Research Article

Determination of Lactate Thresholds in Maximal Running Test by Heart Rate Variability Data Set

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Abstract

Background: Heart rate variability (HRV) data set has been used to identify HRV thresholds during exercise. However, the relationship between HRV thresholds and the first (LT1) and second lactate threshold (LT2), has been poorly investigated during maximal running test. In this regard, HRV may act as a useful, less expensive and time consuming method to estimate physiological thresholds.

Objectives: The aim of the present study was to verify the validity and reproducibility of the heart rate variability (HRV) method to determine first and second lactate thresholds during a maximal running test.

Methods: Nineteen male runners (30.4 ± 4.1 years; body mass of 74.3 ± 8.5 kg; height of 176 ± 6.4 cm and body fat of 13.8 ± 4.6 %) performed two progressive maximal tests on a treadmill, with initial speed at 5 km.h^{-1} and 1 km.h^{-1} increments every 3 minutes, until exhaustion. Measures of HRV and blood lactate concentrations were obtained during the tests and physiological thresholds were identified as lactate thresholds by using fixed concentrations (2.0 and 3.5 mmol.L^{-1}) and Dmax method, as well as HRV thresholds.

Results: There were no differences between the first physiological thresholds identified as lactate (2.0 mmol.L^{-1} = 11.9 ± 2.9 km.h^{-1} and Dmax = 12.3 ± 1.5 km.h^{-1}) or HRV threshold (11.6 ± 1.6 km.h-1). Accordingly, there were no differences between the second physiological thresholds identified as lactate threshold (3.5 mmol.L^{-1} = 14.2 ± 2.6 km.h^{-1}) or HRV threshold (14.2 ± 2.0 km.h^{-1}). Furthermore, HRV thresholds showed good agreement between test and retest.

Conclusions: Physiological thresholds identified by using HVR data set provided reliable and practical estimation of LT$_1$ and LT$_2$ during a maximal running test.

Keywords: Heart Rate Variability, Blood Lactate Concentrations, Autonomic Modulation

1. Background

The relationship between parasympathetic (PNS) and sympathetic nervous system (SNS) activation can be assessed with heart rate variability (HRV) analysis (1, 2). Increasing the exercise intensity may thus reflect a reduction in HRV during the initial stages of a maximal incremental exercise test, thereafter a significant PNS activation withdrawal occurs between 50% - 60% of the maximal oxygen uptake (VO$_{2MAX}$) (3). That intensity has been related with the first physiological threshold (i.e. first lactate or ventilatory threshold - LT$_1$ and VT$_1$, respectively) (4). In contrast, there is a gradual increment in SNS activation from intensities corresponding to LT1 and VT$_1$, with remarkable increase at exercise intensities related to the second physiological threshold (i.e. second lactate and ventilatory threshold - LT$_2$ and VT$_2$, respectively) (3, 5). As a result, studies have suggested that HRV analysis may be a po-

tential and useful tool to estimate the exercise intensity of the first and second physiological thresholds (4, 6-8).

Using a cycling exercise mode, Cottin et al. (7) found that nonlinear increases in HRV data, assessed by high frequency (HF) and peak frequency spectrum (fHF) of the beat-to-beat RR intervals, identified VT$_1$ and VT$_2$. They also showed that instantaneous HF multiplied by fHF (HF.fHF) provided a reliable and accurate index to identify these thresholds. In a subsequent study, these authors expanded these findings to running exercise mode. Using an exhaustive incremental running test performed on a 200 m track, Cottin et al. (8) showed that VT$_1$ and VT$_2$ could be assessed through a simple HRV monitor. Recently, these results were further confirmed in obese individuals (9), corroborating the usefulness of the HRV method to identify the first and second physiological threshold in different individuals, during different exercise modes.

However, despite the support to use HRV data set when determining VT_1 and VT_2 (7-9), some aspects should be addressed. No study has currently verified the agreement between breakpoints identified through HRV and LT_1 and LT_2, as no lactate measures were included in those previous studies (7, 8). In this regard, it should be highlighted that lactate thresholds (10-13) is considered as a gold standard method when predicting running aerobic performance. The agreement between ventilatory and lactate thresholds is still an ongoing debate, as these indices are used to identify identical exercise intensities. Since work by Gaesser and Poole (14) nearly thirty years ago, studies have shown different results when comparing VT_1 and VT_2 to LT_1 and LT_2, respectively (15-17).

