Exercise Physiology

Intrarater reliability of different methods of heart rate variability threshold analysis and postexercise parasympathetic reactivation in young women

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Abstract - Objective: To evaluate the reliability of HRVT and postexercise parasympathetic reactivation analysis after a submaximal exercise test in young women. **Methods:** Twenty-four young women [21.1 (20.1, 24.7) years; 21.4 (20.1, 23.1) kg/m^2] underwent three incremental exercise tests on a treadmill on occasions separated by 48 h. R-R intervals were continuously recorded during the incremental tests and throughout 5 min of post-exercise active recovery for HRVT and parasympathetic reactivation analysis, respectively. HRVT was identified using two methods: a) the intensity where no significant reduction of SD1 HRV index was identified by visual inspection of the graphic ($HRVT_{visual}$), b) the first stage to present SD1 value < 3ms ($HRVT_{3ms}$). Postexercise parasympathetic reactivation was assessed at each minute during five minutes of recovery using SD1 and r-MSSD indexes. Absolute and relative reliability were assessed using the coefficient of variation (CV) and the intraclass correlation coefficient (ICC), respectively. **Results:** Good (ICC = 0.81, CV = 17.3) and excellent (ICC = 0.90, CV = 4.6) reliability were observed for $HRVT_{3ms}$ and $HRVT_{visual}$, respectively. On the postexercise period, good reliability was observed for both SD1 (ICC = 0.82-89, CV = 22.1-28.9) and r-MSSD (ICC = 0.82-89, CV = 21.1-28.6), with a high correlation between indexes in all-time points of analysis (r = 0.96-0.99). **Conclusions:** HRVT may be reproducibly assessed in women, mainly when $HRVT_{visual}$ is used for analysis. In addition, SD1 and r-MSSD provide reliable and redundant measures of postexercise parasympathetic reactivation.

Keywords: Autonomic nervous system, exercise, reproducibility, heart rate variability.

Introduction

Heart rate variability (HRV) consists of the temporal oscillations between successive heartbeats, defined by the distance between two R waves (R-R interval- iRR)[1]. As previously demonstrated by the pharmacological blockade, HRV reflects primarily the autonomic activity on the sinus node, emerging as a valid and noninvasive method for cardiac autonomic assessment at resting condition, during a dynamic exercise, or in the post-exercise recovery phase[2-4].

During exercise, an exponential reduction of different indices of HRV occurs as a consequence of parasympathetic reduction[4,5]. The point of the incremental exercise test where the HRV is stabilized is commonly called "Heart rate variability threshold" (HRVT) and emerges as a practical alternative to anaerobic threshold determination[6-9]. On the other hand, post-exercise recovery is characterized by a progressive increase in the root mean square of successive differences between the adjacent normal R-R intervals (r-MSSD) due to cardiac parasympathetic reactivation[2]. Hence, in a clinical and experimental setting, the cardiac parasympathetic reactivation is considered an independent predictor for cardiovascular prognostic and is often used to evaluate the effects of different pharmacological and non-pharmacological interventions on the cardiac autonomic nervous system[10-12].

However, although constant recommendations to HRV assessment during and after exercise to training load determination or to assess acute and chronic effects of exercise training on the cardiac autonomic modulation, the knowledge about the reproducibility of this measure, essential for a proper interpretation of results, remain underexplored. It is important to note that HRVT and parasympathetic reactivation are assessed in dynamics conditions, while reproducibility of HRV has been investigated primarily in stationary conditions[13-17]. Thus, the

analysis of HRV reproducibility during and after exercise will provide important advancement in knowledge in the cardiac autonomic modulation field.

Another important current limitation is the lack of studies investigating HRV reproducibility in women. Previous studies assessed HRV reproducibility in men[14,15,18,19] or men and women[20] allocated in the same experimental group. Although the relevant scientific contributions of these studies, it is well established that women present different metabolic, cardiovascular and autonomic response to exercise compared to men, as lower sympathetic activation and responsivity, lower metaboreflex activation, and attenuated increase in heart rate (HR), stroke volume and blood pressure[21-23]. Thus, the sex differences observed on cardiovascular control during exercise becomes indisputable the necessity of HRV reproducibility assessment in an experimental group composed exclusively of women.

Lastly, different methods of HRVT determination are available in the literature. In this scenario, the visual graphical inspection of SD1 or r-MSSD stabilization during exercise and the first stage of incremental test to present values of SD1 less than 3 ms is the criteria classically adopted to HRVT identification[7,19,24]. Since different criteria of HRVT can identify parasympathetic stabilization at different exercise intensities[25], it is necessary to know the reproducibility of these different methods of HRVT analysis and the grade of agreement between them, especially in women.

Considering the aforementioned questions, this study aimed to evaluate the reliability of different methods of HRVT and postexercise parasympathetic reactivation analysis after a submaximal exercise test in young women. Additionally, the agreement between different methods of HRVT identification and the correlation between the different indices used for parasympathetic reactivation analysis were investigated. Based on previous studies with men[19], we hypothesize that good reliability of HRVT and parasympathetic reactivation will be observed in young women.

