

Journal of Clinical and Basic Cardiology

An Independent International Scientific Journal



Journal of Clinical and Basic Cardiology 2003; 6 (Issue 1-4), 29-33

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Repeatability of Heart Rate Variability Measured via Spectral Analysis in Healthy Subjects

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Objective was to characterize reproducibility of heart rate variability measurements via spectral analysis during repeated measurements in young healthy subjects. Up to now, reproducibility of heart rate variability has been characterized in a variety of disease states but not extensively in healthy subjects. In a cross-sectional design, healthy volunteers from the hospital staff of a university medical school were recruited. The measurement error of short-term coarse-graining spectral analysis was calculated by 2 methods. Repeated measurements during controlled breathing were performed in seven healthy subjects three times a day during a single session. Such sessions were repeated on a median of 4 days for each subject. Total spectral power in the high and low frequency domain was measured and log transformed. Repeatability was calculated from intra-individual standard deviation as described by Bland and Altman and was also expressed as the intra-class correlation coefficient (ICCC). A total number of 73 observations was obtained for each frequency domain. The intra-class correlation coefficient ranged from 0.658 to 0.807 in the supine and from 0.326 to 0.567 in the standing position. Repeatability ranged from 1.366 to 1.733 ln (ms²) in the supine position and from 1.857 to 2.205 ln (ms²) in the standing position. As a conclusion, a poor reproducibility is obtained in the standing position. In supine position, a medium reproducibility is obtained in the low frequency domain, but a good reproducibility in the high frequency domain. Measurements on different days do not contribute to the variance of spectral power measurements. *J Clin Basic Cardiol 2003; 6: 29–33.*

Key words: autonomic nervous system, spectral analysis, heart rate variability, reproducibility of results

Heart rate variability as a measure of cardiac autonomic function may be assessed by a variety of methods. Time domain measures of 24 hour recordings provide a good view of total power [1]. In such long-term recordings, 95 % of the total power is accounted for by the very low and ultra low frequency spectrum, and only 5 % are accounted for by the low frequency and high frequency spectrum, both being recognized as correlates of sympathetic and parasympathetic modulations. Spectral analysis of 24 hour recordings is also influenced by physical activity, heart rate responses to physical activity, and time of the day.

Short-term frequency domain measurements provide a better discrimination of frequency domains representing sympathetic and parasympathetic modulation [1]. In frequency domain spectral power analysis, application of either the autoregressive model or the nonparametric fast Fourier Transform model is possible. The frequency domain is well assessed by coarse-graining spectral analysis based on a fast Fourier transform (FFT).

Measurement error of the latter method and reproducibility have been tested in patients with metabolic diseases with and without clinical signs of neuropathy. References and comments will be given in the discussion. Little is known about the intra-individual variance and test reproducibility in healthy subjects. Most studies have included patients with metabolic or cardiovascular diseases with various degrees of cardiac autonomic neuropathy (CAN). In such studies, a cohort of patients without clinical signs of CAN were defined as the reference group. Most studies were performed without synchronization of the ventilation during the recording period. The respiratory interval has been demonstrated to greatly influence the high frequency domain of heart rate spectral analysis [2]. Thus, the measurement error of spectral power analysis might not have been characterized appropriately.

The aim of the present study was to characterize the measurement error of short-term coarse-graining spectral analysis in healthy subjects under clinical, non-laboratory conditions. Ventilation was synchronized by an external trigger to 0.2 Hz. This controlled breathing protocol was applied to avoid respiratory modulatory effects on the high frequency domain representing parasympathetic activity [2] and to avoid resonance effects on the 0.1 Hz baroreflex control loop in the domain representing sympathetic modulation [3]. Repeated measurements on a single day and on different days of the same month were tested for repeatability and intraclass correlation coefficient.

Methods

Subjects

A cohort of seven healthy controls of either sex aged 20 to 28 years were recruited for repeated measurements of heart rate variability. None of the subjects was on regular drug treatment, all were nonsmokers, normotensive and physically fit to a high level. The test persons gave consent not to consume coffee or any other drug on the day of examination.

