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**Title:** Within day and between day reproducibility of baroreflex sensitivity in healthy adult males

**Key words:** Reliability; repeatability; sequence; spectral; supine; tilt

## **Abstract**

Within day and between day reproducibility of supine and tilt baroreflex sensitivity was investigated utilising sequence and spectral indices in 46 healthy adult males employing three repeat measures; baseline, + 60 min and + 24 h. Reproducibility was assessed via the 95% limits of agreement and by the technical error of the measurement. For spectral parameters, the limits of agreement indicated same day was marginally better than between day reproducibility. For sequence parameters, between day had marginally better agreement than same day reproducibility. Tilt markedly improved reproducibility across all outcome measures. Precision expressed by the technical error of the measurement for all spectral outcomes was good in both supine and tilt baroreflex sensitivity ( $< 6\%$ ). Precision was lower, but acceptable, for sequence baroreflex sensitivity outcomes in both positions ( $< 11\%$ ). Baroreflex sensitivity transfer gain provided the best agreement and reproducibility during supine and tilt conditions. These findings suggest time and spectral techniques may be employed to assess within day and between day baroreflex sensitivity changes in healthy individuals. The inclusion of a tilt manoeuvre may improve the reproducibility of the outcome measure which may aid in the detection of modest baroreflex sensitivity changes in studies employing limited sample sizes.

## **Introduction**

Changes in baroreflex sensitivity (BRS) have been found to be of clinical value for diagnostic [15] and prognostic [20] purposes and ongoing clinical research supports a link between BRS and disease and health outcomes [27]. Diminished BRS appears to be linked to unfavourable health outcomes [8,17,35,37,38] while conversely, enhancements in BRS may be beneficial to cardiovascular health. One factor that appears to enhance BRS in some circumstances is exercise [6,23,44]. However, if findings are to be of practical use an understanding regarding the magnitude of error or level of variance present in the outcome measure is required as such error or level of variance is a component of the effect size [28,47]. A reproducibility study allows the exploration of the magnitude of error or level of variance providing a basis for future research testing [28].

Reproducibility may be determined on the same day and/ or following a specified period of time i.e.,  $\geq 24$  h and should have reference to the repeated measures protocol to be incorporated in future research. The BRS assessment technique has been implicated in the variability of the BRS outcome measure [11,19,31] with improved reproducibility reported with low frequency (LF) BRS spectral techniques compared to high frequency (HF) BRS spectral techniques [31] during spontaneous breathing and, in some spectral techniques compared to sequence measures [11,19]. The posture position of the participant may also influence the reproducibility and variability of the measure with improved reproducibility reported during standing compared to measures achieved under supine resting conditions [14,19,25]. The improved reproducibility of spontaneous BRS in standing over the mid (1 wk) to long term (1 y) suggested small significant changes (3 or/to 5 ms/mmHg) could be detected

via small sample size (24 - 4 participants), in both the time and spectral domains in follow-up studies [19]. This may be an important finding because in some experimental studies it is only practically possible to recruit small participant numbers, and the previously reported significant changes in BRS following various interventions and events have been small [10,16,24,32,33,41].

Although the spontaneous non-invasive BRS techniques have been routinely employed for baroreflex testing, there are only a few studies that have assessed the absolute reproducibility of these techniques [11,12,19,25,29,31]. Absolute reproducibility provides information regarding the intraindividual variability of a measurement [31]. Limitations in BRS reproducibility studies have been reported [31] and include low sample size [11,19,25,29], limited choice of BRS technique [25] and lack of protocols for between day reproducibility [11,12,19,29]. In the one study which has included a large sample size, a wide selection of BRS parameters and a protocol for between day reproducibility, it did not include same day reproducibility or an orthostatic manoeuvre [31]. Different statistical techniques have also been employed to assess absolute reproducibility and include the coefficient of variation (CV) and the limits of agreement (LOA) [7]. The use of the correlation coefficient has been criticised [2,26] because it provides information regarding the degree of association between the measures and is unable to distinguish between different linear relationships [7,30]. Thus it is possible to have good correlation but a lack of agreement between the measures. The CV requires heteroscedasticity to be explored and quantified before assuming its presence and is considered to be a liberal measure as only 68% of the variability is described [2]. Sample heterogeneity and systematic bias are possible problems with regression analysis in reproducibility investigations