Materials and Methods

Participants and ethical issues

This is a nonexperimental methodological study[26] conducted on 24 young non-athlete women. As inclusion criteria, the participants should be between 20 and 30 years old, body mass index between 19.9 and 29.9 kg/m^2, use oral contraceptives, do not be diagnosed with any disease, being under drug treatment (except contraceptives), present any limitations that make the exercise test impracticable or reported any chemical dependency (i.e., smoking, alcohol and drugs). The use of oral contraceptives was adopted since it is one of the most prevalent methods for family planning in women of reproductive age[27]. As exclusion criteria, we adopted the failure to comply with the guidelines of the study, pregnancy, high systolic (> 140 mmHg) or diastolic (> 90 mmHg) blood pressure, HR < 50 or > 100 bpm, start drug treatment during the study, or the excessive identification artifacts beats in the iRR segments (> 1%)[4].

The present study agreed with the Brazilian National Research Systems and declaration of Helsinki and was approved by the Institutional Ethical Committee on Human Research. All participants were informed about the study's procedures, risks, and benefits and provided the written informed consent before the tests.

Experimental approach to the problem

All measurements were performed in a quiet laboratory at the same time of the day, between 2:00 and 5:00 p.m. Firstly, before the experimental session, all volunteers were submitted to anthropometrical measures (body mass, height, and abdominal circumference) and clinical anamnesis to ensure inclusion/exclusion criteria. The physical activity level was assessed using the International Physical Activity Questionnaire (IPAQ)[28]. The participants were oriented to abstain from stimulants, alcoholic beverages, medicine, and physical activity for at least 48 h before the experimental procedures.

Baseline data

After 10 min of resting in the supine position, HR and blood pressure were systemically measured using a HR monitor (Polar®, model v800) and auscultatory method, respectively. Two measurements of blood pressure were performed in the right arm of participants, and the average of measurements was calculated. If the difference between measurements was greater than 5 mmHg, a new measurement was performed, and the two measurements with difference < 5 mmHg were considered for analysis. In addition, the subjects remained to breathe spontaneously and had their respiratory rate (RR) visually monitored and counted from the number of chest expansion and retraction over 1 min.

R-R intervals acquisition and processing

All iRR time-series were obtained using a valid HR monitor (Polar® v800, Finland)[29,30]. After iRR segments acquisition, all measures were visually verified, and occasional artifacts were manually or automatically removed using an HRV software (Kubios®, version 3.1, Kuopio, Finland). The automated artifact identification and removal was performed using the threshold method that consists of select iRR that are larger or smaller than 0.45 s (very low), 0.35 s (low), 0.25 s (medium), 0.15 s (strong), or 0.05 s (very strong) compared to average iRR [31]. A filter capable of removing the artifacts without compromising the normal iRRs was selected for individual artifact

correction. All artifact correction and HRV analysis were performed using Kubios HRV software (version 3.3.1, Kuopio, Finland).

Heart rate variability during exercise test

The incremental treadmill exercise test consisted of a 2 min warm-up at a constant load of 3 km/h and 2.5% of slope, followed by the increase of 1 km/h each minute until the participant reached 85% of his maximum HR (HR_{max})[19]. The HR_{max} was predicted by the formula: HR_{max} (bpm) = 208 - 0.7 x age[32].

During the exercise test, the parasympathetic dynamic was assessed using the standard deviation of instantaneous iRR variability perpendicularly to the line of identity in the Poincaré Scatter Plot (SD1)[4]. At each stage of the exercise test, a 60 s iRR segment was analyzed, and the HRVT was defined using 2 different methods: a) the load corresponding to the point of stabilization of HRV assessed by visual graphic evaluation of SD1 dynamic during the exercise test ($HRVT_{visual}$), b) the first stage during exercise test to present SD1 value < 3 ms ($HRVT_{3ms}$) (Figure 1). From a physiological perspective, HRVT assessed by SD1 represents the stabilization of parasympathetic deactivation during an incremental exercise test[4].

To characterize participant's responses to the incremental exercise test were assessed the load corresponding to $HRVT_{visual}$ and $HRVT_{3ms}$, the load reached 85% of HR_{max} ($Load_{peak}$), and the HR observed at end of the incremental exercise test (HR_{peak}). All participants were able to reach the target heart rate during the exercise test. Thus, no tests were interrupted for any other reason.

Post-exercise heart rate variability analysis

After achieving 85% of predicted HR_{max} (HR_{peak}), the exercise protocol was interrupted, and an active 5-minute recovery phase was immediately started, with the speed of the treadmill reduced to 2.4 km/h but keeping the incline at 2.5%, as described previously[33]. Then, the r-MSSD and SD1 indices were calculated at each minute (r-$MSSD_1$ to r-$MSSD_5$ and $SD1_1$ to $SD1_5$) of active recovery to assess the post-exercise parasympathetic reactivation[2,4].

The same researcher conducted all exercise tests. The exercise and postexercise iRR data were analyzed blindly by a researcher with over 9 years of experience in HRV analysis.

