Optimal testing interval in the squatting test to determine baroreflex sensitivity

Syunsuke Ishitsuka[1], Natsuyo Kusuyama[1], Makoto Tanaka[2]

[1]Department of Anesthesia, University of Tsukuba Hospital, Tsukuba-city, Ibaraki, Japan

[2] to whom correspondence should be addressed:

Makoto Tanaka, M.D., Department of Anesthesia, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba-city, Ibaraki 305-8575, Japan

e-mail: mtanaka@md.tsukuba.ac.jp

Telephone: 81-29-853-3285

FAX: 81-29-853-3092

Running heads: squatting test and baroreflex

Summary

Recently-introduced “squatting test” utilizes simple postural change to perturb blood pressure, and to assess baroreflex sensitivity (BRS) with various testing sequences. This study was designed to determine the reproducibility and the optimal testing interval of the squatting test in healthy volunteers. Thirty-four subjects free of cardiovascular disorders and taking no medication were instructed to perform repeated squatting test at 30-s, 1-min and 3-min intervals in duplicate in a random sequence, while systolic blood pressure and pulse intervals were determined by using Finapres (Finometer MIDI®). BRS were determined by plotting reflex increases and decreases in systolic blood pressure and succeeding pulse intervals during stand-to-squat and during squat-to-stand maneuvers, respectively. Correlations between duplicate BRS data at each testing interval were analyzed by Pearson’s correlation coefficient, while agreements were assessed by Bland-Altman plots. Except for the maneuvers at 30-sec interval, two measurements of BRS during stand-to-squat and during squat-to-stand maneuvers demonstrated significant correlations at both 1-min and 3-min intervals, while there appears to be no extreme outliner in the Bland-Altman plot during any of the maneuvers at 1-min and 3-min intervals. Correlation coefficient becomes consistently greater in each maneuver as the measurement interval is lengthened from 30 s to 3 min. Our results suggest that the testing interval of the squatting test should be at least 1 min, but ideally longer than or equal to 3 min, to assess baroreflex function.

Keywords: Autonomic nervous system・Baroreflex sensitivity・Squatting test・Vagal nerve activity

Introduction

The importance of cardiovagal function, such as arterial baroreflex response and heart rate variability, in the control of beat-to-beat blood pressure is undisputed. More importantly, evaluations of cardiovagal function have been shown to provide prognostic value after life-threatening disorders [1, 2], as well as useful information regarding short-term morbidity and long-term mortality in surgical patients [3-5].

In order to assess baroreflex function, pharmacological method using vasoactive drugs has been extensively used for human and animal studies [6]. More sophisticatedly, the neck chamber method using computer-driven, pressure-suction device has been developed to study carotid-cardiac baroreflex responses in humans [7, 8]. However, these methods have limited clinical use, especially in outpatients, because of a need for an intravenous access, artificial perturbation in blood pressure, and a sophisticated equipment for research which is not always available.

Recently-introduced squatting test, on the other hand, uses simple posture changes which can be practiced daily to induce blood pressure alterations sufficient to elicit reflex changes in R-R intervals, and thus may be performed easily and non-invasively at bedside or outpatient clinic [9]. Indeed, it has been used to assess successfully cardiovagal function in diabetic patients with autonomic neuropathy [10, 11]. More importantly, changes in R-R interval elicited by blood pressure perturbations during repeated stand-squat maneuvers have been shown to reflect baroreflex mechanism [12], and thus may be used to calculate baroreflex sensitivity (BRS) in humans. However, repeated stand-squat maneuvers have been used at different frequencies using various protocols depending on the investigations, and lack of standard testing method may impede wide-spread use of this method. Accordingly, this study was designed to determine reproducibility and the optimal testing interval of the squatting test in healthy volunteers free of cardiovascular or autonomic nervous system disorders.

Methods

All procedures used in this study were approved by the University of Tsukuba Hospital Ethics Committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from each subject.

