

Influence of aerobic fitness on the correspondence between heart rate variability and ventilatory threshold

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Abstract

The aims of this study were to verify the correspondence between heart rate variability (HRV) and ventilatory thresholds during a progressive exercise test and the relationship with low and high aerobic fitness levels. Twenty male volunteers (29.5±6.2 years; 75.9±13.0 kg; 175.0±7.4 cm) were recruited. The subjects were allocated to two groups according to their VO_2max <48.8 ml•kg⁻¹•min⁻¹ (low cardiorespiratory fitness group) (n=10) and >48.81 ml•kg⁻¹•min⁻¹ (high cardiorespiratory fitness group) (n=10). A progressive test was performed, consisting of 3-min stages beginning at 25 watts and increasing by 25 watts every 3-min. The HRV threshold (HRVT) and ventilatory threshold (VT) analyses were performed through visual inspection. The comparisons with RMSSD values in percentage of maximum workload resulted in a higher effect size (ES) than the SDNN values. The VO_2 in the high cardiorespiratory fitness group at VT (+32%), $\text{HRVT}_{\text{RMSSD}}$ (+27%), and $\text{HRVT}_{\text{SDNN}}$ (+31%) was significantly higher compared to the group with low cardiorespiratory fitness. Higher values were observed for relative load ($\text{W}\cdot\text{kg}^{-1}$) at VT and $\text{HRVT}_{\text{SDNN}}$ in the high cardiorespiratory fitness group in comparison with the low cardiorespiratory fitness group ($P<0.05$), but no difference for VT and $\text{HRVT}_{\text{RMSSD}}$. Significant correlations between at VT and $\text{HRVT}_{\text{SDNN}}$ ($r=0.77$) were found only in the low cardiorespiratory fitness group. Cardiorespiratory fitness should be regarded as a factor for HRVT evaluation. The $\text{HRVT}_{\text{SDNN}}$ was closer to the VT in the low cardiorespiratory fitness group than the $\text{HRVT}_{\text{RMSSD}}$, however, the use of vagal modulation assessed using the HRV parameter was more sensitive to observe possible differences regarding cardiorespiratory fitness.

KEYWORDS: Cardiac autonomic responses; Incremental test; Heart rate variability analysis; Cardiorespiratory fitness.

Introduction

The autonomic nervous system (ANS) is the primary physiological pathway which regulates heart rate (HR) by means of sympathetic and parasympathetic activities on the sinus node¹. In response to exercise stress, HR is accelerated due to cardiac vagal withdrawal concomitant with increased sympathetic outflow². The beat-to-beat oscillation of HR [i.e., heart rate variability (HRV)] is a non-invasive method to assess changes in the balance between the ANS branches³⁻⁵. Heart rate

variability at rest and during low-intensity exercise has been shown to be positively correlated with aerobic fitness⁶ and responsive to aerobic and sports training effects^{4,7,8}.

In response to the physiological stress caused by a progressive exercise test, pulmonary ventilation increases in a non-linear fashion, as a reflection of the subtle increase in the muscular anaerobic contribution to ATP resynthesis⁹. In theory, the ventilatory threshold

(VT) corresponds to the rise in blood lactate above resting levels [i.e., lactate threshold (LT)]¹⁰. Recently, HRV responses during incremental tests have been used to determine the heart rate variability threshold (HRVT), which is purported to coincide with VT and LT¹¹. According to KARAPETIAN¹¹, HRVT can be defined as the point at which there is no further decline in time-domain HRV indices, thus indicating vagal withdrawal, however, the correspondence between HRVT, VT, and LT was tested in subjects with a wide variation in aerobic fitness. A close examination of figure 3 in of the Karapetian's¹¹ paper reveals considerable individual discrepancies in the comparison between autonomic and metabolic thresholds. This suggests that the high correlation between HRVT and VT ($r = 0.89$; ranging from $-0.8 - 2.5$ L/min of oxygen output considering means of HRVT and VT) may be weaker in a more homogeneous groups (i.e., it is possible that there is a low correlation between the VT and HRVT when analyzed separately between individuals with low and high means of HRVT and VT). It is suggested that increases in sympathetic-adrenal activity and subsequent catecholamine release are responsible for stimulating glycolysis

and ventilation breakpoints¹¹. Although it is tempting to mechanistically link HRVT, LT, and VT, it is accepted that in some cases this correspondence may fail, indicating that factors other than sympathetic outflow and anaerobic glycolysis can stimulate pulmonary ventilation^{12,13}, such as the vagal autonomic tone resulting in fast parasympathetic withdrawal in oscillations of HR in individuals with higher aerobic fitness^{14,15}, during progressive tests. This hypothesis could indicate reduced influence of the respiratory-metabolic mechanisms linked to the occurrence of HRVT in individuals with higher aerobic fitness.