Statistical analysis

The normality hypothesis was confirmed for HRVT data but rejected in some post-exercise variables by the Shapiro-Wilk test. Thus, a descriptive statistic was performed using the median and interquartile range, and the nonparametric Friedman test was used for intragroup comparisons. Differences between repeated measures were considered significant when the type I error probability was less than or equal to 5% ($p \leq 0.05$). All HRV data were transformed by taking the natural logarithm before reproducibility analysis. Relative reproducibility was assessed using the intraclass correlation coefficient (ICC) (two-way mixed) and was interpreted as excellent (ICC > 0.90), good (ICC ≥ 0.75 - ≤ 0.90), moderate (ICC = ≥ 0.50 - < 0.75) or poor (ICC < 0.50)[34]. Absolute reproducibility was assessed calculating the coefficient of variation (CV = standard deviation/mean x 100).

The Bland-Altman method was used to verify the agreement between two methods used for HRVT determination ($HRVT_{visual}$ and $HRVT_{3ms}$). However, before Bland-Altman analysis, the absence of correlation between the mean and difference (bias) between methods was confirmed by the Pearson correlation coefficient, an assumption of Bland-Altman analysis. A one-sample t-test

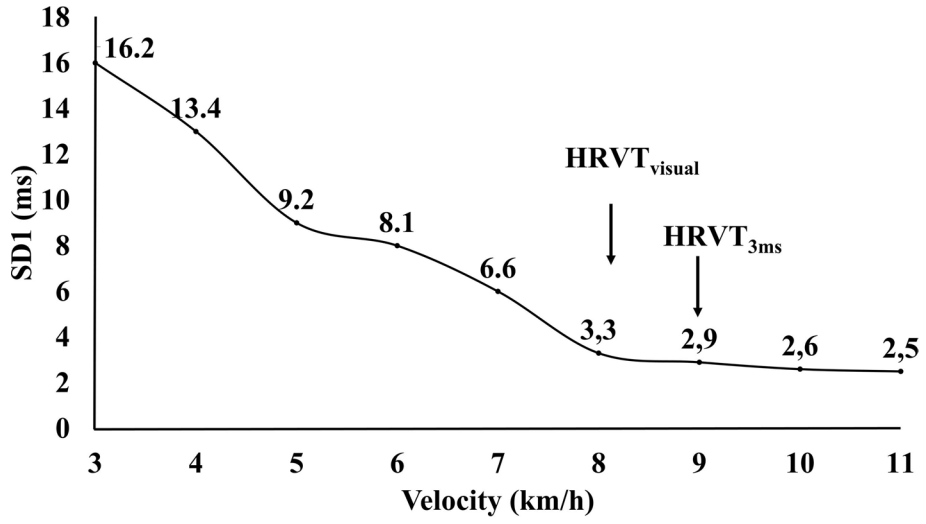

Figure 1 - Representation of heart rate variability threshold determination using visual ($HRVT_{visual}$) and mathematical ($HRVT_{3ms}$) methods.

was used to test the hypothesis that the bias between measurements was zero. Pearson correlation coefficient was used to analyze the correlation between postexercise SD1 and r-MSSD indices. Agreement and correlation analysis was performed using data obtained at the first trial.

Considering an effect size of 0.30 and an alpha error of 0.05 was identified a power of 0.88 to identify possible differences between trials in this study. The statistical power calculation and statistical analysis were performed using the software GPower (v.3.7.1) and the Statistical Package for the Social Sciences (SPSS-v.21), respectively. Figure composition was performed using Graphpad Prism software (Graphpad®, version 5.0).

Results

The participants of this study aged 21.1 (20.1, 24.7) years and had body mass index of 21.4 (20.1, 23.1) kg/m^2. Considering the physical activity level, 66.6% (n = 16) of participants were active, 20.8% (n = 5) minimally active, and 12.5% (n = 3) very active. No difference between trials were observed for resting HR (p = 0.98), RR (p = 0.42), SBP (p = 0.64) or DBP (p = 0.75) (Table 1). Similarly, no differences between trials were identified for the load corresponding to $HRVT_{visual}$ (p = 0.92) and $HRVT_{3ms}$ (p = 0.37), $Load_{peak}$ (p = 0.72) HR_{peak} (p = 0.61), or for any measure of parasympathetic reactivation [r-$MSSD_1$ to r-$MSSD_5$ (p = 0.11-0.51), $SD1_1$ to $SD1_5$ (p = 0.11 to 0.19)] during active recovery phase (Table 2).

In the reliability analysis, ICCs of 0.90 and 0.81 were identified for $HRVT_{visual}$ and $HRVT_{3ms}$, respectively. Considering the postexercise HRV analysis, the ICCs ranged from 0.82 to 0.89 for r-MSSD and SD1 indices (Table 3).

No significant bias was observed between $HRVT_{visual}$ and $HRVT_{3ms}$ methods (p = 0.38) (Figure 2a). Lastly, high correlations (r = 0.96-0.99) were observed between SD1 and r-MSSD indices assessed at all time points of the post-exercise active recovery phase (Figure 2 panels b-f).

Discussion

Confirming the initial hypothesis of this study, good to excellent reliability of HRVT analysis was observed,

Table 1 - Median and interquartile range of hemodynamic and respiratory variables were assessed on three occasions.