[2] and all of these techniques are reported in dimensionless ratios. The LOA plot overcomes these limitations, providing a schematic measurement of error utilising the bias (mean difference across subjects) and 95% limits of agreement (test-retest differences across 95% of population) and aids in identifying heteroscedasticity and skewness in the data [7]. Overall, reproducibility assessed via CV (%) ranged from 14 to 52% (11,12,25,29) and the measurement error in LOA (ms/mmHg) ranged from 8 to 26 ms/mmHg in supine BRS measures and 3 to 4 ms/mmHg in standing BRS measures (11,19). Spectral indices were markedly improved in BRS measures incorporating the LF component compared to those measures incorporating HF (11,29,31) with spectral BRS measures providing better reproducibility than time (sequence) BRS measures. A further statistical technique is the technical error of the measurement (TEM) which estimates the level of precision (or imprecision) associated with the measure i.e., the level of error of the method due to biological and technical factors (34]. The identification and quantification of TEM enhances the opportunity to find genuine change and contributes to a valid interpretation of the results following testing in future studies.

The present study included a large sample size, a range of BRS parameter selection and an orthostatic manoeuvre to comprehensively assess the same day and between day reproducibility of BRS in a healthy adult male population. Reproducibility was assessed via the LOA (actual unit of measure) to assess the level of agreement between the measures and by the TEM.

## Methods

### *Participants*

The forty six non-smoking healthy male participants (18 – 35 y) (table 1) who volunteered to participate had no history of diabetes, hypertension or cardiac disease, showed no signs of disease, were not taking medication and were undertaking regular exercise (moderate exercise  $5 \pm 2 \text{ h}\cdot\text{wk}^{-1}$ ). All participants completed health screening and subsequently provided informed consent. All procedures conformed to those approved and cleared by the University Research Ethics Committee and were in accordance with recognised ethical standards and national/ international laws [18].

**\*\*\*\*Table 1 near here**

### *Study Design*

A test-retest reproducibility study of the procedure for assessment of BRS was conducted. Testing was undertaken in controlled laboratory conditions and across all tests (mean  $\pm$  SD): air temperature  $22.8 (\pm 1)^{\circ}\text{C}$ ; humidity  $36 (\pm 9) \%$ ; barometric pressure  $1009 (\pm 13) \text{ hPa}$ . Each participant was required to visit the laboratory on three separate occasions. Visit 1 allowed determination of resting HR and brachial BP and familiarisation with equipment and testing procedures. During visits 2 and 3, data collection in supine and tilt positions was undertaken at baseline, + 60 min and + 24 h respectively. Participants were requested not to: drink alcohol 24 h before each test; not to drink caffeine on day of each test; not to eat 3 h before each test; not to drink 1 h before each test and, not to exercise 48 h before each test beyond normal daily activities. All participants confirmed that they had complied with the pretesting guidelines. Testing at baseline and + 24 h was scheduled at the same time of day to



avoid a circadian influence. During supine conditions, participants lay on a tilt bed (Model 501, Plinth 2000, Stowmarket, Suffolk, UK) in a horizontal position. The tilt manoeuvre employed a 60° upright tilt in accordance with consensus protocols [4,43]. All tilt manoeuvres followed supine data collection to ensure cardiovascular outcomes were not influenced prior to tilt conditions. Participant breathing was not controlled during testing procedures as consistently improved reproducibility has not been found following paced breathing, albeit  $BRS_{aHF}$  determination [12,31], and LF spectral analysis avoids most respiratory influence.