Subjects and protocol

Thirty-four healthy, non-smoking volunteers were recruited. All subjects were free of cardiovascular or autonomic disorders and taking no medication that could affect cardiovascular system. They abstained from caffeine-containing beverages and alcohol for at least 24 h before the study, and arrived at the laboratory at 8:00 AM after 8-10 h fast. They were familiarized with the environment and interventions before the study, which commenced at 9:00 AM. The ambient temperature was held at 25℃.

Systolic blood pressure (SBP) was measured non-invasively at the middle finger of the right hand using Finapres (Finometer MIDI®), and beat-to-beat pulse interval (PI) was obtained from the waveform. The hand and arm was supported securely with a custom-made vest-sling system to ensure stability of the pressure recordings during the stand-squat maneuvers, while the reference was positioned on the anterior chest at the level of the heart. After at least 10-min rest in the sitting position, subjects were instructed to perform repeated stand-to-squat and squat-to-stand maneuvers at 30-s, 1-min and 3-min intervals in duplicate. Three testing intervals, each consisting of duplicate measurements of each maneuver, were randomized, ie. approximately one-sixth of subjects performed maneuvers according to one of six possible interval sequence combinations. During squatting, subjects could take either a tiptoe or a feet-flat position, depending on their preference for a comfortable performance. During transition between squatting and standing, they were instructed to breathe normally to avoid confounding effect of Valsalva maneuver.

Data acquisition and calculation of baroreflex sensitivity

SBP and PI were determined beat by beat, digitized using a 16-bit analog-digital converter, stored at a sampling rate of 200 Hz in a computer, and subsequently analyzed offline. Calculation of BRS was accomplished by least-square linear regression analysis between SBP and PI in linear relationship during each maneuver, when PI was plotted as a function of the preceding SBP (one offset). Only sequences in which successive SBP differed by at least 1 mmHg were analyzed. We attempted to determine BRS by both transitions of stand-to-squat as well as squat-to-stand maneuvers, but only a pair of BRS data with both correlation coefficients (R) > 0.8 was accepted for further analysis. Percent difference between the two BRS data during each maneuver at each interval was calculated as a fractional difference in BRS measurements over a greater BRS value as a denominator.

Statistics

Comparisons of data among the three testing intervals were first made using repeated-measures ANOVA followed by paired-*t* test with Bonferroni's correction as a *post-hoc* testing. Correlations and agreements between two measurements of BRS associated with stand-to-squat or squat-to-stand maneuvers were analyzed by Pearson's correlation coefficient and Bland-Altman plots, respectively. All data are presented as mean ± SD, and a P value less than 0.05 was considered statistically significant.

Results

The mean age, weight, and height of the subjects were 24 ± 7 yr, 60.6 ± 9.2 kg, and 166 ± 8 cm, respectively. Eighteen subjects were male. Typical SBP and PI responses were obtained in most subjects with acceptable correlation (R > 0.8) during both stand-to-squat and squat-to-stand maneuvers (Fig. 1). In some subjects, however, BRS could not be calculated because of poor correlations (Table 1). No significant difference was seen between BRS values determined in duplicate at all measurement intervals in both maneuvers, thus BRS data are presented as an average of duplicate data for each maneuver at each interval (Table 1). Similarly, there was no significant difference in BRS values during stand-to-squat maneuvers between three intervals, except that BRS during squat-to-stand maneuver at 30-s interval was significantly greater than that at 3-min interval.

Significant positive correlations were demonstrated between duplicate BRS measurements at most of the intervals during both maneuvers (Table 1, Fig. 2, $P < 0.05$). However, clinically acceptable correlations were only demonstrated at 3-min intervals during both postural changes and at 1-min interval during squat-to-stand maneuver, while marginal correlation was obtained at 1-min interval during stand-to-squat maneuver (Table 1, R = 0.44). At 30-s intervals during both maneuvers, correlations between duplicate BRS measurements were poor ($R < 0.4$). Bland-Altman plots showed that most of between-measurements differences were within limits of agreement, and no extreme outliner was found in any of our series (Fig. 3).