Variables	Trial 1	Trial 2	Trial 3	p	ES
$HR_{resting}$ (bpm)	70 (65, 80)	74 (62, 80)	70 (65, 74)	0.98	0.20
$RR_{resting}$ (Cycles/min)	17 (16, 19)	18 (17, 21)	17 (16, 21)	0.42	< 0.10
$SBP_{resting}$ (mmHg)	112 (104, 120)	110 (106, 120)	108 (103, 122)	0.64	0.13
$DBP_{resting}$ (mmHg)	70 (66, 78)	64 (60, 80)	70 (59, 78)	0.75	0.15

Nonparametric Friedman Test. HR= heart rate, RR= respiratory rate, SBP= systolic blood pressure, DBP= diastolic blood pressure.

Table 2 - Median and interquartile range of heart rate variability thresholds and parasympathetic reactivation analysis over the post-exercise active recovery phase.

Variables	Trial 1	Trial 2	Trial 3	p	ES
Vel_{peak} (km/h)	9 (8, 10)	9 (8, 10)	9 (8, 10)	0.72	<0.10
HRpeak (bpm)	163 (160, 164)	163 (160, 164)	163 (161, 164)	0.61	<0.10
$HRVT_{visual}$ (km/h)	7 (7, 8)	7.5 (7, 8)	7.5 (7, 9)	0.92	0.19
$HRVT_{<3}$ (km/h)	7 (7, 8)	8 (7, 9)	8 (7, 9)	0.37	<0.10
$SD1_1$ (ms)	1.6 (2.3, 3.3)	1.9 (2.5, 3.7)	1.9 (2.8, 4.1)	0.11	<0.10
$SD1_2$ (ms)	2.9 (3.6, 6.8)	3.3 (5.3, 8.4)	2.7 (5.8, 7.9)	0.16	<0.10
$SD1_3$ (ms)	3.1 (4.8, 7.1)	4.1 (5.7, 8.8)	2.9 (6.1, 8.4)	0.19	<0.10
$SD1_4$ (ms)	3.1 (4.3, 7.3)	3.7 (6.5, 7.9)	2.9 (6.6, 8.2)	0.16	<0.10
$SD1_5$ (ms)	3.3 (4.5, 7.4)	3.9 (5.8, 8.7)	3.2 (6.1, 8.1)	0.18	<0.10
r-$MSSD_1$ (ms)	2.2 (3.3, 5.3)	2.7 (3.5, 5.3)	2.7 (3.9, 5.6)	0.51	0.10
r-$MSSD_2$ (ms)	4.1 (5.1, 9.6)	4.7 (7.5, 11.9)	3.9 (8.1, 11.1)	0.31	<0.10
r-$MSSD_3$ (ms)	4.4 (6.7, 9.8)	5.8 (8.1, 12.5)	4.1 (8.4, 11.8)	0.16	<0.10
r-$MSSD_4$ (ms)	4.3 (6.1, 10.3)	5.2 (9.1, 11.2)	4.1 (9.3, 11.7)	0.16	<0.10
r-$MSSD_5$ (ms)	4.5 (6.1, 10.5)	5.5 (8.2, 12.3)	4.9 (8.6, 11.2)	0.11	<0.10

Nonparametric Friedman test. $HRVT_{visual}$= Heart rate variability threshold assessed using the visual method, $HRVT_{3ms}$= Heart rate variability threshold assessed using the mathematical method, SD1= standard deviation of instantaneous iRR variability perpendicular to the line of identity in the Poincaré Scatter Plot, r-MSSD= root mean square of successive differences between normal R-R intervals, 1-5= First to the fifth minute of active recovery.

Table 3 - Absolute (CV) and relative (ICC) reliability of different methods of heart rate variability threshold and parasympathetic reactivation analysis over the post-exercise active recovery phase.

	ICC (IC 95%)	CV% (IC 95%)
$HRVT_{visual}$ (km/h)	0.90 (0.81, 0.95)	4.6 (−6.1, 15.2)
$HRVT_{3ms}$ (km/h)	0.81 (0.61, 0.91)	17.3 (−28.2, 62.84)
$SD1_1$ (ms)	0.89 (0.78, 0.94)	22.1 (−3.51, 47.53)
$SD1_2$ (ms)	0.84 (0.69, 0.92)	28.3 (−8.43, 64,9)
$SD1_3$ (ms)	0.84 (0.70, 0.93)	28.9 (1.84, 55.2)
$SD1_4$ (ms)	0.82 (0.65, 0.91)	25.5 (−4.58, 55.5)
$SD1_5$ (ms)	0.88 (0.76, 0.94)	22.9 (0.8, 44.8)
r-$MSSD_1$ (ms)	0.89 (0.78, 0.95)	21.1 (−4.66, 46.5)
r-$MSSD_2$ (ms)	0.84 (0.69, 0.92)	28.6 (−7.92, 65)
r-$MSSD_3$ (ms)	0.84 (0.70, 0.93)	28.6 (2, 55.1)
r-$MSSD_4$ (ms)	0.82 (0.65, 0.91)	25.6 (−4.49, 55.6)
r-$MSSD_5$ (ms)	0.87 (0.74, 0.94)	23.9 (−0, 47.8)

