



Ventilatory threshold and heart rate variability in adolescents

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ABSTRACT

The analysis of blood lactate concentration and pulmonary gas exchanges are methods traditionally employed to identify the transition in the muscle metabolism energy production. However, the analysis of heart rate variability has been recently suggested as an alternative method. The objective of the present study was to compare the heart rate variability threshold (HRVT) with the ventilatory threshold (VT) in a sample of adolescents. Forty-one subjects (22 boys and 19 girls) with age between 15 and 18 years were submitted to a maximal exercise test in a treadmill (modified Bruce protocol). The VT was identified using the ventilatory equivalent of oxygen by means of ergospirometry resources. The heart rate variability was analyzed from the R-R intervals, through the Poincaré plot, which provides information with regard to the standard deviation of the instantaneous beat-to-beat variability (SD1), the standard deviation of the long-term continuous R-R intervals (SD2) and the SD1/SD2 ratio. The HRVT was identified according to three criteria: (1) difference lower than 1 ms in the SD1 between two consecutive exercise levels; (2) SD1 lower than 3 ms; and (3) occurrence of both criteria simultaneously. Through the analysis of the results it was verified that the R-R intervals and the SD2 reduced progressively at each 10% interval of $\dot{V}O_{2peak}$ until the end of the physical effort ($0.05 < p < 0.01$). The SD1 reduced significantly since 20% until 50% of $\dot{V}O_{2peak}$. From 60% until the $\dot{V}O_{2peak}$ there was no significant difference in the SD1. The SD1/SD2 ratio began to increase at 60%. The VT occurred at $54.4 \pm 8.8\%$ of $\dot{V}O_{2peak}$, while the HRVT occurred at $52.4 \pm 12.5\%$, $57.0 \pm 14.1\%$ and $57.8 \pm 13.8\%$ of $\dot{V}O_{2peak}$ for criteria 1, 2 and 3, respectively. No significant differences between the VT and the three criteria used for HRVT identification were observed. Significant product-moment correlation coefficients were found between the HRVT identified by the three criteria and the VT, when $\dot{V}O_2$ absolute values were analyzed. However, there was no statistically significant correlation between the HRVT and the VT when expressed in proportion of $\dot{V}O_{2peak}$. In short, it seems early to apply the HRVT as an alternative method in the adolescents VT identification.

INTRODUCTION

In maximal exercise tests with progressive increase on the workload, the energy production at intensities up to 50 to 60% of the peak oxygen consumption ($\dot{V}O_{2peak}$) is predominantly originated from the aerobic metabolism. With the increased metabolic demand

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through the elevation on the physical effort intensity, the anaerobic metabolism begins to supplement the aerobic energy production⁽¹⁻³⁾. The determination of the physical effort intensity in which the aerobic-anaerobic transition occurs in the muscular metabolism plays important role in the exercise physiology area, being widely employed for the evaluation of the physical fitness directed to long-term endurance, the prescription of aerobic exercises intensities and the monitoring of modifications in aerobic indicators induced by training programs^(4,5).

The methods traditionally used to identify this transition in the muscular metabolism are the analysis of blood lactate concentration and the pulmonary gas exchanges⁽⁶⁻⁸⁾ that allow identifying the lactate threshold (LT) and the ventilatory threshold (VT), respectively. However, other non-invasive and more accessible methods have been recently proposed to identify the physical effort intensity in which the aerobic-anaerobic transition occurs⁽⁹⁻¹²⁾. The analysis of heart rate variability (HRV) is among the available alternative methods⁽¹³⁻¹⁹⁾.

The analysis of HRV allows quantifying the modulation of the autonomic nervous system in the sinoatrial node triggering frequency⁽²⁰⁾. Studies using HRV in incremental physical efforts have attempted to demonstrate that the parasympathetic modulation trends to decrease progressively up to its full removal in approximately 50 to 60% of the $\dot{V}O_{2peak}$ ⁽²¹⁻²⁴⁾. Experiments conducted by Tulppo *et al.*^(16,17) and Yamamoto *et al.*^(13,14) suggest that the effort intensity corresponding to the end of the vagal withdrawal and to the beginning of the more significant participation of the sympathetic modulation coincides with VT. The study of Lima and Kiss⁽¹⁹⁾ compared the LT with the physical effort intensity in which the vagal withdrawal finished, called by the author as the heart rate variability threshold (HRVT). A coincidence between LT and HRVT was verified, providing evidences of the occurrence of a possible causal relation between the autonomic and metabolic events.

