Repeatability of spectral components of short-term blood pressure and heart rate variability during acute sympathetic activation in healthy young male subjects

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1. Changes in the low-frequency (LF) components of blood pressure and heart rate variability and in the ratio of LF to high-frequency (HF) components of heart rate variability (LF/HF ratio) are used to assess acute changes in sympathetic control of blood pressure or heart rate and in sympathovagal balance that occur in response to physiological or pharmacological stimuli. Before these spectral indexes can be used to assess the effects of drug therapy or other clinical interventions on reflex sympathetic activity, their repeatability must be evaluated.

2. Intra-observer repeatability was studied by analysing changes in the LF components (expressed as absolute or normalized units) of cardiovascular variability and in the LF/HF ratio during sympathetic activation induced by nitroglycerin infusion (n = 10 subjects) or 60° head-up tilt (n = 13 subjects) repeated on two occasions, 2 days and 1 week apart respectively, in healthy young male volunteers. Repeatability was estimated as recommended by Bland and Altman.

3. Bland and Altman’s plots of the repeatability of changes in the LF components and LF/HF ratio showed that measurements were sufficiently repeatable to be used over periods of time of up to 1 week in clinical studies.

4. The sample-size tables derived from our results show that expression of spectral components as normalized units, and use of a cross-over design, minimize the number of subjects to be included in clinical studies conducted using similar designs and LF component changes as endpoints.

INTRODUCTION

Power spectral analysis of systolic blood pressure (SBP) and heart rate (HR) variability measured using a Finapres device may provide non-invasive and accurate assessment of autonomic nervous system modulation of cardiovascular function [1, 2]. Low-frequency (LF ~0.1 Hz) SBP oscillations reflect rhythmic changes in vasomotor activity that are thought to be mediated by the sympathetic control loop of the baroreflex [3]. LF HR oscillations reflect a combination of sympathetic and parasympathetic influences, whereas high frequency (HF ~0.25 Hz) HR oscillations provide an index of parasympathetic activity [1, 2]. In resting conditions, satisfactory repeatability of these spectral indexes during periods of up to 1 month has been reported in healthy volunteers [4, 5].

An inability of the autonomic nervous system to reflexively modulate sympathetic and parasympathetic activity in response to stressful conditions has been incriminated in the pathogenesis of many cardiovascular diseases, including sudden death, myocardial infarction and syncope [6, 7]. Cardiovascular reflex tests based on blood pressure (BP) and HR variability have been developed to evaluate autonomic cardiovascular modulation. Changes in the LF components of cardiovascular variability and in the ratio of the LF to HF components of HR variability (LF/HF ratio) have been shown to reflect acute changes in the sympathetic control of HR or BP [8, 9] and in the sympathovagal balance [3] that occur in response to pharmacological or physiological stimuli. These indexes have been used to assess reflex sympathetic activation induced by nitroglycerin (NTG) infusion [3, 10] and head-up tilt [11, 12]. Before they can be used to assess the effects of drug therapy or other clinical interventions on reflex sympathetic activity, their repeatability must be evaluated.

The aim of this study was to evaluate intra-observer repeatability of changes in the spectral components of SBP and HR variability during sympathetic

Key words: blood pressure, heart rate, methodology, repeatability, spectral analysis, variability.

Abbreviations: BP, blood pressure; Di, difference between two measurements; HF, high frequency; HR, heart rate; i.v., intravenous; LF, low frequency; NTG, nitroglycerin; RC, repeatability coefficients; SBP, systolic blood pressure.

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activation induced by a pharmacological stimulus, i.e. infusion of NTG, and a physiological stimulus, i.e. head-up tilt, in healthy subjects.

METHODS

Selection of subjects

Twenty-three healthy male subjects were selected among medical students and their relatives. They were considered healthy volunteers if their medical history and physical examination showed no evidence of cardiovascular, pulmonary, renal or hepatic disease and if their ECG revealed no abnormalities. Subjects having presented any previous medical history of vaso-vagal symptomatology were excluded. None of the study subjects were taking any drugs at the time of the study. Subjects who smoked more than five cigarettes a day were excluded. Subjects were instructed to refrain from drinking beverages containing caffeine or alcohol and from smoking for at least 12 h before each study day. The study was conducted in a quiet room with a controlled temperature of 22°C.

All subjects gave their written informed consent to participate in the study, which was approved by the local Committee for the Protection of Human Subjects in Biomedical Research of our institution.