ICC= intraclass correlation coefficient, CV= coefficient of variation, $HRVT_{Visual}$= heart rate variability threshold assessed using the visual method, $HRVT_{3ms}$= Heart rate variability threshold assessed using the mathematical method, SD1= standard deviation of instantaneous iRR variability perpendicular to the line of identity in the Poincaré Scatter Plot, r-MSSD = root mean square of successive differences between normal R-R intervals, 1-5= First to the fifth minute of active recovery.

with a high value of ICC and low CV observed when the visual method was used for HRVT analysis. Additionally, good reliability was observed on all post-exercise HRV indices obtained from the first to the fifth minute of active recovery, regardless of the index used for parasympathetic reactivation analysis (r-MSSD or SD1). In the secondary analysis, non-significant bias and large limits of agreement were observed between $HRVT_{visual}$ and $HRVT_{3ms}$ methods, and a strong correlation was observed between post-exercise SD1 and r-MSSD analysis.

Our results corroborated with previously reported data by Cruz et al. (2017)[19]. The authors observed high reliability of HRVT, assessed using the visual method, in a sample of young men submitted to an identical incremental exercise test protocol (ICC = 0.92; 0.82 to 0.97). Furthermore, the relative reliability observed in our study is close to the observed in healthy subjects submitted to an incremental shuttle walk test (ICC = 0.92; 0.82 to 0.96)[35]. Thus, these data indicate good to excellent reliability of HRVT assessed on a treadmill regardless of gender.

It is important to note that lower reliability was observed for $HRVT_{3ms}$ than $HRVT_{visual}$ in the present study. As didactically demonstrated by Cruz et al. (2019)[25], and shown in Figure 1, a small variation (< 1 ms) in the SD1 is sufficient to change the stage corresponding to $HRVT_{3ms}$ (i.e., 3.3 to 2.9) despite visible stabilization of this index, which may explain the lower reproducibility of this method identified in our study. In fact, higher reproducibility of $HRVT_{visual}$ compared to mathematical methods was also confirmed in young men exercising on a cycle ergometer[18,25]. Thus, despite the advantage of reducing the possible influence of the evaluator's expectation on the HRVT analysis, these results indicate that the mathematical methods commonly used in HRVT assessment need to be carefully interpreted.

Despite the non-significant bias, large limits of agreement (~2 km/h) were observed between $HRVT_{visual}$ and $HRVT_{3ms}$. Thus, since both $HRVT_{visual}$ and $HRVT_{3ms}$ are recommended as practical alternatives to estimate the lactate or ventilatory thresholds[7,24], it is necessary to identify which of these measures best represent the anaerobic threshold in future studies. In this sense, Queiroz et al. (2018)[7] observed similar limits of agreement for $HRVT_{3ms}$ and $HRVT_{visual}$ when contrasted with the oxygen consumption at ventilatory threshold (despite lower bias on $HRVT_{3ms}$ method) in overweight and obese young individuals. However, the best method of HRVT analysis for parasympathetic stabilization or anaerobic threshold analysis remains underexplored, mainly in women.

Good reproducibility was observed for r-MSSD and SD1 assessed in all-time points of postexercise active recovery. These results are partially agreeing with previous findings in young men, where moderate to excellent reliability (ICC = 0.69 to 0.95) of r-MSSD was observed throughout five minutes of recovery walking on a treadmill after a submaximal exercise test[19]. On the other hand, large random variation was previously observed for HRV analysis from the fifth to the tenth minute of passive recovery after a maximal exercise test[36], suggesting that postexercise HRV reliability may be protocol-dependent.

Another aspect that should be observed is the similarity of ICCs and CVs for r-MSSD and SD1 and the high correlations observed between these indices in all post-exercise HRV analyses. Corroborating with Ciccone et al. (2017)[37], these data suggest that SD1 and r-MSSD indices provide reproducible and redundant information about the post-exercise parasympathetic dynamic. Notably, Cruz et al. (2017)[19] observed an identical dynamic of SD1 and r-MSSD during an incremental exercise test, confirming the hypothesis of redundancy between these indices. Thus, these data indicate that resting, exercise, and post-exercise parasympathetic dynamics can be sufficiently assessed using a single HRV index (SD1 or r-MSSD).

Absolute reliability demonstrates a relative variation from 21 to 28.9% of the HRV analysis in the recovery phase; this aspect should be considered when comparing groups or the effects of different interventions on the post-exercise cardiac parasympathetic modulation. However, as previously discussed[14], the relative analysis of HRV indices can overvalue a low absolute variation and should be cautiously interpreted. In this scenario, despite large relative variation for r-MSSD and SD1 in the postexercise phase observed in the present study, in absolute terms, it corresponds to less than 1 ms, which may not be clinically or functionally relevant.