The lack of correspondence between HRVT and VT may reveal differing mechanistic bases underlying these threshold intensities and raises concerns on the use of the former as a simple and non-invasive alternative to aerobic capacity evaluation and training intensity prescription, to replace VT. Therefore, the aims of this study were threefold: 1) to compare the HRV changes during progressive exercise performed by groups possessing low and high aerobic fitness levels; 2) to compare the HRVT and VT between groups with low and high aerobic fitness levels and; 3) to verify the correspondence between HRVT and VT within each aerobic fitness group.

Methods

The study involved two groups, divided according to aerobic fitness levels (low and high). For each group, oxygen consumption (VO_2) at VT and at HRVT were determined from same progressive cycling test. One subject from the low maximal oxygen consumption (VO_{2max}) group was excluded from the analyses due to abnormal ventilatory responses which precluded determination of VT. The VO_2 at VT and HRVT was compared within and between groups to ascertain the role of aerobic fitness, as assessed by VO_{2max} , on the cardiac autonomic response to exercise and on the correspondence between HRVT and VT. According to the median of ($48.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), the participants were evenly split into two groups: low cardiorespiratory fitness group (range: $37.2 - 44.2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $n = 10$) and high cardiorespiratory fitness group (range: $49.5 - 61.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $n = 10$). The cut-off value established for high cardiorespiratory fitness group was near the 95% percentile of assumed as ~ 50 and $47.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for men aged 25-34 and 35-44¹⁶, respectively.

Twenty male volunteers aged between 20 and 44 years of age (29.5 ± 6.2 years) were recruited. Prior to data collection, all participants were initially surveyed to gather information on their general characteristics. Individuals were included in the study if they met the following inclusion criteria: were healthy with physically active or sedentary lifestyles in the previous six months [using the International Physical Activity Questionnaire criteria (<http://www.ipaq.ki.se/>)], nonsmokers, presented no cardiovascular dysfunction (asked in an anamnesis procedures considering the last medical appointment), and were not under drug administration within at least four weeks before of the incremental test. All participants signed an informed consent and the procedures were approved by the local Ethics Committee (process number 091/2013).

TABLE 1 displays the physical and physiological characteristics of the low and high cardiorespiratory fitness groups. There were no significant differences between groups regarding age, height, and HRmax. The cardiorespiratory fitness (and maximal power output

- W_{max}) was higher for the group with high aerobic fitness in comparison with the group with low aerobic fitness. Body weight and BMI were also higher in the lower aerobic fitness group.

TABLE 1 - Physical and physiological characteristics of the low cardiorespiratory fitness group; (n = 10) and high cardiorespiratory fitness group; (n = 10) from the median of VO_{2max} ($48.7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Mean values, (\pm standard deviation) and confidence intervals [CI 95%].

	Low cardiorespiratory fitness group	High cardiorespiratory fitness group	<i>p</i>
Age (years)	30.3 \pm 6.1 [25.9 – 34.6]	28.6 \pm 6.4 [23.9 – 33.2]	0.55
Weight (kg)	83.9 \pm 13.8 [74.0 – 93.8]	67.8 \pm 4.7 [64.4 – 71.2]	<0.02*
Height (cm)	174.9 \pm 8.4 [168.0 – 180.2]	175.8 \pm 6.4 [171.3 – 180.4]	0.61
BMI ($\text{kg}\cdot\text{m}^{-2}$)	27.7 \pm 3.9 [24.9 – 30.4]	22.0 \pm 2.0 [20.5 – 23.4]	<0.01*
VO_{2max} ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	41.3 \pm 3.5 [38.8 – 43.8]	56.2 \pm 4.1 [53.3 – 59.1]	<0.01*
VO_{2max} ($\text{L}\cdot\text{min}^{-1}$)	3.4 \pm 0.4 [3.1 – 3.7]	3.8 \pm 0.3 [3.6 – 4.1]	<0.05*
HRmax (bpm)	189.1 \pm 11.2 [181.1 – 197.1]	188.8 \pm 10.9 [181.0 – 196.5]	0.95
W_{max} (W)	267.2 \pm 28.6 [246.8 – 287.7]	300.0 \pm 29.8 [278.7 – 321.3]	<0.03*

* Significant difference using independent student T-test;
BMI = body mass index;
 VO_{2max} = maximum oxygen output;
HRmax = maximal heart rate;
 W_{max} = maximal power from incremental test.

Procedures

Exercise testing

Subjects were instructed to avoid food or beverages containing caffeine for 24 hours and vigorous exercise for 48 hours prior to testing. Upon arrival at the laboratory, body mass and height were measured with a calibrated scale and a stadiometer to the nearest 0.02 kg and 0.1 cm, respectively. The body mass index (BMI)

was calculated by dividing the weight (kg) by the square of the height (cm).