Information associated to the HRV in different conditions and pathologies have been generally observed using linear mathematical methods^(20,25). However, studies that involved the linear analyses methods in physical exercises found inconsistent results⁽²⁶⁻²⁸⁾. The non-linear Poincaré plot method provides useful information with regard to the cardiac autonomic modulation during the performance of physical efforts not easily detected by linear analyses^(29,30), and by means of the use of this analysis, the possibility of identifying the LT has been observed⁽¹⁹⁾.

The objective of the present study was to identify HRVT by means of the Poincaré plot quantitative analysis and to compare it with the occurrence of VT in a sample of healthy adolescents from both genders. The hypothesis of the present study is that the cardiac autonomic responses during incremental physical effort are associated to metabolic and ventilatory responses that occur in the transition of the muscle metabolism energy production. Thus, the HRV analysis may be an alternative method to identify this transition.

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METHODS

Subjects

Forty-one adolescents (22 boys and 19 girls) with ages ranging from 14 to 18 years were studied. The subjects were randomly recruited in local public schools. All subjects were healthy, with normal blood pressure and underwent no medicinal treatment at the moment of the evaluations. The adolescents and their parents and/or responsible were previously informed with regard to the experimental procedures to be employed and signed a free consent form for the participation. The intervention protocols were approved by the Ethics Research Committee of the Londrina State University and followed the norms of the resolution 196/96 of the National Health Council on researches involving human beings.

Test of maximum physical effort

The subjects were oriented not to ingest caffeine-based beverages for four hours before the test of maximum physical effort (TPE), to ingest a light meal two hours before and to avoid intense physical efforts at the day before. The tests were performed between 3 pm and 6 pm in laboratory with room temperature kept close to 20 and 23°C.

The TPE was performed in treadmill (*Inbrasport/Millennium*). The adolescents remained one minute at rest in orthostatic position. Following this, the test of physical effort took place using the modified Bruce protocol. This protocol promotes progressive increment of the workload each three minutes. All subjects reached stage in which they needed to run. Verbal encouragement was employed in the attempt to obtain physical effort close to maximum. The test was interrupted by means of voluntary exhaustion.

The minute volume (VE), the oxygen intake ($\dot{V}O_2$) and the carbon dioxide production ($\dot{V}CO_2$) were continuously recorded at rest and during TPE through the analysis of the pulmonary gas exchanges (metabolic analyzer VO2000 – *Aerosport Inc.*). Based on these information, the respiratory exchange ratio ($R = \dot{V}CO_2/\dot{V}O_2$), the oxygen ventilatory equivalent ($VE/\dot{V}O_2$) and the carbon dioxide ventilatory equivalent ($VE/\dot{V}CO_2$) were determined. The ventilatory variables were collected each minute. The equipment used for the analysis of the gas exchange was previously calibrated at the beginning of each TPE. The calibration was performed with samples of room gas (20.9% of O_2 and 0.04% of CO_2) and with samples obtained from a cylinder with known concentration of O_2 (17%) and CO_2 (5%). Additionally, the gases flow of the device was calibrated using a 3-liters syringe, according to recommendations of the manufacturer.

The $\dot{V}O_{2peak}$ was established as the highest oxygen intake reached during TPE. The VT was identified at the physical effort intensity in which the $VE/\dot{V}O_2$ reached its minimum value before presenting progressive increases without concomitant increases on the $VE/\dot{V}CO_2$ ⁽³¹⁾. When the $VE/\dot{V}O_2$ response could not provide VT unequivocally, the V-Slope method was used for confirmation⁽³⁾. The VT was determined independently by three different evaluators. The results were compared and when discrepancies higher than 5% were observed, the graphics were reevaluated⁽³²⁾. The average value presented by all evaluators was adopted as the VT.

Collect of the beat-to-beat heart rate (R-R intervals)

The R-R intervals were continuously recorded by a cardio frequencimeter (*Polar Electro Oy* – model S810) at rest and during TPE. The data were collected with sampling frequency of 1,000 Hz. The R-R intervals recordings were manually edited through visual inspection in the attempt of avoiding that artifacts would contaminate the analysis. Following, the recordings were automatically filtered by the *Polar Precision Performance* software (version 3.02.007). Any R-R interval with difference above 20% of the previous interval was automatically filtered⁽¹³⁾. All recordings presented less than 1.5% of random error.