Experimental protocol

Study I design: repeatability of sympathetic activation induced by NTG infusion. This study was conducted in ten volunteers aged 21–29 years (mean ± SD; 24 ± 2.3 years). After their arrival at the clinical investigation unit, the subjects were asked to lie supine. An indwelling catheter was placed in a left forearm vein, which was kept patent by an infusion of 0.9% NaCl in water. Subjects rested for 30 min. After baseline recordings, 0.45 mg of NTG (Lenitral, Besins-Iscovesco Laboratories, Paris, France) was infused intravenously in 1 min, and HR and BP were recorded for 10 min. Twenty-four hours later the subjects returned to the laboratory, and the same protocol was repeated.

Study II design: repeatability of sympathetic activation during head-up tilt. This study was conducted in 13 volunteers aged 22–40 years (mean ± SD, 25 ± 4.9 years). After their arrival at the clinical investigation unit, the subjects rested supine on a tilt-table, with their feet against a foot-rest and their waist secured by a loose belt around the table. After a 30 min rest, the table was tilted to 60° head-up over a period of 20 s and left in that position for 10 min. The subjects returned to the laboratory 1 week later and the same protocol was repeated under the same conditions and at the same time.

Signal processing and spectral analysis of BP and HR variability

Signal processing. BP was monitored continuously and non-invasively using a Finapres device (model 2300; Ohmeda, Trappes, France). This method is based on servo-plethysmomanometry using the volume clamp technique [13, 14] and allows detection of arterial pulsations. The Finapres cuff was fitted to the third finger of the right hand, which was maintained at the level of the heart on an adjustable support throughout the study. Before beginning the baseline recordings, BP was simultaneously measured using an automated intermittent BP recording device (Dinamap 1846 SXP Critikon, Tampa, FL, U.S.A.) placed on the left arm. Finapres device recording was started only when the difference between SBP values measured by each device was less than 15 mmHg. BP and HR were recorded continuously during a 5 min rest period immediately before the NTG infusion or head-up tilt, and for 10 min after the NTG infusion or during the head-up tilt.

The analogue output from the Finapres was connected to an analogue-to-digital converter to permit data acquisition, storage and analysis using a PC computer. The BP signal was digitized using a 12-bit analogue-to-digital converter at a rate of 500 Hz, then processed by an algorithm based on feature extraction for the detection and measurement of the characteristics of an arterial pressure cycle, using the computer program Anapres 3.0 (Notocord Systems, Croissy/Seine, France). A sampling rate of 2 Hz was chosen because this window was well suited to the HR of the healthy subjects, in whom the number of beats/min was usually in the 55–120 range. Raw data for systolic and diastolic BP and for HR were stored on a hard disk, and variations in the frequency domain were analysed later.

Frequency domain analysis of HR and BP variability. The equal time interval sampling of systolic and diastolic BP and HR allowed direct spectral analysis of each distribution using a fast Fourier transform algorithm on 512-point stationary time series, using the computer program Anapres 3.0 (Notocord Systems). This corresponded to a period of 4 min 16 s at our sampling rate of 2 Hz. Only stationary periods free from ectopic beats or missing data were analysed. A square window function was used. The stationary nature of the recordings was carefully verified by visual inspection and was confirmed by comparing the means and SDs for the two consecutive 256-point time series used for the analysis. After NTG infusion or head-up tilt, spectral analysis was performed over a period of 256 s, starting 50–70 s after discontinuation of the NTG infusion and 90 s after the 60° head-up position had been reached, which were the times needed to reach stationary haemodynamic conditions.

Each spectral band was characterized by its frequency and by its modulus. Modulus reflects the amplitude of oscillations at the frequency being considered. The frequency of the oscillation scale (abscissa) was analysed up to 0.5 Hz. The integration of the values of consecutive bands was computed to permit estimation of the different
components of BP or HR variability. The total area under the curve was taken as the overall variability and was obtained by integrating the spectral bands from 0.004 to 0.5 Hz. The LF component of BP or HR variability was obtained by integrating the values of 16 consecutive bands from 0.066 to 0.129 Hz of the BP or HR spectrum. The HF component of HR variability was obtained by integrating the values of seven consecutive bands detected within the 0.2–0.5 Hz range and centred around the respiratory peak. This respiratory peak was defined as the frequency at the maximum spectral peak in this frequency region in the HR spectrum and agreed with the breathing rate of the subject. During the NTG test, the breathing rate was determined during each recording period. During the tilt-test, the breathing rate was continuously monitored using a nasal/oral airflow sensor (Edentec, Nellcor, Jouyen-Josas, France).