On the other hand, Karapetian et al. (4) have suggested the identification of a HRV threshold by using time domain indexes (standard deviation - SD and mean successive difference – MSD), which may match lactate and ventilatory thresholds during maximal incremental cycling test. Furthermore, it is important to point out that those studies have reported a nonlinear pattern in HRV data during maximal incremental exercise tests, thus making possible the identification of a HRV threshold when visually inspecting abrupt breakpoints in HRV responses during maximal incremental exercises. Consequently, it has been suggested that the HRV threshold, regardless of the method used for its determination, may be used to estimate the first and second physiological thresholds with different methodologies of HRV data analysis such as frequency domain (7, 8), time domain (4) or Poincare plot (18). Unfortunately, however, an important aspect that remains to be confirmed is the possibility of estimating LT_1 and LT_2 by using HRV data set in incremental running test.

The use of HRV data set for estimation of these traditional thresholds may be of interest, as this method is less expensive and time consuming, and requires uncomplicated and accessible HR monitors. Traditional VT_1 and VT_2 determination requires sophisticated and expensive metabolic gaseous exchange analyzers, while the LT_1 and LT_2 require invasive procedures with multiple blood sample collections, making these methods impractical for clinical assessment and daily endurance training sessions. Therefore, the study of alternative HRV methods for estimation of the first and second physiological threshold has practical relevance.

Reliability of the HRV thresholds method has been provided during maximal incremental cycling tests (18), but no study has been designed yet, to verify the HRV thresholds' validity and reproducibility when determining LT_1 and LT_2 in maximal incremental running test. Thus, the purpose of this study was to determine if HRV thresholds could identify the lactate thresholds' intensity during a maximal, incremental running test. We expected that HRV thresholds may elicit reliability and reproducibility when identifying lactate thresholds in treadmill running test.

2. Objectives

Therefore, the first aim of this study was to verify if the HRV method would determine valid HRV breakpoints during running test and if these corresponds to the first and second physiological threshold such as LT_1 and LT_2. The second aim of this study was to verify the reproducibility of the HRV method in running test.

3. Methods

3.1. Participants

Nineteen recreational long-distance male runners (30.4 ± 4.1 years; body mass of 74.3 ± 8.5 kg; height of 176 ± 6.4 cm and body fat of 13.8 ± 4.6 %) volunteered to participate in this study. All participants were healthy, non-smokers, with no cardiovascular or neuromuscular diseases. They were informed about risks and benefits before participating in the study, thereafter they provided their written informed consent. The study protocol was approved by the institutional ethics committee of the University of São Paulo, and conducted according to international standards (19).

3.2. Study Design

The experimental design consisted of two maximal incremental running tests interspersed by a washout period of 3 - 7 days. The tests were performed at the same time of the day and in standard laboratory conditions (humidity of ≈ 50% and temperature of ≈ 22°C). Participants were asked to avoid intense exercises, alcohol and caffeine beverages 24 hours before each test, and to consume a light meal 3 hours before the tests. Before starting the experimental procedures in each test session day, we asked participants about the accomplishment of these recommendations so that only participants who had followed them were evaluated.

3.4. Maximal Incremental Running Test Protocol

Participants wore a cardio belt for beat-to-beat heart rate (HR) measures (s810 Polar®, Kempele, Finland). Thereafter, the participants rested for 20 min (10 min supine + 10 min sitting) for baseline measures of HR and lactate concentrations. Lactate concentrations [La] were determined from a 25 μL blood sample, drawn from the tip of the forefinger (YSI 1500, Yellow Springs, OH, USA). Then the participants moved to the treadmill (CEFISE TK35, Nova Odessa,

Brazil) and warmed up for 3 min at 5 km.h⁻¹ and 1% gradient. The test started at 5 km.h⁻¹, with 1 km.h⁻¹ increases every 3 min, until exhaustion [10]. The HR data set were recorded continuously throughout the tests, while 25 μL of blood samples were collected during the last 30 s of every stage, while the participant was running.

