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Reproducibility of non-invasive measurement and of short-term variability of blood pressure and heart rate in healthy volunteers

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1 Spectral analyses of blood pressure and heart rate oscillations are increasingly used to assess the influences of diseases and drugs on the autonomic nervous system. Such influences can only be interpreted in view of the spontaneous variability of these oscillations. We therefore studied the reproducibility of power spectral analyses of blood pressure and heart rate fluctuations measured by a non-invasive finger plethysmographic method in 24 healthy volunteers.

2 Intra-observer reproducibility was assessed from measurements obtained on 3 consecutive days and 1 month later in each subject. Inter-observer reproducibility was assessed by comparing measurements made by two observers on one occasion.

3 There was no significant difference in standard haemodynamic and spectral analysis parameters (low frequency: 60–130 mHz and high frequency: respiration rate ± 30 mHz) measured on 3 consecutive days and 1 month later in each subject. The standard deviation of differences between systolic blood pressure or heart rate oscillations on different occasions was in the 150–200 and 50–100 mm Hg $Hz^{-1/2}$ or beats min^{-1} $Hz^{-1/2}$ range for low frequency and high frequency oscillations respectively. Similar results were found when inter-observer reproducibility was considered.

4 From these results, we derived a sample-size table giving the number of subjects to be included in studies of cross-over or parallel design in order to detect a non-random difference in spectral analysis parameters. For example, detection of a 200 mm Hg $Hz^{-1/2}$ difference in low frequency oscillations of systolic blood pressure at an alpha and a beta risk of 0.05 and 0.10 respectively would require the inclusion of approximately 15 subjects in a cross-over design study and 50 subjects in a parallel design study.

5 We conclude that, under our experimental conditions, non-invasive measurements of blood pressure and heart rate oscillations are reproducible. This technique can be used to assess the influences of diseases and drugs on the autonomic nervous system using a reasonably small number of subjects.

Keywords method variability blood pressure heart rate spectral analysis

Introduction

Analysis of short-term variability of haemodynamic parameters is increasingly used as a method for the assessment of autonomic nervous system activity in investigational studies. Power spectrum analyses of heart rate (HR) and blood pressure (BP) fluctuations are used to measure pathophysiological or pharmacological influences on the sympatho-vagal balance [1–3]. Spectral analysis of BP and HR oscillations

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has been used to define prognosis indices in patients following myocardial infarction, congestive heart failure and coronary artery disease and for the diagnosis of autonomic neuropathy in patients with diabetes mellitus or uraemia [4, 5]. Power spectral analysis of BP and HR variations is not only useful in physiological and pharmacological studies but, because the method is readily available from commercial devices, may also be used in daily clinical practice.

Non-invasive plethysmographic finger blood pressure recording has recently been extensively used for the short-term continuous measurement of BP and HR. This method gives information similar to that obtained from invasive methods [6–8]. However, limited information is available on the reproducibility of this and other techniques of power spectral analyses of BP and HR variations [4]. Such reproducibility studies are critical in designing investigational studies because, for any parameter considered, the significance of the difference attributable to an intervention depends on the spontaneous variability of this parameter which, therefore, influences the statistical power of clinical trials assessing the effects of drugs or diseases on BP and HR oscillations.

The primary objective of the present study was to examine the spontaneous short-term and mid-term intra-observer reproducibility of spectral analyses of BP and HR oscillations determined by using a non-invasive recording device. A secondary objective was to use the results to construct a sample size table of the number of subjects to be included in pharmacological studies testing the influence of drugs on BP and HR oscillations. An additional objective was to study inter-observer reproducibility.

Methods

Subjects

Twenty-four healthy subjects were included in this study. They were selected from medical students and their relatives. They were 18 males and 6 females with ages ranging from 19 to 29 years (mean ± s.d.: 24.2 ± 2.6 years). Subjects had no clinical sign of organic or systemic disease and all had a normal electrocardiogram. Their respiratory rate was superior to 10 min^{-1}. Subjects smoking more than five cigarettes a day or taking any drug were excluded from the study. They were instructed to come to the Clinical Pharmacology Unit after a light breakfast and to avoid caffeine containing beverages during the 8 h preceding the recordings. 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.