Each spectral component was expressed in absolute units [mmHg/Hz^{1/2} for BP and beats min^{-1}/Hz^{1/2} for HR] and as a percentage of the total area under the curve (normalized units). The LF/HF ratio of the HR variability spectrum was also calculated. Systolic and diastolic BP, and HR measurements, are given as the mean values of the 256 s recordings used for spectral analysis.

Statistical analysis

Data are reported as means±SEM. The paired Wilcoxon’s test was used to determine the significance of differences between spectral components at rest and during sympathetic activation. Differences were considered significant for \( P<0.05 \).

The parameters whose repeatability was analysed were changes in the LF component of SBP and HR variability and percentage changes in the LF/HF ratio of HR variability. When two series of paired measurements were compared, the results were analysed in two steps as recommended by Bland and Altman [15]. First, we used the paired t-test to compare the means of the differences between the two series of measurements with zero. Then, we plotted the relative (positive or negative) differences within each pair of measurements (Di) against the mean of the pair, to verify the assumption that Di did not vary in any systematic way over the range of measurements.

Repeatability coefficients (RC), as defined by the British Standards Institution [16], were calculated using the formula \( RC = \sqrt{\frac{\sum Di^2}{n}} \), where Di is the observed difference between two measurements in a pair and \( n \) the sample size. This coefficient is the SD of the estimated difference between two repeated measurements. The 95% confidence interval of the expected difference was calculated as \( \pm 1.96RC \).

Construction of power tables

We used the variances estimated in these two studies to calculate the number of subjects to be included in future studies conducted using either a cross-over design (comparing the changes in LF components of BP and HR variability over time or according to treatment) or a parallel-group design (comparing the variance of these parameters across two groups of subjects). Sample sizes were calculated to detect a difference in spectral components expressed as absolute and normalized units, assuming a power of 0.80 (beta = 0.20).

RESULTS

Haemodynamic status at rest and during sympathetic activation

Baseline SBP, diastolic BP and HR did not differ significantly between study days in either study: 118±5/7±10 mmHg and 63±3 beats/min compared with 115±5/5±4 mmHg and 64±4 beats/min for the NTG study, and 118±9/5±5 mmHg and 62±8 beats/min compared with 117±11/5±6±4 mmHg and 68±8 beats/min for the head-up tilt study. None of the healthy subjects experienced syncope or presyncope symptoms during head-up tilt. The changes in SBP and diastolic BP induced by sympathetic activation between the two stationary recordings used for fast Fourier transform analysis did not differ significantly between the two study days in either test (+9±4/+3±4 mmHg compared with +6±5/+3±2 mmHg for the NTG test, and +1±5/+10±3 mmHg compared with −1±7/+9±4 mmHg for the head-up tilt test). Similarly the increases in HR did not differ significantly between the two study days in either study: +4±3 and +3±5 beats/min for the NTG study and +23±9 and +24±9 beats/min for the head-up tilt study.

Spectral components at rest and during sympathetic activation

SBP and HR spectral components at rest and during NTG infusion and head-up tilt on each study day are shown in Tables 1 and 2. In neither study did the spectral components differ between control periods on the two study days. The increase in the LF components of SBP and HR variability and in the LF/HF ratio did not differ significantly between the two study days.

Repeatability and construction of power tables

Bland and Altman’s plots of repeatability of the changes in LF components of BP and HR variability are shown in Figure 1 for the NTG infusion study and in Figure 2 for the head-up tilt study. Plots of repeatability of the changes in the LF/HF ratio for the two studies are shown in Figure 3. None of the mean relative differences were significantly different from zero. There were no obvious relationships between the Di measurements and the mean change.
Spectral analysis repeatability

Fig 1. Plots of differences between selected pairs against means of the pairs for the changes in LF components of SBP variability (upper panel) and HR variability (lower panel) induced by an i.v. NTG infusion repeated after a 24 h interval. The corresponding RCs are indicated. Spectral indexes (differences within each pair and mean of the pair) are expressed in absolute values (left panel) and in normalized units (nu) (right panel). Abbreviation: bpm, beats min⁻¹. Horizontal lines indicate the mean differences and their 95% confidence intervals.

Fig 2. Plots of differences between selected pairs against means of the pairs for the changes in LF components of SBP variability (upper panel) and HR variability (lower panel) induced by a 60° head-up tilt repeated after a 1 week interval. The corresponding RCs are indicated. Spectral indexes (differences within each pair and mean of the pair) are expressed in absolute values (left panel) and in normalized units (nu) (right panel). Abbreviation: bpm, beats min⁻¹. Horizontal lines indicate the mean differences and their 95% confidence intervals.
Fig 3. Plots of differences between selected pairs against means of the pairs for the percentage changes in the low-frequency to high frequency ratio (LF/HF) of HR variability, induced by an i.v. NTG infusion repeated after a 24 h interval (left panel) and by a 60° head-up tilt repeated after a 1 week interval (right panel). The corresponding RCs are indicated. Horizontal lines indicate the mean differences and their 95% confidence intervals.