Discussion

A major finding of our study is that a degree of correlation between the duplicate BRS measurements by the squatting test depends on the testing interval as well as the type of maneuvers. More importantly, correlation coefficient becomes consistently less in each maneuver and BRS determined by the squat-to-stand maneuver becomes significantly greater as the measurement interval is shortened from 3 min to 30 s (Table 1). These results indicate that the testing interval should be at least 1 min, but ideally longer than or equal to 3 min, when BRS is determined using the squatting test. Our results are also in agrement with a recent study which showed frequency-dependent characteristics of cardiac baroreflex gain derived from the squatting test between 0.03 and 0.1 Hz [13], although it was not our intension to determine the mechanism underlying the frequency-dependency of cardiac BRS.

Whether or not approximately 30% difference in duplicate BRS measurements by this method represents true physiological phenomenon is unclear. Within-subject variation of 27% has been reported for BRS by the phenylephrine pressor test measured in one to several months apart under the similar environment [14]. Similar extent of intra-individual variability on three different days has been reported for the drug-induced methods using phenylephrine and nitroprusside, and for the spontaneous sequence method [15], suggesting that the extent of variability with respect to duplicate BRS measurements seen in our study may not be inherent in the methodology, *per se*. However, we cannot exclude a possibility that varying degree of background sympathetic activity and central influences that presumably vary within subjects over time might affect central baroreflex control or beat-to-beat vagal control of the heart rate over the course of repeated, strenuous maneuvers [16].

The squatting test has been used in limited number of clinical researches to assess cardiac autonomic function in diabetic patients [10, 11]. Marfella *et al* advocated squatting ratio, R-R interval ratios before and after standing or squatting maneuver, and demonstrated that these ratios correlated well with diabetic duration, discriminated between healthy subjects and diabetic patients to a greater extent than most of the other reflex tests, and identified mild impairment of cardiac autonomic integrity [10]. Nakagawa *et al* also showed that heart rate changes after standing and squatting maneuvers correlated well with BRS determined by the phenylephrine test, and were less in diabetic patients compared with healthy subjects [11]. These studies, however, did not calculate BRS from reflexly changing R-R intervals that accompany blood pressure perturbations by the postural stress. On the other hand, Zhang *et al* reported that repeated stand-squat maneuvers at 5-s and 10-s intervals produced large and coherent oscillations in blood pressure and R-R intervals, and calculated transfer function gain was reduced in the elderly, suggesting the typical effect of aging on reducing BRS [12]. A more recent review article also showed that BRS determined by linear regression during squat-stand maneuvers is reduced in the elderly compared with young subjects [9]. These previous investigations, however, focus on different autonomic variables or perform stand-squat maneuvers at undefined intervals. To make valid and feasible comparisons among similar studies, therefore, a standard testing regimen for the squatting test needs to be established.

Absolute BRS values in our series are comparable to those reported previously using the squatting test [9, 12], but are considerably smaller than those determined by the pharmacological and spontaneous sequence methods. Calculated BRS values may differ depending on the methods used, the sites of baroreceptors stimulated, and speed and extent of blood pressure alterations. BRS determined by various methods may not be summarized comprehensively in a single number [13, 17]. Indeed, carotid-cardiac BRS elicited by neck pressure-suction ramps were reportedly one-fifth to one-sixth of integrated BRS determined by the phenylephrine pressor test or spontaneous sequence method [18-21], and BRS determined by the squatting test and the modified Oxford method showed poor concordance [13]. In addition, increasing and decreasing preload/central blood volume produced by squatting and standing maneuvers, respectively, may have exerted complex effects on baroreflex-mediated cardiac responses from cardiopulmonary receptors [22, 23]. These considerations together with previous reports suggest that BRS determined by the different methodologies may represent different aspects of cardiac vagal responses and may not be used interchangeably.