Study design

Each volunteer came five times to the Clinical Pharmacology Unit to have blood pressure and heart rate variability recordings: three times at 24 h intervals (day 1, D1; day 2, D2; day 3, D3), once the next week (week 1, W1) and once the next month (month 1, M1). In order to limit external sources of parameter variations, including circadian variability, the D1, D2, D3, M1 recordings were performed by the same observer under the same experimental conditions of clock time, devices, room temperature in each volunteer in order to study intra-observer reproducibility. The W1 recording was performed twice successively in a random order by two different observers at 20 min time interval for the study of inter-observer reproducibility. Studies were carried out in a quiet room with a controlled temperature of 20 to 24° C after 20 min rest in the supine position.

Blood pressure and heart rate recordings

Finger arterial pressure was measured continuously by using a non-invasive plethysmographic recording device (Finapres® model 2300, Ohmeda, Maurepas, France) [9]. The cuff was fitted to the third finger of the dominant hand and placed at heart level. An automated intermittent blood pressure recording device (Dinamap® model 1846 SXP, Critikon Inc., Tampa Fla.) was positioned on the opposite arm. Before starting each recording we proceeded to a standardization of the measures of systolic blood pressure (SBP) obtained by the Finapres and the Dinamap device. Recordings were started only when the difference in SBP given by both devices was less than 15 mm Hg [6]. Five minute recordings of BP and HR variabilities were then performed with the subject remaining in the supine position while breathing spontaneously.

Parameters studied

For each recording and each subject, mean and s.d. of SBP diastolic BP (DBP) and HR were computed over 256 s of continuous recording. Spectral analyses parameters of SBP, DBP and HR variations based on the fast Fourier transform on 256 points time series were calculated by using the computer software Anapres 3.0 (Notocord Systems, Igny, France). This corresponded to a 4 min 16 s recording period at a 1 Hz sampling rate. The low frequency (LF) oscillations of HR and BP (i.e. Mayer wave) [10] were defined as the area of the 66–129 mHz frequency band [11, 12]. High frequency (respiratory) oscillations (HF) were arbitrarily chosen as a band of 30 mHz width centred on the subject's spontaneous respiratory frequency which was measured by counting the number of inspirations over 5 min of recording. Areas under the power spectrum *vs* frequency curve of LF and HF oscillations were calculated for SBP, DBP and HR.

Measurement of reproducibility

We prospectively studied short-term intra-observer reproducibility by comparing the results obtained on D3 with those obtained on D2, the latter being con-

sidered as the standard recording. D1 recording was not chosen as the standard because we wanted to test the hypothesis that a training test was required to obtain stable results. For this purpose, D1 recordings were compared with D2 recordings. Mid-term intra-observer reproducibility was studied by comparing results obtained on M1 with those of D2.

Inter-observer reproducibility was studied on W1 by comparing the results of the recordings performed by the two observers. The order of the two operators was randomized. The recordings performed by each observer were separated by a 20 min interval. The second operator repeated the experimentation from the beginning, i.e. including the placement of the finger cuff and the SBP calibration. This part of the study included only 23 subjects because one subject could not be studied that day due to a technical problem unrelated to the study.

Data analysis

The number of subjects to be included in this study was estimated from the results of a previous study performed in 11 subjects [13]. In this study, the mean increase in LF oscillations of systolic BP associated with the reflex sympathetic stimulation induced by nitroglycerin infusion was approximately 150 mm Hg $Hz^{-1/2}$ The s.d. of LF oscillations of systolic BP under control conditions was approximately 140 mm Hg $Hz^{-1/2}$. In the present study, the number of subjects to be included was calculated in order to detect a mean difference of 80 mm Hg $Hz^{-1/2}$ with a 0.80 power (beta risk of 0.20) and an alpha risk of 0.05. The difference of 80 mm Hg $Hz^{-1/2}$ was chosen arbitrarily as one which would be the minimum difference relevant to most clinical pharmacology studies of power spectral changes. The assumption was that if the present study was able to detect such a difference, then the technique would be of limited applicability in clinical pharmacology studies.