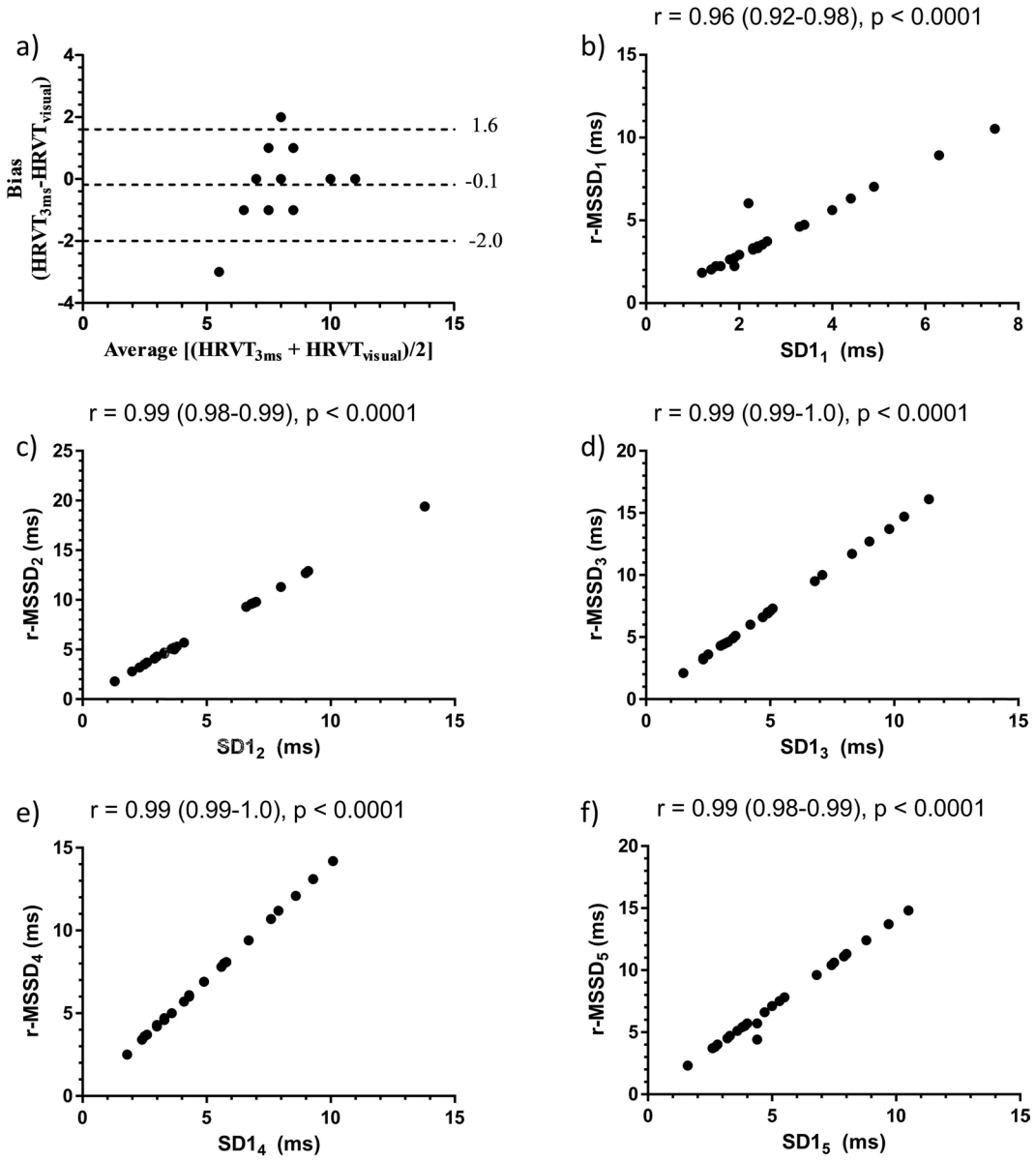

Figure 2 - Agreement between $HRVT_{visual}$ and $HRVT_{3ms}$ (a) and correlations (95% confidence intervals) between r-MSSD and SD1 indices assessed at 1st (b), 2nd (c), 3rd (d), 4th (e), and 5th (f) minute of postexercise active recovery. Pearson correlation coefficient (r, $p< 0.05$).

The identification of HRVT is an important approach in clinical and exercise settings. It has been shown that the intensity corresponding to parasympathetic stabilization during exercise coincides with different invasive and/or expensive traditional indicators of the anaerobic threshold[7,9,24], an important predictor of exercise performance[38], and one of the most robust parameters for individualizing de cardiorespiratory training[39]. From a clinical perspective, HRVT allows the assessment of the effects of different pharmacological and nonpharmacological interventions on the cardiac parasympathetic dynamic during exercise, an important approach due to the antiarrhythmic effect of cardiac parasympathetic activation[40]. In this scenario, our results provide innovative information indicating that HRVT can be reproducibly assessed in young women, an important methodological criterion poorly explored so far.

Postexercise cardiac HRV analysis has been used to investigate the acute and chronic effects of exercise training and nutritional interventions on cardiac autonomic responsivity. However, although the validity of this approach has already been confirmed in studies using pharmacological blockade[4], the reliability studies have been limited to samples composed of men or men and women allocated in the same experimental group. Thus, the present study adds important information to current

literature demonstrating a good reproducibility of SD1 and r-MSSD analysis, proxy measures of parasympathetic reactivation during the postexercise active recovery in young women.

