UDC 612.833

S. ISHITSUKA,[1] N. KUSUYAMA,[1] and M. TANAKA[2]

OPTIMAL TESTING INTERVALS IN THE SQUATTING TEST TO DETERMINE BAROREFLEX SENSITIVITY

Received 30.09.13

The recently introduced "squatting test" (ST) utilizes a simple postural change to perturb the blood pressure and to assess baroreflex sensitivity (BRS). In our study, we estimated the reproducibility of and the optimal testing interval between the STs in healthy volunteers. Thirty-four subjects free of cardiovascular disorders and taking no medication were instructed to perform the repeated ST at 30-sec, 1-min, and 3-min intervals in duplicate in a random sequence, while the systolic blood pressure (SBP) and pulse intervals were measured. Baroreflex sensitivity was estimated by plotting reflex increases and decreases in the SBP and succeeding pulse intervals during stand-to-squat and squat-to-stand maneuvers, respectively. Correlations between duplicate BRS data at each testing interval were analyzed by the Pearson's correlation coefficient, while agreements were assessed by Bland-Altman plots. Two measurements of BRS during stand-to-squat and squat-to-stand maneuvers demonstrated significant correlations at both 1-min and 3-min intervals, while at 30-sec intervals correlation was poor. Correlation coefficients became considerably greater in each maneuver as the measurement interval was increased from 30 sec to 3 min. Our results suggest that the testing interval in the ST should be at least 1 min long, but ideally it should be longer than or equal to 3 min, to assess the baroreflex adequately.

Keywords: autonomic nervous system, baroreflex sensitivity, squatting test, vagal nerve activity.

INTRODUCTION

The importance of the cardiovagal functions (manifested, e.g., in the arterial baroreflex response and heart rate variability) in the control of the beat-to-beat blood pressure is undisputable. More importantly, evaluations of the cardiovagal function have been shown to provide a significant prognostic value in life-threatening disorders [1, 2] and for estimation of short-term morbidity and long-term mortality in surgical patients [3-5].

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

A recently introduced squatting test (ST), on the contrary, uses simple postural changes that can be practiced daily to induce blood pressure alterations sufficient to elicit reflex changes in the R-R intervals. Thus, it may be performed easily and noninvasively at bedside or outpatient clinics [9]. Indeed, it has been used to assess successfully the cardiovagal function in diabetic patients with autonomic neuropathy [10, 11]. More importantly, changes in the R-R intervals elicited by blood pressure perturbations during repeated stand-squat maneuvers have been shown to reflect the baroreflex mechanism [12]. Thus, these phenomena may be used to calculate the baroreflex sensitivity (BRS) in humans. However, repeated

[1] Department of Anesthesia, University of Tsukuba Hospital, Tsukuba-city, Japan.
Correspondence should be addressed M. Tanaka
(e-mail: mtanaka@md.tsukuba.ac.jp).

stand-squat maneuvers were realized at different repetition frequencies according to various protocols used (depending on the aims of the investigation). Lack of a standard testing procedure may impede widespread use of this method. Accordingly, our study was designed to improve the reproducibility of the ST and to evaluate the optimum intertest interval in the in healthy volunteers free of cardiovascular or autonomic nervous system disorders.

METHODS

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

The systolic blood pressure (SBP) was measured noninvasively at the middle finger of the right hand using Finapres (Finometer MIDI®), and beat-to-beat pulse intervals (PIs) were obtained from the waveform. The hand and arm were supported securely with a custom-made vest-sling system to ensure stability of the pressure recordings during the stand-squat maneuvers; the reference was positioned on the anterior chest at the level of the heart. After at least 10-min-long rest in the sitting position, subjects were instructed to perform repeated stand-to-squat and squat-to-stand maneuvers at 30-sec-, 1-min-, and 3-min-long intervals in duplicate. Three testing intervals, each consisting of duplicate measurements of each maneuver, were randomized. Approximately one-sixth of the 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 breath normally (to avoid a confounding effect of the Valsalva maneuver).