The results of our study should be interpreted with some caution. First, whether the squatting test can replace the conventional methods remains to be seen. In other words, correlations between BRS determined by the squatting test and those by other methods need to be validated, although BRS determined by the squatting test has been reported to possess some of the characteristics typical to baroreflex responses, such as the inhibitory effect of aging [9, 12]. Second, only young, healthy individuals were assigned in our study, while involving variety of subjects with various degrees of autonomic impairment or those with disorders know to affect autonomic nervous system might have led to better insight into autonomic disorders detected by the squatting test. Third, BRS could not be determined by this method in approximately 10% of subjects due to inadequate correlation between reflexly changing PI and SBP perturbations. Moreover, this method may not be suitable for extreme elderly or disabled subjects, who have difficulties in performing repeated stand-squat maneuvers. Fourth, we did not study intervals longer than 3 min. Whether more than 3 min intervals would show superior reproducibility in duplicate measurements remain unclear. However, correlation coefficients between duplicate BRS determined at

3 min intervals were considered clinically sufficient, and within-subjects variations in our series were similar to those reported previously [3, 15]. Lastly, although both cardiac and sympathetic efferents play an important role in controlling arterial blood pressure, both arms of baroreflex function do not correlate within healthy normotensive humans [24].

In conclusion, BRS were measured in duplicate by the repeated stand-squat maneuvers at 30-s, 1-min and 3-min intervals in healthy volunteers free of cardiovascular or autonomic nervous system disorders. We have found that two measurements of BRS during stand-to-squat and squat-to-stand maneuvers demonstrated significant correlations at both 1- and 3-min intervals without extreme outliner by the Bland-Altman plot, while correlation coefficient becomes consistently greater in each maneuver as the measurement interval is lengthened from 30 s to 3 min. These results suggest that the testing interval should be at least 1 min, but ideally longer than or equal to 3 min, when BRS is determined using the squatting test.

Acknowledgements

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. M. T. La Rovere, J. T. Bigger Jr, F. I. Marcus, et al., “Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators,” *Lancet*, **351,** 478-484 (1998).
2. T. G. Robinson, S. L. Dawson, P. J. Eames PJ, et al., “Cardiac baroreceptor sensitivity predicts long-term outcome after acute ische mic stroke,” *Stroke*, **34**, 705-712 (2003).
3. T. W. Latson, T. H. Ashmore, D. J. Reinhart, et al., “Autonomic reflex dysfunction in patients presenting for elective surgery is associated with hypotension after anesthesia induction,” *Anesthesiology*, **80,** 326-337 (1994).
4. M. Filipovic, R. V. Jeger, T. Girard T, et al., “Predictors of long-term mortality and cardiac events in patients with known or suspected coronary artery disease who survive major non-cardiac surgery," *Anaesthesia*, **60,** 5-11 (2005).
5. A. T. Mazzeo, E. La Monaca, R. Di Leo, G. Vita, et al., “Heart rate variability: a diagnostic and prognostic tool in anesthesia and intensive care,” *Acta. Anaesthesiol. Scand.*, **55**, 797-811 (2011).
6. K. G. Cornish, M. W. Barazanji, T. Yong and J. P. Gilmore, “Volume

expansion attenuates baroreflex sensitivity in the conscious nonhuman primate," *Am. J. Physiol.*, **257,** R595-598 (1989).

7. D. L. Eckberg DL, V. A. Convertino, J. M. Fritsch and D. F. Doerr DF, "Reproducibility of human vagal carotid baroreceptor-cardiac reflex responses," *Am. J. Physiol.*, **263**, R215-220 (1992).
8. D. L. Eckberg DL and J. M. Fritsch JM, "How should human baroreflexes be tested?," *News Physiol. Sci.*, **8**, 7-12 (1993).
9. A. J. Scheen and J. C. Philips, "Squatting test: a dynamic postural manoeuvre to study baroreflex sensitivity," *Clin. Auton. Res.*, **22**, 35-41 (2012).
10. R. Marfella, D. Giugliano, G. di Maro, et al., "The squatting test. A useful tool to assess both parasympathetic and sympathetic involvement of the cardiovascular autonomic neuropathy in diabetes," *Diabetes*, **43,** 607-612 (1994).
11. M. Nakagawa, T. Shinohara, F. Anan, et al., "New squatting test indices are useful for assessing baroreflex sensitivity in diabetes mellitus," *Diabet. Med.*, **5**, 1309-1315 (2008).
12. R. Zhang, J. A. Claassen, S. Shibata, et al., "Arterial-cardiac baroreflex function: insights from repeated squat-stand maneuvers," *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **297,** R116-123 (2009).
13. H. M. Horsman, K. C. Peebles, D. C. Galletly and Y. C. Tzeng, "Cardiac baroreflex gain is frequency dependent: insight from repeated sit-to-stand

maneuvers and the modified Oxford method," *Appl. Physiol. Nutr. Metab.*, **38,** 753-759 (2013).

