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Within-Session Stability of Short-Term Heart Rate Variability Measurement

by
Lukas Cipryan[1]

The primary aim of this study was to assess the retest stability of the short-term heart rate variability (HRV) measurement performed within one session and without the use of any intervention. Additionally, a precise investigation of the possible impact of intrinsic biological variation on HRV reliability was also performed. First, a single test-retest HRV measurement was conducted with 20-30 min apart from one another. Second, the HRV measurement was repeated in ten non-interrupted consecutive intervals. The lowest typical error (CV = 21.1%) was found for the square root of the mean squared differences of successive RR intervals (rMSSD) and the highest for the low frequency power (PLF) (CV = 93.9%). The standardized changes in the mean were trivial to small. The correlation analysis revealed the highest level for ln rMSSD (ICC = 0.87), while ln PLF represented the worst case (ICC = 0.59). The reliability indices for ln rMSSD in 10 consecutive intervals improved (CV = 9.9%; trivial standardized changes in the mean; ICC = 0.96). In conclusion, major differences were found in the reliability level between the HRV indices. The rMSSD demonstrated the highest reliability level. No substantial influence of intrinsic biological variation on the HRV reliability was observed.

Key words: *heart rate variability, reliability, typical error, autonomic nervous system.*

Introduction

Homeostasis of the human body is maintained by the intrinsic rhythms of a number of systems, such as the cardiac and respiratory systems. All these rhythms are evinced as an oscillation which may be influenced by several factors. The rate of the oscillation can provide information on the state of the complex systems involved (Gernot, 2014). The oscillation of consecutive heart beats, heart rate variability (HRV), has become a powerful method which helps athletes to understand body responses to exercise and consequently significantly improves the training process and sport performance. The measurement simplicity and non-invasiveness are the main advantages of the HRV monitoring, making this measurement attractive for everyday use in training practice (Buchheit, 2014; Kiviniemi et al., 2014; Plews et al., 2013).

The methodological approach to the HRV measurement has been standardized since the Task Force was published in 1996. However, certain doubts about HRV analysis and following interpretation still exist (Farah et al., 2014; Pagani et al., 2012; Paso et al., 2013; Wallén et al., 2012). For instance, the reliability of the HRV measurement is not definite, even if its determination is fundamental for assessing the HRV measurement sensitivity. This deficiency might be explained by various methodological designs of the reliability studies (Boullosa et al., 2014; Cipryan and Litschmannova, 2013; Sandercock et al., 2005), as well as by a number of factors influencing HRV results (Task Force, 1996) or by various statistical approaches to the reliability analysis (Hopkins et al., 2009).

Paradoxically, the within-session

[1] - *Human Motion Diagnostic Centre, Ostrava University, Czech Republic*

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reliability (stability) of the widely used short-term HRV measurement in supine rest, in healthy young individuals and without the use of a cardiovascular reflex has not been established. The recent studies focused on the day-to-day reliability of the HRV parameters (Sinnreich et al., 1998; Tarkiainen et al., 2005). However, these results do not provide information about the stability of the single HRV measurement, which we do not intend to repeat on other days. Identifying the influence of a single intervention on the HRV measurement conducted within one laboratory session would be limited if the measurement error was larger than the expected change (Hopkins, 2000). Therefore, the main aim of this study was to assess the within-session stability of such HRV measurement. Additionally, we also aimed at a precise investigation of the possible impact of the intrinsic biological variation (i.e. the natural fluctuation of the autonomic nervous system activity) on HRV reliability.

Material and Methods

Participants

Recreationally active participants volunteered for this study. None of the participants was clinically diagnosed with any chronic or acute cardiovascular, metabolic or respiratory disease. Those excluded from the study included smokers and those on medication and/or nutritional supplements of any type. Prior to the participant's involvement, the local Ethics Committee of the Ostrava University approved the experimental protocol and the investigation conformed to the principles outlined in the Declaration of Helsinki. All the participants were fully informed about the study objectives and provided written informed consent.