3.5. Determination of Physiological Thresholds

LT_1 and LT_2: The speed corresponding to a 2 mmol.L⁻¹ blood lactate concentration was used to determine LT_1 [20], while the speed corresponding to 3.5 mmol.L⁻¹ identified the LT_2 [10].

D_{max}: The lactate threshold was further determined according to the D_{max} method (LT_{Dmax}) suggested by Cheng et al. [11], thereby providing individualized lactate threshold values. Briefly, LT_{Dmax} reflects the longest perpendicular distance between [La] values predicted by a 3° order polynomial function over actual [La] values and values derived from a linear regression calculated with the outermost lactate values (i.e. the first and last values of that curve).

HRV thresholds: Beat-to-beat RR interval data were analyzed for aberrant values. A RR interval value was interpreted as a premature beat if it deviated from the previous interval by more than 30% [1, 4]. The Poincare plot analysis was performed on RR interval time series to calculate the standard deviation of the instantaneous (SD1) and continuous long-term RR intervals (SD2) [3]. Poincare plot is a nonlinear measure of temporal correlations within time series of inter-beat intervals in which an RR interval is plotted against its first predecessor. The SD1 has been shown to correlate strongly with vagal tone (PNS) and SD2 has been shown to correlate with the PNS and SNS. Thereafter, raw RR intervals recorded during the last 60 s of each stage of exercise were normalized by the mean RR interval and multiplied by 1000 ($SD1_n$ and $SD2_n$) [1]. Thus, in accordance with behavior of these variables in previous studies [3, 18], $SD1_n$ and $SD2_n$ were visually used by three evaluators to determine two HRV thresholds. The first ($HRVT_1$) and second ($HRVT_2$) HRV thresholds were identified at the first breakpoint in $SD1_n$ and $SD2_n$ curves, respectively. Figure 1 illustrates typical $SD1_n$ and $SD2_n$ curves and the HRV thresholds.

All physiological thresholds, LT_1, LT_2, LT_{Dmax}, $HRVT_1$ and $HRVT_2$ were expressed in absolute speed (km.h⁻¹), HR (bpm), milliseconds beat-to-beat RR interval (ms) and blood lactate concentration (mmol.L⁻¹). Additionally, thresholds were expressed relatively (%) to maximal values.

3.6. Statistics

Values were expressed as mean and standard deviation ($\pm$ SD). After ensuring Gaussian data distribution (normality and homoscedasticity), a repeated-measure design
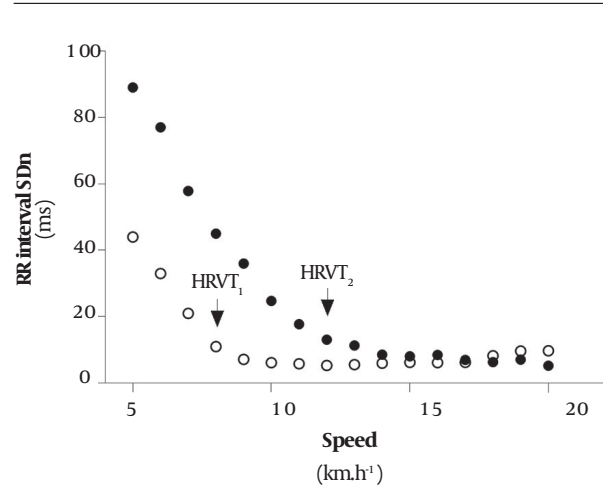

Figure 1. The First and Second HRV Thresholds ($HRVT_1$ and $HRVT_2$) Visually Determined by $SD1_n$ and $SD2_n$, Respectively

ANOVA with Bonferroni's post-hoc test were used to verify possible differences among different thresholds identified in maximal incremental running test. Typical error of measurement [21] was used to verify the test-retest HRV breakpoints' reproducibility, as well as to compare them with lactate thresholds. The Bland and Altman plot further provided a visual analysis of the data set bias [22], in addition to the reliability analysis with intra-class coefficient correlation (ICC). All analyzes were performed with SPSS (19.0), and with a spreadsheet designed to specifically calculate the reliability (available on www.sportsci.org). Results were interpreted as significant when P was < 0.05.