#### *Data collection*

Participants rested supine for 20 min before data collection. Continuous 10 min collections of R-R interval data and beat by beat BP data were undertaken while participants were in supine and tilt positions. A three lead ECG (Absolute Aliens Oy, Turku, Finland) was attached to the participants' chest and R-R interval measures were determined from the recorded ECG. The collection of beat-by-beat BP signal data was determined via finger servo-plethysmomanometry (Portapres Model-2, FMS, Finapres Medical Systems BV, Amsterdam, The Netherlands) with the hand kept at heart level throughout the measurement process. Full signal acquisition was achieved via the physiocal immediately prior to each supine and each tilt measurement.

#### *Data analysis*

The signal data were fed into an acquisition system (WinAcq, Absolute Aliens Oy, Turku, Finland) where the signals were interpolated and relayed to a laptop computer (Tecra S1, Toshiba, Finland) using a sampling rate of 800 Hz and stored for later analysis. The data were processed with dedicated software (WinCPRS, Absolute

Aliens Oy, Turku, Finland) and both time ( $BRS_{UpUp}$  and  $BRS_{DownDown}$ ) and spectral ( $BRS_{\alpha LF}$  and  $BRS_{TFTG}$ ) analyses of BRS were undertaken. The WinCPRS software was utilised to calculate the moving average of the signal over the data range (0.05 s) for the BP data. The ECG data was filtered using a Butterworth low pass filter at 45 Hz to reduce noise and minimise any measurement error. R-R intervals were calculated from ECG signals and the data was visually inspected to identify and correct any irregular or missing R-R intervals. Three analysis techniques for BRS determination were employed; one technique in the time domain (sequence) and two techniques in the spectral domain ( $\alpha$  coefficient and transfer gain) [5,13,22,25,36,37,39,40,45,48].

### *Statistical analysis*

The reproducibility analyses were undertaken in Microsoft Excel using a scatter plot to display agreement and LOA employing the technique of Bland and Altman [7]. This technique is known to be affected by heteroscedastic data, so initially the relationship between the mean of the two repeat measures and the mean absolute difference of the two repeat measures was plotted and quantified (equation of line of best fit). Given the weak relationship and low slope, the bias and LOA were determined with the standard approach. The LOA plots provided a visual examination of the agreement between the measures; narrow confidence intervals (CI) suggested good agreement (lesser variability in the measure) while wide CI's suggested poor agreement (greater variability in the measure). An alternative approach of estimating the TEM to assess reproducibility was also undertaken in accordance with an accepted assessment protocol [34]. Criterion levels [3,34,46] for reproducibility (TEM) may be interpreted as good ( $\leq 6\%$ ), 'fair' (7 - 20%) or 'poor'

(> 20%) as measurement error for experimental testing purposes i.e., anthropometric assessment processes suggest a satisfactory level of precision for skinfold measurement is  $\leq 5\%$  while biomechanical investigations have reported precision levels of  $\leq 20\%$ . Sample size calculation for follow-up studies to detect a given change in tilt BRS was defined with the formula  $n = 8s^2/d^2$ , where  $n$  is the sample size,  $s$  is the typical error (95% CI) and  $d$  is the required meaningful change ( $\Delta$  BRS) [21].

