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Ultra-short heart rate variability recording reliability: The effect of controlled paced breathing

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Abstract

Background: Recent studies have reported that Heart Rate Variability (HRV) indices remain reliable even during recordings shorter than 5 min, suggesting the ultra-short recording method as a valuable tool for autonomic assessment. However, the minimum time-epoch to obtain a reliable record for all HRV domains (time, frequency, and Poincare geometric measures), as well as the effect of respiratory rate on the reliability of these indices remains unknown.

Methods: Twenty volunteers had their HRV recorded in a seated position during spontaneous and controlled respiratory rhythms. HRV intervals with 1, 2, and 3 min were correlated with the gold standard period (6-min duration) and the mean values of all indices were compared in the two respiratory rhythm conditions.

Results: rMSSD and SD1 were more reliable for recordings with ultra-short duration at all time intervals (r values from 0.764 to 0.950, $p < 0.05$) for spontaneous breathing condition, whereas the other indices require longer recording time to obtain reliable values. The controlled breathing rhythm evokes stronger r values for time domain indices (r values from 0.83 to 0.99, $p < 0.05$ for rMSSD), but impairs the mean values replicability of domains across most time intervals. Although the use of standardized breathing increases the correlations coefficients, all HRV indices showed an increase in mean values (t values from 3.79 to 14.94, $p < 0.001$) except the RR and HF that presented a decrease ($t = 4.14$ and 5.96 , $p < 0.0001$).

Conclusion: Our results indicate that proper ultra-short-term recording method can provide a quick and reliable source of cardiac autonomic nervous system assessment.

KEYWORDS

autonomic nervous system, cardiac autonomic regulation, controlled paced breathing, heart rate variability, rMSSD, ultra-short-term recording

1 | INTRODUCTION

Heart rate variability (HRV) is a commonly noninvasive autonomic biomarker used in clinical protocols (Beauchaine & Thayer, 2015; Task Force, 1996; Thayer & Lane, 2009; Thayer, Åhs, Fredrikson, Sollers, & Wager, 2012). The quantitative values obtained by

R-R intervals analysis has reliability to distinguish patients with obesity (Iliescu, Tudorancea, Irwin, & Lohmeier, 2013; Yadav et al., 2017), diabetes (Arroyo-Carmona et al., 2016; Dimitropoulos, 2014), psychiatric disorders (Chalmers, Quintana, Abbott, & Kemp, 2014), and others specific situations (for a review see Thayer & Lane, 2009; Thayer et al., 2012;), when the impairment of vagal

tone regulation is reported as a common biomarker for these clinical conditions (Laborde, Mosley, & Thayer, 2017; Thayer & Lane, 2000, 2009). In order to obtain reliable data for the HRV quantitative analysis, it is recommended to carry out recordings with 5 min or longer, according to the research design (Laborde et al., 2017; Task Force, 1996).

Recent studies have reported that some time-domain HRV indices remain reliable even in recordings shorter than 5 min, proposing the ultra-short recording method (Esco & Flatt, 2014; Nakamura et al., 2015; Thong, Li, McNames, Aboy, & Goldstein, 2003; Voss, Schulz, Schroeder, Baumert, & Caminal, 2009). In these experiments, the comparison of HRV indices, such as root mean square of standard deviation (rMSSD) or standard deviation of RR interval (SDNN), showed strong correlations between random periods shorter than 1 min (10, 30, and 60 s) and standard gold intervals (longer than 5 min; Munoz et al., 2015; Nussinovitch et al., 2011). The reliability of ultra-short recordings goes beyond measures at rest, being stable also to discriminate autonomic adaptability to physical training in elite athletes (Nakamura et al., 2015; Pereira, Flatt, Ramirez-Campillo, Loturco, & Nakamura, 2016).

The time-domain reliability is not replicated to the frequency domain indices, this may occur because most studies do not control the participants' breathing rhythm (Munoz et al., 2015; Nussinovitch et al., 2011). The interindividual changes in respiratory rate during long-term recording may impair frequency domain analysis based on the Fast Fourier Transform (FFT), so the alteration of HRV oscillatory morphology may produce inconstant data segments for analysis (Nussinovitch et al., 2011). To avoid this effect, some researchers have performed HRV recordings with standardized respiratory rhythms by using breathing pacer (Flatt & Esco, 2013).

Although breathing pacer can standardize heart rate oscillations, there are no evidences of its effects in data reliability improvement during ultra-short recordings. In this sense, the aims of this study were: (a) To analyze ultra-short recordings reliability including time, frequency, and Poincare geometric measures domains at different time intervals (1, 2, and 3 min) compared to the gold standard interval; and, (b) To compare the reliability of these indices in the

spontaneous and controlled breathing condition (6 breaths/min) besides also evaluate the effects of controlled breathing in HRV indices.

2 | METHODS

2.1 | Participants

HRV recordings were carried out in 20 healthy male volunteers, aged between 18–28 years old (23.65 ± 3.24). The following exclusion criteria were applied: (a) Diagnostic of cardiac arrhythmia; (b) The intake of caffeine or energetic drinks three hours prior to data collection; (c) Alcoholic drinking 24 hr before the experiment; (d) Sleep deprivation in the night before the research; (e) Report of discomfort when performing the 6 breaths/min breathing rate. This study was approved by a local Ethics Committee, and was conducted according to the Helsinki Declaration. All participants were informed of experimental risks and research benefits, and signed an informed consent form agreeing with the procedures.

