**Title**

Reproducibility of 24-hour ambulatory blood pressure and measures of autonomic function.

**Running head**

Reproducibility of ABPM and autonomic function.

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**Conflicts of interest**

None declared

**Abstract**

**Objectives:**

Determining the number of familiarisation sessions required for accurate recordings of ambulatory blood pressure monitoring (ABPM) and autonomic function is a pre-requisite for the appropriate design of intervention studies. The benefit of familiarisation trials remains largely unexplored. The objective of the current investigation was to assess the reproducibility of 24-hour ABP, 24-hour HRV and resting measurements of heart rate variability (HRV) and blood pressure variability (BPV).

**Methods:**

Eleven pre-hypertensive and hypertensive adults participated. Ambulatory blood pressure and HRV were measured across 24-hours on 4 occasions. In addition, five minute resting measures of HRV and BPV were recorded and analysed. Variability between consecutive pairs of trials was calculated.

**Results:**

The typical error (TE) induced by ambulatory recordings of systolic blood pressure (SBP) reduced over time (3.8-2.8mmHg). The greatest effect of familiarisation was observed at night. Ambulatory HRV was more reproducible than resting measures. The most reproducible markers were rMSSD (CV; 13.2-10%) and HF nu (CV; 15.2-6.4%) with pNN50% showing the poorest reproducibility (CV; 23.9-20.7%). Overall BPV (SD) was more reproducible than the frequency domain LF component.

**Conclusions:**

Familiarisation trials are required for the most accurate recordings of both 24-hour ABPM and HRV. Ambulatory HRV provide superior reproducibility to resting measurements.

**Keywords:** ambulatory blood pressure monitoring, heart rate variability, blood pressure variability, reproducibility

## Introduction

Hypertensive adults display a persistent elevation of systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) alongside a deterioration of autonomic functioning [1]. The detection of meaningful changes in these variables is a pre-requisite for the proper interpretation of post treatment/intervention findings. As these physiological markers are sensitive to changes in mood, time of day and nutritional intake, isolating the true biological effects of a therapeutic intervention is problematic.

Blood pressure has traditionally been measured within a clinic environment. This method is commonly associated with white-coat and masked hypertension. In addition, the placebo effect and observer bias reduce the suitability of this measurement for clinical research [2]

Ambulatory blood pressure monitoring (ABPM) could be more representative of BP because it provides measurements during daily activities. Ambulatory blood pressure has also been shown to be more reproducible than clinic measurements [2]. However, the novelty of wearing an ambulatory device for the first time might initiate a pressor effect [3]. Therefore, a limitation of previous studies is the measurement of APBM across two monitoring periods only.

Heart rate variability (HRV) and blood pressure variability (BPV) provide valuable information on autonomic function [4]. Despite BPV being increasingly recognised as a risk factor for cardiovascular disease [5], the assessment of its absolute reliability is not commonplace. In contrast, the reliability of HRV has received considerable attention.

Relative reliability of HRV is frequently reported as good-excellent [6]. However, large intra subject variability and therefore poor absolute reliability has also been detected [6]. Longer recordings have shown lower variability [7]. However, the effect of familiarisation on reliability remains unexplored.

In light of the limited data, this study aims to assess whether familiarisation improves the reproducibility of resting autonomic function (BPV and HRV) and 24-hour ABPM and HRV in pre-hypertensive and hypertensive adults.

## Methods

Eleven (9 females, 2 males) pre-hypertensive and hypertensive adults (SBP; 141.7±8.9mmHg, DBP; 83±10.9mmHg) with a mean age of 67.2±4.7yrs, body mass of 67.5±12kg and height 162.4±8.3cm participated. Four participants were taking anti-hypertensive medication. Those with SBP >160mmHg or <130mmHg, a history of cardiovascular events or diabetes were excluded. Buckinghamshire New University ethics board approved this investigation. All participants provided written informed consent.

### Research design

Participants attended the laboratory on four occasions for repeat measurements. Pre-visit conditions, day and timing of measurement was standardised.

### Assessment of short-term BPV and HRV

Lying supine, a continuous BP measurement was recorded to assess short-term BPV (Finapres, TNO Instruments, Amsterdam, The Netherlands). An ECG recording was obtained simultaneously using a 2-lead (Cardiotens, Meditech, Hungary) or 3 lead (Card(X)plore, Meditech, Hungary) configuration. All recordings took place during 10 minutes rest and the final 5 minutes was analysed.