## Results

Data for BRS outcome measures, bias, standard deviation, 95% LOA and CI's are provided in table 2, data for TEM are provided in table 3 and LOA plots are provided in figures 1 - 4. In general, same day reproducibility and between day reproducibility was similar i.e., supine (mean)  $BRS_{UpUp}$ : 30 ms/mmHg vs. 28 ms/mmHg and 10% vs 9% respectively (tables 2 and 3; figures 1 - 2). The bias (mean difference across subjects) was small (i.e., close to zero) for all BRS outcomes at all time points. Thus because there were no consistent differences between the first and second measurements, the data could be used to assess reproducibility. Overall, poorer agreement (i.e., lower reproducibility) and between subject heterogeneity was observed for parameters in supine position (7 – 30 ms/mmHg) (i.e., figures 1 and 3) compared to tilt position (3 – 5 ms/mmHg) (i.e., figures 4 - 5) with all BRS outcomes. In the supine position the sequence outcomes provided poorer agreement and heterogeneity (14 – 30 ms/mmHg) (i.e., figures 1) than the spectral outcomes (7 – 11 ms/mmHg) (i.e., figures 3). The adopted criteria suggested there was markedly better (good) agreement and reduced heterogeneity with all BRS outcomes in the tilt position (3 – 5 ms/mmHg) (table 2). In practice, to be confident of genuine change post-intervention, change must be greater than the measurement error. For example,

in this study population post-intervention change should be greater than 9 ms/mmHg in supine  $BRS_{aLF}$  and/ or greater than 4 ms/mmHg in tilt  $BRS_{aLF}$  (mean  $\pm$  95% LOA ms/mmHg). The findings of lesser variability in all BRS measures under tilt conditions suggest reproducibility was better following an orthostatic manoeuvre compared to resting supine BRS measures. The findings for TEM under the adopted criteria suggested precision for all spectral outcomes was good in both supine and tilt ( $< 6\%$ ) although precision was lower for the time (sequence) outcomes in both positions (4 – 10%) (table 3). Sample sizes for follow-up studies intended to detect given changes in BRS are provided in table 4.

\*\*\*\***Figure 1 near here**

\*\*\*\***Figure 2 near here**

\*\*\*\***Figure 3 near here**

\*\*\*\***Figure 4 near here**

\*\*\*\***Figure 5 near here**

\*\*\*\***Table 2 near here**

\*\*\*\***Table 3 near here**

\*\*\*\***Table 4 near here**

## **Discussion**

The present study investigated the same day and between day reproducibility of the measurement of BRS in a healthy adult male population and the influence of a tilt manoeuvre on the reproducibility of BRS outcome measures. Key findings included minimal differences between same day reproducibility and between day

reproducibility and a marked improvement in reproducibility in all BRS outcome measures in the tilt position.

Same day reproducibility was assessed previously in two studies [11,29] and reproducibility over various time periods have included 24 h (between day) [25,31] and up to one year [12,19,29]. Although previous studies assessing reproducibility > 24 h were not directly applicable to the present study, they did provide additional evidence for the reproducibility of BRS and for the usefulness of an orthostatic manoeuvre during testing [19]. Differences between study procedures (i.e., sample size, BRS parameter selection, re-testing time lag and testing manoeuvres) did not allow direct comparison between studies however overall, the measurement error assessed via 95% LOA (ms/mmHg) ranged from 8 to 26 ms/mmHg in supine BRS measures [11,19] and 3 to 4 ms/mmHg in standing BRS measures [19]. The present study observed similar findings with 95% LOA ranging from 7 to 30 ms/mmHg in supine BRS measures and 3 to 5 ms/mmHg in tilt BRS measures. None of the previous studies had included an assessment of TEM which indicates the level of precision of the measure. The present study observed good precision for the spectral measures in supine ( $\leq 6\%$ ) and for all measures in tilt ( $\leq 5\%$ ) (table 3). Although same day reproducibility and between day reproducibility was similar, same day reproducibility was minimally better than between day reproducibility for the spectral parameters while between day reproducibility was minimally better than same day reproducibility for the sequence parameters (tables 2 and 3). This was an unexpected finding and no explanation could be found for this result.