Measurement Device

All measurements were performed with the Varia Pulse TF3 System by Sima Media Olomouc Ltd., Czech Republic. This device is suitable for short-term sampling and recording of heart rate and processing of the data via coarse-graining spectral analysis according to Yamamoto [4]. The electrocardiographic heart actions were recorded by a pair of epidermal electrodes connected to an integrated ultrahigh frequency-transmitter (wave length 950 nm). Electrodes and transmitter were attached to the epigastrium of the test person and fixed by a belt. The single channel ECG data were telemetrically transferred to a receiver connected with an IBM

Received: August 19th, 2002; accepted: January 14th, 2003.

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compatible computer. The analysis of heart rate variability was performed by the computer software of the TF3 system.

Study Protocol

Measurements of a particular person were performed on different days, mainly 4 days (1 to 5) within one month. On a particular day, three successive measurements were performed on each person during a single recording session. The recordings were performed in a quiet room under stable conditions in the afternoon. The number of days from each subject ranged between 1 and 5 because not all subjects were ready to come on several days. Nevertheless, all subjects were included into the study. The exact protocol of recordings is shown in Table 1.

To synchronize the breathing cycle of all subjects to the same frequency, a light signal gave a short blink at 5 second intervals. This results in 12 breaths per minute. Test persons were instructed to start each inhalation with the light signal and adjust ventilation to their comfort. Each recording unit comprised measurements during supine, standing and supine position. Each recording unit comprised 900 heart beats, in particular 300 in a supine position (phase 1), 300 heart beats in a standing position (phase 2), 300 heart beats in a supine position (phase 3). On a particular day, this recording unit was repeated 3 times without interruption of the recording session. The duration of a single cycle depended on the heart rate and lasted for about 15 minutes. The whole recording session on a particular day amounted to about 1 hour.

Processing of Data

Heart rate variation data were collected at a sampling rate of 500 Hz and analyzed by short-term spectral power analysis. The spectral power variables were non-normally distributed. Therefore, natural log transform was applied due to the positively skewed distribution of the original variables.

Distortions of the very low frequency domain (1/f spectrum) were excluded by applying the coarse-graining method by Yamamoto and Hughson [4]. The coarse-graining algorithm is a method to eliminate the influence of a very low frequency band near 0.02 Hz that implies noise on the two defined frequency bands (LF and HF). The coarse-graining analysis algorithm is integrated into the computer software of the Varia Pulse TF3 system.

Measurement Parameters and Abbreviations

The analysis of heart rate spectra is principally based upon a fast Fourier transform (FFT) and modified by coarse-graining spectral analysis according to Yamamoto and Hughson [4]. Heart rate variability in the high frequency (H) domain (0.15–0.50 Hz) and a low (L) frequency domain (0.06–

0.15 Hz) were analyzed separately. The spectral power (ms^2) of the high frequency (HF) domain is generally attributed to modulation by the parasympathetic nervous system and spectral power of the low frequency (LF) domain is attributed to a combined modulation by both the sympathetic and parasympathetic nervous system. The combination of frequency band and measurement phase resulted in 6 variables L1, L2, L3 and H1, H2, H3. The variables L1, H1, L3, H3 were obtained in supine position, the former before and the latter after tilt. The variables L2 and H2 were obtained in standing position.

Statistical Analysis

The number of all observations from all persons was 73. For calculation of repeatability, analysis of variance (ANOVA) with random effects was used. In a first step, a two-way analysis of variance was performed to analyze whether the day of examination contributes to the variance of the spectral parameters. This was not the case. The variance component of the day was zero in all cases. Hence the factor day was removed from the models leading to the application of one-way analysis of variance.

Measurement error was assessed by two different statistical methods, both described by Bland and Altman [5, 6]. Repeatability [5] was principally calculated from the intra-individual SD. The intraclass correlation coefficient (ICCC) [6] was calculated from the inter-individual and intra-individual variance components.

The repeatability coefficient (RC) was calculated by means of $\text{RC} = 2.77\text{sw}$, where sw is the within-subject standard deviation estimated by using ANOVA with random effects as described above [5]. The difference between 2 measurements of the same individual is expected to be less than the repeatability coefficient for 95 % of the pairs of observations. The intraclass correlation coefficient (ICCC) is the proportional contribution of the between-subject variance to the total variance, ie, $\text{ICCC} = \text{sb}^2 / (\text{sb}^2 + \text{sw}^2)$, where sb^2 and sw^2 are the estimated variance components of the ANOVA model described above [7]. The ICC is the average correlation between all possible pairs of observations from the same individual [6].