2.2 | Physiologic recording

Participants' chest skin was cleaned with isopropyl alcohol (70%) skin swab, followed by the fixation of a comfortable elastic straps to the chest for the acquisition of RR intervals (iRR, in ms) by a PolarH7 heart rate monitor (Polar, Finland). All acquisitions have occurred in the period between 9 and 12 hr in a bright and quiet room. The resting baseline was made in sitting position, followed by a breathing paced condition (6 breaths/min), both periods were 6 min long.

The Kubios software (v.2.2) (Tarvainen, Niskanen, Lipponen, & Ranta-aho, & Karjalainen, 2014) was used to analyze the RR intervals data and artifacts that were manually filtered by visual inspection. Ectopic beats and artifacts were automatically detected and replaced by interpolated adjacent beats by applying an adaptive filter to generate normal-to-normal (NN) interval time series (as seen in Abad et al., 2017; Nakamura et al., 2015; Pereira et al., 2016; Tarvainen et al., 2014). HRV was analyzed in time, frequency and Poincare geometric

FIGURE 1 Timeline of epochs extraction. The numbers represent the time interval (1, 2, and 3 min) and the letters an analysis period (A, first epoch; B, second epoch; C, third epoch; D, fourth epoch; E, fifth epoch; F, sixth epoch)

TABLE 1 Pearson correlation results (Bonferroni corrected *p*-values) between all-time intervals (1, 2, and 3 min) and the gold standard period (6 min) for spontaneous breathing condition

Heart rate variability indices	1A	1B	1C	1D	1E	1F	2A	2B	2C	3A	3B
RR	0.885* (0.001)	0.894* (0.001)	0.981* (0.001)	0.945* (0.001)	0.927* (0.001)	0.864* (0.001)	0.298 (1.0)	0.984* (0.001)	0.926* (0.001)	0.972* (0.001)	0.958* (0.001)
SDNN	0.822* (0.001)	0.448 (1.0)	0.598 (0.38)	0.543 (0.98)	0.514 (1.0)	0.577 (0.45)	0.826* (0.001)	0.676 (0.08)	0.775* (0.001)	0.841* (0.001)	0.798* (0.001)
rMSSD	0.891* (0.001)	0.919* (0.001)	0.875* (0.001)	0.906* (0.001)	0.890* (0.001)	0.768* (0.001)	0.934* (0.001)	0.950* (0.001)	0.890* (0.001)	0.960* (0.001)	0.924* (0.001)
pNN50	0.842* (0.001)	0.917* (0.001)	0.855* (0.001)	0.813* (0.001)	0.810* (0.001)	0.472 (1.0)	0.920* (0.001)	0.901* (0.001)	0.869* (0.001)	0.958* (0.001)	0.874* (0.001)
LF	0.291 (1.0)	0.569 (0.5)	0.829* (0.001)	0.101 (1.0)	0.275 (1.0)	0.605 (0.29)	0.281 (1.0)	0.729* (0.001)	0.544 (0.96)	0.637 (0.15)	0.436 (1.0)
HF	0.777* (0.001)	0.546 (0.95)	0.826* (0.001)	0.387 (1.0)	0.223 (1.0)	0.448 (1.0)	0.761* (0.001)	0.879* (0.001)	0.398 (1.0)	0.900* (0.001)	0.443 (1.0)
LF:HF	0.512 (1.0)	0.740* (0.001)	0.690* (0.02)	0.346 (1.0)	0.525 (1.0)	0.005 (1.0)	0.905* (0.001)	0.639 (0.31)	0.686* (0.01)	0.952* (0.001)	0.538 (0.65)
SD1	0.869* (0.001)	0.921* (0.001)	0.867* (0.001)	0.965* (0.001)	0.905* (0.001)	0.764* (0.003)	0.933* (0.001)	0.932* (0.001)	0.869* (0.001)	0.955* (0.001)	0.884* (0.001)
SD2	0.776* (0.001)	0.377 (1.0)	0.440 (1.0)	0.426 (1.0)	0.415 (1.0)	0.487 (1.0)	0.766* (0.004)	0.508 (1.0)	0.644 (0.15)	0.647 (0.15)	0.548 (0.72)
D2	0.623 (0.21)	0.433 (0.42)	0.628 (0.70)	0.796* (0.001)	0.351 (1.0)	0.307 (1.0)	0.698* (0.04)	0.825* (0.001)	0.463 (1.0)	0.886* (0.001)	0.457 (1.0)

Notes. (Number and Letter) The numbers represent the time interval (1, 2, and 3 min) and the letters an analysis period (A, first epoch; B, second epoch; C, third epoch; D, fourth epoch; E, fifth epoch; F, sixth epoch); D2 correlation dimension; HF: high frequency (0.15–0.4 Hz); HR: heart rate; LF: low frequency (0.04–0.15 Hz); LF:HF: low frequency-to-high frequency ratio; pNN50: % of RR intervals with difference in successive RR intervals longer than 50 ms; RR: mean of RR intervals; rMSSD: root mean square of standard deviation; SD: standard deviations; SD1: perpendicular points dispersion to the line of instantaneous identity; SD2: points dispersion of the along the long-term identity line; SDNN: standard deviation of RR interval.