Paired Wilcoxon's tests were used for all comparisons. Differences were considered significant when the probability of erroneously rejecting the null hypothesis of no difference was inferior to 5%.

Reproducibility was estimated according to the recommendations of Bland & Altman [14]. This method consists of calculating the mean of the repeated measures and the standard deviation of the differences and plotting the difference between measurements as a function of the mean value in each subject. The smaller the standard deviation of the difference, the greater the reproducibility. Before studying the inter-observer reproducibility, we looked for an order-observer interaction. Results are reported as mean ± 1 s.d. unless otherwise specified.

Construction of power tables

Using the variances estimated in the present study, we derived tables of sample sizes to be included in studies, either of cross-over or of parallel design, with variable degrees of differences of power spectrum parameters as endpoints. For a parallel design, we used the variance of M1-D2 and for a cross-over design, we used the variance of [(M1–D2) – (D3–D2)] and calculated the sample size from usual formulas.

Results

Short-term and mid-term intra-observer reproducibility

Figure 1 shows the mean values of SBP, DBP and HR over the 256 s of continuous plethysmographic recordings in each individual on the four study periods used for intra-observer reproducibility. No significant difference between D2 and D3 or between D2 and M1 were found for both means and s.d.s of SBP, DBP and HR. Mean values of SBP, DBP and HR ranged from 115 ± 8 mm Hg to 120 ± 13 mm Hg, 58 ± 7 mm Hg to 60 ± 10 mm Hg and 65 ± 6 beats

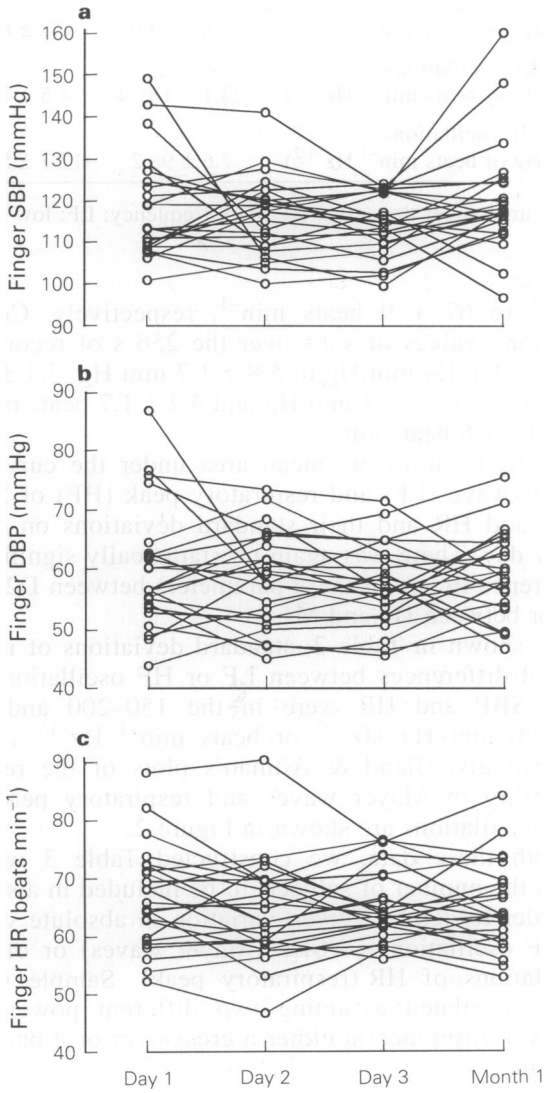

Figure 1 Individual results of standard haemodynamic parameters. Mean systolic blood pressure (SBP; a), diastolic blood pressure (DBP; b) and heart rate (HR; c) values measured with a non-invasive finger plethysmographic method over 256 s are shown at each study period in each individual.

