, ventilatory- and lactate-established threshold points. The strength of correlations was categorized based on the following criteria: <0.1 (trivial), <0.3 (small), <0.5 (moderate), <0.7 (large), <0.9 (very large) and <1 (nearly perfect) [39]. Cohen's effect sizes (*d*) were also calculated and interpreted as follows: ~0.2 (small), ~0.5 (medium) and ~0.8 (large) [40]. In the next step, sample one T-tests were performed to determine which variables met the requirements of a Bland-Altman test. Only variables for which nonsignificant one-sample T-test results were found were further analyzed via the Bland-Altman technique. This was followed by the presentation of the Bland and

Altman plots [36] that were used to compare the HRV-, ventilatory- and lactate-determined threshold points. The mean ±2 times the standard deviation of the difference was taken as the 95% range of agreement for the sets of measurements. Since the Bland-Altman plot analysis may bring proportional bias, linear regression analyses were also performed. Statistical significance was set at $p < 0.05$. Variables for which proportional bias was identified, were recalculated by determining the natural logarithm of these variables. This was again followed by a sample one T-test, paired-sample T-test and correlation analyses, the Bland and Altman plot method and a linear regression analysis.

3. Results

The descriptive statistics of the General Recovery and Hydration Status Questionnaire results revealed the following: Athletes on average slept for 6.74 ± 1.49 hours the night before the test and indicated that their quality of sleep was 3.05 ± 1.49 on average. The average for muscle soreness scores was revealed to be 0.81 ± 1.49 and the color of their urine to be 1.50 ± 0.89. Last-mentioned results indicate that the athletes were in a fully hydrated and recovered state before the start of the $\dot{V}O_{2max}$ test.

Table 1 provides descriptive statistics of the standard incremental maximal oxygen uptake test results.

Table 1. Descriptive statistics of the standard incremental maximal oxygen uptake test results of the endurance athletes

Variables	Mean ± SD	Minimum	Maximum
VT_1 ($\dot{V}O_2$ (L/min))	3.54 ± 0.53	2.70	4.85
VT_1 ($\dot{V}O_2$ (ml/min/kg)	50.9 ±7.08	38.94	61.24
VT_1 (%$\dot{V}O_{2max}$)	71.83 ± 9.42	47.37	92.12
VT_1 BL (mmol/L)	1.96 ± 0.48	1.10	2.70
VT_1 (W)	245 ± 51.73	170.00	349.00
VT_1 (W/kg)	3.52 ± 0.68	2.26	4.48
VT_1 (km/h)	13.00 ± 0.94	11.00	14.00
VT_1 (bpm)	161.86 ± 12.22	137.00	179.00
VT_1 HRV (msec)	386.43 ± 47.14	327.31	481.48
VT_1 (%HR_{max})	83.00 ± 5.8	70.20	90.77
RCP ($\dot{V}O_2$ (L/min))	4.83 ± 0.61	3.36	5.96
RCP ($\dot{V}O_2$ (ml/min/kg))	62.55 ± 10.61	49.57	93.71
RCP (%$\dot{V}O_{2max}$)	88.02 ± 11.53	65.39	117.63
RCP BLa (mmol/L)	4.62 ± 1.64	2.40	8.00
RCP (W)	321.97 ± 62.33	184.00	471.00
RCP (W/kg)	4.63 ± 0.8	2.45	5.61
RCP (km/h)	16.6 ± 0.97	15.00	18.00
RCP (bpm)	182.86 ± 7.96	170.00	198.00
RCP HRV (msec)	347.23 ± 33.77	294.96	401.57
RCP (%HR_{max})	93.79 ± 3.45	85.86	96.88
LT_2 (W)	322.2 ± 61.28	181.00	455.00
LT_2 (W/kg)	4.62 ± 0.75	2.41	5.40
LT_2 (km/h)	16.1 ± 0.88	15.00	17.00
LT_2 (bpm)	182.76 ± 7.22	173.00	198.00
LT_2 (%HRmax)	93.73 ± 2.66	88.67	100.00
BLa_{max} (mmol/L)	11.84 ± 3.98	5.30	18.20
HR_{max} (bpm)	195.1 ± 8.39	180.00	212.00
$\dot{V}O_{2max}$ (L/min)	4.97 ± 0.7	3.65	6.71
$\dot{V}O_{2max}$ (ml/kg/min)	71.16 ± 7.2	56.58	83.21
W_{max} (W)	383.09 ± 66.82	200.00	516.00
W_{max} (W/kg)	5.50 ± 0.85	2.66	6.41
$\dot{V}e/\dot{V}O_2max$ (L/min)	37.71 ± 3.55	30.70	44.61
$\dot{V}e/\dot{V}CO_2max$ (L/min)	35.20 ± 4.14	28.60	41.32
RER_{max}	1.08 ± 0.07	1.00	1.20
$\dot{V}e_{max}$ (L/min)	194.22 ± 28.95	132.60	246.96