Sequence outcome measures had poorer reproducibility compared to spectral measures under supine conditions (i.e., figures 1 - 2; table 2) with 95% LOA ranging from 14 to 30 ms/mmHg compared to 7 to 11 ms/mmHg respectively. The greater magnitude and variability of the supine sequence measure may be attributed in part to two practical features; the influence of respiration on the measure and the lack of suitable sequences to achieve an accurate measure. Sequence BRS is determined over a wide frequency range including the HF domain (0.15 – 0.4 Hz), the frequency band which is synchronized with respiratory rate [9]. Thus sequence measures may be influenced by respiration because respiration affects the naturally occurring oscillations which modulate BP and HR [1]. However, when spectral BRS measures are undertaken in the LF domain only, respiratory influence is attenuated. Reproducibility of different spectral techniques was markedly reduced in BRS HF measures (under spontaneous breathing) compared to those measures incorporating LF only [11,29,31]. Therefore the findings in the present study are consistent with previous research i.e., as respiration was not controlled and LF only was employed for spectral measures, poorer reproducibility was found in sequence outcome measures compared to spectral measures. In conditions of low BP variability, only a few acceptable sequences may be obtained resulting in sequence BRS measures having limited accuracy [5,22,37,42]. The number of recognised sequences may be increased by a longer recording length but such action may have practical implications i.e., satisfaction of stationarity requirements for spectral analysis and the servo-adjustment (Physiocal) requirements of the Portapres over time [30] and an incompatibility with study design requirements where multiple short time measures are required. The achievement of greater BP variability and increased identifiable sequences may be induced by an imposed modification i.e., tilt. The present study found greater

identification of acceptable sequences under tilt conditions compared to supine conditions. These findings suggest the selection of the BRS technique may be an important consideration in research testing because the magnitude of the outcome measure for BRS may be highly variable under the same condition, in the same population and under the same testing environment [31]. Such variability may obscure the detection of genuine change and provide comparability issues between studies.

In the present study a marked improvement in reproducibility in all tilt BRS outcome measures was found (LOA 3 - 5 ms/mmHg; TEM < 2) (i.e., figures 4 - 5; tables 2 and 3) and may be due to a reduction of neural influences of internal and external stimuli on the symphthalic balance, mitigating the variability in the measure [19,25]. These findings could have implications for future study design considerations [19] and the ability to find a significant effect, if such an effect is indeed present. This is because small participant numbers are often recruited for experimental research studies and modest significant changes in BRS (3 – 6 ms/mmHg) have been observed from natural [33,41] and imposed [32] events and interventions [10,16,24]. Sample size estimation (table 4) implies BRS changes (3 or to 5 ms/mmHg) may be found utilising tilt BRS via small sample sizes (26 – 2 participants) in time and spectral domains. This suggests tilt BRS may offer the opportunity to detect meaningful changes in follow-up studies incorporating small participant numbers.

## Limitations

Intra-participant control was achieved via testing guidelines although it was impossible to be sure participants followed the routine exactly. Participants were

healthy adult males (18 – 35 y) undertaking regular exercise thus the findings should be limited to this study population. Spontaneous respiration may have influenced sequence BRS determination by a greater extent than spectral  $BRS_{LF}$  and it was not possible to assess the extent of any respiratory influence.

## **Conclusion**

The employment of a tilt manoeuvre markedly improved the reproducibility of both time and spectral BRS measures for both same day and between day reproducibility and overall the  $BRS_{TFTG}$  technique had the highest reproducibility under both supine and tilt conditions. The improvement in BRS reproducibility during tilt suggests the inclusion of the manoeuvre may be of benefit for future studies, especially where participant sample sizes are limited.



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Table 1. Participant physical characteristics

Characteristic (n = 46)	Mean $\pm$ (SD)
Age (y)	22 ( $\pm$ 5)
Mass (kg)	79.3 ( $\pm$ 11)
Stature (m)	1.80 ( $\pm$ 0.1)
Resting HR (b·min <sup>-1</sup> )	67 ( $\pm$ 11)
Resting BP(br) (mmHg)	
Systolic	124 ( $\pm$ 9)
Diastolic	69 ( $\pm$ 6)