Study overview

Two trials were performed in order to fulfil the study aims:

a) Trial 1 (N = 31; 14 males, 17 females; age 21.86 ± 1.45 years; body mass index 21.90 ± 0.88)

This trial consisted of the test-retest HRV measurements with one repetition. Retest measurements were performed under identical laboratory conditions and 20-30 minutes after the first HRV measurement. The participants remained at rest between the test and retest. However, standing up, walking or talking were allowed.

b) Trial 2 (N = 33, 17 males, 16 females; age 22.07 ± 1.54 years; body mass index 22.13 ± 1.02)

The second trial took place on a different day than Trial 1. Ten consecutive, non-interrupted intervals of short-term HRV measurement were conducted. The participants stayed at rest in the supine position throughout the entire ECG recording.

Participants were asked to maintain all standard requirements, i.e. to refrain from any strenuous physical activity for 48 h, and avoid eating for at least 2 h or caffeine drinks at least 12 h prior to the session. In order to minimize circadian variation, all sessions were conducted between 7 and 10 am. Participants were instructed to breath regularly during the ECG recording. Although the respiratory rate may influence the magnitude of the HRV parameters related to the HF spectral component (Task Force, 1996), it was not controlled in order to increase the applicability of the study results in the field.

HRV measurement and analysis

HRV parameters were calculated from the 5 min resting (supine) periods using the diagnostic system VarCor PF8 (Dimea Group Ltd, Czech Republic). ECG was sampled at 1000 Hz and the accuracy of the measurements was 1 ms. The RR data was visually validated prior to frequency analysis, i.e. assessment for stationarity, ectopic, missing data or aberrant beats. Ectopic beats were excluded. The power spectrum was obtained by transforming the time data (the duration of the RR intervals) into frequency values. Three main spectral components were distinguished: a very low frequency (VLF) < 0.04 Hz, a low frequency (LF) 0.04-0.15 Hz and a high frequency (HF) > 0.15-0.40 Hz (Task Force, 1996). The spectral variables Total spectral power (PT), LF power (PLF), HF power (PHF), ratio LF/HF and LF power in normalized units (LFnu) [PLF / (PT − PVLF) x 100] were employed in this research. In the time domain, the square root of the mean squared differences of successive RR intervals (rMSSD) was also calculated.

Statistical analysis

The magnitude-based inferences were used for the statistical analysis, since the null-hypotheses tests seemed to have serious

shortcomings (Batterham and Hopkins, 2005; Wilkinson, 2014). All HRV indices were log-transformed prior to analysis since an impaired distribution and heterogeneity of variance were assumed. The outliers were detected, removed and not included in the statistical analysis. The absolute and relative reliability of the HRV measurement was assessed using the standard error of measurement (typical error, TE), standardized change in the mean between measurements (effect size, ES) and the intraclass correlation coefficient (ICC) (Hopkins et al., 2009). The TE is also expressed relatively as the coefficient of variation (CV, %). The 90% confidence intervals (CI) were calculated. The threshold values for ES statistics were <0.2 (*trivial*), ≥0.2 (*small*), ≥0.6 (*moderate*), ≥1.2 (*large*), ≥2.0 (*very large*), ≥4.0 (*nearly perfect*). The exact probabilities were expressed and the magnitude of the difference was also evaluated qualitatively as follows: 25-75% *possibly*, 75-95% *likely*, 95-99% *very likely*, >99% *almost certain* (Batterham and Hopkins, 2005). The smallest significant change/difference was considered 0.2 of the between-measurement standard deviation. If the chance of higher or lower differences was >5%, then the true difference was assessed as *unclear*. The following criteria were used to interpret the magnitude of the correlation (r) between the measurements: <0.1 (*trivial*), ≥0.1 (*small*), ≥0.3 (*moderate*), ≥0.5 (*large*), ≥0.7 (*very large*), and ≥0.9 (*almost perfect*). If the 90% CI overlapped small positive and negative values, the magnitude of the correlation was considered *unclear*; otherwise, the magnitude of the correlation was deemed to be the observed magnitude (Hopkins et al., 2009). Statistical analyses were performed using a statistical spreadsheet (Hopkins, 2007).

Results

Single test-retest reliability

Reliability indices for the test-retest HRV measurement are reported in Table 1. The lowest typical error (CV = 21.1%) was found for ln rMSSD and the highest for ln PLF (CV = 93.9%). The standardized change of the mean was *possibly* or *likely trivial* for the ln rMSSD, ln PHF, and ln PT. *Possibly small* differences were revealed in Mean RR, ln LF/HF and ln LFnu. The correlation analysis indicated the highest level for ln rMSSD (ICC = 0.87), while ln PLF represented the worst case (ICC = 0.59).