14. B. Gribbin, T. G. Pickering, P. Sleight and R. Peto, “Effect of age and high blood pressure on baroreflex sensitivity in man,” *Circ. Res.*, **29**, 424-431 (1971).
15. J. Parlow, J. P. Viale, G. Annat, et al., “Spontaneous cardiac baroreflex in humans: comparison with drug-induced responses,” *Hypertension*, **25**, 1058-1068 (1995).
16. A. S. Tadepalli, E. Mills and S. M. Schanberg, “Depression and enhancement of baroreceptor pressor response in cats after intracerebroventricular injection of noradrenergic blocking agents: dependence on supracollicular areas of the brain,” *Circ. Res.*, **39**, 724-730 (1976).
17. H. Yang and J. R. Carter, “Baroreflex sensitivity analysis: spontaneous methodology vs. Valsalva’s maneuver,” *Clin. Auton. Res.*, **23**, 133-139 (2013).
18. D. L. Eckberg and M. J. Eckberg, “Human sinus node responses to repetitive, ramped carotid baroreceptor stimuli,” *Am. J. Physiol.*, **242**, H638-644 (1982).
19. T. J. Ebert, J. J. Hayes, J. Ceschi, et al., “Repetitive ramped neck suction: a quantitative test of human baroreceptor function,” *Am. J. Physiol.*, **247**, H1013-1017 (1984).

20. M. Tanaka, G. Nagasaki and T. Nishikawa, “Moderate hypothermia depresses arterial baroreflex control of heart rate during, and delays its recovery after, general anesthesia in humans,” *Anesthesiology*, **95,** 51-55 (2001).
21. M. Tanaka and T. Nishikawa, “The concentration-dependent effects of general anesthesia on spontaneous baroreflex indices and their correlations with pharmacological gains,” *Anesth. Analg.*, **100**, 1325-1332 (2005).
22. H. O. Stinnett, V. S. Bishop and D. F. Peterson, “Reduction in baroreflex cardiovascular responses due to venous infusion in the rabbit,” *Circ. Res.*, **39,** 766-772 (1976).
23. J. A. Pawelczyk and P. B. Raven, “Reductions in central venous pressure improve carotid baroreflex responses in conscious men,” *Am. J. Physiol.*, **257**, H1389-1395 (1989).
24. A. P. Dutoit, E. C. Hart, N. Charkoudian, et al., “Cardiac baroreflex sensitivity is not correlated to sympathetic baroreflex sensitivity within healthy, young humans,” *Hypertension*, **56**, 1118-1123 (2007).