4. Results

Baseline HRV values (423.1 $\pm$ 28 ms vs. 994.6 $\pm$ 139.3 ms; P < 0.05), but not baseline lactate values (1.34 $\pm$ 0.5 mmol.L⁻¹ vs. 1.18 $\pm$ 0.3 mmol.L⁻¹. P > 0.05), were significantly different between test and retest. With regard to values recorded at the exhaustion in maximal incremental running tests, no significant difference was observed between test and retest (P > 0.05) in speed (16.5 $\pm$ 1.8 km.h⁻¹ vs. 16.6 $\pm$ 1.7 km.h⁻¹), HR (192 $\pm$ 6 bpm vs. 191 $\pm$ 6 bpm) and blood lactate concentration (9.2 $\pm$ 1.9 mmol.L⁻¹ vs. 9.2 $\pm$ 1.9 mmol.L⁻¹).

4.1. Identification of Physiological Thresholds

Overall results were that neither the first nor the second. Physiological threshold was different when determined by lactate and HRV thresholds expressed in absolute as well as in relative values. For example, while LT_1 matched HRV_1, LT_2 matched HRV_2. Interestingly, LTDmax

matched the first (LT_1 and HRV_1), but not the second physiological threshold (LT_2 and HRV_2.). Table 1 shows in full results of the first and second physiological thresholds identified during the maximal incremental running test.

Pearson correlation coefficient analysis in thresholds expressed as speed revealed significant correlation ($P < 0.01$) between $HRVT_1$ and LT_1 ($r = 0.71$), $HRVT_1$ and LT_{Dmax} ($r = 0.68$), $LT1$ and LT_{Dmax} ($r = 0.75$), $HRVT_2$ and LT_{Dmax} ($r = 0.65$). Additionally, significant correlation was observed between $HRVT_2$ and LT_2 ($r = 0.86$).

Figure 2 shows the magnitude of differences and bias between HRV and lactate thresholds. A good agreement was observed between $HRVT_1$ and LT_1 (-0.26 ± 2.07 km.h^{-1}), as well as between $HRVT_2$ and LT_2 (0.00 ± 1.29 km.h^{-1}), given the small bias reported.

4.2. Reproducibility of the HRV Thresholds

Figure 3 presents the Bland-Altman plot with bias analysis for the $HRVT_1$ and $HRVT_2$ reliability. There was a reasonable random distribution in test-retest residuals for $HRVT_1$ and $HRVT_2$, thus suggesting a homoscedastic distribution as a function of the speed. Results further suggested a consistent reproducibility for thresholds determined through the HRV data set, given the typical error of measurement reported at $\approx 6\%$ (≈ 0.45 km.h^{-1}) and the ICC showed high correlations ($P < 0.001$) (Table 2).

5. Discussion

The main findings of the present study were that the HRV data set provided fair estimation of LT_1 and LT_2 through $HRVT_1$ and $HRVT_2$, respectively, during maximal running test. Furthermore, HRV thresholds elicited good reproducibility with low bias during incremental running test.