### 24-hour Ambulatory measurements

The Cardiotens and Card(X)plore devices were used for ambulatory BP and HRV recordings. Following resting measurements, a pneumatic cuff was attached to the participant’s non-dominant arm. Chest electrodes were retained from earlier measurements. The device was programmed to record BP every 30 minutes between 06.00-22.00 and every hour between 22.00-06.00. Participants record sleep and waking hours.

### Data processing

*Heart rate variability:* ECG signals were analysed by the Cardiovisions software (Meditech, Hungary) using time and frequency domain analysis [4]. Beat to beat intervals were considered valid if they were different from the previous interval by less than 20%. Overall variability (SDNN) and variables representative of vagal tone (rMSSD, pNN50%, HF) were analyzed.

*Blood pressure variability:* Powerspectral analysis of continuous BP recordings was carried out using online software (Cardioseries, v2.4). All tachograms were visually inspected for abnormally shaped and ectopic beats; these were replaced by a linear interpolation algorithm. Data was resampled at 2Hz and Fast Fourier transform was applied to 128-point sections. Overall variability (SD) and low frequency (LF) were analysed.

*24-hour blood pressure:* Total average, day and night average were calculated for both SBP and DBP.

### Statistical analysis

A spreadsheet was used to assess the reproducibility [8]. Consecutive pairs of measurements (1-2, 2-3, 3-4) were analysed and the TE was calculated. If the measurement did not meet the criteria for normality the TE was calculated on log transformed data (ln) and reported as a coefficient of variation (CV%). Statistical analysis was conducted using IBM SPSS statistics 23 (IBM Corporation, Somers, New York). A one way ANOVA with repeated measures was carried out to determine significant differences between each measurement.

## Results

Descriptive statistics for all variables (mean ±SD) are presented in Table 1. There were no significant differences detected. Variation between measurements is displayed in Table 2. The TE within SBP measurements showed a small but progressive decline across trials. Typical error within the DBP was smaller with only night-time measurements showing a decline across trials. As compared with 24-hour measurements, resting HRV displayed larger magnitudes of error (Table 2). The CV(%) between 24-hour measures of rMSSD reduced over time. Similarly, HF (nu) displayed the smallest CV(%) during average and night time recordings between trials 3-4. The SD component of BPV also showed the smallest CV(%) during trials 3-4.

## Discussion

The first novel finding is that TE for SBP decreased across measurements. Importantly, the increased sensitivity was most evident during the night, a period which has been shown to display the most variability across consecutive measurements [2]. These findings indicate that habituation to the procedure is beneficial. The TE of SBP was lower in the present study compared with those previously reported in hypertensives [2]. This likely reflects the lower baseline BP of the current participants. Variability in blood pressure is generally proportional to BP values and therefore variability may increase as the severity of hypertension increases.

Although the reproducibility of DBP improved across consecutive night measurements a smaller magnitude of error was found as compared with SBP (Table 2). This finding is consistent with previous studies [2,9]. There has been limited discussion in the literature with regards to the reasons for this. However, increases in sympathetic activity during daily tasks such as reading and talking have been shown to influence SBP to a greater extent [9]. In addition, the current participants displayed a predominance of isolated systolic hypertension; a reduced/plateaued DBP may have contributed to smaller variability.

In relation to HRV, this study found lower CV in all 24-hour HRV parameters in comparison to 5-minute resting recordings. Large day-to-day variations in resting recordings is consistent with previous research [6]. Despite large variations, the reproducibility of time domain parameters was similar to that of other clinical populations [6] .

The most reproducible markers of vagal tone over 24-hours were rMSSD and HF nu with pNN50% displaying the largest CV (Table 2). Typical error during night-time recordings of rMSSD and HF nu progressively declined across trials indicating a habituation to wearing the device whilst sleeping. Other research found similar variation between 2 consecutive measurements [10]. The improvements in reproducibility following familiarisation are novel to the current research.

Good reproducibility of 24-hour HRV as determined by the SDNN parameter was found. This is in contrast to findings from Pitzalis and colleagues who found larger CV (20%) in young healthy adults [7]. It is well known that HRV is reduced in clinical and older populations [1] and may therefore display more reproducibility.