Figure legends

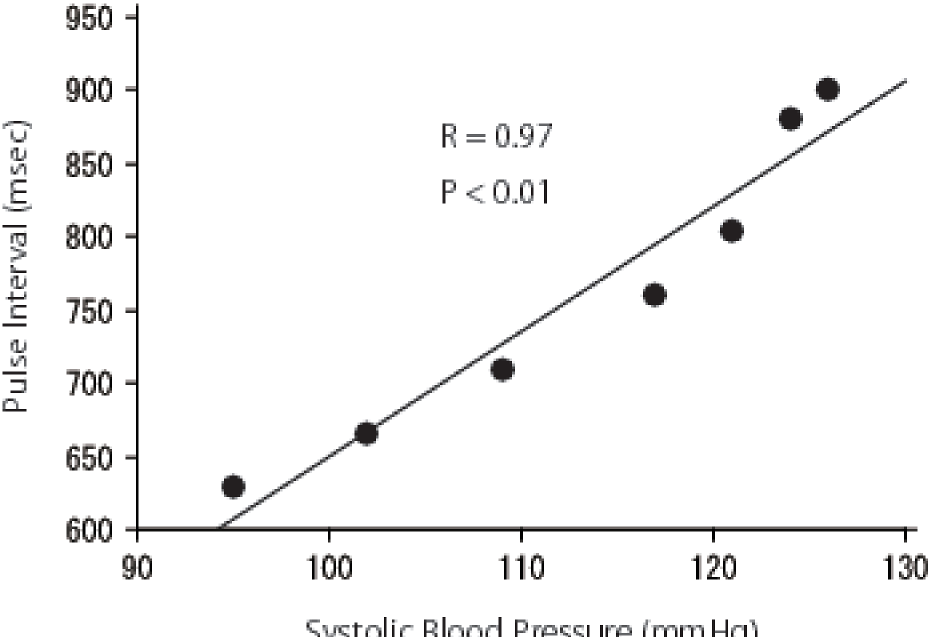
Figure 1 Typical blood pressure and pulse interval responses elicited by postural changes (from standing to squatting and from squatting to standing) in a healthy volunteer determined at 3-min interval.

Figure 2 Least-square regression of baroreflex sensitivities determined in duplicate from standing to squatting (top panel) and from squatting to standing (bottom panel) maneuvers at 3-min interval. A broken line indicates the line of equality, and a solid line indicates the line of regression in each figure.

Figure 3 Reproducibility of baroreflex sensitivities during two maneuvers (from standing to squatting and from squatting to standing) determined at 3-min intervals. Bland-Altman plots showed no major relation between the differences in baroreflex sensitivities determined in duplicate (y axis) versus the mean of the two measurements (x axis). A solid line indicates the mean difference (bias), and broken lines indicate limits of agreements (mean ± 1.96*SD) of the two maneuvers. Note that no extreme outliner exists in our series.

Figure 1

Typical response during stand-to-squat maneuver



Typical response during squat-to-stand maneuver

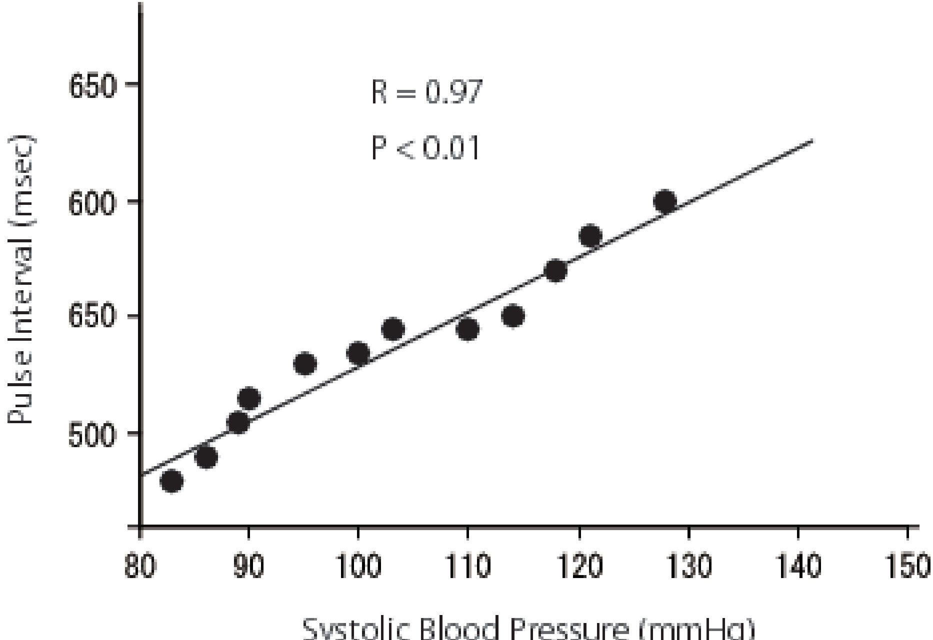
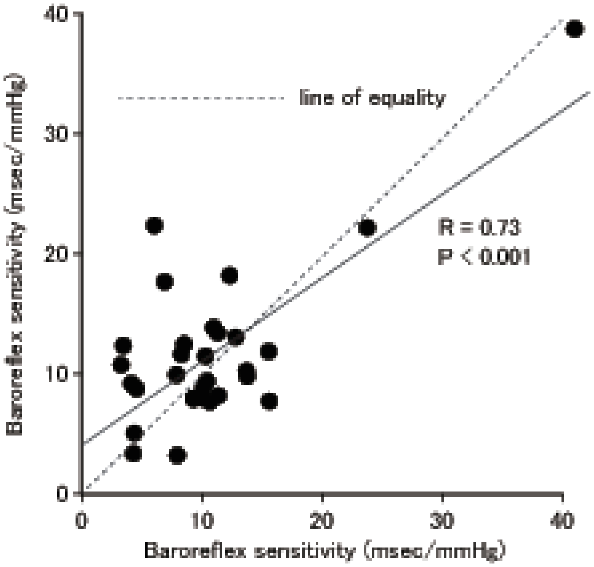