**p* < 0.05 for Bonferroni corrected Pearson correlation analysis.

TABLE 2 Pearson correlation results (Bonferroni corrected *p*-values) between all-time intervals (1, 2, and 3 min) and the gold standard period (6 min) for controlled breathing condition

Heart rate variability	1A	1B	1C	1D	1E	1F	2A	2B	2C	3A	3B
RR	0.950* (0.001)	0.977* (0.001)	0.969* (0.001)	0.966* (0.001)	0.972* (0.001)	0.933* (0.001)	0.983* (0.001)	0.963* (0.001)	0.970* (0.001)	0.991* (0.001)	0.986* (0.001)
SDNN	0.923* (0.001)	0.923* (0.001)	0.926* (0.001)	0.895* (0.001)	0.785* (0.001)	0.704* (0.03)	0.952* (0.001)	0.941* (0.001)	0.850* (0.001)	0.980* (0.001)	0.920* (0.001)
rMSSD	0.905* (0.001)	0.904* (0.001)	0.921* (0.001)	0.987* (0.001)	0.849* (0.001)	0.825* (0.001)	0.955* (0.001)	0.970* (0.001)	0.906* (0.001)	0.973* (0.001)	0.949* (0.001)
pNN50	0.925* (0.001)	0.916* (0.001)	0.939* (0.001)	0.867* (0.001)	0.901* (0.001)	0.775* (0.003)	0.965* (0.001)	0.963* (0.001)	0.938* (0.001)	0.981* (0.001)	0.969* (0.001)
LF	0.184 (1.0)	0.637 (0.16)	0.674 (0.07)	0.204 (1.0)	0.447 (1.0)	0.157 (1.0)	0.632 (0.18)	0.570 (0.57)	0.276 (1.0)	0.794* (0.001)	0.355 (1.0)
HF	0.355 (1.0)	0.601 (0.33)	0.508 (1.0)	0.403 (1.0)	0.277 (1.0)	0.300 (1.0)	0.461 (1.0)	0.592 (0.39)	0.143 (1.0)	0.773* (0.004)	0.267 (1.0)
LF:HF	0.129 (1.0)	0.729* (0.01)	0.692* (0.04)	0.333 (1.0)	0.189 (1.0)	0.096 (1.0)	0.096 (1.0)	0.561 (0.66)	0.450 (1.0)	0.048 (1.0)	0.485 (1.0)
SD1	0.905* (0.001)	0.904* (0.001)	0.921* (0.001)	0.896* (0.001)	0.850* (0.001)	0.826* (0.001)	0.955* (0.001)	0.970* (0.001)	0.909* (0.001)	0.973* (0.001)	0.948* (0.001)
SD2	0.922* (0.001)	0.921* (0.001)	0.923* (0.001)	0.896* (0.001)	0.781* (0.003)	0.691* (0.04)	0.950* (0.001)	0.942* (0.001)	0.849* (0.001)	0.976* (0.001)	0.918* (0.001)
D2	0.546 (0.83)	0.321 (1.0)	0.264 (1.0)	0.215 (1.0)	0.094 (1.0)	0.507 (1.0)	0.632 (0.18)	0.360 (1.0)	0.518 (1.0)	0.597 (0.36)	0.485 (1.0)

Notes. (Number and Letter) The numbers represent the time interval (1, 2, and 3 min) and the letters an analysis period (A, first epoch; B, second epoch; C, third epoch; D, fourth epoch; E, fifth epoch; F, sixth epoch); D2 correlation dimension; HF: high frequency (0.15–0.4 Hz); HR: heart rate; LF: low frequency (0.04–0.15 Hz); LF:HF: low frequency-to-high frequency ratio; pNN50: % of RR intervals with difference in successive RR intervals longer than 50 ms; RR: mean of RR intervals; rMSSD: root mean square of standard deviation; SD: Standard Deviations; SD1: perpendicular points dispersion to the line of instantaneous identity; SD2: points dispersion of the along the long-term identity line; SDNN: standard deviation of RR interval.

**p* < 0.05 for Bonferroni corrected Pearson correlation analysis.

TABLE 3 Comparison between mean heart rate variability (HRV) indices (*p*-values) of all-time intervals (1, 2, and 3 min) and the gold standard period (6 min) for spontaneous breathing condition