Table 1 Spectral analysis parameters (mean ± s.d.)

	Study day 1	*Study day 2*	*Study day 3*	*Study month 1*
Systolic blood pressure				
AUC LF oscillations (mm Hg $Hz^{-1/2}$)	555 ± 193	533 ± 200	538 ± 142	533 ± 157
AUC HF oscillations (mm Hg $Hz^{-1/2}$)	153 ± 124	145 ± 65	133 ± 66	119 ± 59
Diastolic blood pressure				
AUC LF oscillations (mm Hg $Hz^{-1/2}$)	372 ± 105	372 ± 124	353 ± 92	385 ± 114
AUC HF oscillations (mm Hg $Hz^{-1/2}$)	71 ± 57	72 ± 44	63 ± 36	74 ± 57
Heart rate				
AUC LF oscillations (beats min^{-1} $Hz^{-1/2}$)	576 ± 236	523 ± 226	539 ± 199	543 ± 227
AUC HF oscillations (beats min^{-1} $Hz^{-1/2}$)	269 ± 148	268 ± 138	265 ± 120	241 ± 102

AUC: area under the curve; HF: high frequency; LF: low frequency (see text for definitions).
No difference was statistically significant across study periods.

Table 2 Differences between study periods (mean ± s.d.)

	Systolic blood pressure			*Heart rate*		
	D1–D2	*D3–D2*	*M1–D2*	*D1–D2*	*D3–D2*	*M1–D2*
Absolute value (mm Hg or beats min^{-1})	1.8 ± 13.0	1.8 ± 6.5	3.7 ± 12.9	2.1 ± 6.1	0.9 ± 6.7	0.5 ± 4.6
AUC LF oscillations (mm Hg or beats min^{-1} $Hz^{-1/2}$)	22.1 ± 181.4	4.5 ± 158.1	0.5 ± 209.5	52.7 ± 175.4	15.6 ± 165.8	19.2 ± 186.7
AUC HF oscillations (mm Hg or beats min^{-1} $Hz^{-1/2}$)	7.6 ± 98.2	−12.7 ± 51.4	−26.4 ± 63.1	14.4 ± 101.7	−19.3 ± 99.5	−42.9 ± 119.2

AUC: area under the curve; HF: high frequency; LF: low frequency (see text for definitions).

min^{-1} to 67 + 9 beats min^{-1}, respectively. Corresponding values of s.d.s over the 256 s of recording were 5.3 ± 1.4 mm Hg to 5.8 ± 1.7 mm Hg, 3.1 ± 0.8 mm Hg to 3.4 ± 1.4 mm Hg and 4.7 ± 1.7 beats min^{-1} to 4.9 ± 1.6 beats min^{-1}.

Table 1 shows the mean area under the curve of Mayer wave (LF) and respiratory peak (HF) of SBP, DBP and HR and their standard deviations on each study day. There was again no statistically significant difference in any of these parameters between D2 and D3 or between D2 and M1.

As shown in Table 2, standard deviations of inter-period differences between LF or HF oscillations of both SBP and HR were in the 150–200 and the 50–100 mm Hg $Hz^{-1/2}$ or beats min^{-1} $Hz^{-1/2}$ range respectively. Bland & Altman's plots of the reproducibility of Mayer waves and respiratory peak of SBP oscillations are shown in Figure 2.

With these data, we constructed Table 3 which gives the number of subjects to be included in a study in order to detect a given variation in absolute value of LF oscillations of SBP (Mayer waves) or of HF oscillations of HR (respiratory peak). Sample sizes were calculated assuming two different powers to detect a difference in either a cross-over or a parallel design.