Table 1

Reliability of HRV variables

	N	Standardized differences (90% CL)	% chances of higher/trivial/lower differences	Rating of the difference	TE (90% CL)	CV (90% CL)	ICC (90% CL)
Mean RR (ms)	30	+0.24 (0.05; 0.42)	57/42/0	*possibly small*	0.41 (0.34; 0.53)	5.2 (4.3; 6.7)	0.86 (0.76; 0.92)
ln rMSSD(ms)	29	+0.16 (-0.02; 0.34)	40/60/0	*possibly trivial*	0.40 (0.33; 0.52)	21.1 (17.1; 28.0)	0.87 (0.77; 0.93)
ln PHF (ms^2)	28	+0.08 (-0.11; 0.28)	12/87/1	*likely trivial*	0.43 (0.35; 0.55)	45.1 (35.7; 61.8)	0.86 (0.74; 0.92)
ln PT (ms^2)	27	+0.16 (-0.05; 0.38)	26/74/0	*possibly trivial*	0.46 (0.38; 0.60)	41.2 (32.6; 56.6)	0.83 (0.70; 0.91)
ln PLF (ms^2)	29	-0.09 (-0.47; 0.30)	6/66/27	*unclear*	0.86 (0.71; 1.11)	93.9 (72.5; 134.4)	0.59 (0.34; 0.76)
ln LF/HF	29	-0.20 (-0.46; 0.06)	0/64/35	*possibly small*	0.58 (0.48; 0.75)	74.23 (57.9; 104.2)	0.76 (0.59; 0.86)
ln LFnu (%)	28	-0.30 (-0.52; -0.08)	0/33/67	*possibly small*	0.48 (0.40; 0.62)	37.23 (29.7; 50.6)	0.82 (0.69; 0.90)

N – number of participants (without outliers); CL – confidence limits;
TE – typical error; CV – coefficient of variation (%);
ICC – intraclass coefficient of correlation

Table 2

Reliability of ln rMSSD in 10 consecutive 5 min intervals

Measurement comparison	N	Standardized differences (90% CL)	% chances of higher/trivial/lower differences	Rating of the difference	TE (90% CL)	CV (90% CL)	ICC (90% CL)
1 vs. 2	30	+0.08 (0.01; 0.14)	0/100/0	*almost certain trivial*	0.14 (0.11; 0.18)	7.3 (6.0; 9.4)	0.98 (0.97; 0.99)
2 vs. 3	33	+0.06 (-0.05; 0.16)	1/99/0	*very likely trivial*	0.26 (0.21; 0.32)	13.4 (11.0; 17.2)	0.94 (0.90; 0.97)
3 vs. 4	30	+0.06 (-0.01; 0.12)	0/100/0	*almost certain trivial*	0.15 (0.13; 0.20)	7.7 (6.3; 9.9)	0.98 (0.96; 0.99)
4 vs. 5	31	+0.04 (-0.04; 0.12)	0/100/0	*almost certain trivial*	0.19 (0.16; 0.24)	9.6 (7.9; 12.4)	0.97 (0.94; 0.98)
5 vs. 6	31	-0.06 (-0.13; 0.01)	0/100/0	*almost certain trivial*	0.16 (0.14; 0.21)	8.3 (6.8; 10.7)	0.98 (0.96; 0.99)
6 vs. 7	32	+0.08 (-0.02; 0.19)	2/98/0	*very likely trivial*	0.25 (0.21; 0.32)	12.6 (10.3; 16.2)	0.94 (0.90; 0.97)
7 vs. 8	33	+0.01 (-0.09; 0.10)	0/100/0	*almost certain trivial*	0.23 (0.19; 0.29)	10.9 (9.0; 13.9)	0.95 (0.92; 0.97)
8 vs. 9	32	-0.04 (-0.12; 0.05)	0/100/0	*almost certain trivial*	0.21 (0.17; 0.26)	9.2 (7.6; 11.8)	0.96 (0.93; 0.98)
9 vs. 10	32	-0.14 (-0.26; -0.02)	0/84/16	*likely trivial*	0.27 (0.23; 0.35)	12.1 (10.0; 15.6)	0.93 (0.88; 0.96)
1 vs. 10	32	+0.26 (0.02; 0.50)	56/44/0	*possibly small*	0.56 (0.47; 0.71)	23.8 (19.4; 31.1)	0.77 (0.62; 0.87)