The progressive test was performed on an electromagnetically braked cycle ergometer (LODE, Excalibur Sport, Groningen, Netherlands) in a laboratory with a controlled temperature ($\sim 23^{\circ}\text{C}$), and a fan directed to the participant when required, in order to improve thermal comfort during the test. The testing protocol consisted of 3-min stages¹¹ beginning at 25 watts and increasing 25 watts every 3-min¹⁵.

The 3-min stages were chosen for the progressive test as this enables a higher quality stationary signal of RR intervals to be obtained. Subjects were instructed to maintain a pedal cadence of between 60 and 90 revolutions per min (rpm). The exercise test duration ranged from 15 to 40 min, depending on each subject's cardiorespiratory fitness. Subjects finished the test when they reached volitional exhaustion or felt unable to maintain the pedal cadence >60 rpm. Throughout the test, participants were verbally motivated to give their best performance.

Determination of heart rate variability threshold (HRVT)

Heart rate variability was measured using a telemetric device (Polar RS800 Electro Oy, Kempele, Finland) used to record each subject's RR intervals (beat-to-beat fluctuation in HR) throughout the test. The RR interval data were stored in the receiving watch and subsequently uploaded to a computer for analysis using Polar Pro Trainer software (version 5.0). All analyses were performed using Kubios HRV Analysis Software 2.0 (Biosignal Laboratory, University of Kuopio, Finland). The RR intervals from the final 2 min of each progressive test stage were used for analysis of HRV¹¹, avoiding the influence of non-stationary RR intervals on HR oscillations, commonly observed in the early stages. The RR interval was automatically interpolated in cases where it deviated from the previous interval by >30%, followed by visual inspection to correct for noise¹⁵. The standard deviation of all RR intervals (SDNN), which represents both sympathetic and parasympathetic activity and the square root of the mean squared differences of successive RR intervals (RMSSD) in milliseconds (ms), as a parasympathetic activity indicator, were retained for analysis¹. To determine the HRVT, the RMSSD and SDNN for each stage were plotted against power output (W) or percentage of maximum workload (%W). The exercise intensity at which no further decline in SDNN and RMSSD values is observed defines the occurrence of HRVT (HRVT_{SDNN} and HRVT_{RMSSD}, respectively). Two experienced researchers (with at least 2 years of practical experience in assessments of cardiopulmonary parameters in exercise tests) with no access to the identity of the subjects performed the HRVT analyses. In the case of disagreement between power outputs, a third independent researcher performed the HRV determination. In all cases, the third result coincided with one of the previous results and this intensity was thus defined as the individual's HRVT, or the median those three outcomes. High inter-rater intraclass

correlation coefficient (0.80) between the researchers considering HRVT was found.

Determination of ventilatory threshold (VT) and VO₂max

Respiratory gas exchange was measured continuously breath-by-breath using a metabolic cart (Quark CPET, Cosmed, Italy) and averaged each 20-s in order to obtain VT and VO₂max. Prior to each test, the equipment was calibrated using ambient air and gases of known O₂ (16%) and CO₂ (5%) concentrations. The turbine flow-meter was calibrated using a 3-L syringe. The calibration procedures followed the manufacturer's instructions.

The individual VO₂max was determined as the highest value achieved in 20-s averages close to the end of the test. Although no secondary criterion was used to validate VO₂max¹⁷, all subjects reported >19 rating of perceived exertion on the 6-20 Borg scale, determined in the final 30 seconds of each stage¹⁸. The VT was determined at the power output corresponding to the increase in VE/VO₂ without a concomitant increase in VE/VCO₂¹⁶. The power output and VO₂ at VT have previously been considered a valid measure with high intraclass correlations using tests with increments of 25W per stage¹⁹. Each individual's VT was assessed by two experienced researchers involved in the study, who were blinded to the participants' identity and group allocation²⁰. In cases of disagreement between the two researchers, a third researcher arbitrated. The HR, VO₂, and RPE at VT were retained for analyses. The heart rate (HR) and VO₂ at VT and HRVT were calculated as the mean values from the final 2-min of the stage.

Statistical analysis

The Shapiro-Wilk's test indicated normal distribution of data. Thus, the independent Student's t test was applied for comparisons between groups (low versus high cardiorespiratory fitness group) for age, anthropometric variables (weight, height, and BMI), physiological responses from progressive testing (VO₂max, HRmax, and Wmax), physiological variables at VT, HRVT_{RMSSD}, and HRVT_{SDNN} (VO₂, %VO₂max, HR, %HRmax, RPE), load (watts), and relative load (watts•kg⁻¹). One way ANOVA was used to compare the physiological and perceptual responses within each group at VT, HRVT_{RMSSD}, and HRVT_{SDNN} (VO₂, %VO₂max, HR, %HRmax, RPE), load (watts), and relative load (watts•kg⁻¹). The two-way repeated measures ANOVA with Bonferroni correction was used to compare the values of RMSSD and SDNN (ms) at

the percentages of maximum workload corresponding to from 10% to 100% between the high and low cardiorespiratory fitness groups. The sphericity was checked using Mauchly's test and whenever the test was violated the necessary technical corrections were performed with the Greenhouse-Geisser test. Data are presented as mean±standard deviation (SD) and the significance level was set at 5% ($P<0.05$). Pearson's correlation was performed to assess the relationship between VO_2 ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) values at ventilatory