The HRV analysis was performed by means of the Poincaré plot quantitative analysis. This analysis consists of the plotting of each R-R interval in function of the previous interval. The Poincaré analysis provides information with regard to: (1) the standard deviation of the beat-to-beat instantaneous variability (SD1), characterized as marker of the parasympathetic modulation^(16,29); (2) the long-term standard deviation of continuous R-R intervals (SD2), characterized as marker of the parasympathetic and sympathetic modulation^(16,29,30); and (3) the SD1/SD2 ratio that, during incremental physical effort may be used as indicative of the increase on the sympathetic modulation⁽¹⁶⁾.

The R-R intervals were grouped in 1-minute sequences for the HRV analysis. The first minute of physical effort was excluded from the analysis due to the sudden increase on the heart rate and transitory reduction on the HRV attributed to the sudden vagal withdrawal⁽³³⁾. The HRTV was determined by means of three criteria separately: (1) according to Tulppo *et al.*⁽¹⁷⁾, the end of the vagal withdrawal occurs at the first stage where the difference between SD1 of two consecutive stages is lower than 1 ms; (2) according to Lima and Kiss⁽¹⁹⁾, the HRTV is determined when SD1 reaches value lower than 3 ms; and (3) the combination of both previous criteria. Through criterion (3), the HRTV occurred when both criteria, (2) and (3), were fulfilled.

Statistical treatment

For the study of the autonomic modulation, the values of variables $\dot{V}O_2$, R-R intervals, SD1, SD2 and SD1/SD2 at each minute were interpolated at 0.08 Hz (software *Microcal Origin 6.0*) in the attempt of reporting information associated to HRV according to the relative $\dot{V}O_2$ of effort intensities corresponding to 20, 30, 40, 50, 60, 70, 80, 90 and 100% of the $\dot{V}O_{2peak}$.

The statistical analyses were conducted using a commercial software (*Statistica 5.5*). The data parametric distribution was verified through the Shapiro Wilk test. VT and HRTV identified by means of the three criteria were compared by means of the One-Way analysis of variance for repeated measures. The Pearson product-moment correlation coefficient was employed for the analysis of the statistical associations between thresholds. As the HRV at different physical effort intensities reported as $\dot{V}O_{2peak}$ proportion presented non-parametric distribution, the values of SD1, SD2 and SD1/SD2 were submitted to logarithmic transformation. The autonomic modulation during physical effort reported as $\dot{V}O_{2peak}$ proportion was also analyzed by means of the One-Way analysis of variance for repeated measures. When the analyses of variance identified significant differences, the Newmann Keuls *post-hoc* test of multiple comparisons was employed.

RESULTS

The anthropometrical and functional characteristics of the subjects involved in the present study are presented in table 1.

TABLE 1
Average values ± standard deviations of anthropometrical and functional characteristics of the adolescents evaluated

Age (years):	15.3 ± 0.8
Body weight (kg):	59.6 ± 9.9
Stature (cm):	166.7 ± 8.8
Body mass index (kg/m ²):	21.4 ± 2.6
Rest heart rate (bpm):	91.0 ± 16.2
Peak heart rate (bpm):	191.6 ± 9.4
$\dot{V}O_{2peak}$ (ml/kg/min):	40.9 ± 7.9
Time of test (min):	14.2 ± 2.8

Figure 1 shows behavior observed in R-R intervals and in SD1, SD2 and SD1/SD2 indexes from the Poincaré analysis reported as $\dot{V}O_{2peak}$ proportion. The R-R intervals and the SD2 index decreased

progressively since 20% up to the $\dot{V}O_{2peak}$ ($0.05 < p < 0.01$ between consecutive intensities). The SD1 index decreased progressively and presented significant differences between consecutive intensities since 20% up to 50% of the $\dot{V}O_{2peak}$ ($p < 0.01$). From

60% up to the $\dot{V}O_{2peak}$, no significant differences occurred between consecutive intensities. The SD1/SD2 ratio reached the lowest value in 60% of the $\dot{V}O_{2peak}$. From this intensity on, a progressive increase occurred.