N – number of participants (without outliers); CL – confidence limits; TE – typical error; CV – coefficient of variation (%); ICC – intraclass coefficient of correlation

Ln rMSSD stability over 10 consecutive intervals

The course of the within-session stability indices for ln rMSSD in 10 consecutive intervals (Table 2) was characterized by the average TE throughout all the intervals of 0.20 (90% CL: 0.18; 0.22) and an average CV of 9.9% (8.8; 11.3). The standardized changes in the mean in all consecutive comparisons were *likely* or *almost certainly trivial* and the average ICC was 0.96 (0.94; 0.98).

When the first and tenth intervals were compared, the standardized difference of the mean was *possibly small*. The CV was similar (23.8%) to the previous analysis (Trial 1). The magnitude of the correlation between these two measurements was *very large* (ICC = 0.77).

Discussion

To the best of our knowledge, this is the first study to analyse the within-session stability level of the short-term HRV measurement without the use of any cardiovascular reflex. Since the HRV measurement is frequently used in sport and exercise investigations as well as in training practice, its standardization is of high importance. The presented reliability results might be, therefore, used for monitoring and estimating the magnitude of individual response to exercise and training, comparing the precision of HRV measures and equipment, and estimating the sample size in experiments (Hopkins, 2000).

Previous reliability studies have been primarily designed as a test-retest with repeating the HRV measurement(s) after several days (Al Haddad et al., 2011; Farah et al., 2014), weeks (Nunan et al., 2009) or months (Salo et al., 2001; Tarkiainen et al., 2005). These reliability study designs are definitely suitable for the repetitive inter-day HRV monitoring. However, the conditions and participants in the reliability study ought to be similar to those in the intended experiment. The time between the consecutive pairs of trials in the reliability study also has to be similar to the time between the pre and post-

measurement in the experiment (Hopkins, 2000). It is therefore debatable if these reliability study designs provide appropriate information about the reliability level for a single or repeated HRV measurement conducted during one laboratory session which is usual for a large cohort studies or for the pre vs. immediate post-intervention HRV observation.

Test-retest stability

The absolute and relative reliability levels were assessed in this study. The most important measure of absolute reliability is the within-subject standard deviation (typical error, TE) which represents the expected variation in an individual´s value from measurement to measurement. The possible source of TE may be a technological error or biological variation (Hopkins, 2000). The latter one is considered the more important, since the technological error of the apparatus or operator is believed to be unlikely in this within-session stability design.

The inherent "biological" fluctuation of the autonomic nervous system (ANS) activity can be, however, expected owing to its high sensitiveness to various internal or external factors, particularly when the HRV measurement is repeated after several hours to months. The retest reliability studies of the HRV measurement with the repetition after days to months might be influenced by several factors, such as sleep quality (Jackowska et al., 2012; Stein and Pu, 2012), daily physical activity (Hautala et al., 2010), circadian rhythms (Bonnemeier et al., 2003; Boudreau et al., 2012; Chen, 2011) or diet (Lima-Silva et al., 2010). All these factors are a potential source for the measurement bias and do not have to be considered if the test-retest, with the relatively short time between measurements, is performed during a single laboratory session.

The study results revealed meaningful TE differences between the HRV indices. The coefficient of variation (CV) ranged from 21.1% for ln rMSSD to 93.9% for ln PLF (Table 1). This finding of the lower CV for the time domain index (ln rMSSD) and a substantially higher CV for the frequency domain indices is in accordance with previous reliability studies, in which the CV for the time and frequency domain indices ranged between 4-18% and 7-82%, respectively (Al Haddad et al., 2011; Toyry et al., 1995). However, Sinnreich et al. (1998) and Tarkiainen et al. (2005) did not observe such significant differences between the time (CV 6-11%) and frequency domain indices (CV 7-15%). A slightly lower CV for the frequency versus time domain indices was even reported by Farah et al. (2014) who also presented the highest CV for the ratio indices.