VT_1 = First ventilatory threshold; RCP = Respiratory compensation point; HRV = Heart rate variability mean R-R intervals (msec); $\dot{V}O_{2max}$ = maximal oxygen uptake; Wmax = maximal power output; $\dot{V}e$ = minute ventilation; $\dot{V}O_2$ = oxygen uptake; BLamax = Maximal blood lactate concentration; HRmax = maximal heart rate; LT = Lactate threshold, BLa = Blood lactate; RER = Respiratory exchange ratio; $\dot{V}e/\dot{V}O_2$ = ventilatory equivalent for oxygen; $\dot{V}e/\dot{V}CO_2$ = ventilatory equivalent for carbon dioxide; W/kg = Watts per kilogram; km/h = kilometers per hour; bpm = beats per minute; L/min = Liters per minute; ml/min/kg = milliliters per minute per kilogram; mmol/L = millimole per liter.

Results from Table 1 indicate that athletes obtained VT_1 at an average power output of 245 W, RCP at a power output of 321.97 W, and $\dot{V}O_{2max}$ at 71.16 ml/kg/min. The LT_2 was obtained at 322.2 W, while the maximal BLa was observed to be 11.84 mmol/L. Results indicate that athletes reached $VT_1\dot{V}O_2$ at 50.9 ml/kg/min and $RCP\dot{V}O_2$ at 62.55 ml/kg/min. Tables 2, 3 and 4 present the descriptive statistics of the absolute power outputs at the VT_1, RCP, LT_2 and the HRV-established thresholds of the endurance athletes, respectively.

The results in Table 2 indicate that VT1DFAα1 (W) was obtained at an average absolute power output of 233.15 W, compared to VT_1RMSSD (W) which shows a value of 236.01 W. VT_1SD_1 (W) delivered a very similar value of 237.11 W compared to VT_1HF power (W), which obtained a much higher average value of 254.74 W.

Table 3 indicates that RCPDFAα1 (W) occurred at 335.87 W compared to RCPHF power (W) which occurred at 352.89 W.

Results from Table 4 show that LT_2DFAα1 (W) occurred at 335.87 W compared to LT_2HF power (W) which obtained a higher average value of 352.89 W.

The Shapiro-Wilk test for data normality revealed no significant p-values for the variables that were included in the study. Therefore, data are normally distributed.

The paired-sample T-test and correlation analyses delivered the results that are presented in Table 5. The researchers could not detect VT_1HF power in two participants while VT_1SD_1 and RCPHF power could not be detected in one participant. Therefore, the last-mentioned participants' threshold values were excluded from the paired-sample T-test and correlation analyses.

The results in Table 5 show that most comparisons between the absolute power outputs at the different ventilatory-, lactate- and HRV-established thresholds delivered significant differences. The comparisons between VT_1 (W) and VT_1DFAα1 (W), VT_1 (W) and VT_1RMSSD (W) as well as between VT_1 (W) and VT_1SD_1 (W) were the only variables that did not produce significant differences. The effect size categorizations of the last-mentioned differences all revealed small values. On the other hand, the correlations between all threshold values obtained significant large to near-perfect values.

Table 2. Descriptive statistics of the absolute power outputs at the first ventilatory and HRV-determined thresholds of the endurance athletes

Variables	VT1 (W)	VT_1DFAα1 (W)	VT_1RMSSD (W)	VT_1HF power (W)	VT_1SD_1 (W)
Threshold values	227.8 ±36.84	233.15 ±55.27	236.01 ±42.24	254.74 ±53.10	237.11 ±45.94

VT_1 = First ventilatory threshold; W = watts; $DFA_{\alpha1}$ = exponent alpha 1 of the non-linear detrended fluctuation analysis method; RMSSD = squared root of the mean squared differences between successive R-R intervals; HF power = High-frequency power; SD1 = Standard descriptor 1.