(VT) and heart rate variability thresholds ($\text{HRVT}_{\text{RMSSD}}$ and $\text{HRVT}_{\text{SDNN}}$). The aforementioned analyzes were performed in SPSS version 20.0 software for Windows. Additionally, Cohen's effect size (ES) was used to interpret the magnitude of difference between the high and low $VO_{2\text{max}}$ groups considering the RMSSD and SDNN values at each percentage (from 10 to 100 % of maximum workload). The threshold values for Cohen's ES statistics were 0.20 – 0.50 (small), 0.50 – 0.80 (moderate), and >0.80 (large)²¹.

Results

The HRV (RMSSD and SDNN parameters) responses to the progressive test between the groups with different cardiorespiratory fitness levels are displayed in FIGURE 1. The pairwise comparisons between low and high cardiorespiratory fitness showed there were no main effect significant differences for RMSSD and SDNN at the percentage of maximum workload corresponding to from 10% to 100% of $VO_{2\text{max}}$. Two-way repeated measures ANOVA demonstrated main effects only when considering the percentage of maximum workload factor for RMSSD ($F=27.3$; $P<0.01$) and SDNN ($F=54.4$; $P<0.01$). The group and interaction effects between the percentage of maximum workload and group were not significant for either RMSSD ($F=0.81$; $P>0.05$) or SDNN ($F=0.02$; $P>0.05$). FIGURE 1 illustrates the HRVT identification of two typical subjects from the low (Panel C) and high (Panel D) cardiorespiratory fitness groups. In the low cardiorespiratory fitness group, it was easier to visually determine the $\text{HRVT}_{\text{RMSSD}}$ and $\text{HRVT}_{\text{SDNN}}$, and in this case they were coincident. On the other hand, in the high cardiorespiratory fitness group, it was more difficult to clearly determine the HRVT thresholds, and the $\text{HRVT}_{\text{RMSSD}}$ and $\text{HRVT}_{\text{SDNN}}$ did not coincide in most cases.

TABLE 2 presents the comparisons between VO_2 at VT and at the heart rate variability thresholds ($\text{HRVT}_{\text{RMSSD}}$ and $\text{HRVT}_{\text{SDNN}}$). The VO_2 at VT (+32%), $\text{HRVT}_{\text{SDNN}}$ (+31%), and $\text{HRVT}_{\text{RMSSD}}$ (+27%), as well as the relative workloads to body mass at VT (+41%), $\text{HRVT}_{\text{SDNN}}$ (+33%), and $\text{HRVT}_{\text{RMSSD}}$ (+39%), were higher in the group with high cardiorespiratory fitness compared to the group with low cardiorespiratory fitness. There were no significant differences between the low and high cardiorespiratory fitness groups for the percentage of $VO_{2\text{max}}$ ($\%VO_{2\text{max}}$) at VT (67.2 ± 5.7 vs 63.8 ± 10.3 ; $P=0.12$), $\text{HRVT}_{\text{RMSSD}}$ (61.5 ± 8.7 vs

56.7 ± 8.4 ; $P=0.73$), or $\text{HRVT}_{\text{SDNN}}$ (70.0 ± 9.8 vs 66.1 ± 9.4 ; $P=0.99$), respectively.

The power output at VT (144.4 ± 20.8 W) did not differ from the power output at $\text{HRVT}_{\text{RMSSD}}$ (125.0 ± 25.0 W; $P=0.06$) or $\text{HRVT}_{\text{SDNN}}$ (152.8 ± 29.2 W; $P=0.35$) within the low cardiorespiratory fitness group. However, a significant difference was found between power output at $\text{HRVT}_{\text{RMSSD}}$ and $\text{HRVT}_{\text{SDNN}}$ ($P<0.01$) in this group. Similarly, in the high cardiorespiratory fitness group the power output at VT (165.0 ± 42.8) was not different from the power output at $\text{HRVT}_{\text{RMSSD}}$ (135.0 ± 29.3 W; $P=0.07$) or $\text{HRVT}_{\text{SDNN}}$ (170.0 ± 34.9 W; $P=0.05$). The power output was different ($P<0.01$) between $\text{HRVT}_{\text{RMSSD}}$ and $\text{HRVT}_{\text{SDNN}}$.