HRV	6A	1A	1B	1C	1D	1E
RR	809.8 ± 36.7	821.6* ± 46.4 (0.02)	816.7 ± 46.4 (0.16)	811.7 ± 39.8 (0.29)	804.6* ± 35.9 (0.04)	803.7* ± 35.2 (0.04)
SDNN	36.4 ± 8.02	32.02* ± 12.34 (0.01)	28.7* ± 7.21 (0.004)	31.99* ± 10.1 (0.02)	32.4* ± 9.7 (0.05)	32.1 ± 11.6 (0.06)
rMSSD	20.1 ± 4.8	21.7 ± 7.6 (0.13)	20.6 ± 5.6 (0.57)	20.1 ± 5.4 (0.81)	20.2 ± 5.5 (0.81)	19.7 ± 4.6 (0.27)
pNN50	2.1 ± 2.7	3.7 ± 6.4 (0.10)	2.55 ± 3.8 (0.31)	2.07 ± 3.09 (0.69)	2.27 ± 1.74 (0.75)	1.7 ± 2.08 (0.27)
LF	33.5 ± 12.8	40.3 ± 19.7 (0.14)	42.9* ± 18.6 (0.01)	41.9* ± 19.6 (0.003)	51.1* ± 19.7 (0.002)	47.3* ± 18.3 (0.004)
HF	14.6 ± 8.7	20.9* ± 19.7 (0.005)	27.47* ± 15.9 (0.004)	17.23 ± 13.07 (0.14)	18.7 ± 10.76 (0.10)	20.01 ± 13.16 (0.10)
LF:HF	3.06 ± 1.8	2.9 ± 2.6 (0.89)	3.01 ± 2.7 (0.90)	3.9 ± 3.02 (0.08)	4.1 ± 3.4 (0.15)	3.8 ± 3.3 (0.22)
SD1	14.3 ± 3.4	15.4 ± 5.4 (0.10)	14.6 ± 4.03 (0.43)	14.3 ± 3.88 (0.99)	14.6 ± 4.1 (0.50)	14.08 ± 3.2 (0.40)
SD2	50.7 ± 12.5	42.07* ± 17.5 (0.002)	37.5* ± 10.7 (0.003)	42.6* ± 14.6 (0.02)	43.1* ± 14.21 (0.02)	42.8* ± 16.8 (0.04)
D2	1.1 ± 0.8	1.02 ± 0.8 (0.38)	0.87 ± 0.6 (0.08)	1.1 ± 0.8 (0.90)	1.5 ± 1.5 (0.09)	1.2 ± 0.9 (0.79)

Notes. (Number and Letter) The numbers represent the time interval (1, 2, and 3 min) and the letters an analysis period (A, first epoch; B, second epoch; C, third epoch; D, fourth epoch; E, fifth epoch; F, sixth epoch); D2 correlation dimension; HF: high frequency (0.15–0.4 Hz); HR: heart rate; LF: low frequency (0.04–0.15 Hz); LF:HF: low frequency-to-high frequency ratio; pNN50: % of RR intervals with difference in successive RR intervals longer than 50 ms; RR: mean of RR intervals; rMSSD: root mean square of standard deviation; SD: Standard Deviations; SD1: perpendicular points dispersion to the line of instantaneous identity; SD2: points dispersion of the along the long-term identity line; SDNN: standard deviation of RR interval.

**p* < 0.05 values for paired Student's *t*-test between 6 min epoch and the respective time interval.

measures domain (see Tarvainen et al., 2014 for detailed HRV indices calculation). The time domain included mean RR intervals (RR), standard deviation of RR interval (SDNN), root mean square of standard deviation (rMSSD), % of RR intervals with difference in successive RR intervals longer than 50 ms (pNN50). The frequency domain features were performed by Fast Fourier Transform (FFT), ascribing the % of low frequency (LF) (0.04–0.15 Hz), % of high frequency (HF) (0.15–0.4 Hz), and the low frequency-to-high frequency ratio (LF:HF). The Poincare geometric measures domain was composed by the perpendicular points dispersion to the line of instantaneous identity (SD1), points dispersion of the along the long-term identity line (SD2) and correlation dimension (D2) (Brennan, Palaniswami, & Kamen, 2001; Kamen, Krum, & Tonkin, 1996; Tarvainen et al., 2014; Task Force, 1996).

For each HRV recording (6 min), the quantitative data analysis was performed for all time intervals (1, 2, 3, and 6 min) in the rest and controlled breathing conditions. Thus, for each HRV recording we extracted 1-min (*n* = 6), 2-min (*n* = 3), and 3-min epochs (*n* = 2; Figure 1).

2.3 | Statistical analysis

The Pearson correlation was performed between the HRV indices with different time intervals (1, 2, 3, and 6 min) and the gold standard (6 min). The statistical test was performed separately for each respiratory rhythm condition. The reproducibility of time intervals mean values was verified by paired *t*-test between the gold standard time intervals and other time epochs (1, 2, and 3 min). A paired *t*-test was used to investigate the difference in HRV indices during the two breathing conditions, for this procedure the gold standard interval was used. All statistical procedures were performed in Stata 14©, for *t*-tests the significance level was set at *p* < 0.05 and Pearson correlations *p*-values were adjusted by Bonferroni correction.

3 | RESULTS

Data from the correlation between ultra-short time intervals and gold standard time during the breathing conditions for all HRV indices are presented in Tables 1 and 2. For baseline spontaneous breathing period the time domain indices (mean RR, rMSSD and pNN50) showed better correlation results (*r* values from 0.298 to 0.972) and higher frequencies of *p* < 0.05 for 1-min epochs, with emphasis in rMSSD for improved *r* values (from 0.768 to 0.919, *p* < 0.05) in 1-min epochs and for all time intervals (*r* values from 0.768 to 0.960, *p* < 0.05). The frequency domain indices (LF, HF, and LF:HF) was poorly correlated in the 1-, 2-, or 3-min periods (*r* values from 0.223 to 0.952). For nonlinear indices, the SD1 presented similar results to rMSSD (*r* values from 0.764 to 0.965, all time intervals with *p* < 0.05), and the correlation of the other nonlinear indices (SD2 and D2) showed few significant *p*-values for all time intervals (as seen in Table 1).