The ranges of medium and good reproducibility are arbitrary to some extent. For the ICC, from the statistical point of view, a value of 1 means complete reproducibility, 0 means no reproducibility. Values between 0.1–0.3 indicate virtually no reproducibility, values between 0.8–1.0 a very high reproducibility; the range 0.4–0.7 is traditionally regarded as medium.

For the “repeatability“, the interpretation is not a statistical but a clinical one. It depends on the system measured and on how relevant a deviation of the measured value is for the clinician. Because of this, we have shown how the measurement error might lead to another classification of neuropathy stage in the discussion.

Ethical Considerations

The study was approved by the local Ethical Committee. All subjects gave informed consent.

Results

Descriptive statistics of log transformed data are given in Table 2. In supine position, log transformed spectral power $\ln(\text{ms}^2)$ in the high frequency domain before (H1) and after (H3) tilt were 8.02 and 8.11. In the standing position (H2), the value dropped to 6.72. Again in supine position, log transformed spectral power in the low frequency domain before (L1) and after (L3) tilt were 7.39 and 7.33, respectively. During standing, L2 amounted to only 6.37. Repeatability analy-

Table 1. Protocol of spectral analysis sessions

Patient number	Measurements on day				E
	A	B	C	D	
1	1, 2, 3	4, 5, 6	7, 8, 9	10, 11, 12	–
2	1, 2, 3	4, 5, 6	7, 8, 9	10, 11, 12	–
3	1, 2, 3	4, 5, 6	7, 8, 9	10, 11, 12	–
4	1, 2, 3	4, 5, 6	7, 8, 9	10, 11, 12	13
5	1, 2, 3	4, 5, 6	7, 8, 9	–	–
6	1, 2, 3	–	–	–	–
7	1, 2, 3	4, 5, 6	7, 8, 9	10, 11, 12	–

Subsequent days are listed as A to E; recording units (supine, standing, supine) are indicated in subsequent Arabian numbers; on days A–D three recording units were performed in sequence

sis of natural log transformed spectral power parameters is shown in Table 3.

In supine position, ICCC of variables of the high frequency domain (H1 and H3) were 0.807 and 0.775. ICCC of H2 in the standing position was 0.326. In supine position, ICCC of variables of the low frequency domain (L1 and L3) were 0.658 and 0.686, respectively. ICCC of L2 in the standing position was 0.567.

In supine position, repeatability of variables of the high frequency domain (H1 and H3) were 1.366 and 1.600. Repeatability of H2 in the standing position was 2.205. In supine position, repeatability of variables of the low frequency domain (L1 and L3) were 1.675 and 1.733. Repeatability of L2 in the standing position was 1.857.

Discussion

So far, only a few papers have addressed the question of repeatability of heart rate variability measurements in healthy subjects, in particular with an appropriate statistical method. In a frequency domain study by Bernardi [8] including 11 healthy subjects with 6 breaths per minute and a fixed ventilation volume, coefficients of variance were 15.3 % for cross correlation data. Several studies have applied time domain or frequency domain analyses to 24 hours recordings [9–11]. By collecting data over a 24 h period, deterioration by various degrees of physical activity, heart rate responses and ventilation may occur. Maybe for that reason, in a study by Van Hoogenhuyze [9], day to day variation within healthy subjects was high (up to 40 %).

In a previous study [12] including 10 healthy subjects tested under tilt table conditions, reproducibility was calculated by an inappropriate statistical method, because mean values of all individuals of a group were compared. Moreover, non-significant results were misinterpreted as a proof of no difference.