Heart rate expressed both in absolute values (bpm) and as a percentage of maximal heart rate ($\%HR_{\text{max}}$) was significantly different ($P<0.05$) in the low cardiorespiratory group between the VT (80.8 ± 4.2 $\%HR_{\text{max}}$; 153.0 ± 13.5 bpm) and the $\text{HRVT}_{\text{RMSSD}}$ (77.7 ± 5.4 $\%HR_{\text{max}}$; 146.8 ± 9.8 bpm), and between the VT and the $\text{HRVT}_{\text{SDNN}}$ (84.1 ± 4.7 $\%HR_{\text{max}}$; 159.2 ± 12.3 bpm). In the high cardiorespiratory fitness group, there were no differences ($P>0.05$) in heart rate between the VT (78.2 ± 7.2 $\%HR_{\text{max}}$; 147.8 ± 17.7 bpm) and $\text{HRVT}_{\text{RMSSD}}$ (77.7 ± 5.4 $\%HR_{\text{max}}$; 139.9 ± 10.0 bpm), or between the VT and $\text{HRVT}_{\text{SDNN}}$ (80.3 ± 6.9 $\%HR_{\text{max}}$; 151.1 ± 11.4 bpm).

Differences were found in $\text{HRVT}_{\text{RMSSD}}$ and $\text{HRVT}_{\text{SDNN}}$ in both groups for $\%VO_2$ ($P<0.01$). No differences were detected between the low and high cardiorespiratory fitness groups regarding power output at VT ($P=0.21$), $\text{HRVT}_{\text{RMSSD}}$ ($P=0.44$), or $\text{HRVT}_{\text{SDNN}}$ ($P=0.26$). No differences in $\%HR_{\text{max}}$ at VT, $\text{HRVT}_{\text{RMSSD}}$, or $\text{HRVT}_{\text{SDNN}}$ were identified between groups ($P=0.36$, 0.21 , 0.18 , respectively). The RPE at $\text{HRVT}_{\text{RMSSD}}$ and $\text{HRVT}_{\text{SDNN}}$ were significantly

different ($P < 0.01$) in both groups.

In addition to these findings, the rating of perceived exertion (RPE) was not different between the groups with low and high cardiorespiratory fitness at VT (12.8 ± 1.2 vs 13.9 ± 3.1), $HRVT_{RMSSD}$ (11.8 ± 2.2 vs 12.7 ± 3.4), or $HRVT_{SDNN}$ (13.2 ± 2.5 vs 14.8 ± 2.5) ($P > 0.05$), respectively.

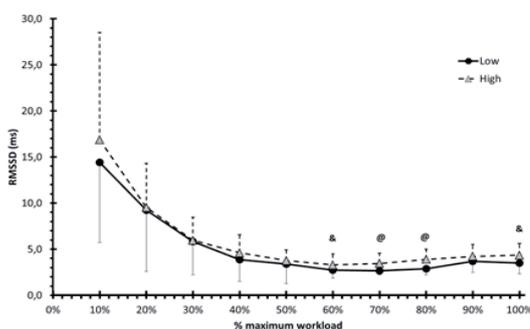
Considering the HRV analyzes assuming the percentage of maximum workload from the progressive test, the Cohen's ES of the pairwise comparison was large at 70% (ES: 0.94) and 80% (ES: 1.10), and moderate at 60% (ES: 0.52) and 100% (ES: 0.68) for RMSSD. Regarding the SDNN values, the ES was small from 10 to 100 % of maximum workload. Significant differences ($P < 0.05$) and a large Cohen's ES were observed when comparing the low and high cardiorespiratory fitness for relative workloads with VT (ES: 1.50), $HRVT_{RMSSD}$ (ES: 1.22), and $HRVT_{SDNN}$ (ES: 1.32). The Cohen's ES between VT and $HRVT_{RMSSD}$ within groups was 0.69 (moderate)

and 0.81 (large) for the low and high cardiorespiratory fitness groups, respectively (see TABLE 2). When the data from both groups were pooled and power output was expressed relative to body mass ($W \cdot kg^{-1}$), the ES in the comparison between VT (2.09 ± 0.58) and $HRVT_{RMSSD}$ (1.76 ± 0.49) was (ES: 0.61) and between $HRVT_{RMSSD}$ and $HRVT_{SDNN}$ (2.20 ± 0.61) (ES: 0.74) was moderate ($P < 0.05$). No difference and a small ES were observed between VT and $HRVT_{SDNN}$ (ES: 0.18; $P > 0.05$).

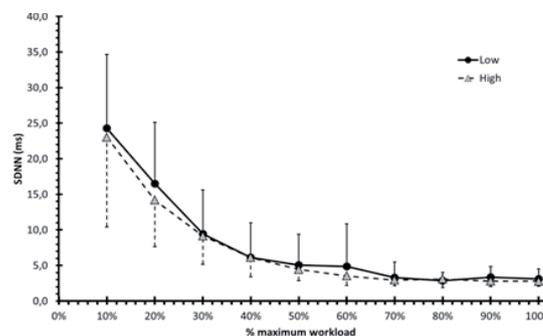
TABLE 3 displays the Pearson's correlation coefficients (r) between VO_2 values at VT, $HRVT_{RMSSD}$ and $HRVT_{SDNN}$. The correlations were higher in the low cardiorespiratory fitness group than in the high cardiorespiratory fitness group. VO_2 at VT (0.84, $P < 0.05$ and 0.62, $P = 0.06$), $HRVT_{RMSSD}$ (0.61, $P = 0.07$ and 0.48, $P = 0.15$), and $HRVT_{SDNN}$ (0.76, $P < 0.05$ and 0.67, $P < 0.05$) demonstrated higher correlations with in the low cardiorespiratory fitness group than the high cardiorespiratory fitness group, respectively.