Figure 2

Correlation between baroreflex sensitivities determined in duplicate during stand-to-squat maneuver at 3-min interval



Correlation between baroreflex sensitivities determined in duplicate during squat-to-stand maneuver at 3-min interval

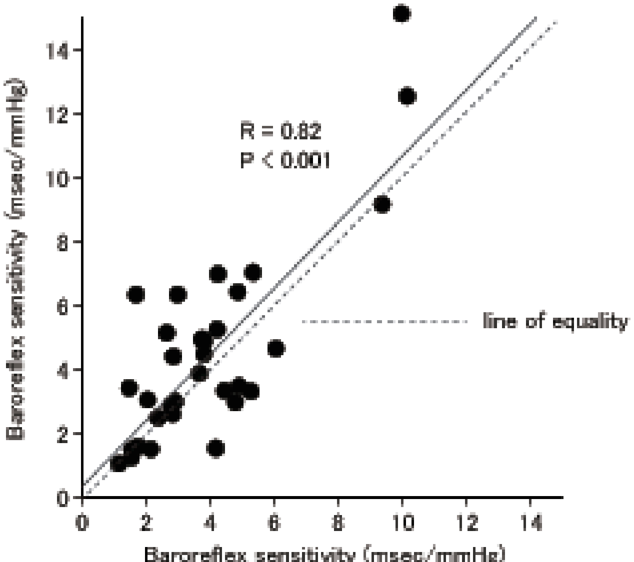
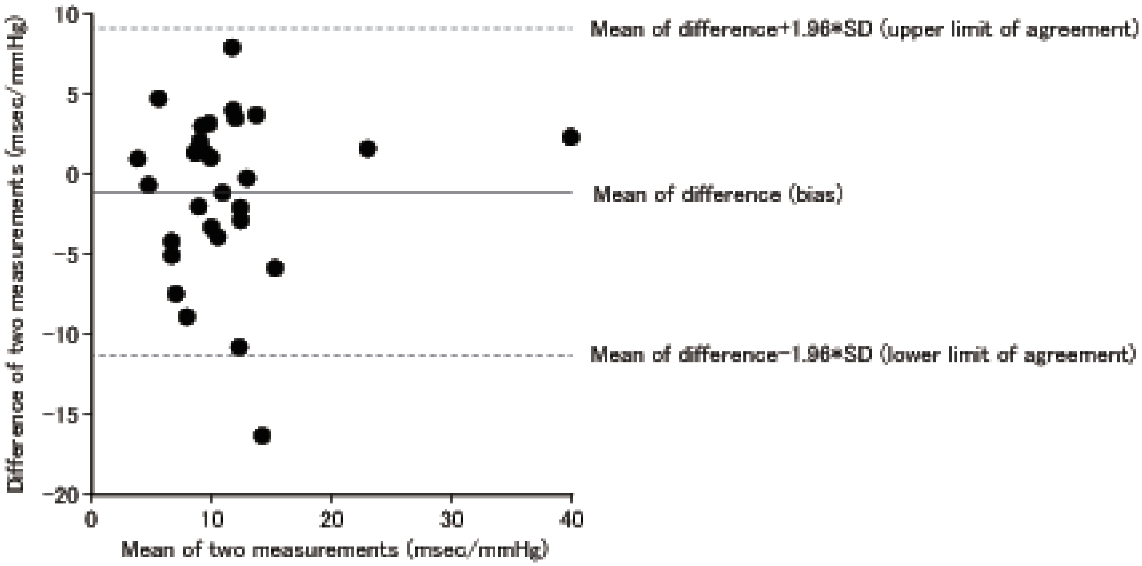