In the vast majority of studies, reproducibility of heart rate variation parameters was assessed in patients with cardiovascular [13] or metabolic diseases. In some studies, diabetic patients who lack clinical signs of neuropathy were included [14–16]. Such patients may not necessarily be defined as healthy subjects. This is also suggested by the finding that spectral power is higher in our cohort of healthy subjects than in the reference group without CAN published by Howorka [15]. They used the same measurement device as we did. In our study, mean values of spectral power were 8.02 and 8.11 in the high frequency domain, and 7.39 and 7.33 in the low

Table 2. Descriptive statistics of natural log transformed data of spectral power

Variable	N	Min	Max	Mean	SD
L1	73	5.37	9.50	7.39	0.98
H1	73	5.60	9.90	8.02	1.05
L2	73	3.36	8.44	6.37	0.98
H2	73	3.17	8.60	6.72	0.95
L3	73	4.36	9.06	7.33	1.06
H3	73	4.80	9.91	8.11	1.15

L1 = low frequency domain first supine phase; L2 = low frequency domain standing phase; L3 = low frequency domain second supine phase; H1 = high frequency domain first supine phase; H2 = high frequency domain standing phase; H3 = high frequency domain second supine phase; N = number of observations; Min = minimum value of spectral power, natural log transformed, expressed as $\ln(\text{ms}^2)$; Max = maximum value of spectral power, natural log transformed, expressed as $\ln(\text{ms}^2)$; SD = standard deviation of spectral power, natural log transformed, expressed as $\ln(\text{ms}^2)$

frequency domain. The corresponding mean values in the study by Howorka were 6.2 in the high and 7.1 in the low frequency domain.

The study by Burger [14], as another point of criticism, assessed reproducibility using the Pearson correlation coefficient and repeated measures analysis of variance. This procedure, in particular hypothesis tests based upon repeated measures analysis of variance, is an invalid statistical method for the problem.

Healthy subjects have been included in the following studies: Breuer [17] studied 10 healthy subjects and revealed a poor reproducibility. Coefficients of variation were 36–74 %. Ziegler [18] included 20 healthy subjects measured twice on two consecutive days. Reproducibility was shown as standard deviation factors. The intra-individual standard deviation factor was 1.58 and 1.50 in the high and low frequency band, respectively. A critical point of the study is the lack of using a synchronized ventilation cycle, because both the high and low frequency domain are influenced largely by fluctuation of the vagal modulation during respiration. This might have distorted the reproducibility of the measurements. Thus, measurement error of spectral power analysis might not have been characterized appropriately in previous studies.

The present study included a homogenous cohort of young and physically fit healthy subjects. Heart rate variability was analyzed by short-term coarse-graining spectral power analysis [4]. To overcome the influence of non-standardized ventilation on HF power and to improve the discrimination of the HF and LF power segments [2, 19], we decided to perform the study with a standardized ventilation cycle synchronized by an external trigger. Controlled respiration at 12–15 breaths/min is able to separate better the peaks of spectral power of the low and high frequency band [2, 3, 12, 19, 20].

Table 3. Descriptive statistics of natural log transformed data of spectral power

	log L1		log H1	
	Variance	SD	Variance	SD
Between subjects	0.70263	0.83823	1.01393	1.00694
Within subject	0.36584	0.60485	0.24302	0.49298
Total	1.06847	1.03367	1.25695	1.12114
ICCC	0.658		0.807	
Repeatability		1.675		1.366
	log L2		log H2	
	Variance	SD	Variance	SD
Between subjects	0.58737	0.76640	0.30586	0.55304
Within subject	0.44923	0.67024	0.63364	0.79602
Total	1.03660	1.01813	0.93950	0.96928
ICCC	0.567		0.326	
Repeatability		1.857		2.205
	log L3		log H3	
	Variance	SD	Variance	SD
Between subjects	0.85560	0.92498	1.15124	1.07296
Within subject	0.39142	0.62564	0.33364	0.57762
Total	1.24702	1.1167	1.48489	1.21856
ICCC	0.686		0.775	
Repeatability		1.733		1.600

L1 = low frequency domain first supine phase; L2 = low frequency domain standing phase; L3 = low frequency domain second supine phase; H1 = high frequency domain first supine phase; H2 = high frequency domain standing phase; H3 = high frequency domain second supine phase; ICCC = intraclass correlation coefficient; SD = standard deviation; Variance and Repeatability are expressed as $\ln(\text{ms}^2)$

The aim of our respiratory protocol was to produce a clear and reproducible HF spectral power. This might at the same time be a disadvantage in terms of the LF component [21]. The phenomenon has been mentioned by the Task Force [1]. However, since repeatability was quite good for the high frequency domain variables, controlled breathing does not seem to reduce reproducibility of spectral power measurements *per se*.