5.1. HRV Data Set and the Identification of Lactate Thresholds

The results of this study showed that HRVT1, identified by analysis on time domain of the HRV data matched LT_1 and LT_{Dmax}, either expressed in absolute or relative values of speed and HR. The significant vagal withdrawal during incremental exercises has been well documented in the literature using time domain HRV analysis (4, 18, 23). In fact, $HRVT_1$ showed relative values of speed around 70.6%, close to values (74.6%) reported by Candido et al. (18) when using a cycle ergometer and similar methods for HRV analysis. In the present study, although the correlation between either $HRVT_1$ and LT1 or $HRVT_1$ and LT_{Dmax} (expressed as km.h^{-1}) has elicited significant coefficient values ($r = 0.71$ and $r = 0.68$, respectively), this was lower than the correlation reported by a previous study by Karapetian et al. ($r = 0.82$)
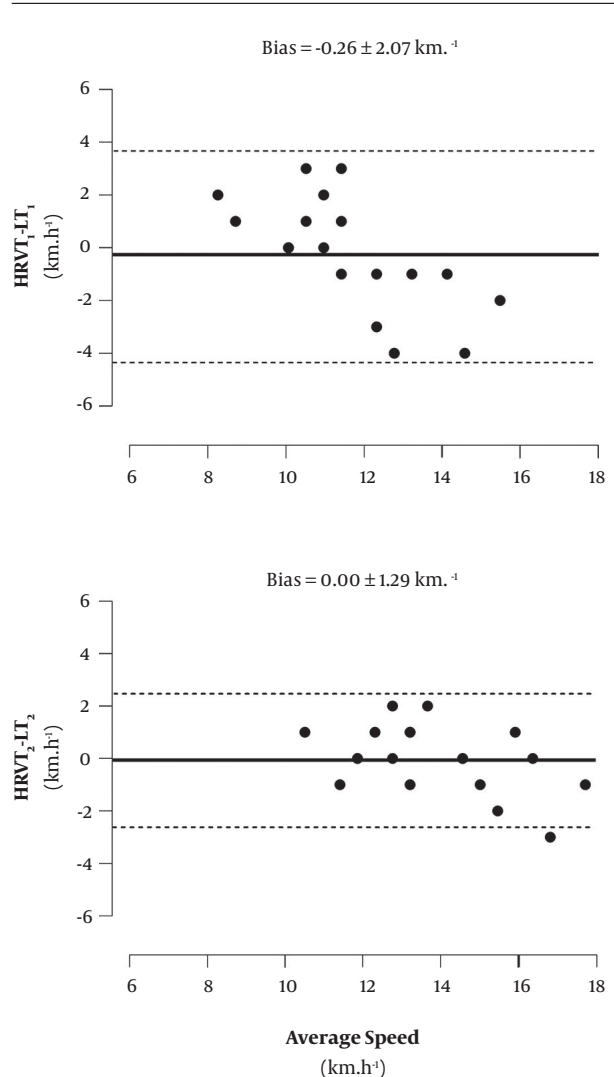

Figure 2. Bland and Altman Plot for the Agreement Between HRVT and LT_1 (A) and LT_2 (B) Methods.

(4) which used similar visual analysis to identify the vagal withdrawal, that is the point at which the HRV showed no further decrease. However, it is important to note that in contrast to the present study which used a treadmill running incremental test, Karapetian et al. (4) used a cycle ergometer, thus limiting deeper comparisons as the values were expressed as power output (watts) instead of speed (km.h^{-1}).

Furthermore, others have shown that HRV is a useful method to identify the second physiological breakpoint (6-8). Accordingly, we showed that $HRVT_2$ was similar to the second physiological threshold identified through the traditional lactate method (LT_2). The variable SD2 was used because it represents the parasympathetic and sym-

Table 1. Physiological Thresholds Expressed in Absolute Speed, Milliseconds Beat-to-Beat RR Interval, Heart Rate, and Blood Lactate Concentrations During Maximal Running Test Among Recreational Long-Distance Male Runners[a]

	Speed		RR		HR		Lactate	
	(km.h^{-1})	(%)	(ms)	(%)	(bpm)	(%)	(mmol.L^{-1})	(%)
LT$_1$	11.9 ± 3[b,c]	71.4 ± 11[b,d]	392.8 ± 48[e]	92.9 ± 11[e]	155 ± 17[b,d]	80.8 ± 8[b,d]	1.97 ± 0.33[b,d]	30.3 ± 10.6[b,d]
LT$_2$	14.2 ± 3	85.7 ± 7	348.4 ± 25	82.5 ± 5	173 ± 12	90.2 ± 5	3.20 ± 0.51	48.9 ± 15.4
LT$_{Dmax}$	12.3 ± 1	74.5 ± 4[b,c]	376.5 ± 27	89 ± 4	161 ± 12[b,c]	83.5 ± 5[b,c]	2.25 ± 0.67[b,d]	32.6 ± 7.2[b,d]
HRVT$_1$	11.6 ± 2[b,d]	70.6 ± 6[b,d]	384.2 ± 38[e]	90.8 ± 4e	155 ± 15[b,d]	80.7 ± 7[b,d]	2.02 ± 0.68[b,d]	29.3 ± 7.8[b,d]
HRVT$_2$	14.2 ± 2	86.1 ± 6	349.6 ± 21	82.8 ± 4	173 ± 9	90.4 ± 5[e,d]	3.42 ± 1.20	50.9 ± 18.3

[a]Values are expressed as mean ± SD.
[b]Significant differences in relation to LT$_2$ and HRVT$_2$ methods.
[c]P < 0.05.
[d]P < 0.01.
[e]Significant differences in relation to LTDmax methods. (Percentage values (%) are relative to baseline (RR) or maximal values (speed, HR and lactate).