A detailed description of sex differences in cardiovascular responses to exercise is beyond the scope of this study, but some mechanisms need to be highlighted. It has been demonstrated that women present attenuated increase cardiovascular and autonomic response during exercise. These differences are partially attributed to the attenuated increase in sympathetic nerve activity and responsivity, lower cardiovascular sensibility to catecholamines, and blunted exercise-induced mechanoreflex and metaboreflex activation[22]. From a mechanistic perspective, it has been proposed that estrogen reduces the sensibility and expression of alpha-1 receptors (vasoconstrictors), increases the sensitivity of beta-adrenergic receptors (vasodilators), and reduces the sympathetic innervation[22]. Thus, the analysis of HRVT and cardiac parasympathetic reactivation in a group composed exclusively of pre-menopausal women was an important and necessary approach adopted in this study and needs to be considered in future investigations.

The main limitations of this study are the sample restricted to users of oral contraceptives and the absence of lactate or ventilatory threshold analysis. Since HRVT coincides with different methods of anaerobic threshold determination[7-9], it is plausible to infer that the reliability of HRVT may be dependent on the participants' aerobic capacity. On the other hand, our results add important information to current literature since the reliability of different methods of HRVT determination and postexercise indices of HRV were investigated. Additionally, under our knowledge, this is the first study to investigate the reliability of HRVT and postexercise parasympathetic reactivation in a sample composed exclusively of young women.

From future perspectives, we highlight the importance of investigating the reliability of different methods of HRV analysis during and after exercise in men and women, conditions in which the effect of different interventions on the cardiac autonomic nervous system are commonly investigated. In addition, it is important to know if variables as age, cardiorespiratory fitness level, health, nutritional status, anthropometrical profile, and the environment of iRR acquisition might influence HRV reliability.

Conclusion

We conclude that HRVT may be reproducibly assessed in young women during incremental tests on the treadmill, mainly when the visual method identifies cardiac parasympathetic stabilization. On postexercise HRV analysis, good and similar reproducibility was observed for r-MSSD and SD1 indices, showing that both markers allow a reproducible analysis of postexercise parasympathetic reactivation in women. Lastly, the high correlation observed between SD1 and r-MSSD indicates that these measures provide redundant information about the postexercise parasympathetic dynamic.

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References

1. Ferreira M Jr, Zanesco CL. Heart rate variability an important approach for assessing autonomic modulation. Motriz: J Phys Ed. 2016;22:3-8.
2. Goldberger JJ, Le FK, Lahiri M, Kannankeril PJ, Ng J, Kadish AH. Assessment of parasympathetic reactivation after exercise. Am J Physiol Heart Circ Physiol. 2006;290:2446-52.
3. Polanczyk CA, Rohde LE, Moraes RS, Ferlin EL, Leite C, Ribeiro JP. Sympathetic nervous system representation in time and frequency domain indices of heart rate variability. Eur J Appl Physiol Occup Physiol. 1998;79:69-73.
4. Tulppo MP, Makikallio TH, Takala TE, Seppanen T, Huikuri HV. Quantitative beat-to-beat analysis of heart rate dynamics during exercise. Am J Physiol. 1996;271:244-52.
5. Tulppo MP, Hautala AJ, Makikallio TH, Laukkanen RT, Nissila S, Hughson RL, et al. Effects of aerobic training on heart rate dynamics in sedentary subjects. J Appl Physiol. 2003;95:364-72.
6. Leprêtre PM, Bulvestre M, Ghannem M, Ahmaidi S, Weissland T, Lopes P. Determination of ventilatory threshold using heart rate variability in patients with heart failure. Surgery. 2013;12:2-6.
7. Queiroz MG, Arsa G, Rezende DA, Sousa LCJL, Oliveira FR, Araújo GG, et al. Heart rate variability estimates ventilatory threshold regardless body mass index in young people. Sci sports. 2018;33:39-46.
8. Shiraishi Y, Katsumata Y, Sadahiro T, Azuma K, Akita K, Isobe S, et al. Real-Time Analysis of the Heart Rate Variability During Incremental Exercise for the Detection of the Ventilatory Threshold. J Am Heart Assoc. 2018;7:1-12.
9. Vasconcellos F, Seabra A, Montenegro R, Cunha F, Bouskela E, Farinatti P. Can heart rate variability be used to estimate gas exchange threshold in obese adolescents? Int J Sports Med. 2015;36:654-60.
10. Duarte A, Soares PP, Pescatello L, Farinatti P. Aerobic training improves vagal reactivation regardless of resting vagal control. Med Sci Sports Exerc. 2015;47:1159-67.
11. Michael S, Jay O, Graham KS, Davis GM. Longer exercise duration delays post-exercise recovery of cardiac parasympathetic but not sympathetic indices. Eur J Appl Physiol. 2017;117:1897-906.
12. Rolim PS, Matos RAC, Soares EMKK, Molina GE, Cruz CJG. Caffeine increases parasympathetic reactivation without altering resting and exercise cardiac parasympathetic