In clinical practice, the central question of the interpretation of measurements is, in how far the measured data would allow the classification to one of the current neuropathy classes, ie marginal (incipient) or overt (advanced) neuropathy. The measurement error should be small enough to allow the classification of the test person to a class or group on the basis of a single measurement. It would be an ideal situation if 90 % of measurements from healthy persons would not exceed the 90th percentile of measurement from patients with incipient neuropathy. But up to now, we do not have enough knowledge about the distribution of individual measurements in each class of CAN and of the degree of overlap between the classes. Most studies indicated the mean of all measurements of a class and the standard error of the mean (SEM), but did not present the data in detail. Another problem of the reference values is a pronounced age dependency of heart variability parameters. A decline of spectral power with age has been demonstrated [8, 18, 22, 23] which is in accordance with own observations [24]. To estimate the degree of overlap, a large number of subjects at different ages in each neuropathy class would be required. It is also an open question whether within a class of neuropathy, there is a weighted or a normal statistical distribution. So, information that would allow the attachment of individual heart rate variability data to a particular class of neuropathy is not available.

The other point that would imply an error to classification and diagnosis of a particular patient is the measurement error. Information about the measurement error of the here applied method has been assessed by calculating the repeatability and the intraclass correlation coefficient. The ICC values are principally easy to interpret as the values range between -1 and +1. In the standing position, ICC values of both the low and high frequency domain amount to 0.567 and 0.326 which indicates a medium to bad repeatability. In the supine position, the ICC values are in the range of 0.658 to 0.807, representing a medium or good reproducibility. The high frequency domain measured in supine position almost exhibits a good ICC value of 0.807 in the first supine phase (H1) and 0.775 in the second supine phase (H3). Intra-individual variance was always lower than inter-individual variance except for the standing position (H1 and H3) where the inter-subject variance was lower than intra-individual variance. This means that the difference of repeated measurement values between different subjects was even smaller than the measurement differences within one subject.

To give an impression of what our repeatability data mean, we would like to compare our data from healthy subjects with data from diabetic patients with various degrees of neuropathy. Such data are available from a study by Howorka [15] who assessed heart rate variability with the same measurement device as we did for spectral power analysis in the frequency domain. Diabetic patients with no, incipient, and severe neuropathy were included: In the high frequency band, log transformed spectral power expressed as $\ln(\text{ms}^2)$ and SEM in different groups of neuropathy were 6.2 ± 0.3 (no CAN), 5.1 ± 0.2 (incipient CAN), 3.7 ± 0.5 (severe CAN). Mean spectral power in our cohort of young healthy subjects was 8.02 ± 1.05 ($n = 73$) in the high frequency band. Assuming the hypothetical case that a single measurement in

this group was 8.02, the repeatability of 1.366 would mean that in 95 % of cases, the following measurements lie within a range of 6.654–9.386. The lower values of this range touch the range that is expected from the diabetic patients with neuropathy. But the same repeatability, applied to the class of incipient neuropathy as defined by Howorka, could lead to a substantial overlap of measurements from different neuropathy classes.

The heart rate variability measurements of persons with neuropathy exhibits almost a good reproducibility in some studies [11, 14]. In contrast to that, young healthy subjects, as included in the study by Van Hoogenhuyze [9], showed a maximal day-to-day variability of 40 %, whereas in cardiac patients, the day to day variability was only 19 %. This may be attributed to the reduced variability in sympathetic and parasympathetic modulation with increasing damage of autonomic nerves. In contrast to that, the validity of the method for healthy persons and the ability of the method to discriminate healthy persons from early stages of neuropathy is questionable and requires further research. As a conclusion, measurements of heart rate variability in healthy persons may produce misleading results of particular parameters due to a medium or poor repeatability and ICC. Unacceptably low reproducibility was found in the standing position, assessed by both repeatability and intraclass correlation coefficient. This may in part be attributed to the condition of our controlled breathing protocol. A medium to good reproducibility of spectral power is found in supine position for both current frequency bands representing parasympathetic and sympathetic modulation. In the supine position, the best reproducibility was found in the high frequency domain which represents parasympathetic activity. Measurements on different days do not contribute to the variance of spectral power parameters.

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