Inter-observer reproducibility

SBP, DBP and HR measurements and corresponding spectral analysis parameters performed on W1 by two different observers did not significantly differ. For the systolic blood pressure, inter-observer difference was 1 ± 10 mm Hg for the absolute value, 59 ± 209 mm Hg $Hz^{-1/2}$ for the low-frequency component of spectral power and 10 ± 53 mm Hg $Hz^{-1/2}$ for the HF component of spectral power. Corresponding values for the diastolic blood pressure were 4 ± 7 mm Hg, 2 ± 100 mm Hg $Hz^{-1/2}$ and 7 ± 27 mm Hg $Hz^{-1/2}$. Corresponding values for the heart rate were 1 ± 3 mm Hg, 74 ± 229 beats min^{-1} $Hz^{-1/2}$ and 19 ± 94 beats min^{-1} $Hz^{-1/2}$. There was no order-observer interaction. No inter-observer difference was statistically significant.

Discussion

Our results demonstrate that, under our experimental conditions, measurements of BP and HR oscillations using the Finapres device and spectral analysis based in fast Fourier transform are highly reproducible over periods of 1 day to 1 month. This is true for all parameters studied and for both intra- and inter-observer reproducibility.

Spectral analysis assesses the non-random components of blood pressure and heart rate variability and gives quantitative indices of the autonomic nervous system activity. There are two major oscillations of BP and HR: the LF oscillation is centred at about 100 mHz and the HF oscillation, which is synchronous of respiration, is centred at about 200 mHz. When considering HR variability, the HF component is

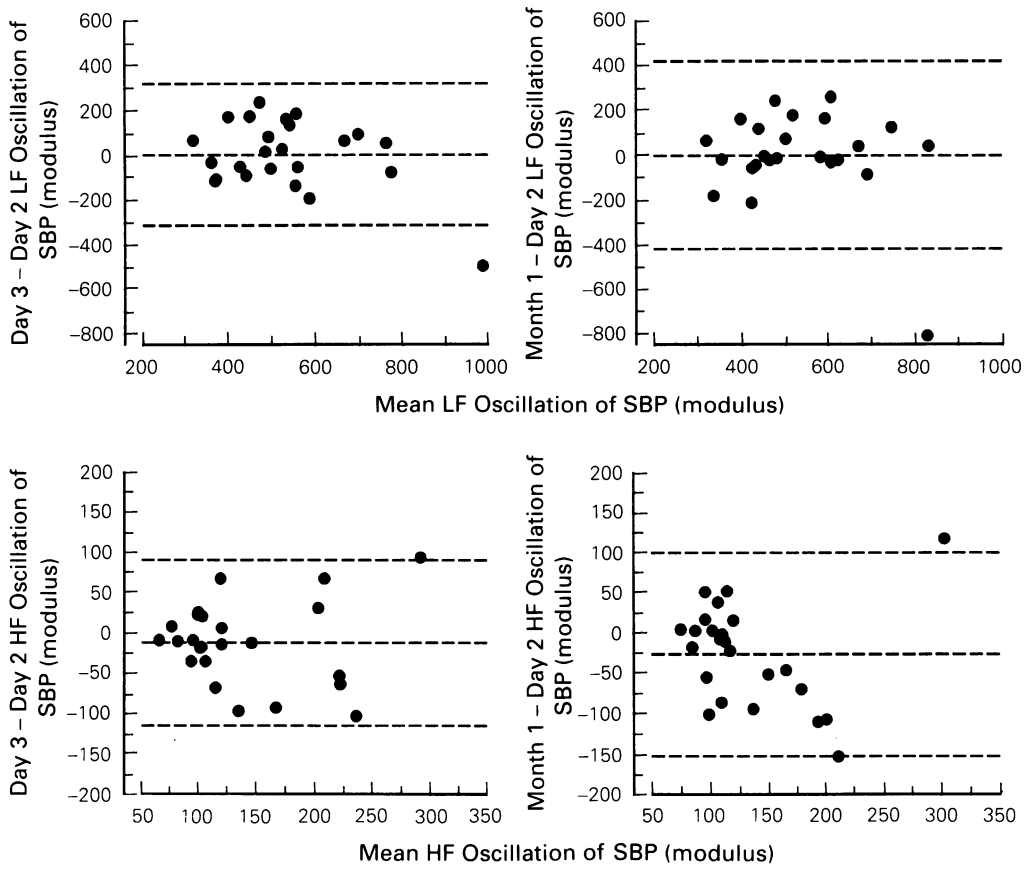

Figure 2 Plots of differences between selected pairs of study periods as a function of the mean value for systolic blood pressure oscillations. Plots give a visual index of the reproducibility of low frequency (upper panels) and high frequency (lower panels) oscillations of systolic blood pressure. Horizontal lines indicate mean and 95% confidence interval of the difference between two given study periods.