Following Pearson correlation, the controlled breathing period did not change the frequency domain of reliability indices (*r* values from 0.184 to 0.674) and did not improve the frequency of significant *p*-values (as seen in Table 2). The time domain indices (RR, rMSSD, and pNN50) maintained their reliability for all time intervals, where rMSSD remained the most reliable due to higher *r* values (*r* values from 0.825 to 0.987, *p* < 0.05), whereas the SDNN index had its reliability increased for all time intervals (*r* values from 0.704 to 0.980, *p* < 0.05 for all time intervals). For nonlinear indices, the results were similar in that all indexes presented replicability for 3 or 3 min intervals, but the SD2 index had its reliability increased for all time intervals (*r* values from 0.691 to 0.976, *p* < 0.05) when compared with spontaneous breathing (*r* values from 0.415 to 0.776, only *r* = 0.776 has *p* < 0.05). It was only observed that the controlled breathing condition increased the *r* values for indices all domains (time, frequency, and nonlinear), keeping

1F	2A	2B	2C	3A	3B
804.1 ± 39.4 (0.21)	800.6 ± 86.2 (0.62)	807.7 ± 36.7 (0.16)	802.8* ± 34.9 (0.03)	816.3* ± 41.8 (0.01)	803.7* ± 34.6 (0.01)
29.48* ± 11.9 (0.004)	33.6* ± 9.9 (0.03)	33.6 ± 8.5 (0.07)	33.6* ± 9.4 (0.05)	34.5 ± 9.1 (0.09)	33.9* ± 8.4 (0.04)
18.49* ± 3.7 (0.01)	21.2 ± 6.4 (0.12)	20.2 ± 5.2 (0.87)	19.1* ± 4.1 (0.04)	20.9 ± 5.8 (0.13)	19.6 ± 4.4 (0.10)
0.64* ± 1.2 (0.01)	3.1 ± 4.8 (0.07)	2.1 ± 2.9 (0.94)	1.1* ± 1.3 (0.03)	2.7 ± 4.07 (0.08)	1.5 ± 1.9 (0.09)
43.4* ± 16.8 (0.009)	37.5 ± 15.5 (0.31)	37.5 ± 18.2 (0.16)	40.6* ± 13.6 (0.02)	34.6 ± 14.1 (0.69)	37.3 ± 9.01 (0.16)
21.4 ± 12.05 (0.01)	19.6* ± 14.9 (0.03)	16.2 ± 10.12 (0.17)	17.78 ± 12.3 (0.25)	17.27* ± 11.52 (0.03)	15.24 ± 9.06 (0.77)
2.6 ± 2.1 (0.52)	3.1 ± 2.1 (0.79)	3.9 ± 4.4 (0.27)	3.3 ± 2.6 (0.44)	2.9 ± 2.06 (0.34)	3.6 ± 2.2 (0.22)
13.17* ± 2.7 (0.02)	15.07 ± 4.5 (0.10)	14.3 ± 3.7 (0.95)	13.6* ± 2.8 (0.05)	14.8 ± 4.1 (0.12)	13.7 ± 3.8 (0.14)
39.2* ± 17.5 (0.004)	44.7* ± 14.2 (0.008)	45.1* ± 12.2 (0.05)	45.4* ± 13.6 (0.004)	47.7 ± 14.8 (0.25)	47.1 ± 13.76 (0.21)
0.7* ± 0.6 (0.03)	1.04 ± 0.7 (0.39)	1.05 ± 0.6 (0.27)	1.1 ± 0.8 (0.79)	1.06* ± 0.7 (0.03)	1.1 ± 0.7 (0.95)

significantly r values from indices with $p < 0.05$ in spontaneous breathing condition and improving deSDNN and SD2 reliability.

The reproducibility of mean HRV indices during spontaneous breathing condition for 1-, 2-, and 3-min was tested by paired samples t -test between 6-min interval and other time epochs (Table 3). For time domain indices, rMSSD and pNN50 presented few significant differences between different time intervals (only one time interval presented a significant difference from 6-min epoch). Similar results were observed in LF:HF, SD1 and D2 indices. For controlled breathing condition, all indices presented an increase in the frequency of statistically different values, except the HF values that were reproducible at all time intervals (Table 4).

According with paired t -test, significant changes were observed in all HRV indices in time, frequency, and Poincare geometric measures domains ($p < 0.001$) in the comparison between rest and controlled breathing conditions (Table 5). Accordingly, a significant increase in SDNN ($t = 11.52$, $p = 0.0001$), rMSSD ($t = 7.41$, $p = 0.0001$), pNN50 ($t = 6.36$, $p = 0.0001$), LF ($t = 14.94$, $p = 0.0001$), LF:HF ($t = 11.03$, $p = 0.0001$), SD1 ($t = 7.41$, $p = 0.0001$), SD2 ($t = 9.75$, $p = 0.0001$), and D2 ($t = 3.79$, $p = 0.001$) were noticed and associated with a significant decrease in the RR ($t = 4.14$, $p = 0.0001$) and HF ($t = 5.96$, $p = 0.0001$) in the controlled breathing condition.