modulation: A balanced placebo design. Eur J Sports Sci. 2019; 19:490-8.
13. Cipryan L, Litschmannova M. Intra-day, and inter-day reliability of heart rate variability measurement. J Sports Sci. 2013;31:150-8.
14. Cruz CJG, Porto LGG, Silva Rolim P da, Souza Pires D de, Garcia GL, Molina GE. Impact of heart rate on the reproducibility of heart rate variability analysis in the supine and standing positions in healthy men. Clinics. 2019;74:1-6.
15. Nakamura FY, Pereira LA, Esco MR, Flatt AA, Moraes JE, Cal Abad CC, et al. Intra- and inter-day reliability of ultra-short-term heart rate variability in rugby union players. J Strength Cond Res. 2017;31:548-51.
16. Sandercock GR, Bromley PD, Brodie DA. The reliability of short-term measurements of heart rate variability. Int J Cardiol. 2005;103:238-47.
17. Silva CC, Bertollo M, Reichert FF, Boullosa DA, Nakamura FY. Reliability of Heart Rate Variability in Children: Influence of Sex and Body Position During Data Collection. Pediatr Exerc Sci. 2017;29:228-36.
18. Candido N, Okuno NM, Silva CC da, Machado FA, Nakamura FY. Reliability of the Heart Rate Variability Threshold using Visual Inspection and Dmax Methods. Int J Sports Med. 2015;36(13):1076-80.
19. Cruz CJG, Rolim PS, Pires DS, Mendes CMO, Paula GM de Porto LGG, et al. Reliability of heart rate variability threshold and parasympathetic reactivation after a submaximal exercise test. Motriz: J Phys Ed. 2017;23:65-70.
20. Pinna GD, Maestri R, Torunski A, Danilowicz-Szymanowicz L, Szwoch M, La Rovere MT, et al. Heart rate variability measures: a fresh look at reliability. Clin Sci (Lond). 2007;113:131-40.
21. Koenig J, Thayer JF. Sex differences in healthy human heart rate variability: A meta-analysis. Neurosci Biobehav Rev. 2016;64:288-310.
22. Smith JR, Koepp KE, Berg JD, Akinsanya JG, Olson TP. Influence of Sex, Menstrual Cycle, and Menopause Status on the Exercise Pressor Reflex. Med Sci Sports Exerc. 2019;51:874-81.
23. Wheatley CM, Snyder EM, Johnson BD, Olson TP. Sex differences in cardiovascular function during submaximal exercise in humans. Springerplus. 2014;3:1-13.
24. Sales MM, Campbell CS, Morais PK, Ernesto C, Soares-Caldeira LF, Russo P, et al. Noninvasive method to estimate anaerobic threshold in individuals with type 2 diabetes. Diabetol Metab Syndr. 2011;3:1-8.
25. Cruz CJG, Porto LGG, Silva RAS, Molina GE. Is a short-stage protocol during an incremental exercise test reliable for heart rate variability threshold analysis? Motriz: J Phys Ed. 2019;25:1-7.
26. Page P. Research designs in sports physical therapy. Int J Sports Phys Ther. 2012;7:482-92.
27. United Nations, Department of Economic and Social Affairs, Population Division. Contraceptive use by method 2019: data booklet; 2019.
28. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381-95.
29. Caminal P, Sola F, Gomis P, Guasch E, Perera A, Soriano N, et al. Validity of the Polar V800 monitor for measuring heart rate variability in mountain running route conditions. Eur J Appl Physiol. 2018;118:669-77.
30. Giles D, Draper N, Neil W. Validity of the Polar V800 heart rate monitor to measure RR intervals at rest. Eur J Appl Physiol. 2016;116:563-71.
31. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO, Karjalainen PA. Kubios HRV—heart rate variability analysis software. Computer Meth Progr Biomed. 2014;113:210-20.
32. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. J Am Coll Cardiol. 2001;37:153-6.
33. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. N Engl J Med. 1999;341:1351-7.
34. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med. 2016;15:155-63.
35. Dourado VZ, Guerra RL. Reliability and validity of heart rate variability threshold assessment during an incremental shuttle-walk test in middle-aged and older adults. Braz J Med Biol Res. 2013;46:194-9.
36. Dupuy O, Mekary S, Berryman N, Bherer L, Audiffren M, Bosquet L. Reliability of heart rate measures used to assess post-exercise parasympathetic reactivation. Clin Physiol Funct Imaging. 2012;32:296-304.
37. Ciccone AB, Siedlik JA, Wecht JM, Deckert JA, Nguyen ND, Weir JP. Reminder: RMSSD and SD1 are identical heart rate variability metrics. Muscle & Nerve. 2017;56:674-8.
38. Borszcz FK, Tramontin AF, Souza KM de, Carminatti LJ, Costa VP. Physiological Correlations With Short, Medium, and Long Cycling Time-Trial Performance. Res Q Exerc Sport. 2018;89:120-5.
39. Pymer S, Nichols S, Prosser J, Birkett S, Carroll S, Ingle L. Does exercise prescription based on estimated heart rate training zones exceed the ventilatory anaerobic threshold in patients with coronary heart disease undergoing usual-care cardiovascular rehabilitation? A United Kingdom perspective. Eur J Prev Cardiol. 2020;27:579-89.
40. Ng GA. Vagal modulation of cardiac ventricular arrhythmia. Exp Physiol. 2014;99:295-9.

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