Table 3. Descriptive statistics of the absolute power outputs at the RCP and HRV-determined thresholds of the endurance athletes

Variables	RCP (W)	RCP DFAα1 (W)	RCP HF power (W)
Threshold values	302.99 ±51.62	335.87 ±85.12	352.89 ±70.64

RCP = Respiratory compensation point; W = watts; DFAα1 = exponent alpha 1 of the non-linear detrended fluctuation analysis method; HF power = High-frequency power.

Table 4. Descriptive statistics of the absolute power outputs at the lactate and HRV-determined thresholds of the endurance athletes

Variables	LT_2 (W)	LT_2 DFAα1 (W)	LT_2 HF power (W)
Threshold values	299.66 ±55.64	335.87 ±85.12	352.89 ±70.64

LT_2 = Second Lactate threshold point; W = watts; $DFA_{\alpha1}$ = exponent alpha 1 of the non-linear detrended fluctuation analysis method; power HF = High-frequency power.

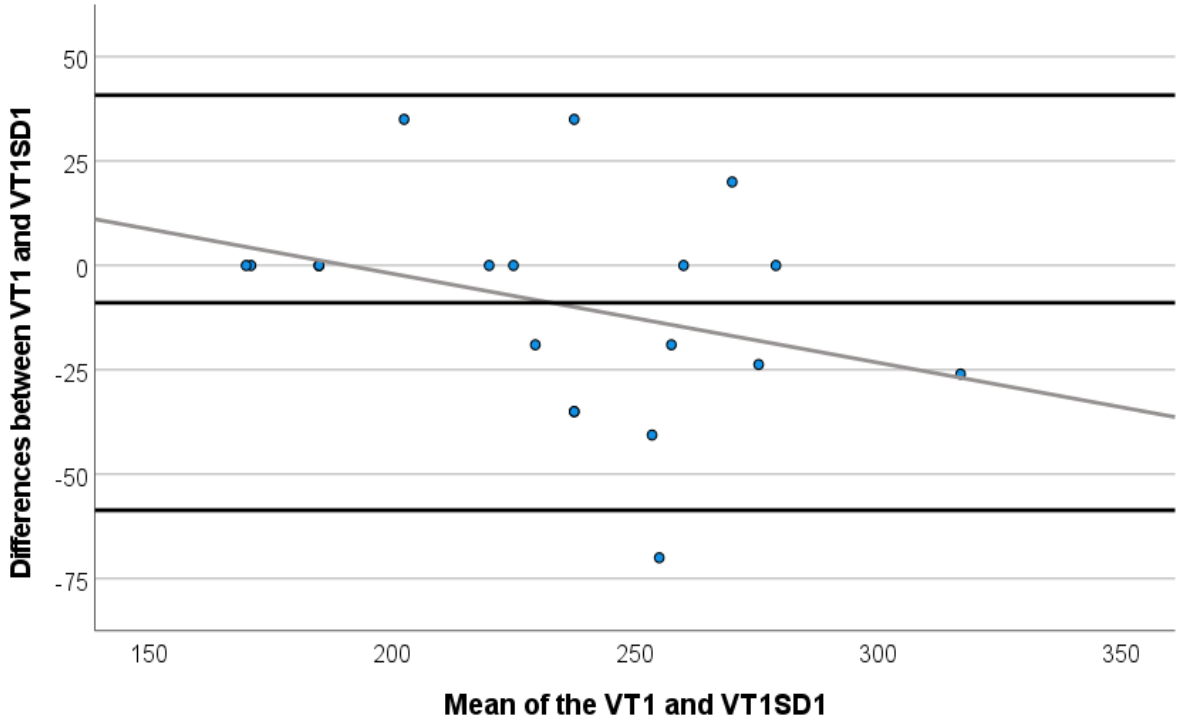
Table 5. The paired-sample T-test and correlation analyses results of the comparison between the absolute power outputs at the different ventilatory-, lactate- and HRV-determined thresholds of the endurance athletes