Figure 3

Stand-to-squat maneuver at 3 min interval



Squat-to-stand maneuver at 3 min interval

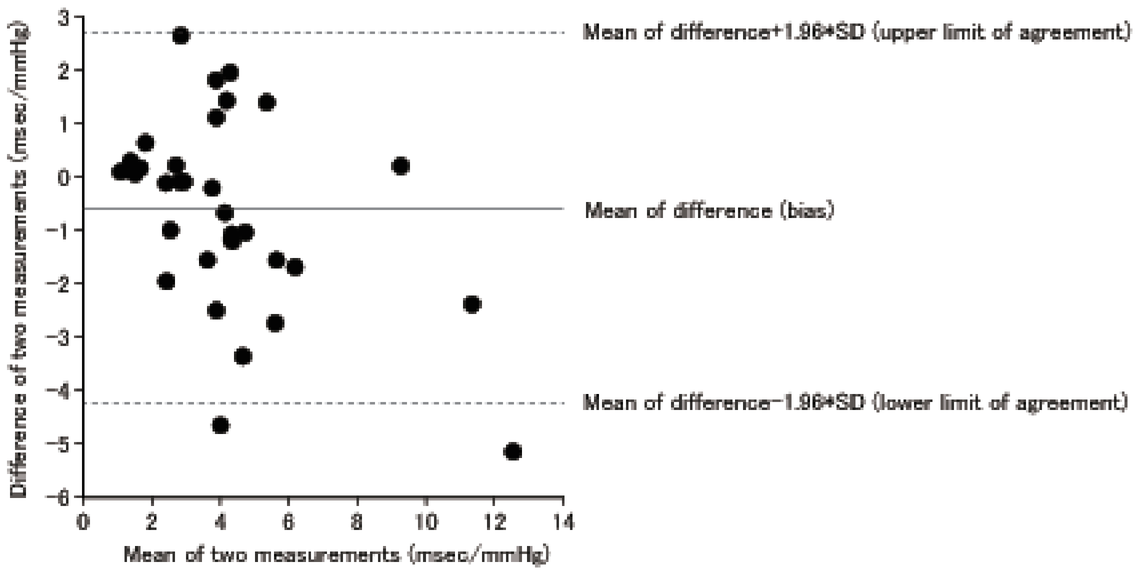


Table 1. Percent difference, correlation coefficient, *P* value, bias and limit of agreement between two BRS measurements by the squatting test

	3-min		1-min		30-sec	
	stand-to-squat	squat-to-stand	stand-to-squat	squat-to-stand	stand-to-squat	squat-to-stand
Number of subjets	30	33	32	30	31	31
BRS (ms/mmHg)	11.3 ± 7.0	4.3 ± 2.7	11.0 ± 5.9	4.7 ± 2.5	11.1 ± 4.5	5.2 ± 2.6*
% difference between measurement	30 ± 21	26 ± 18	30 ± 22	26 ± 15	30 ± 19	31 ± 21
Correlation coefficient	0.73	0.82	0.44	0.71	0.25	0.38
p value	<0.001	<0.001	0.01	<0.001	0.17	0.04
Bias	-1.1	-0.7	1.0	0.0	-1.4	-0.1
Limit of agreement	9.0〜-11.2	2.8〜-4.2	13.1〜-11.1	3.7〜-3.6	9.4〜-12.3	5.5〜-5.8

Data are mean ± SD. BRS, baroreflex sensitivity (ms/mmHg).*P < 0.05 *versus* squat-to-stand maneuver at 3-min interval.