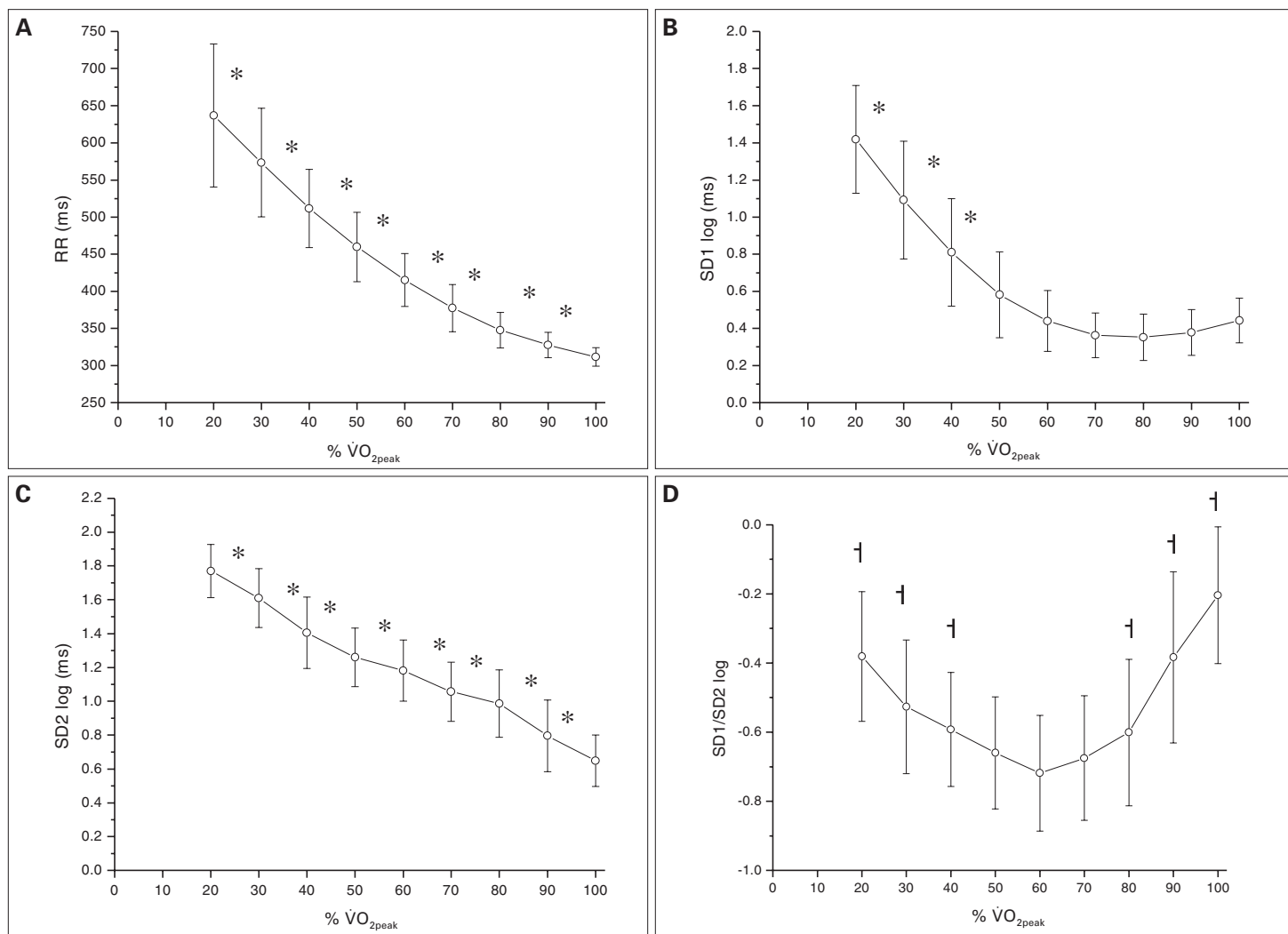


Fig. 1 – Behavior of variables associated to HRV during the performance of physical effort reported as $\dot{V}O_{2peak}$ proportion. **A)** R-R intervals; **B)** standard-deviation of the beat-to-beat instantaneous variability (SD1); **C)** long-term standard-deviation of continuous R-R intervals (SD2); **D)** SD1/SD2 ratio. * significant difference between consecutive intensities ($0.05 < p < 0.01$), † significant difference in relation to the value corresponding to 60% of the $\dot{V}O_{2peak}$ ($0.05 < p < 0.01$).