4 | DISCUSSION

This study investigated the effects of controlled breathing rhythm on short-term HRV indices reliability. The results suggest that rMSSD and SD1 have the higher r coefficients and more replicable mean values for all time intervals in spontaneous breathing conditions. The breathing rate standardization impairs the HRV indices

reliability, except in the case of HF. Other HRV indices showed poorly r coefficients and high frequency of mean values with statistically significant difference (when compared to 6-min epochs), suggesting that the gold standard time interval (>5 min) are required for reliable recordings. The comparison between mean HRV indices during breathing conditions suggests that controlled breathing protocol presents a significant effect on all HRV indices.

Previous studies that compare HRV indices in different time intervals support the same relation between time domain indices and higher replicability in short-term time intervals. The comparison between HRV recordings with 10, 60, and 300 s, showed that only rMSSD presents reliability to short-term measurements, whereas other indices (including other time domain indices as SDNN) require longer measurements (Nussinovitch et al., 2011; Thonget et al., 2003). Important to say, although these studies found significant correlations only in rMSSD, they have analyzed only a random time interval within the total sample, which can cause artifact epochs to be chosen for analysis by decreasing the r values of the indices that depend of the signal morphology. Recent studies, with large samples ($n = 3,387$) using more than one signal epoch for short-term comparisons, found that rMSSD presents reliability to short-term comparison, requiring at least 30 s for replicable data (Munoz et al., 2015).

The rMSSD higher reliability makes it commonly reported in studies using ultra-short recordings. Studies that compare elite athletes in rest and postexercise conditions showed that the reliability to this measurement modality can discriminate physical adaptation to train load and fatigue (Esco & Flatt, 2014; Nakamura et al., 2015; Schmitt, Regnard, & Millet, 2015). As in elite sport training, short-term rMSSD values have been used to clinical samples, such as observed in patients with diabetes (Chen, Yang, Liu, & Tang, 2015) or mental stress (Salahuddin, Cho, Jeong, & Kim, 2007).

TABLE 4 Comparison between mean heart rate variability (HRV) indices (*p*-values) of all-time intervals (1, 2, and 3 min) and the gold standard period (6 min) for controlled breathing condition

HRV	6A	1A	1B	1C	1D	1E
RR	754.4 ± 47.1	781.4* ± 52.04 (0.001)	761.8* ± 54.2 (0.01)	747.6* ± 52.4 (0.03)	746.1* ± 44.8 (0.007)	743.8* ± 48.6 (0.006)
SDNN	73.1 ± 11.7	75.9 ± 16.06 (0.08)	71.7 ± 15.7 (0.37)	69.6* ± 13.5 (0.005)	68.7* ± 12.3 (0.002)	70.2 ± 9.8 (0.09)
rMSSD	33.2 ± 6.08	37.1* ± 7.5 (0.001)	34.2 ± 7.5 (0.19)	32.4 ± 7.5 (0.23)	31.2* ± 6.1 (0.004)	31.7* ± 5.2 (0.04)
pNN50	13.6 ± 7.5	19.3* ± 10.5 (0.001)	14.6 ± 9.8 (0.27)	12.9 ± 7.9 (0.29)	12.2 ± 8.6 (0.16)	11.7* ± 6.9 (0.01)
LF	81.4 ± 6.2	87.7* ± 8.4 (0.008)	84.7 ± 13.4 (0.18)	85.02* ± 8.7 (0.02)	79.5 ± 16.6 (0.6)	85.1 ± 11.4 (0.12)
HF	2.8 ± 0.9	3.07 ± 1.9 (0.64)	4.3 ± 1.8 (0.24)	2.8 ± 1.2 (0.95)	2.6 ± 1.2 (0.44)	2.8 ± 1.3 (0.99)
LF:HF	31.4 ± 11.3	42.3 ± 27.1 (0.09)	31.09 ± 15.9 (0.84)	35.4 ± 15.1 (0.12)	36.1 ± 14.7 (0.18)	35.5 ± 14.1 (0.28)
SD1	23.5 ± 4.3	26.4* ± 5.3 (0.001)	24.3 ± 5.3 (0.13)	23.07 ± 5.4 (0.34)	22.2* ± 4.4 (0.01)	22.57 ± 3.7 (0.07)
SD2	100.6 ± 16.1	104.09 ± 22.3 (0.13)	98.5 ± 21.9 (0.32)	95.7* ± 18.6 (0.006)	94.6* ± 16.9 (0.002)	96.7 ± 13.8 (0.10)
D2	2.1 ± 0.1	1.7* ± 0.2 (0.001)	2.2 ± 2.1 (0.77)	1.7* ± 0.1 (0.001)	1.8* ± 0.1 (0.001)	1.7* ± 0.1 (0.001)

Notes. (Number and Letter) The numbers represent the time interval (1, 2, and 3 min) and the letters an analysis period (A, first epoch; B, second epoch; C, third epoch; D, fourth epoch; E, fifth epoch; F, sixth epoch); D2 correlation dimension; HF: high frequency (0.15–0.4 Hz); HR: heart rate; LF: low frequency (0.04–0.15 Hz); LF:HF: low frequency-to-high frequency ratio; pNN50: % of RR intervals with difference in successive RR intervals longer than 50 ms; RR: mean of RR intervals; rMSSD: root mean square of standard deviation; SD: Standard Deviations; SD1: perpendicular points dispersion to the line of instantaneous identity; SD2: points dispersion of the along the long-term identity line; SDNN: standard deviation of RR interval.