HR, heart rate; BP(br), resting brachial blood pressure



Table 2. Limits of Agreement for supine and tilt BRS measures

BRS (ms/mmHg)	Bias	SD Differences	95% Limits of Agreement (+)	95% Limits of Agreement (-)	Confidence Intervals (+)	Confidence Intervals (-)
	Mean Difference		(ms/mmHg)	(ms/mmHg)	(ms/mmHg)	(ms/mmHg)
<i>Base / +60 min</i>						
Seq UpUp (S)	0.57	15.45	30.84	-29.70	34.79	-33.65
Seq UpUp(T)	0.36	2.24	4.75	-4.04	5.33	-4.61
Seq DownDown (S)	-0.17	11.21	21.80	-22.13	24.67	-19.27
Seq DownDown (T)	-0.38	1.95	3.44	-4.20	3.94	-4.70
$\alpha$ LF (S)	0.85	4.42	9.50	-7.81	10.63	-8.93
$\alpha$ LF (T)	-0.31	1.77	3.17	-3.78	3.62	-4.23
TFTG (S)	1.69	3.34	8.24	-4.85	9.09	-5.07
TFTG (T)	0.09	1.28	2.60	-2.43	2.93	-2.76
<i>Base / +24 h</i>						
Seq UpUp (S)	0.99	14.17	28.76	-26.78	32.38	-30.40
Seq UpUp(T)	-0.52	2.78	4.93	-5.97	5.64	-6.68
Seq DownDown (S)	-0.23	7.45	14.36	-14.83	16.26	-16.73
Seq DownDown (T)	-0.80	1.83	2.78	-4.38	3.25	-4.84
$\alpha$ LF (S)	-0.62	5.45	10.06	-11.30	11.45	-12.69
$\alpha$ LF (T)	-0.54	2.36	4.08	-5.16	4.68	-5.76
TFTG (S)	0.34	4.75	9.65	-8.97	10.86	-10.18
TFTG (T)	-0.15	1.97	3.71	-4.00	4.21	-4.50

Note: BRS, baroreflex sensitivity; Seq, sequence; LF, low frequency; TFTG, transfer function transfer gain; (S), supine; (T), tilt

Table 3. BRS parameters and Technical Error of Measurement				
BRS (ms/mmHg)	TEM	% TEM		
Base / +60 min				
Seq UpUp (S)	10.81	10.41		
Seq UpUp(T)	1.59	4.06		
Seq DownDown (S)	7.84	9.11		
Seq DownDown (T)	1.39	4.83		
$\alpha$ LF (S)	3.15	4.70		
$\alpha$ LF (T)	1.26	3.56		
TFTG(S)	2.62	4.11		
TFTG(T)	0.90	2.77		
Base / +24 h				
Seq UpUp (S)	9.93	9.49		
Seq UpUp(T)	1.98	5.29		
Seq DownDown (S)	5.21	6.06		
Seq DownDown (T)	1.40	5.00		
$\alpha$ LF (S)	3.84	6.00		
$\alpha$ LF (T)	1.69	4.86		
TFTG(S)	3.33	5.44		
TFTG(T)	1.38	4.31		
Note: BRS, baroreflex sensitivity; Seq, sequence;				
LF, low frequency; TFTG, transfer function				
transfer gain; (S), supine; (T), tilt				

Table 4. Number of participants required for follow-up studies with tilt BRS									
Same day reproducibility (+ 60 min)					Between day reproducibility (+ 24 h)				
$\Delta$ BRS	Seq UpUp	Seq DownDown	$\alpha$ LF	TFTG	Seq UpUp	Seq DownDown	$\alpha$ LF	TFTG	
1	155	117	96	51	238	103	171	119	
2	39	29	24	13	59	26	43	30	
3	17	13	11	6	26	11	19	13	
4	10	7	6	3	15	6	11	7	
5	6	5	4	2	10	4	7	5	

Note:  $\Delta$  BRS, change in baroreflex sensitivity (ms/mmHg); Seq, sequence; LF, low frequency; TFTG, transfer function transfer gain