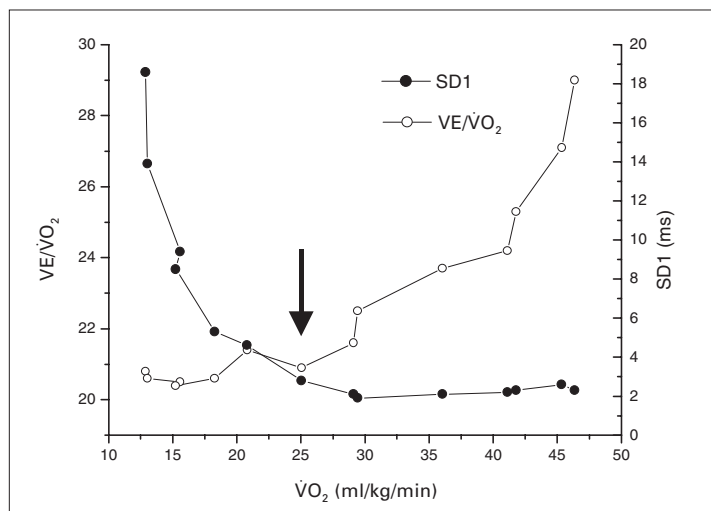


Fig. 2 – Representative example of VT and HRTV identification. Thresholds occurred at the same physical effort intensity. Arrow indicates the value of $\dot{V}O_2$ in which the thresholds were identified.

A representative example with regard to the VT and HRTV identification of one of the boys involved in the study is found in figure 2. VT occurred at the moment in which $\dot{V}O_2$ reached 25.0 ml/kg/min. The HRTV, according to the three criteria considered, which were convergent in this individual, occurred in $\dot{V}O_2$ identical to the $\dot{V}O_2$ of the VT.

The thresholds of the adolescents evaluated are presented in table 2 in values of $\dot{V}O_2$ (ml/kg/min) and $\dot{V}O_{2peak}$ proportion.

TABLE 2
Average values \pm standard deviations of ventilatory threshold (VT) and HRV (HRTV) thresholds reported in absolute values of $\dot{V}O_2$ (ml/kg/min) and in $\dot{V}O_{2peak}$ proportion (% $\dot{V}O_{2peak}$)

	$\dot{V}O_2$ (ml/kg/min)	% $\dot{V}O_{2peak}$
VT	22.0 \pm 4.1	54.4 \pm 8.8
HRTV (Tulppo <i>et al.</i> criterion)	21.0 \pm 5.6*†	52.4 \pm 12.5*†
HRTV (Lima and Kiss criterion)	22.6 \pm 5.3	57.0 \pm 14.1
HRTV (both criteria)	22.9 \pm 5.3	57.8 \pm 13.8

* Significant difference between HRTV (Tulppo *et al.*) and HRTV (Lima and Kiss) ($0.05 < p < 0.01$).

† Significant difference between HRTV (Tulppo *et al.*) and HRTV (both criteria) ($0.05 < p < 0.01$).

Generally, the criterion proposed by Tulppo *et al.*⁽¹⁶⁾ provided lower values, followed by the criterion proposed by Lima and Kiss⁽¹⁹⁾ and by the conjunction of both criteria. No significant differences occurred between VT and the three criteria to identify HRTV. Significant correlation coefficients were identified ($0.05 < p < 0.01$) between VT and the three criteria to identify HRTV when expressed in absolute values of the $\dot{V}O_2$ (table 3). The correlation coefficients between VT and the three criteria to identify HRTV were not significant when the values were expressed in relation to the $\dot{V}O_{2peak}$.

TABLE 3
Product-moment correlation coefficients between VT and HRTV in absolute values of $\dot{V}O_2$ (ml/kg/min) and in values expressed in relation to $\dot{V}O_{2peak}$

	VT	HRTV (Tulppo <i>et al.</i> criterion)	HRTV (Lima and Kiss criterion)	HRTV (both criteria)
VT	–	0.28	0.28	0.23
HRTV (Tulppo <i>et al.</i> criterion)	0.46*	–	0.80*	0.98*
HRTV (Lima and Kiss criterion)	0.43*	0.82*	–	0.98*
HRTV (both criteria)	0.42*	0.87*	0.97*	–

Right upper triangle correlation coefficients equivalent to values expressed in relation to the $\dot{V}O_{2peak}$.
Left lower triangle correlation coefficients equivalent to values of $\dot{V}O_2$ expressed in absolute unit (ml/kg/min).