Threshold comparisons	*T*	95% CI	*p*-Value	Cohen's *d*	*R*
VT_1 (W) – VT_1DFAα1 (W)	-0.61	-23.64 – 12.95	0.55	-0.13^{S}	0.69^{L}*
VT_1 (W) – VT_1RMSSD (W)	-1.43	-20.17 – 3.75	0.17	-0.31^{S}	0.79^{VL}*
VT_1 (W) – VT_1HF power (W)	-3.98	-47.87 – -14.78	0.001*	-0.91^{L}	0.77^{VL}*
VT_1 (W) – VT_1SD_1 (W)	-1.57	-20.79 – 2.95	0.13	-0.35^{S}	0.83^{VL}*
RCP (W) – RCPDFAα1 (W)	-3.32	-53.56 – -12.20	0.003*	-0.72^{L}	0.89^{VL}*
RCP (W) – RCPHF power (W)	-7.18	-61.66 – -33.83	0.00*	-1.61^{L}	0.93^{NP}*
LT_2 (W) – RCPDFAα1 (W)	-3.87	-55.72 – -16.70	0.001*	-0.84^{L}	0.90^{VL}*
LT_2 (W) – RCPHF power (W)	-8.60	-63.71 – -38.78	0.00*	-1.92^{L}	0.94^{NP}*

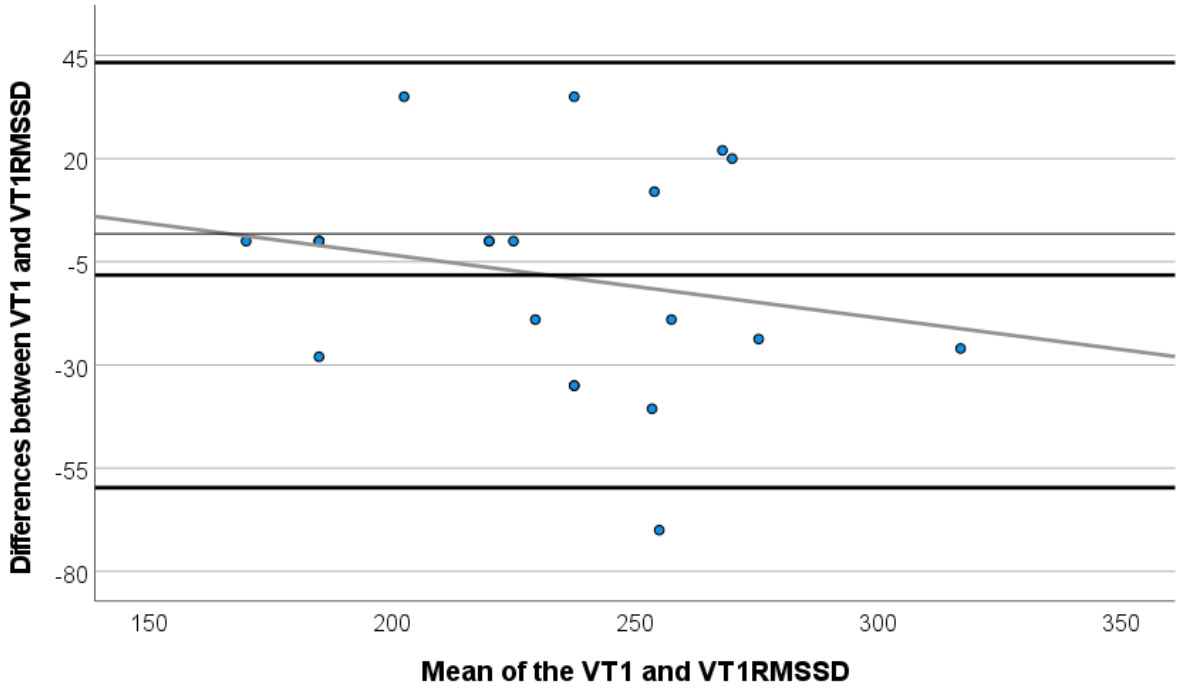
95% CI = 95% Confidence intervals; *p < 0.05; Cohen's *d*: S = Small (~0.2); M = Medium (~0.5), L = Large (~0.8); Correlations coefficient categorization: T = Trivial (<0.1); S = Small (<0.3); M = Medium (<0.5); L = Large (<0.7); VL = Very large (<0.9), NP = Near perfect (<1.0).