The second important component of retest reliability is the change in the mean which consists of a random and systematic change. The random change is represented by TE which is discussed above. A systematic change in the mean is a non-random change between two trials. It may be caused by loss or improvement of individuals´ motivation between trials, by fatigue, the learning or training effect (Hopkins, 2000). All these factors do not immediately concern the presented study design. As expected, the results primarily indicated a *trivial* change in the mean. A *possibly small* change in the mean was observed for Mean RR, ln LF/HF, and ln LFnu (Table 1). It can be stated that the systematic change might be considered negligible in this study.

The relative or inter-individual reliability is expressed by the correlation analysis. The retest correlation demonstrates how closely the values of one trial track the values of another (Hopkins, 2000). The correlation might be considered the most frequently used indicator of the reliability level. Apart from the potential shortcomings of the correlation (e.g. sensitiveness to data heterogeneity and sample size), the correlation does not provide information about the actual variation in the individual´s value from measurement to measurement. Therefore, the correlation cannot be the only marker of reliability. Most ICC values in the present study were deemed *very large*. The highest ICC was in ln rMSSD (ICC 0.87; 90% CL 0.77; 0.93). The worst case was in ln PLF (ICC 0.59; 90% CL 0.34; 0.76) which can be, however, still classified as a *large* correlation. Previous studies investigating resting HRV indices similarly reported the range of the ICC from 0.68 to 0.84 (Guijt et al., 2007; Maestri et al., 2010; Schroeder et al., 2004; Sinreich et al., 1998). Indeed, there needs to be a reminder that caution must always be taken when comparing reliability studies of diverse designs and a different number of participants. Since the same reliability study has not been published yet, the comparisons of the reliability studies throughout the entire discussion include serious limitations.

Ln rMSSD stability over 10 consecutive intervals

The intrinsic biological variation, which is typical for all body systems including ANS (Gernot, 2014), might be a potential factor decreasing the reliability level of the HRV measurement. The short-term HRV measurement was, therefore, repeated ten times without any disruption in order to investigate this presumption and answer the question how the HRV variables would change over time under as stable conditions as possible. The HRV variable ln rMSSD was chosen for this purpose owing to the highest reliability and also to keep this study simple. When the results are assessed successively interval by interval, TE (CV 7.3 – 13.4%) is substantially lower than in the previous single test-retest HRV measurement (ICC 21.1%). However, the TE of the first versus tenth interval (ICC 23.8%) was extremely close to this result. The standardized change in the mean describing systematic error was *trivial* for all the interval by interval comparisons, except for the first versus tenth interval difference (*possibly small*). This means that a systematic bias might be excluded. The correlations between consecutive intervals were *nearly perfect* for all the comparisons. This meaningfully higher level of reliability is not surprising as most of the possible factors influencing the HRV measurement were eliminated in this experimental design. It is also apparent that there was no clear trend in systematic (ES) or random (TE) error for the ln rMSSD throughout the monitoring period.

Limitations of the study

The study results might primarily be applied to healthy young people who regularly participate in sport activities at a recreational level. The caution must be taken when generalizing the present findings to other individuals of different age or training and health status.

In the present study, participants were allowed to breath spontaneously during HRV monitoring. Although this would be expected to have influenced the value of the HRV indices, the main aim of this study was to assess reliability, not the level of the HRV variables. Previous studies have shown that HRV reliability does not differ to any substantial extent between paced and spontaneous breathing (Bertsch et al., 2012).

Conclusions

Increased attention should be paid to selection of an appropriate HRV variable, when HRV is used for assessing meaningful experimental or training changes, as major differences were found in the reliability level. The vagal-related HRV parameter rMSSD should be preferable due to its higher reliability when compared to the other frequency domain indices. Additionally, this study showed that the possible intrinsic HRV oscillation under stable conditions, expressed by ln rMSSD, was *trivial* and therefore, it was not considered within HRV analysis. Researchers and practitioners should be aware of the magnitude of typical errors which might result in a misinterpretation of study conclusions based on the simple pre vs. post-intervention HRV measurement during one session.

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Corresponding author:

Lukas Cipryan, Ph.D.
Ostravska Univerzita, CDLP
Varenska 40a
702 00 Ostrava
Czech Republic
Phone: +420605807925
E-mail: lukas.cipryan@osu.cz