* Correlation coefficients statistically significant ($0.05 < p < 0.01$).

DISCUSSION

The present study shows that the quantitative Poincaré plot analysis may be useful for the analysis of the autonomic modulation during incremental physical effort tests. The SD1 index decreased progressively up to a given physical effort intensity, later presenting stabilization tendency. This physical effort intensity was statistically associated with the intensity in which the VT occurred, being expressed as absolute values of $\dot{V}O_2$. However, when the VT was established by means of the $\dot{V}O_{2peak}$ proportions, no significant correlation coefficients were statistically identified.

Traditionally, the HRV has been analyzed through the power spectral density^(20,25), which is a linear mathematical analysis. However, one of the conditions imposed for its performance is stationary condition of the signal recorded^(20,25). When the mechanisms responsible for the variation of the R-R intervals during the recording period remain unchanged, as the case of the rest situation, the stationary condition may be obtained. However, if eventually these mechanisms do not seem to be stable, for example during incremental physical efforts, the results of the spectral analysis generally becomes inconsistent^(26,27). Braun *et al.*⁽³⁴⁾ suggest that the R-R intervals change in a quite complex and apparently erratic way, exhibiting patterns suggestive of non-linear processes. In this context, one proposes that due to the non-linear components, the R-R intervals temporal series cannot be properly analyzed by means of linear methods such as the spectral analysis.

It has been recently showed that several non-linear methods aimed at the HRV analysis in physical effort provide consistent results with regard to autonomic modulation^(15,21,23,24). One of these methods is the quantitative Poincaré plot analysis^(16,17,19,22,29,30). Tulppo *et al.*⁽¹⁶⁾ studied the effects of the parasympathetic blockage on the SD1, SD2 and SD1/SD2 indexes determined based on the Poincaré analysis at rest and during the performance of physical effort. Incremental doses of atropine induced the progressive reduction of SD1, reaching values close to 0 ms after the full blockage. During the performance of physical effort, the SD1 index remained stable. The SD2 index decreased during atropine administration but remained decreasing during the performance of physical effort after full parasympathetic blockage⁽¹⁶⁾. In other study, the moxonidine administration (central-action sympathetic blockade) also induced reductions on the SD2 index⁽³⁰⁾. These results suggest that the SD1 index reflects the parasympathetic modulation

and the SD2 index reflects both the vagal and the sympathetic modulation. Furthermore, after full vagal blockage, the SD1/SD2 ratio increased during physical effort only after intensities above 60% of the maximum $\dot{V}O_2$ ⁽¹⁶⁾, what suggests that the SD1/SD2 ratio is an indicative of the increase on the sympathetic modulation.

The present study showed that the SD1 index decreased progressively from rest up to approximately 55% of the $\dot{V}O_{2peak}$. This result is in agreement with other findings that attribute the increase on the heart rate up to VT especially to the vagal withdrawal^(13,14,16,17,19). From this intensity on, the SD1 index remained reduced, however, it did not present a second plateau afterwards as observed in the study of Lima and Kiss⁽¹⁹⁾, otherwise, it presented slight tendency towards higher values. The SD2 index decreased progressively in a linear way up to the end of the physical effort. This reduction on the SD2 value up to approximately 60% of the $\dot{V}O_{2peak}$ seems to have occurred especially due to the vagal withdrawal^(16,17,22). In higher intensities, the reduction observed may have occurred due to the progressive increase on the sympathetic modulation^(15,35). The SD1/SD2 index initially decreased due to the faster reduction of SD1 in relation to the SD2. From approximately 60% of the $\dot{V}O_{2peak}$, when SD1 remained stable and SD2 remained decreasing, the SD1/SD2 ratio started to increase probably due to the increase on the sympathetic modulation^(16,30).