The one sample T-test results showed that the means between the following variables obtained significant differences and that these variables could therefore not be included in the Bland-Altman plots: VT_1 (W) and VT_1HF power (W), RCP (W) and RCPDFAα1 (W), RCP (W) and RCPHF power (W), LT_2 (W) and LT_2DFAα1 (W) as well as LT_2 (W) and LT_2HF power (W).

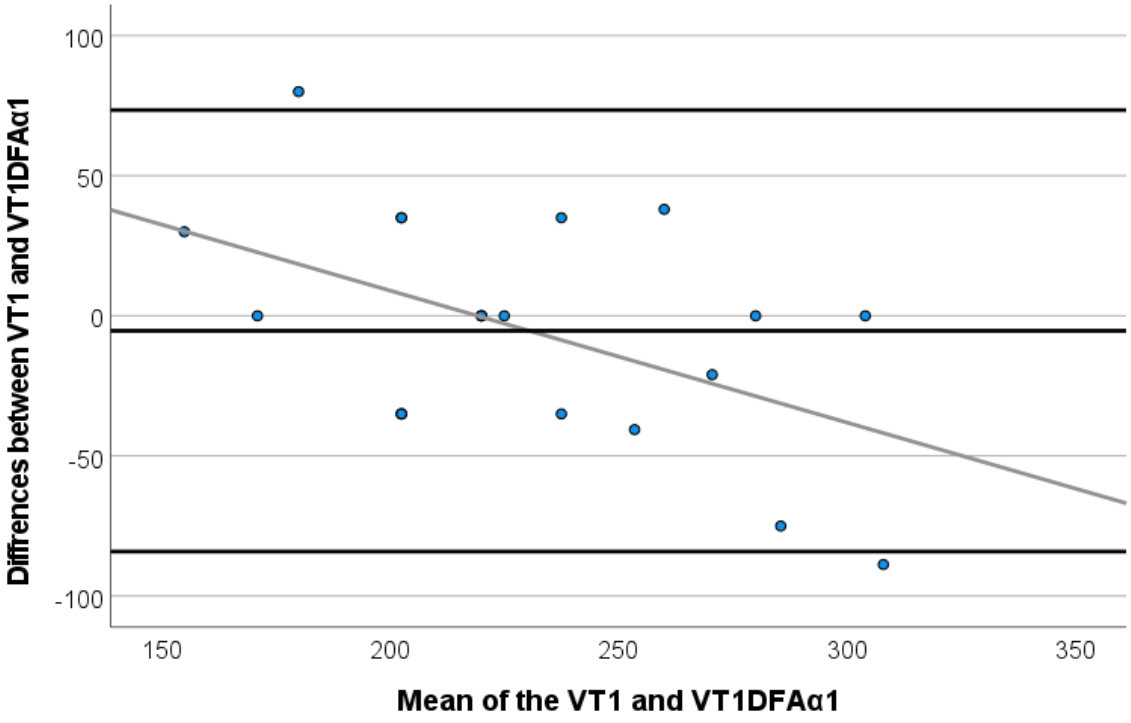
The rest of the variables were included in the Bland-Altman plots that are presented in Figure 1.



A



B



C

Figure 1. A. The Bland-Altman plot of the agreement between the absolute power outputs at VT_1 and VT_1HF power; B. The Bland-Altman plot of the agreement between the absolute power outputs at VT_1 and VT_1RMSSD; C. The Bland-Altman plot of the agreement between the absolute power outputs at VT_1 and VT_1DFAα1

The above-presented Bland-Altman plots reveal negative regression lines for the agreements between the absolute power outputs at VT_1 and the different HRV-established VT_1 absolute power outputs. Furthermore, one participant's values fall outside the 95% range of agreement for the VT1 and $VT1SD_1$ as well as for the VT_1 and VT_1RMSSD comparisons. However, for the comparison between VT_1 and VT_1DFAα1 two participants' values fall outside the 95% range of agreement.

The linear regressions of the differences between the means of the absolute power outputs at VT_1 and VT_1SD_1, VT_1 and VT_1RMSSD as well as between VT_1 and VT_1DFAα1, obtained non-significant p-values (p = 0.146 and p = 0.344) for the first two mentioned groups of variables and a significant p-value of 0.02 for the last-mentioned group of variables. Due to the presence of proportional bias, the natural logarithms of the difference between, and means of VT_1 and VT_1DFAα1 were calculated. This was followed by the calculation of the paired-sample T-test and correlation analyses which delivered a non-significant T-value of -0.21 (p = 0.84), a small effect size of -0.05 and a significant, large correlation coefficient of 0.68 (p = 0.001). Subsequently, a sample one T-test which produced a non-significant p-value of 0.84, was performed. Next, a Bland-Altman plot that is presented in Figure 2 was compiled.