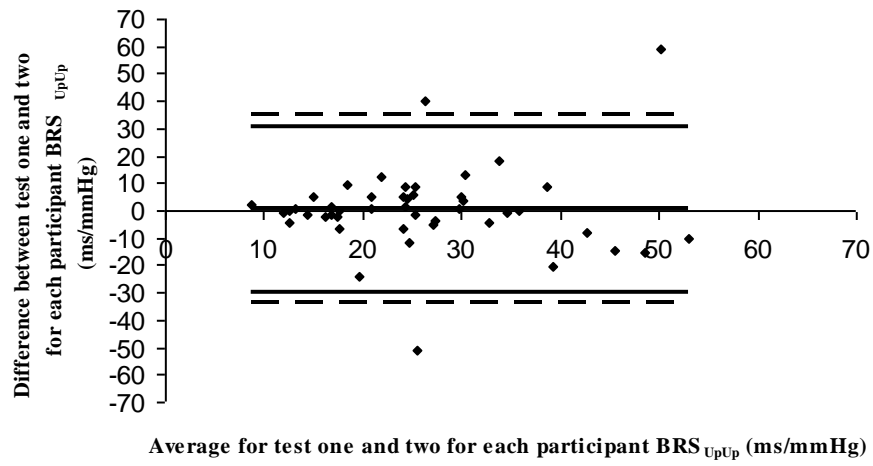


Figure 1. Bland and Altman plot in supine BRS<sub>UpUp</sub> between baseline and + 60 min

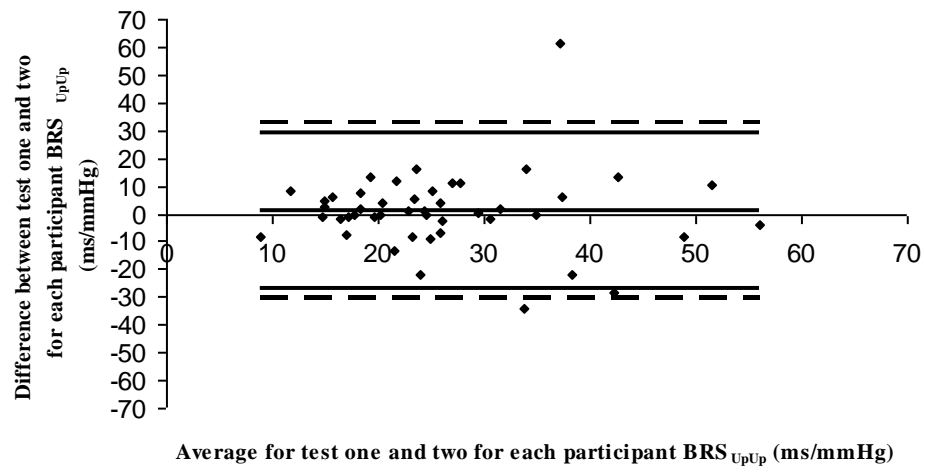


Figure 2. Bland and Altman plot in supine BRS<sub>UpUp</sub> between baseline and + 24 h