The relation between VT and HRV was initially suggested by Yamamoto *et al.*⁽¹³⁾. Using the Poincaré analysis, Tulppo *et al.*⁽¹⁶⁾ showed that the vagal modulation is not present above VT. Tulppo *et al.*⁽¹⁷⁾ suggested that the end of the vagal withdrawal occurs when the difference between SD1 and the two consecutive stages reaches value below 1 ms. On the other hand, Lima and Kiss⁽¹⁹⁾ suggest that the progressive decrease on SD1 stops when SD1 reaches values below 3 ms, thus proposing this point as the HRTV identifier. These same authors correlated HRTV with LT, presenting value of $r = 0.76$.

The results found in the present study show that the HRTV determined by means of criteria proposed by Tulppo *et al.*⁽¹⁷⁾ and Lima e Kiss⁽¹⁹⁾ employed separately or conjointly, presented significant correlation with VT when the absolute values of $\dot{V}O_2$ were used. However, no correlations statistically significant were found between HRTV and the VT identification expressed as $\dot{V}O_{2peak}$ proportion. Thus, it seems early to apply the HRVT as an alternative method in the adolescents VT identification.

Despite the amount of studies on VT and LT found in literature, controversies on the physiological mechanisms responsible for the occurrence of thresholds still remain. Wasserman *et al.*⁽³⁾ consider that the VT occurs as a response to the increase of the H^+ and CO_2 plasma concentration as result of the increase on the lactate production. Rowell *et al.*⁽³⁶⁾ discuss other causal mechanisms for the occurrence of VT such as the increase on the K^+ plasma concentration, the elevation of the body temperature, the muscular chemical reflex mediated by afferent fibers III and IV, the increase on the catecholamines plasma concentration, among others. Regardless the cause, the VT theoretically occurs slightly after LT⁽³⁾, once it is a response to metabolites produced by muscular metabolism.

The literature agrees that the increase on the lactate production during exercise occurs due to the disproportion between the glycolysis velocity and the mitochondrial oxidation rate⁽⁴⁾. Mazzeo and Marshall⁽³⁵⁾ found high correlation between LT and the increase on the catecholamines plasma concentration. Considering that catecholamines stimulate glycolysis and glycogenolysis⁽³⁷⁾, the authors suggest that the increase on the sympathetic activity is primary mechanism that generates glycolysis acceleration, thus increasing blood lactate during incremental effort test. However, studies that compared LT in incremental effort with or without the use of β -adrenergic blockade showed that LT occurs regardless the catecholamines influence^(38,39), not corroborating the causal relation between increase on the sympathetic activity and LT.

In the present study, individuals presenting HRTV after VT as well as individuals presenting the opposite response were identified. These results corroborate findings of studies that showed that the relations between catecholamines, blood lactate and ventilation are not always constant to predict a cause-effect direct relation^(5,38,39). However, the literature agrees that the responses are part of the same phenomenon^(4,5).

Considering that the responses for the homeostasis maintenance during physical effort are part of the same phenomenon, the identification of the metabolic transition is justified by several methods. Perhaps the selection of the most suitable method should take into consideration practical aspects such as equipment availability, specificity, reproducibility, accuracy and whether or not the technique is invasive.

The HRV analysis to determine HRTV, besides being a non-invasive and accessible method, provides important information on the autonomic regulation during physical effort. Information available in literature suggest that conditions related to higher parasympathetic activity are associated to higher heart electrical stability^(40,41). Yet, the high sympathetic activity increases the cardiac vulnerability and the risk of cardiovascular events^(42,43). Thus, the HRTV seems to represent the transition between physical effort intensities with higher probability of cardiovascular events.

One of the limitations of the present study was the physical effort test protocol employed. The test was performed in treadmill

using regular protocol in clinical evaluations. However, information available in literature suggest that this is not the ideal protocol for the identification of thresholds⁽⁶⁾. Despite this limiting factor, significant correlation coefficients were found between VT and HRTV.

CONCLUSIONS

Through the results found in the present study it was verified that the quantitative Poincaré plot analysis seems to have been sufficiently sensible to evaluate the autonomic modulation in incremental physical efforts by means of treadmill protocol employed in routine clinical evaluations. However, information gathered in the study suggest that the possible VT identification by means of HRTV is not recommendable, considering the low statistical correlation found between HRV and the VT estimation, expressed as $\dot{V}O_{2peak}$ proportion. Further studies are required in the attempt to improve the identification criteria associated to HRTV and to compare the accuracy of the quantitative Poincaré plot analysis with other non-linear methods in the identification of the muscular metabolism transition.

All the authors declared there is not any potential conflict of interests regarding this article.

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