Figure 2 again reveals a negative regression line for the agreement between the absolute power outputs at $logVT_1$ and $logVT_1$DFAα1. A further analysis of the plot also shows that the values of one participant fall outside the 95% range of agreement for the last mentioned set of measurements. The linear regression analysis of the differences between, and means of the absolute power outputs at $logVT_1$ and $logVT_1$DFAα1 obtained a significant p-value of 0.03. This means that proportional bias remained despite the recalculations of the difference between, and means of VT_1 and VT_1DFAα1 by applying the natural logarithms.

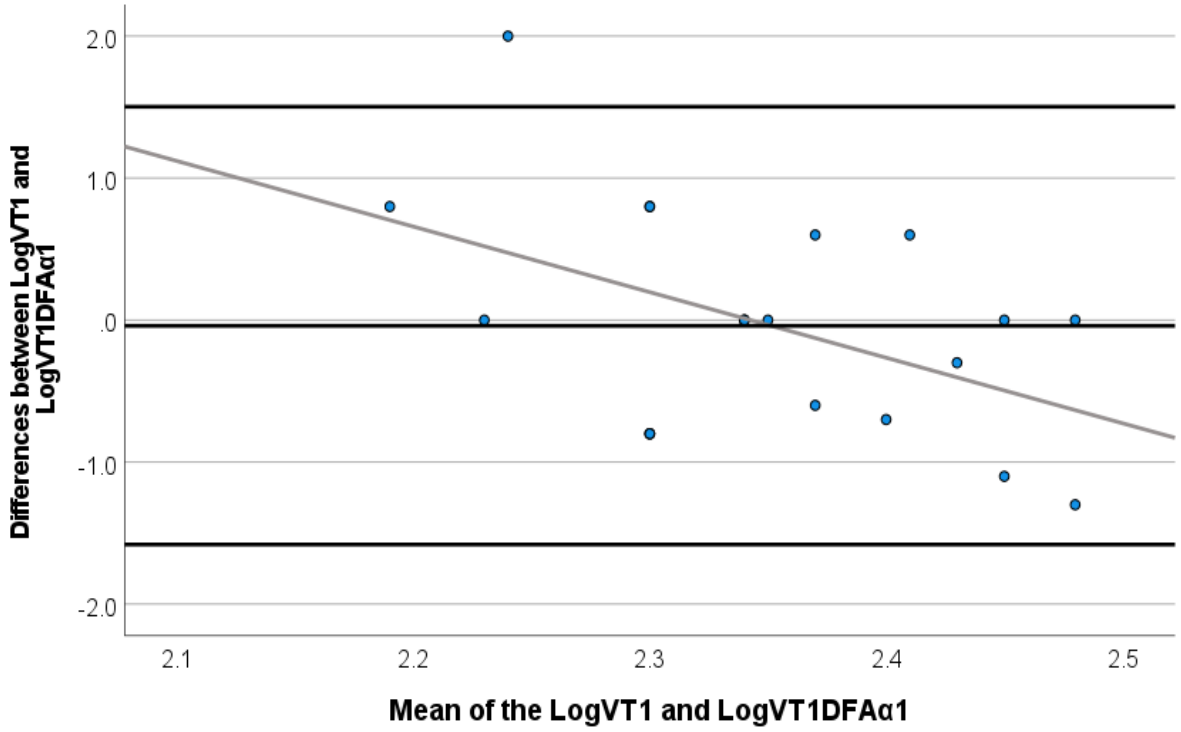


Figure 2. The Bland-Altman plot of the agreement between the absolute power outputs at logVT_1 and logVT_1DFAα1