Table 2. Reliability Analysis for HRVT$_1$ and HRVT$_2$

Reliability Variables	HRVT$_1$	HRVT$_2$
TEM		
Absolute, km.h^{-1}	0.45	0.46
Relative, %	6.0	6.0
ICC		
Magnitude	0.889	0.884
P Value	0.000	0.000

[z]Abbreviations: ICC, intra-class coefficient correlation; TEM, typical error of measurement.

pathetic nervous system activity, and this variable shows a non-linear pattern in intensities close to heavy and severe domains (3). At intensities above the first physiological threshold as the LT$_1$ the sympathetic system starts to intensify its activation (24), so that a significant activation occurs in intensities close to the second physiological threshold, the LT$_2$. Analyzing the results of Tulppo et al. (3), it was observed a breakpoint in SD2 very nearby intensities ≈ 80% - 90% VO$_{2MAX}$ during maximal progressive cycling test, having the participants being submitted or not to a parasympathetic system activity blockage before the test. In the present study, HRVT$_2$ showed relative values of speed and HR around 86.1% and 90.5%, respectively. These values are close to intensities related to the second physiological threshold (5, 24).

Despite some criticism, the identification of two physiological thresholds using different variables is possible; due to a cascade of integrated neurophysiological events that occurs in organs and tissues responsible for locomotion (16). In maximal incremental exercises, the first metabolic transition zone matches a significant PNS with-

drawal. Concurrently, there are increases in CO$_2$ production derived from the bicarbonate buffering in active muscles at the same time (4, 25). The identification of a plateau in SD1, an index referred to the vagal component of the autonomic balance, has been coincident with the intensity around the first metabolic transition zone (P > 0.05) (3).

From the first physiological threshold during incremental exercises there is an increase in muscle and blood pH levels. Together, there is a greater CO$_2$ production as the exercise progresses, thus intensifying the activation of higher respiratory control centers which leads to an abrupt increase in ventilation minute at the second threshold. Changes in HRV are mediated mainly by alterations in the respiratory sinus arrhythmia and the hyperventilation mechanical effects on the sinus node at this point (26). As a result, there are concomitant changes in respiratory, muscular and CNS systems. In this regard it is important to point out that, regardless of the debate involving physiological thresholds, the present study showed a consistent agreement between different methods identifying physiological thresholds, thus indicating that HRV data set is a valuable tool for physiological assessments used in exercise intensity prescription.

5.2. Reproducibility of the HRV Thresholds

Results of ICC (≈ 0.88; P < 0.001) and Bland and Altman analysis indicated good agreement between test-retest HRV thresholds, with acceptable typical error of measurement between them (≈ 6%). Together with others (7, 8), these results show that thresholds identified by HRV data set, even when analyzing HRV in time domain, is a useful and reliable method to determine the first and second lactate thresholds as identified by LT$_1$ (and LT$_{Dmax}$) and LT$_2$ during maximal running test.