FIGURE 1 - Changes in mean and standard deviation of RMSSD (Panel A) and SDNN (Panel B) in percentage of maximum workload of individuals from groups with low (closed black circles) and high (gray triangle) cardiorespiratory fitness levels. HRV vs. workload of an individual from the low aerobic fitness group (Panel C) and an individual from the high aerobic fitness group (Panel D).

Panel A

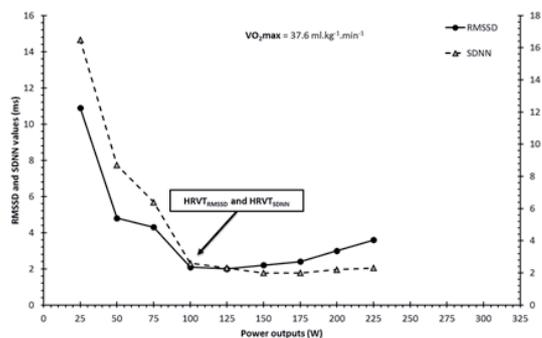


Panel B



©Significant difference in pairwise comparisons between low and high cardiorespiratory fitness using Bonferroni's post-hoc test and large effect size (ES > 0.80).
 §Difference considering moderate effect size (0.50–0.80).

Panel C



Panel D

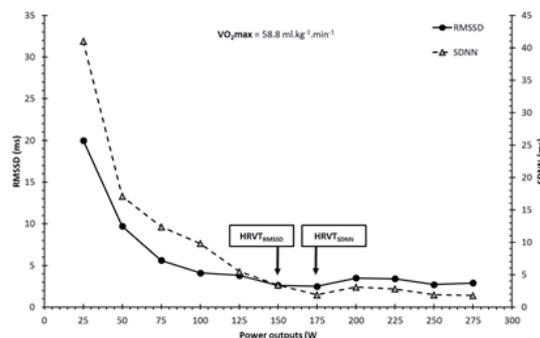


TABLE 2 - Comparisons between low (n=9) and high (n=10) cardiorespiratory fitness groups for VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$), relative workload ($W \cdot kg^{-1}$) and percentage of W_{max} (%) corresponding to ventilatory (VT) and heart rate variability thresholds ($HRVT_{RMSSD}$ and $HRVT_{SDNN}$).

	Low cardiorespiratory fitness group		High cardiorespiratory fitness group		Effect Size (ES): Low - High cardiorespiratory fitness groups	
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)						
VT	27.3	± 3.7 [24.5 – 30.2]	36.3	$\pm 6.9^*$ [31.3 – 41.3]	1.58 (Large)	
$HRVT_{RMSSD}$	25.0	± 4.3 [21.7 – 28.4]	31.9	$\pm 5.4^*$ [28.0 – 35.8]	1.39 (Large)	
$HRVT_{SDNN}$	28.5	$\pm 5.1^{\#}$ [24.6 – 32.5]	37.3	$\pm 6.2^{\#}$ [32.8 – 41.7]	1.51 (Large)	
ES: VT - $HRVT_{RMSSD}$	0.58 (Moderate)		0.71 (Moderate)			
ES: VT - $HRVT_{SDNN}$	0.26 (Small)		0.14 (Trivial)			
ES: $HRVT_{RMSSD}$ - $HRVT_{SDNN}$	0.74 (Moderate)		0.92 (Large)			
Relative to body mass workload ($W \cdot kg^{-1}$)						
VT	1.7	± 0.3 [1.5 – 2.0]	2.4	$\pm 0.6^*$ [2.0 – 2.8]	1.49 (Large)	
$HRVT_{RMSSD}$	1.5	± 0.3 [1.2 – 1.8]	2.0	$\pm 0.5^*$ [1.7 – 2.3]	1.22 (Large)	
$HRVT_{SDNN}$	1.8	± 0.5 [1.5 – 2.2]	2.5	$\pm 0.6^*$ [2.1 – 2.9]	1.32 (Large)	
ES: VT - $HRVT_{RMSSD}$	0.69 (Moderate)		0.81 (Large)			
ES: VT - $HRVT_{SDNN}$	0.29 (Small)		0.18 (Trivial)			
ES: $HRVT_{RMSSD}$ - $HRVT_{SDNN}$	0.85 (Large)		1.01 (Large)			

* Significant different between low vs. high cardiorespiratory fitness group ($P < 0.05$);
Significant difference within low or high cardiorespiratory fitness group in comparison between $HRVT_{RMSSD}$ and $HRVT_{SDNN}$.