4. Discussion

The main purpose of the study was to determine whether the HRV-established thresholds can be used to accurately estimate the VT_1, RCP and LT_2 of endurance athletes. To our knowledge, this is the first study to investigate the accuracy of HRV for the estimation of VT_1, RCP and LT_2 in endurance-trained cyclists as well as middle- and long-distance athletes. The main findings of the study are that absolute power outputs at VT_1SD_1 and VT_1RMSSD, as revealed by the Bland-Altman plots, showed high levels of agreement with the absolute power outputs at VT_1. Therefore, SD_1 and RMSSD may serve as easier, non-invasive, and cost-effective measures to determine the absolute power outputs at the VT_1 of endurance athletes. Although not athletes, a study on chronic heart failure patients also observed a good agreement between the power (W), HR (bpm) and $\dot{V}O_2$ (ml/min) at VT_1RMSSD and VT_1 [42]. The paired-sample correlation analysis in this study also revealed very large significant correlations between the absolute power outputs at VT_1RMSSD, VT_1SD_1 and VT_1. This is similar to the reported correlation coefficient between the speeds at VT_1SD_1 and VT_1 ($r = 0.84$, $p < 0.05$) for older healthy men (age: >40 years) [43]. Then again, lower correlations were reported between the $\dot{V}O_2$ (ml/kg/min) at VT_1SD_1 ($r = 0.38$, $p \leq 0.05$), VT_1RMSSD ($r = 0.47$, $p \leq 0.05$) and VT_1 of healthy men [25]. These correlations can be explained by the VT_1-related shift from parasympathetic to sympathetic predominance which is indicated by the changes in RMSSD and SD_1, and which commonly occurs at 50-65% of the $\dot{V}O_{2max}$ [11,25]. SD_1 is also influenced by the respiration-driven aperiodic fluctuations in heart rate (respiratory sinus arrhythmia) via the vagus nerve [44,45]. RMSSD is regarded to be identical to SD_1 as both reflect vagally mediated changes in HRV [46,47]. According to McMorris and Hale [47] exercise at an aerobic intensity (VT_1) may cause an increase in plasma catecholamines which serves as a stimulant for the vagal pathway. This would explain the sudden changes in SD_1 and RMSSD at the VT_1. Despite this contention, contrasting results were previously found by Blasco-Lafarga *et al.* [49], who did not observe any significant associations between the exercise intensities noted as a percentage of $\dot{V}O_{2max}$ at the VT_1SD_1, VT1RMSSD, and VT_1 for elite young male cyclists.

Another HRV-related measure that was used in the past to identify ventilatory and lactate thresholds is DFAα1. Previously researchers observed a decrease in DFAα1 with an increase in exercise intensity, leading to the notion that DFAα1 may serve as another HRV-related indicator of physiological thresholds [49-51]. DFAα1 decreases due to the significant deactivation of the PNS outflow which occurs at the VT_1 point [5,51]. At the onset of exercise, HR increases due to PNS withdrawal, with a continuation of exercise and an increase in exercise intensity that leads to a further increase in HR due to SNS activation [34,49,52]. In this study, the significant paired-sample correlation coefficient between the absolute power outputs at VT_1 and DFAα1 seems to verify the last-mentioned deduction and agrees with the findings of Rogers *et al.* [5] who also found direct associations between DFAα1, VT_1 HR and VT_1 $\dot{V}O_2$.

A further analysis of our study results through a Bland-Altman plot also revealed a significant agreement between the absolute power outputs at VT_1 and DFAα1. Despite this finding, and recalculations of the differences between, and means of VT_1 and VT_1DFAα1 by applying the natural logarithms, proportional bias remained. Proportional bias can be described as increases or decreases in the differences between values in proportion to the average values [53]. Therefore, we had to conclude that proportional bias caused the significant agreement between the absolute power outputs at VT_1 and DFAα1. One of the possible reasons for the originally identified proportional bias is that two of the participants' values fell outside the 95% range of agreement for the comparison between the absolute power outputs at VT_1 and VT_1DFAα1. Notwithstanding, this deduction the presence of proportional bias casts a shadow of doubt on the validity and accuracy of using the DFAα1 technique to determine VT_1.

Previously, researchers also explored the use of HF power as a HRV-related measure to determine VT_1. However, similar to our findings, no significant correlations were reported between the HRs and speeds at *f*HF and VT_1 of elite ski-mountaineers [1]. In contrast, others observed significant strong correlations between the absolute and relative $\dot{V}O_{2max}$ values of male cyclists and triathletes at the HF-determined threshold point and at VT_1 (r = 0.97; r = 0.92, p < 0.05) [23]. The same group of researchers also reported a significant strong correlation between the running speeds of professional soccer players at the HF-determined threshold point and VT_1 (r = 0.97, p < 0.001) [24].