Table 3. Sample-size table derived from the NTG infusion repeatability study. Absolute units are mmHg/Hz\(^{1/2}\) for the change in the LF component of SBP variability and beats min\(^{-1}/Hz^{1/2}\) for the change in the LF component of HR variability. Normalized units are expressed as percentages.
Spectral analysis repeatability
used increasingly to provide an index of sympathetic activity in healthy subjects [12, 20, 21] and in patients with cardiovascular diseases [22, 23]. In the present study, we used 5 min recordings and stationary haemodynamic conditions. Short-term recordings are more likely to be stationary, allow more precise control of the laboratory conditions under which the measurements are made and facilitate examination of specific short interventions such as head-up tilt or NTG infusion. In addition, use of short-term recordings of 5 min processed by frequency domain methods was recommended in the recent guidelines for measurement of HR variability [24]. The use of longer recordings with an overlapping segment technique could reduce the variance but only at the cost of a deterioration in frequency resolution.

After NTG infusion and during head-up tilt, the higher LF components of HR and SBP variability and the higher LF/HF ratio of HR variability indicated sympathetic activation with a shift in the sympathovagal balance toward sympathetic predominance. These results are in agreement with previous studies using the same tests of sympathetic activation [7, 10–12]. Changes in HR and spectral components of BP and HR variability showed a trend towards a weaker response to NTG infusion. Cardiovascular responses to i.v. NTG infusion and to head-up tilt are complex and different in terms of the pathways involved. The cardiovascular response to a rapid NTG infusion depends primarily on the arterial baroreceptor reflex, whereas the cardiovascular response to a 60° head-up tilt is largely mediated by both the arterial baroreceptor reflex and the cardiopulmonary receptor reflex [25]. The arterial baroreceptor reflex provokes cardiac sympathetic activation and cardiac vagal withdrawal in addition to vasomotor sympathetic activation. The cardiopulmonary receptor reflex causes vasomotor sympathetic activation. The fact that vasomotor sympathetic activity is controlled by both reflexes may explain why the vascular response to sympathetic activation was larger during head-up tilt than during NTG infusion. Nevertheless, the aim of the present

Table 4. Sample-size table derived from the head-up tilt repeatability study. Absolute units are mmHg/Hz^{1/2} for the change in the LF component of SBP variability and beats/min/Hz^{1/2} for the change in the LF component of HR variability. Normalized units are expressed as percentages.

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study was not to compare the responses to different tests of sympathetic activation and our study was not designed to allow such a comparison.

To assess repeatability, we used the widely accepted method developed by Bland and Altman [15], which is based on graphic techniques and simple calculations. The plots showed that repeatability of the changes in the LF components was satisfactory, regardless of whether changes in the LF components were expressed as absolute or normalized units. However, since the normalization procedure minimizes the effects of variation in total spectral power, the choice of normalized units exerts a marked influence on the sample-size tables derived from our results. The choice of normalized units reduced the required sample size (except for the HR LF change induced by NTG infusion). The sample-size tables also show that a cross-over design minimizes the required sample size. These results are in agreement with those of Dimier-David et al. [5], who conducted a repeatability study of HR and BP variability under resting conditions in healthy young male volunteers. The sample-size tables derived from our study can only be used for further studies conducted in healthy male volunteers. Other investigations are needed to develop such tables in other populations, such as patients with cardiovascular diseases. In these patients, the relevance of the LF component of cardiovascular variability to assess sympathetic cardiovascular modulation is limited when variability is reduced, for instance during heart failure or after a myocardial infarction [1, 26]. A saturating effect of persistent sustained sympathetic discharges directed at the heart may result in a marked reduction in HR variability and does not permit the superimposing of HR variability [26].

In conclusion, under our controlled experimental conditions, intra-observer repeatability of changes in LF components of short-term SBP and HR variability, and in the LF/HF ratio of HR variability during sympathetic activation induced by NTG infusion or head-up tilt, was satisfactory. Our sample-size tables may be helpful in designing clinical pharmacology studies conducted in healthy young male volunteers, using head-up tilt or NTG infusion as stimuli and spectral changes in SBP and HR variability as endpoints.

REFERENCES