TABLE 3 - Pearson product-moment correlation coefficients between VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) values at ventilatory (VT) and heart rate variability thresholds ($HRVT_{RMSSD}$ and $HRVT_{SDNN}$).

	Low cardiorespiratory fitness group (n=9)			High cardiorespiratory fitness group (n=10)			
	VO_2 at VT	VO_2 at $HRVT_{RMSSD}$	VO_2 at $HRVT_{SDNN}$	VO_2 at VT	VO_2 at $HRVT_{RMSSD}$	VO_2 at $HRVT_{SDNN}$	
VO_2 at VT	-----	0.52	0.77*	-----	0.38	0.39	
VO_2 at $HRVT_{RMSSD}$	0.52	-----	0.85*	0.38	-----	0.80*	
VO_2 at $HRVT_{SDNN}$	0.77*	0.85*	-----	0.39	0.80*	-----	

* $P < 0.05$.

Discussion

The main result of the study was that the differences between VT, $HRVT_{RMSSD}$, and $HRVT_{SDNN}$ were greater in the high cardiorespiratory fitness group than in the low cardiorespiratory fitness group. Thus, this finding indicates that the use of the threshold as an aerobic capacity assessment tool (first threshold) should be viewed with some caution, especially in individuals with high cardiorespiratory fitness. The rationale between VT and RR oscillations measured through vagal HRV indices is evidenced as being synchronous with respiratory sinus arrhythmia, mediated by the vagus nerve^{22, 23}. However, the greatest differences were observed in individuals who presented higher VO_2 max values, although this was not noted by other authors^{11, 23}. We consider that there is a certain mismatch in physiological responses in HRV and respiratory/ventilatory dynamics in individuals with high aerobic fitness during a progressive test.

Abnormal behavior concerning ventilatory responses in progressive tests have been reported with VT determination²⁴, as well as in one subject in the present study from the low cardiorespiratory fitness group. This fact demonstrates that VT should be analyzed carefully, requiring trained staff and gas exchange analysis equipment, which generates high cost. On the other hand, the HRVT using the visual inspection technique is an easy tool and less costly than VT, besides presenting high reproducibility²⁵. Although the correspondence between metabolic transition thresholds and HRVT has been documented previously^{11, 26}, we found that it may present some disagreement in well-conditioned individuals who demonstrate higher rates of VO_2 max, possibly due to the more pronounced physiological mechanisms underlying vagal autonomic activity being more pronounced in well-trained individuals²⁷. However, in the low cardiorespiratory fitness group, the correspondence between HRVT measured using the RMSSD parameter and VT were similar, as reported by SALES et al.²⁶ comparing HRVT through vagal-mediated indices and VT²⁶.

Considering the differences between HRV indices through progressive exercise testing, the stabilization point using sympathetic or overall parameters (i.e., SDNN) occurs later than vagal-mediated indices^{5, 25}. Similarly, the VO_2 at $HRVT_{RMSSD}$ was lower than VO_2 at $HRVT_{SDNN}$ in both groups. However, no difference was found between VT and $HRVT_{SDNN}$

in the low or high cardiorespiratory fitness groups when expressed in VO_2 . This difference is probably related to the physiological significance of HRV indices which reflect the sympathetic and parasympathetic drives of cardiac autonomic control during exercise^{5, 15}. The RMSSD is considered as a parasympathetic parameter related to the autonomic control perspective, which leads to a faster decay rate during progressive exercise testing, while SDNN is influenced by both sympathetic and parasympathetic pathways^{1, 5} and generally achieves asymptotic values after VT⁵. Similar values were found to our results when the percentage VO_2 max at VT and HRVT (~64%) were analyzed according to parasympathetic parameters²². The HRV by time domain has proven to be a valid way to examine the vagal withdrawal in progressive tests^{5, 11, 22, 28}, and high reliability was observed when ascertaining HRVT using the vagal parameter through visual inspection²⁵, as performed in the present study.

While the VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) at VT, $HRVT_{RMSSD}$, and $HRVT_{SDNN}$ was higher in the high cardiorespiratory fitness group, the percentage (%) VO_2 values were similar between the thresholds analyzed and the groups studied. Differences were found between groups for relative to body mass power output corresponding to VT and $HRVT_{SDNN}$, but no difference was observed for $HRVT_{RMSSD}$. In addition, although $HRVT_{SDNN}$ displayed better correlations in the comparison with VT than $HRVT_{RMSSD}$, the use of the vagally mediated RMSSD throughout the progressive test appears to discriminate cardiorespiratory fitness levels (FIGURE 1, panel A) considering percentages of workloads near to the VT or HRVT (60-80% of VO_2 max). Conversely, SDNN did not differ between groups (FIGURE 1, panel B) at any workload. These results confirm the assumption that vagal indices are higher in individuals with good cardiorespiratory fitness levels during progressive exercise testing¹⁵. This could be due to a physiological concept, the higher 'parasympathetic reserve' present in the high cardiorespiratory fitness group, as previously observed^{5, 6, 15}. In this sense, the use of a vagal HRV parameter analyzed by RMSSD was sensitive to discriminate physical fitness for initial workloads comparing the low and high cardiorespiratory fitness groups. Thus, our results suggest, according to ES, the use of a vagal HRV parameter as a method able to discriminate by