Interestingly, results from our study revealed that none of the HRV-related thresholds was able to accurately estimate the power outputs at RCP and LT_2. In contrast to our findings, researchers reported significant correlations between the *f*HF-determined threshold of Spanish professional basketball players and VT_2 $\dot{V}O_2$ (ml/kg/min) (r = 0.91, p < 0.05), VT_2 HR (bpm) (r = 0.90, p < 0.005), and VT_2 speed (km/h) (r = 0.93, p < 0.005) [12]. Similarly, the HR and speed at the *f*HF-determined threshold of elite ski-mountaineers significantly correlated with RCP (r^2 = 0.92, p < 0.001) [1]. Additionally, the absolute (r = 0.98, p < 0.001) and relative power outputs (r = 0.93, p < 0.001) at the second HF threshold of male cyclists and triathletes obtained significant correlations with the power output values at RCP [23]. Concerning LT_2, researchers revealed a significant correlation between the HF power-determined threshold of high-level swimmers and LT_2 (r = 0.93, p < 0.05) [16]. In addition, Mateo-March *et al.* [22] found that the power output/HR DFAα1-threshold determined value significantly correlated with LT_2 (r = 0.93, p < 0.001) in a group of elite endurance cyclists.

Differences in results between our and previous studies can be attributed to the differences in participants, with our participants being endurance-trained cyclists and athletes, while others made use of young healthy men (age: between 18 and 25 years) [25], older healthy men (age: > 40 years) [43], professional team-sport participants [11] and young elite road cyclists (age: 15.43±0.51 years) [49]. Differences in exercise adaptations, maturity levels and age between participants will give rise to distinct ANS profiles and responses, which may directly influence the relationships between gas analysis- and HRV-determined thresholds. Furthermore, researchers made use of varied testing protocols to determine the thresholds. For example, some preferred the walking shuttle test [43], while others, which included us, used a graded running or cycling exercise test until voluntary exhaustion [11,25,49]. The differences in modes and intensities between testing protocols will trigger varied ANS responses and threshold values, which may cause variations in relationships between different types of thresholds.

5. Conclusions

In conclusion, study results verified the accuracy and validity of using the VT_1SD_1 and VT_1RMSSD to determine the absolute power outputs of endurance-trained cyclists and middle- and long-distance athletes at the VT_1. Furthermore, the agreement between the absolute power outputs at DFAα1 and VT_1 showed proportional bias, which suggests that DFAα1 does not serve as an accurate estimate of endurance-trained athletes' absolute power outputs at VT_1. Surprisingly, and in contrast to what previous researchers suggest, none of the HRV-related techniques could accurately estimate the absolute power outputs at RCP or LT_2. Consequently, we can not verify the use of DFAα1 and HF power to accurately estimate the absolute power outputs of endurance-trained athletes at the RCP and LT_2. Given the last-mentioned results, the hypothesis that the HRV-established thresholds of endurance athletes as determined through changes in DFAα1, SD1, RMSSD, and HF power can be used to accurately estimate VT_1, RCP and LT_2, is only partially accepted. Differences in the type of participants and testing protocols being used between our and previous studies may explain some of the discrepancies between results.

Notwithstanding, the unexpected results, coaches, sport scientists and sport-related professionals are encouraged to use changes in SD_1 and RMSSD to determine the precise VT_1-related training program workloads of athletes for the continuous monitoring of training adaptations in places where expensive and sophisticated testing equipment and laboratories are not available. Even though our results bring valuable information to the fore, it should be interpreted with caution due to the following limitations: The present study only focuses on a selected group of endurance-trained athletes, which may have caused outliers to harm relationships between variables. Consequently, it would be beneficial for future researchers to also include bigger sample sizes of a higher variety of endurance-trained athletes. Furthermore, a

repeated-measures study which aims to verify the different threshold points of athletes by subjecting them to several $\dot{V}O_{2max}$ tests can be recommended.

Acknowledgements

The authors would like to thank the National Lottery Board of South Africa, which provided funding for this work. Deep gratitude is expressed to people who contributed toward the successful completion of this study, most especially Jean Verster (coach of the middle- and long-distance athletics team), and JP van Zyl (manager of the African Continental Development Cycling team).

Declaration of Interest

There are no conflicts of interest.

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