## Limitations and Conclusions

 Blood pressure and autonomic function were only assessed in a small sample of pre-hypertensive and hypertensive adults. The current study observed a predominance of isolated systolic hypertension; a phenomenon common in older populations. Readers should be cautious about generalising these findings to younger hypertensive groups.

It is recommended that ambulatory recordings should be preceded with at least one habituation period. For HRV, 24-hour measurements offer the best reliability with familiarisation trials of some benefit. Although clinic measurements provide quick measurements in controlled conditions; 24-hour measurements offer superior reproducibility and where possible should be utilised in the design of intervention studies.

**Table 1** Descriptive statistics (mean+SD) of ABPM, HRV and BPV.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **24-hr ABPM** | **Trial 1** | **Trial 2**  | **Trial 3**  | **Trial 4**  |
| Average SBPAverage SBP | 136.4 ± 8.9 | 138.3 ± 12.9 | 136 ± 10.7 | 136.4 ± 9.6 |
| Average DBP | 78.2 ± 7.3 | 77.5 ± 8.9 | 76.7 ± 8.1 | 76.6 ± 7.6 |
| Day SBP | 141 ± 11.1 | 142.6 ± 13.5 | 141.4 ± 11.6 | 141.9 ± 10 |
| Day DBP | 81.2 ± 7.6 | 80.4 ± 8.4 | 80 ± 8.7 | 80.6 ± 8.3 |
| Night SBP | 125.6 ± 9.1 | 125.3 ± 12.8 | 120.4 ± 11.1 | 121.9 ± 11.9 |
| Night DBP | 69.4 ± 6.6  | 68.9 ± 9.8 | 67.1 ± 7.7 | 67.6 ± 7.2 |
| **24-hr average HRV** |  |  |  |
| rMSSD (ms) | 24.4±12.3 | 28.9±17.5 | 26.2±14.1 | 27.3±14.4 |
| SDNN (ms) | 71.3±17.1 | 74.1±21.4 | 73.7±23.7 | 70.0±17.8 |
| pNN50 (%) | 5.0±7.4 | 7.3±10 | 5.7±8 | 6.2±8 |
| HF nu  | 25.5±9.3 | 29.4±10.2 | 26.8±9.0 | 28.0±9.9 |
| **Daytime HRV** |  |  |  |  |
| rMSSD (ms) | 22.1±11.3 | 26.0±15.8 | 24.0±26.5 | 24.9±14.1 |
| SDNN (ms) | 71.7±17.6 | 74.8±22.7 | 74.8±26.5 | 68.0±19.1 |
| pNN50 (%) | 3.5±4.8 | 5.6±7.7 | 4.3±6.2 | 4.6±5.4 |
| HF nu | 27.3±10 | 28.7±9.4 | 28.4±8.5 | 28.1±8.3 |
| **Night-time HRV** |  |  |  |  |
| rMSSD (ms) | 28.4±14.6 | 29.4±16.2 | 30.6±16.8 | 32.2±17.2 |
| SDNN (ms) | 70.3±19.6 | 73.6±19.9 | 72.0±20.1 | 73.8±17.1 |
| pNN50 (%) | 8.1±12.3 | 10.8±15.4 | 8.7±12.1 | 9.4±14.0 |
| HF nu  | 25.2±10.5 | 28.5±13.3 | 26.5±10.1 | 27.2±13.4 |
| **Short-term HRV**  |  |  |  |  |
| rMSSD (ms) | 21.7±12.6 | 22.5±16.9 | 21.5±15.7 | 23.2±18.6 |
| SDNN (ms) | 31.8±15.3 | 35.4±19.2 | 33.9±18 | 35.6±17.3 |
| pNN50% | 4.1±7.4 | 6.1±13.1 | 4.3±7.1 | 7.8±12.9 |
| HF nu | 43.1±13.8 | 41.4±14.2 | 33.7±12.8 | 36.6±15.5 |
| **Short-term BPV** |  | 41.4±14.2 | 33.7±12.8 | 36.6±15.5 |
| SD mmHg | 5.6±1.4 | 5.9±1.6 | 5.6±1.0 | 6.7±1.6 |
| LF (%) mmHg | 46.8±13.2 | 45±10.3 | 41.7±10.7 | 50.3±7.1 |

SBP, systolic blood pressure; DBP, diastolic blood pressure; rMSSD, root mean square of successive differences; SDNN, standard deviation of all NN intervals; pNN50%, the percentage of adjacent NN intervals differing by more than 50ms; LF, low frequency; HF, high frequency; nu, normalised units; SD, standard deviation.