**p* < 0.05 values for paired *t*-test between 6 min epoch and the respective time interval.

The high replicability of rMSSD during ultra-short recordings can be understood by its mathematical formula. Calculated by the difference of consecutive R intervals (Task Force, 1996), the rMSSD values are poorly influenced by fluctuations in heart rate values, making the measurement more stable even in periods with different heart oscillations (Saboul, Pialoux, & Hautier, 2013). The identical mathematical metrics between rMSSD and SD1 explains the similar reliability of these indices (Ciccone et al., 2017). Furthermore, indices such as SDNN or those of the frequency domain that are not derived from the difference between RR intervals, have a direct influence on fluctuations of heart rate values, so that small changes in one specific period of the record are not replicable in the others (Nussinovitch et al., 2011).

Although the frequency and Poincare geometric measures domain indices are more influenced by heart rate oscillations, the results suggest that breathing standardization does not improve the indices reliability during short-term recordings. This may have occurred because there is no synchronization between waves peaks and valleys of different epochs, a factor that directly influences the values based on FFT (Laborde et al., 2017). Therefore, the lack of synchrony between the beginning of respiratory cycle can contribute to reduce the reliability of these indices at different epochs. In addition, there is no certainty that all individuals have been able to adapt equally to the breathing pace and maintain it for a prolonged time, which may justify impairment in some time domain indices (rMSSD and pNN50).

Important to say, these results are supported by the correlation analysis, despite the mean values comparison of HF suggests that all time intervals values are similar during controlled breathing

condition. Thus, our hypothesis is that HF decrease during controlled respiration condition may reduce the interindividual variation, as all participants have a significant reduction (nearly around to zero values). Although there is better replicability of mean values, the robust effect of controlled respiration in HF may impair some experimental effects (the participant may still be influenced by the controlled breathing effect). Thus, the assessment of HF under these breathing conditions may only reflects the individual's adaptability to the breathing rhythm and not to reflect the proper resting baseline values.

During the fixed breathing pacer of 6 breaths/min there is a sinusoidal variation induction in RR intervals (Vaschillo, Vaschillo, & Lehrer, 2006). This pattern is related to the synchrony between heart rate and breathing frequency caused by resonant frequency rhythm (Lehrer, Vaschillo, & Vaschillo, 2000; Lehrer et al., 2004), denominated Respiratory Sinus Arrhythmia (ASR), a phenomenon that represents the increase in HR during inspiration and its reduction during expiration (Billman, Huikuri, Sacha, & Trimmel, 2015; Yasuma & Hayano, 2004).

According to our findings, studies comparing controlled and spontaneous breathing rhythm conditions reported significant changes in all HRV domains (Barth & Vecchio, 2014; Tavares et al., 2016). These findings suggest that if breathing rate control is adopted during a baseline rest record, the values obtained will already be influenced by a variable that may change the HRV values, compromising the investigation of other variables that would have the potential to change the HRV indices. In addition, the induction of a fixed breathing rhythm may evoke different effects on participants who are familiar with paced respiration, such as yoga or meditation practitioners (Krygier et al., 2013), with a significant increase in the data variability.

1F	2A	2B	2C	3A	3B
746.8* ± 45.5 (0.06)	771.3* ± 52.2 (0.001)	743.1* ± 54.1 (0.003)	745.3* ± 46.2 (0.002)	763.1* ± 51.6 (0.001)	745.5* ± 45.01 (0.001)
68.4* ± 11.9 (0.03)	74.9 ± 15.7 (0.18)	69.8* ± 12.3 (0.002)	69.9* ± 9.3 (0.03)	74.4 ± 14.2 (0.12)	69.8* ± 10.08 (0.006)
32.06 ± 6.4 (0.16)	35.7* ± 7.1 (0.001)	31.9* ± 6.5 (0.001)	31.9* ± 5.4 (0.03)	34.6* ± 7.1 (0.003)	31.8* ± 5.5 (0.04)
11.8 ± 7.9 (0.15)	16.9* ± 9.7 (0.001)	12.3* ± 7.5 (0.01)	11.7* ± 6.6 (0.006)	15.5* ± 8.8 (0.005)	11.7* ± 6.6 (0.005)
83.5 ± 15.9 (0.57)	87.9* ± 8.2 (0.003)	78.3 ± 11.4 (0.15)	82.03 ± 12.01 (0.83)	84.6* ± 8.6 (0.01)	82.8 ± 9.03 (0.48)
3.2 ± 1.8 (0.42)	2.6 ± 1.07 (0.44)	2.8 ± 0.9 (0.67)	2.9 ± 1.7 (0.81)	3.06 ± 1.6 (0.45)	2.8 ± 0.9 (0.96)
32.8 ± 17.5 (0.75)	36.9* ± 12.8 (0.05)	30.3 ± 9.6 (0.62)	34.6 ± 16.3 (0.35)	32.6 ± 12.5 (0.40)	33.1 ± 12.4 (0.54)
22.8 ± 4.6 (0.23)	25.3* ± 5.1 (0.001)	22.6* ± 4.6 (0.002)	22.7* ± 3.8 (0.04)	25.5* ± 5.03 (0.002)	22.5* ± 3.9 (0.004)
94.1* ± 16.6 (0.03)	103.8 ± 21.9 (0.26)	95.6* ± 17.3 (0.001)	96.09* ± 13.06 (0.02)	101.8 ± 20.1 (0.36)	96.2* ± 13.8 (0.006)
1.8* ± 0.2 (0.001)	1.8* ± 0.2 (0.001)	2.01* ± 0.1 (0.04)	2.07* ± 0.2 (0.05)	1.9* ± 0.2 (0.01)	2.09 ± 0.3 (0.87)