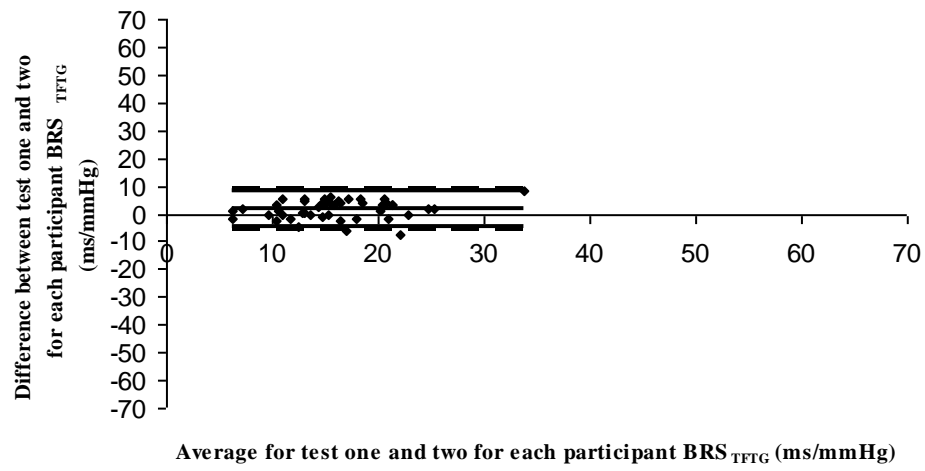


Figure 3. Bland and Altman plot in supine  $BRS_{TFTG}$  between baseline and + 60 min

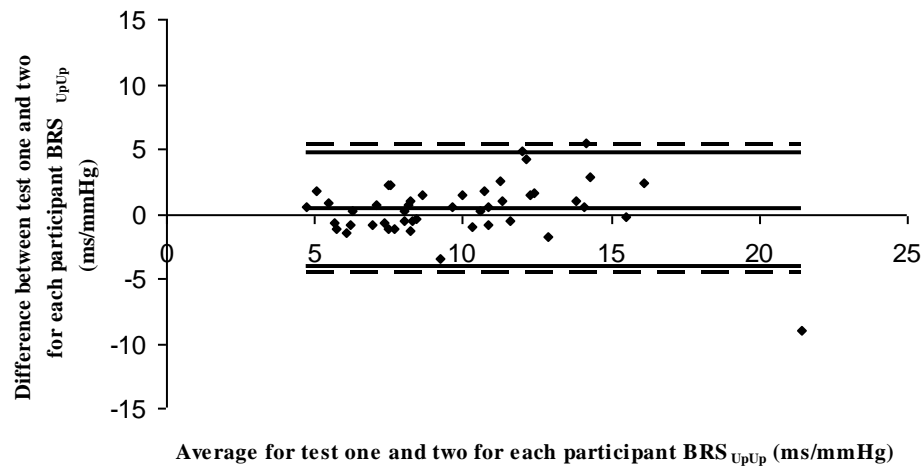


Figure 4. Bland and Altman plot in tilt BRS<sub>UpUp</sub> between baseline and + 60 min

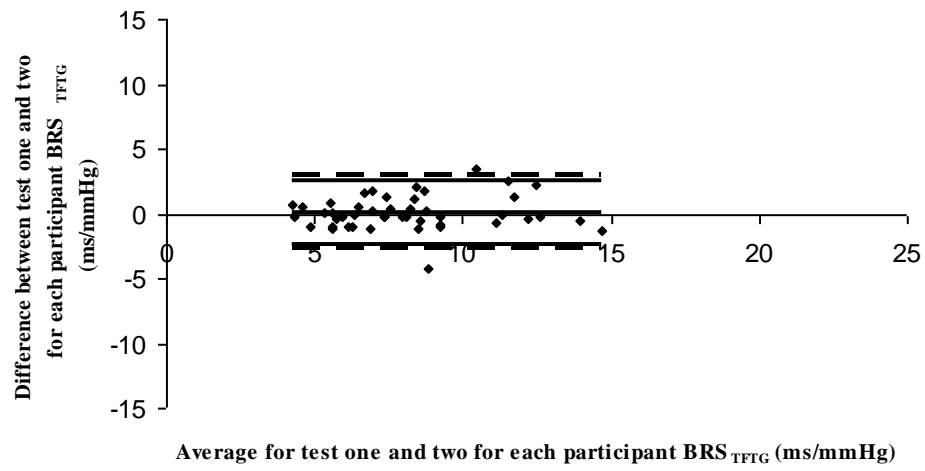


Figure 5. Bland and Altman plot in tilt  $BRS_{TFTG}$  between baseline and + 60 min