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 2** Reproducibility of 24-hour ambulatory blood pressure, 24-hour heart rate variability and short term blood pressure variability and heart rate variability.  | **Trial 2-1 (TE±90% CI)** | **Trial 3-2 (TE±90% CI)** | **Trial 4-3 (TE±90% CI)** |
| **24 hour SBP** |  |  |  |
| Average | 3.8 (2.8-6.1) | 3.1 (2.3-4.9) | 2.8 (2.1-4.4) |
| Daytime  | 4.9 (3.7-7.9) | 3.9 (2.8-6.2) | 2.8 (2.1-4.5) |
| Night-time | 5.4 (3.9-8.5) | 4.4 (3.2-7.0) | 3.6 (2.7-5.8) |
| **24hour DBP** |  |  |  |
| Average  | 1.7 (1.3-2.7) | 1.9 (1.4-3.0) | 1.5 (1.0-2.3) |
| Daytime | 1.4 (1.0-2.2) | 2.1 (1.6-3.4) | 2.0 (1.5-3.2) |
| Night-time | 3.8 (2.8-6.0) | 2.7 (2.0-4.3) | 1.7 (1.3-2.8) |
|  | **Trial 2-1 (CV±90% CI)** | **Trial 3-2 (CV±90% CI)** | **Trial 4-3(CV±90% CI)** |
| **24 hour average HRV**rMSSD ln (ms)  | 13.2 (9-20) | 11.2 (8-28) | 10.3 (8-16) |
| SDNN  | 7.4 (5-12) | 6.6 (5-11) | 9.4 (7-15) |
| pNN50% | 23.9 (17-39) | 20.8 (15-33) | 20.7 (15-33) |
| HF nu ln (%) | 12.1 (9-19) | 15.2 (11-24) | 6.4 (5-10) |
| **24 hour daytime HRV** |  |  |  |
| rMSSD ln (ms)  | 11.4 (8-18) | 9.9 (7-16) | 13.6 (10-22) |
| SDNN  | 9.1 (7-14) | 8.6 (6-14) | 12 (9-19) |
| pNN50% | 20.9 (15-33) | 16.4 (12-26) | 21.4 (16-34) |
| HF nu ln (%) | 11.9 (9-19) | 11.2 (8-18) | 14.1 (10-23) |
| **24 hour night-time HRV** |  |  |  |
| rMSSD ln (ms)  | 14.9 (11-25) | 9.5 (7-16) | 6.9 (5-11) |
| SDNN  | 11.9 (9-19) | 7.9 (6-13) | 9.2 (7-15) |
| pNN50% | 24.3 (19-40) | 30.5 (23-49) | 20.8 (15-33) |
| HF nu ln (%) | 16.9 (12-27) | 20.5 (15-33) | 9.3 (7-15) |
| **Short-term HRV**  |  |  |  |
| rMSSD ln (ms) | 18 (13-29) | 18 (13-29) | 21.8 (16-35) |
| SDNN ln (ms) | 24.8 (18-39) | 26.1 (19-42) | 28.4 (21-45) |
| pNN50% ln  | 35.5 (26-57) | 27.4 (20-44) | 31.5 (23-54) |
| HF nu ln (%) | 30.9 (23-49) | 33.4 (25-53) | 18.4 (14-29) |
| **Short-term BPV** |  |  |  |
| SD ln (mmHg) | 15.4 (11-25) | 15.7 (11-26) | 11 (8-18) |
| LF ln (%) | 28.2 (21-45) | 25.3 (18-42) | 24.7 (18-39) |

SBP, systolic blood pressure; DBP, diastolic blood pressure; rMSSD, root mean square of successive differences; SDNN, standard deviation of all NN intervals; pNN50%, the percentage of adjacent NN intervals differing by more than 50ms; LF, low frequency; HF, high frequency; nu, normalised units; ln, log transformed; SD, standard deviation

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