Based in our results, some practices to the ultra-short HRV measurement are suggested: (a) to use the spontaneous breathing rhythm and apply this same condition to all individuals of the research for ultra-short measurements due to the RSA influence on HRV indices; and, (b) to use the rMSSD (orSD1) to perform ultra-short record analysis as it presents greater reliability at different time intervals.

Important to note, this study has some limitations to be considered. First, the sample size of the present manuscript should be larger and diversified (our sample is only composed by 20 healthy young men) in order to investigate the effect of variables associated to HRV such as age, sex, physical activity, sleep quality, and other behavioral data in ultra-short HRV indices. Second, in our experiment the standard gold record adopted was of 6-min duration. We believe that this time interval is sensitive for the assessment of acute conditions (e.g., recovery period after intense physical exercise), although future studies may investigate the stability of the ultra-short recording indices over a day or a week to verify whereas short-term recording is suitable only for acute events or can be extrapolated as a stable autonomic marker of the individual. Last, our sample is only composed of healthy individuals, we propose the investigation of ultra-short HRV recording reliability in clinical samples (e.g., diabetes) compared to healthy individuals, this could encourage the use of HRV in the clinical routine as the short-term HRV recording represent a noninvasive, low-cost, and practical technique.

Heart rate variability is a technique to quantify the autonomic activity, being commonly reported as possible biomarker for several diseases, including psychiatric disorders (Laborde et al., 2017; Thayer & Brosschot, 2005; Thayer & Lane, 2000, 2009; Thayer et al., 2012). This study encourages the use of ultra-short HRV recording

TABLE 5 Comparison between mean heart rate variability values during the spontaneous and controlled breathing conditions

Indices	Spontaneous mean ± SD	Controlled mean ± SD	t	P
RR	809.85 ± 36.78	754.46 ± 47.14	4.14	0.0001*
SDNN	36.48 ± 8.02	73.11 ± 11.73	11.52	0.0001*
rMSSD	20.31 ± 4.89	33.26 ± 6.08	7.41	0.0001*
pNN50	2.14 ± 2.76	13.61 ± 7.56	6.36	0.0001*
LF	33.55 ± 12.89	81.47 ± 6.27	14.94	0.0001*
HF	14.64 ± 8.76	2.88 ± 0.93	5.96	0.0001*
LF:HF	3.06 ± 1.89	31.486 ± 11.35	11.03	0.0001*
SD1	14.38 ± 3.47	23.55 ± 4.30	7.41	0.0001*
SD2	42.07 ± 17.52	100.66 ± 16.18	9.75	0.0001*
D2	1.02 ± 0.18	2.10 ± 0.16	3.79	0.001*

Notes. D2 correlation dimension; HF: high frequency (0.15–0.4 Hz); HR: heart rate; LF: low frequency (0.04–0.15 Hz); LF:HF: low frequency-to-high frequency ratio; pNN50: % of RR intervals with difference in successive RR intervals longer than 50 ms; RR: mean of RR intervals; rMSSD: root mean square of standard deviation; SD: Standard Deviations; SD1: perpendicular points dispersion to the line of instantaneous identity; SD2: points dispersion of the along the long-term identity line; SDNN: standard deviation of RR interval.

* $p < 0.05$ values for paired t-test.

as complementary resource for clinical reasoning, as the HRV data analysis is based in reliable indices and the assessment carried out in appropriated breathing conditions. The 1-min recording do not take much time when compared with classical long-term monitoring systems or require expansive apparatus (to 24 hr recording) doing this technique more accessible to less developed countries. Therefore, the electrocardiogram recording measurement that is

commonly performed in the hospital routine can be used for other clinical areas besides the classical use in cardiology, as the use of short-term registries optimizes the use of the equipment by a clinicians group.

5 | CONCLUSION

The rMSSD and SD1 present the best reliability to ultra-short-term HRV recordings, being stable for the 1-, 2-, and 3-min recordings. For the measurement of frequency and Poincare geometric measures domains are recommended longer recording periods. The controlled breathing rhythm evokes stronger r values for time domain indices, but impairs the mean values replicability across all time intervals. The comparison between HRV indices during the breathing conditions showed that all HRV indices present an increase in mean values, except the HF that significantly decreased. Therefore, our results suggest that rMSSD (or SD1) and spontaneous breathing rate are more appropriate to measure HRV with the ultra-short-term method.

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DISCLOSURES

The authors declare no conflict of interest.

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