Table 3 Sample size table derived from the present study

	Beta = 0.20 (power = 0.80)		*Beta = 0.10 (power = 0.90)*	
	Cross-over design	*Parallel design*	*Cross-over design*	*Parallel design*
Absolute change of SBP LF oscillations to be detected (mm Hg $Hz^{-1/2}$)				
25	654	2208	876	2954
50	164	552	219	740
75	73	246	98	330
100	41	138	55	186
125	28	90	36	120
150	20	62	26	84
175	16	46	20	62
200	13	36	16	48
225	10	30	13	38
250	9	25	11	32
Absolute change of HR HF oscillations to be detected (beats min^{-1} $Hz^{-1/2}$)				
25	188	716	252	958
50	47	180	63	240
75	23	80	30	108
100	14	46	18	60
125	10	32	12	40
150	8	22	9	30
175	6	18	7	22
200	5	14	6	18
225	5	12	5	14
250	4	10	5	12

HF: high frequency; HR: heart rate; LF: low frequency; SBP: systolic blood pressure. To account for the loss of power which may occur with non-parametric analyses, these numbers should be multiplied by 1.16 when such tests are to be used.

generally accepted as a marker of vagal activity [2, 15], whereas the LF component is considered as a marker of sympathetic activity [2, 16, 17]. Thus, the LF-to-HF ratio is used as a marker of the sympatho-vagal balance. When considering arterial blood pressure variability, the LF component also appears to reflect the sympathetic activity [2, 16] whereas the HF component is largely influenced by mechanical changes induced by respiration and cannot be considered as a marker of vagal activity [16–18].

Other methods have been used to assess sympatho-vagal balance in humans [4, 19]. These include biochemical parameters such as noradrenaline plasma levels or various parameters of HR variability obtained in the time domain from ambulatory continuous Holter recordings such as standard deviation of RR intervals, day time and night time heart rates. Spectral analysis of the short-term oscillations of HR and BP has the advantage of being a simple and innocuous method. To our knowledge, evaluation of the reproducibility of BP and HR measurements by finger plethysmography has not yet been reported. Silke *et al.* [20] were especially interested by its accuracy and by the agreement between non-invasive and intra-arterial pressure measurement. Their study only showed that the reproducibility of the method, studied by the coefficient of variation, was similar to that of the intra-arterial pressure for systolic and diastolic blood pressure values.

In our study, the reproducibility of Finapres measurements was very satisfactory for both haemodynamic and power spectrum parameters. Interestingly, under our experimental conditions, the reproducibility of measurements was as good between day 2 and day 3 as between day 2 and month 1, indicating that this method can be used for cross-over investigational studies performed on periods of a few days to 1 month. Also, the absence of significant difference between parameters obtained on study day 1 and study day 2 indicates that no training effect occurs with this technique. Such a good reproducibility may not be found during measurements obtained under dynamic conditions since the accuracy of HR and BP measurements with the finger plethysmography technique may decrease during exercise [20].

From our results, we were able to derive a sample size table for different study designs and powers in order to choose the number of subjects to be included in investigational studies depending on the extent of the difference in LF oscillations of SBP or HF oscillations of HR to be shown under the influence of any pharmacological, physiological or pathological intervention. We chose to base our calculations on these two indices because there is a general agreement on their use for measuring sympathetic and vagal tones respectively [2, 15, 16]. This table will be helpful in designing further studies using spectral analyses of BP and HR fluctuation as an endpoint.

In conclusion, under controlled experimental conditions, intra- and inter-observer reproducibility of short-term BP and HR variability is high and cross-over studies can be performed when using these endpoints.

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