Bias = 0.05 ± 0.97 km. ⁻¹

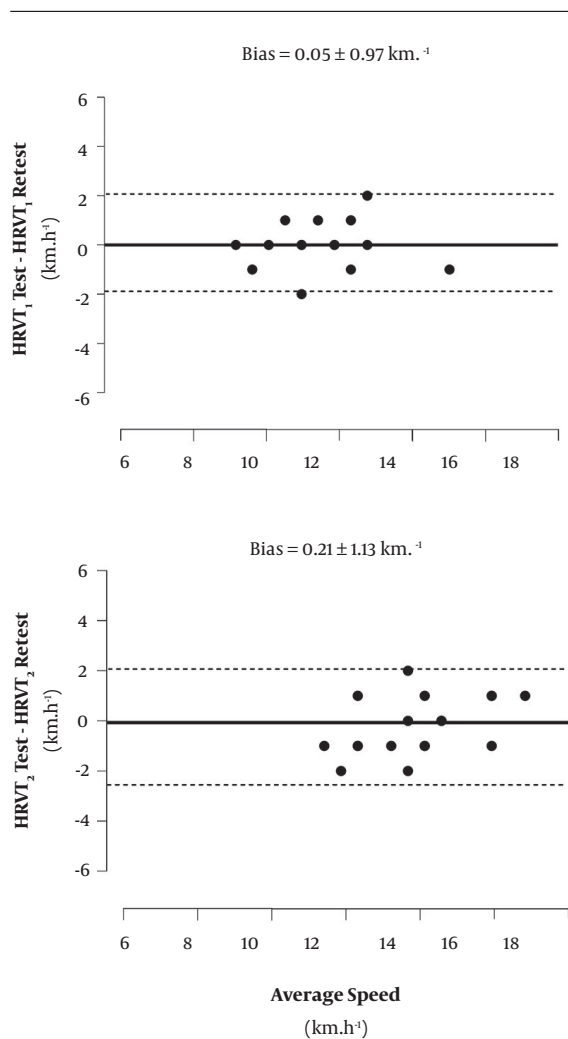Bias = 0.21 ± 1.13 km. ⁻¹

Average Speed

(km.h⁻¹)

Figure 3. Bland and Altman Plot for the Reliability of $HRVT_1$ (A) and $HRVT_2$ (B)

The choice for HRV time domain analysis was based on previous results (27), which reported an unsuccessful spectral analysis in non-stationary HRV data when characterizing the balance between PNS and SNS during exercise. Thus, we used Poincare's plot after normalization of SD1 and SD2 values by the mean RR, multiplied by 1000. This procedure is similar to that previously reported and allowed us to individualize analysis of the HRV (28).

Furthermore, an important aspect should be considered. Instead of using cycling as the exercise mode, we used running and participants performed a maximal incremental running test on treadmill. Most studies have investigated HRV thresholds in cycling. Therefore, these results are novel because they show that the HRV data set may further identify physiological thresholds during running on treadmill.

Some may suggest that the use of fixed blood lactate concentrations to determine LT_1 and LT_2, that is 2.0 and 3.5 mmol.L⁻¹, respectively, may be interpreted as a limitation of the present study. Some have criticized the use of fixed blood lactate concentrations as this may provide arbitrary and non-individualized threshold intensities (29). In the present study we first identified LT_1 and LT_2 individually, by using visual inspection of lactate curves (29). Probably due to a highly exponential lactate-speed relationship, there was a very low agreement between evaluators when determining LT_1, having this threshold been identified at intensities much lower (∼ 40% $Speed_{MAX}$) than those suggested for moderate-well trained middle-distance runners performing maximal running tests, that is between 60% - 70% $Speed_{MAX}$ (24, 29). Thus, we decided to use fixed blood lactate concentrations to determine LT_1 and LT_2, Therefore, we acknowledge that fixed blood lactate concentrations may be limited in some situations, however this method provided non-subjective determination of two thresholds in all participants, thus agreeing with the classical widespread threshold conceptual framework for performance and training prescription (29). In fact, both thresholds were identified within the intensity range suggested for LT_1 (60% - 70% $Speed_{MAX}$ or VO_{2MAX}) and LT_2 (80% - 90% $Speed_{MAX}$ or VO_{2MAX}) in moderate-well trained middle-distance runners (5, 24, 29).

On the other hand, we identified the lactate threshold according to the LT_{Dmax} in order to determine an individualized threshold. While this method has been considered as a valuable tool to determine lactate thresholds, two aspects should be pointed out. First, this method is considerably susceptible to minimal changes in blood lactate concentration responses over time during exercise, thereby causing "artificial changes" in lactate thresholds sometimes, mainly by fluctuations in lactate values during initial and final stages (30). Second, despite providing a non-subjective individualized threshold, only one physiological threshold can be determined by it so that we could have been unable to properly answer the research question of the present study.

5.3. Conclusions

This study showed that HRV could be a feasible, non-invasive method to determine traditional and new lactate thresholds during maximal running test, given the good agreement between thresholds identified by HRV data set, with those identified by traditional and more recent lactate techniques (LT_1 and LT_2 and LT_{Dmax}). In addition, the HRV thresholds showed good reproducibility between test and retest. Thus, thresholds derived from HVR data set may be a reliable and practical tool to determine traditional lactate thresholds during a maximal running test.

Acknowledgments

Footnote

References

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