cardiac vagal modulation withdrawal throughout progressive exercise and its relationship with cardiorespiratory physical fitness, as highlighted by other studies^{14, 15}.

A greater relationship between VT and $HRVT_{RMSSD}$ than the overall (SDNN) HRV parameter as assessed by $HRVT_{SDNN}$ was expected, as observed previously^{5, 11, 22}. However, the present study demonstrated a significant correlation only between the VO_2 at VT and $HRVT_{SDNN}$ ($r=0.77$) for the low cardiorespiratory fitness group and a weaker non-significant correlation ($r=0.39$) for the high cardiorespiratory fitness group. The Pearson's correlation presented lower values between the VO_2 at VT and $HRVT_{RMSSD}$ in the low ($r=0.52$) and high ($r=0.38$) cardiorespiratory fitness groups. This finding confirms the hypothesis that cardiorespiratory fitness level is an important factor to consider in HRVT evaluation and its physiologic mechanisms linked to the VT or metabolic transition should be revised. Different results were found to relate the VT with HRVT, using vagal parameters, ($r=0.69-0.97$) by COTTIN²² in well-trained subjects and ($r=0.89$) by KARAPETIAN¹¹ in non-athletes. This disagreement could be due to the different methods applied to determine HRVT or the profile of the individuals studied.

A strict methodological criterion was applied to determine VT and HRVT in the present study to minimize potential errors by using the visual inspection technique. In this way, physical fitness appears to be the main factor regarding HRVT determination, considering the results of individuals with high cardiorespiratory fitness. It is not possible to state precisely what physiologically led to such a result, whether there is a mechanistic link between cardiac autonomic response and VT during the incremental test. A larger relationship was found between the VO_2 at VT with a vagal HRV parameter (SD1, an equivalent parameter of RMSSD) by TULPPO⁵ and overall HRV parameter (SDNN) by YAMAMOTO²⁹ at the moment that the RR interval variability decreases progressively toward stability (~60% of W_{max}). To summarize, although HRVT has been validated previously with both LT and VT^{1, 22, 26}, the present study demonstrated that only $HRVT_{SDNN}$ was correlated with VT in the low cardiorespiratory fitness group, therefore caution is recommended, especially with individuals with high cardiorespiratory fitness levels.

The increase in heart rate during exercise can be partly explained by the vagal withdrawal. The HR at $HRVTRMSSD$ preceded the HR at VT in the low cardiorespiratory fitness group, as observed by SHIBATA³⁰. In spite of this, in the present study the HR at $HRVT_{RMSSD}$ was not different from that observed in the VT and $HRVT_{SDNN}$. This disagreement may indicate that the physiological mechanisms may be partly distinct and that methodological aspects of VT, LT, and HRVT determination could contribute to mismatched results. The main reason for this is the higher variability of HRV during exercise observed in the high cardiorespiratory fitness group (see FIGURE 1C and 1D as an example), influenced by larger vagal tone¹⁵. Additionally, one advantage of HRVT through visual inspection is related to the fact that VT evaluation is an expensive method and LT is an invasive procedure, whereas HRVT through visual inspection using an HR monitor with RR interval recording (every 2 min per stage), besides being a non-invasive method, is easily applicable and highly reproducible²⁵.

The limitations in the present study must be pointed out. The determination of VT and HRVT by visual inspection is open to some subjectivity, despite presenting higher reliability than HRVT measured by mathematical models²⁵. To reduce this subjectivity, methodological criteria are applied using two, or in some cases three evaluators, which can lower the practicality of this method. In addition, the split of only two groups, the small sample size, and recruitment of only males makes it difficult to generalize our results to different populations. In this way, additional research is warranted to confirm our hypothesis and findings with a larger population using clustering analysis.

In conclusion, the main finding of this study was that cardiorespiratory physical fitness may affect HRVT evaluation and that the $HRVT_{SDNN}$ was closer to VT in the low cardiorespiratory fitness group than the high cardiorespiratory fitness group. However, the use of vagal modulation assessed by HRV, for which we used RMSSD, is more appropriate than SDNN to observe possible differences regarding cardiorespiratory fitness during progressive exercise testing. Thus, the use of HRVT as a tool to assess metabolic transition should be used with caution.

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Conflict of interest

No conflicts of interest are declared by the authors.

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