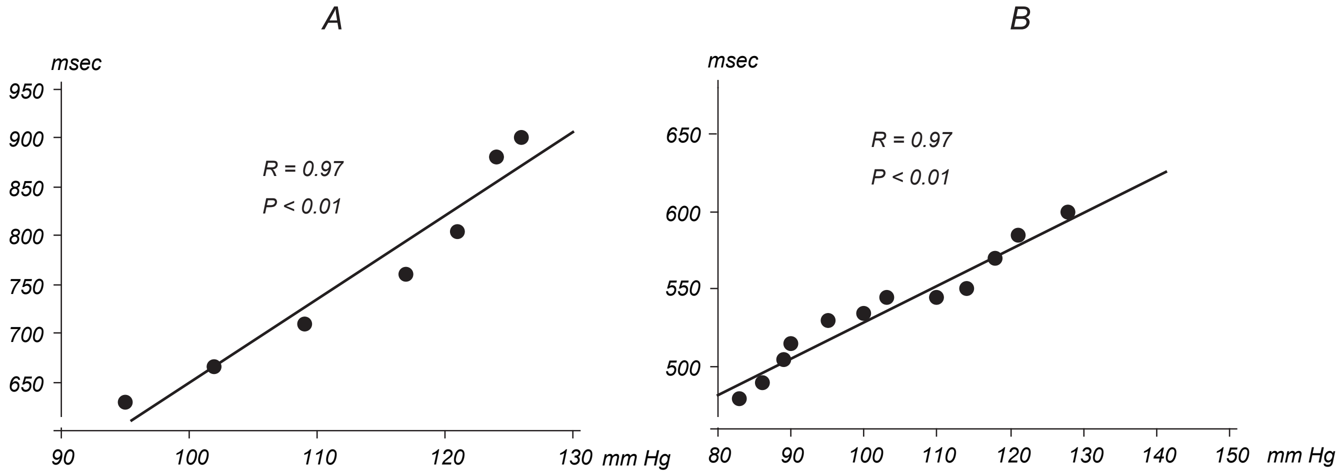
Data Acquisition and Calculation of Baroreflex Sensitivity (BRS). The SBP and PIs were determined beat by beat, digitized using a 16-bit analog-digital converter, stored at a sampling rate of 200 sec^{-1} in a computer, and subsequently analyzed offline. Calculation of BRS was accomplished by least-square linear regression analysis between SBP and PI in a linear relationship during each maneuver, when PIs were plotted as a function of the preceding SBP (one offset). Only sequences in which successive SBP values differed by at least 1 mm Hg were analyzed. We attempted to determine BRS by transitions in both stand-to-squat and squat-to-stand maneuvers, but only pairs of the BRS data with both correlation coefficients (R) above 0.8 were accepted for further analysis. The normalized difference (%) between the two BRS data during each maneuver at each interval was calculated as the fractional difference in BRS measurements over a greater BRS value as a denominator.

Statistics. Comparisons of the data among the three testing intervals were first made using repeated-measures ANOVA followed by the 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 the Pearson's correlation coefficient and Bland-Altman plots, respectively. All data are presented as means ± ± s.d., and a P value below 0.05 was considered statistically significant.

RESULTS

The mean age, body mass, and height of the subjects were 24 ± 7 years, 60.6 ± 9.2 kg, and 166 ± 8 cm, respectively. Eighteen subjects were men. 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 the BRS values determined in duplicate at all measurement intervals in both maneuvers; thus, the BRS data are presented as an average of duplicate data for each maneuver at each interval (Table 1). Similarly, there was usually no significant difference between BRS values during stand-to-squat maneuvers with three intervals; only BRS during a squat-to-stand maneuver at 30-sec-long intervals was significantly greater than that at 3-min-long intervals.

Significant positive correlations were demonstrated between duplicate BRS measurements at most of the



F i g. 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 intertest intervals. Abscissa) Systolic blood pressure, mm Hg; ordinate) pulse interval, msec.

Р и с. 1. Типові зміни кров'яного тиску та кардіоінтервалів, викликані змінами пози (від положення стоячи до присідання, і навпаки) у здорового випробуваного при інтервалі між тестами 3 хв.

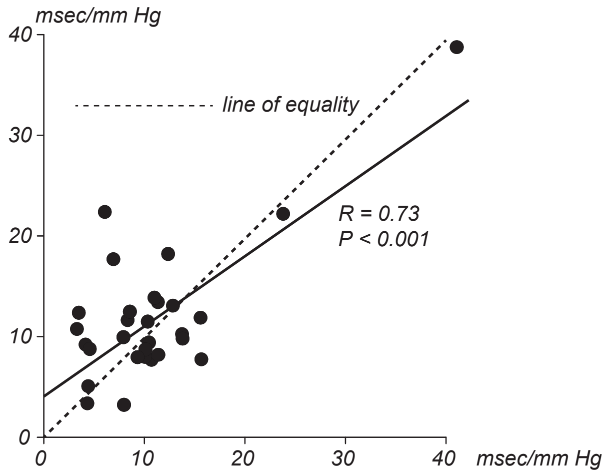
Table 1. Normalized difference (%), correlation coefficient, P value, bias, and limit of agreement between two BRS measurements in the squatting test

Результати визначення барорефлекторної чутливості в тесті присідання

	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 subjects	30	33	32	30	31	31
BRS, msec/mm Hg	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 the measurements, %	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

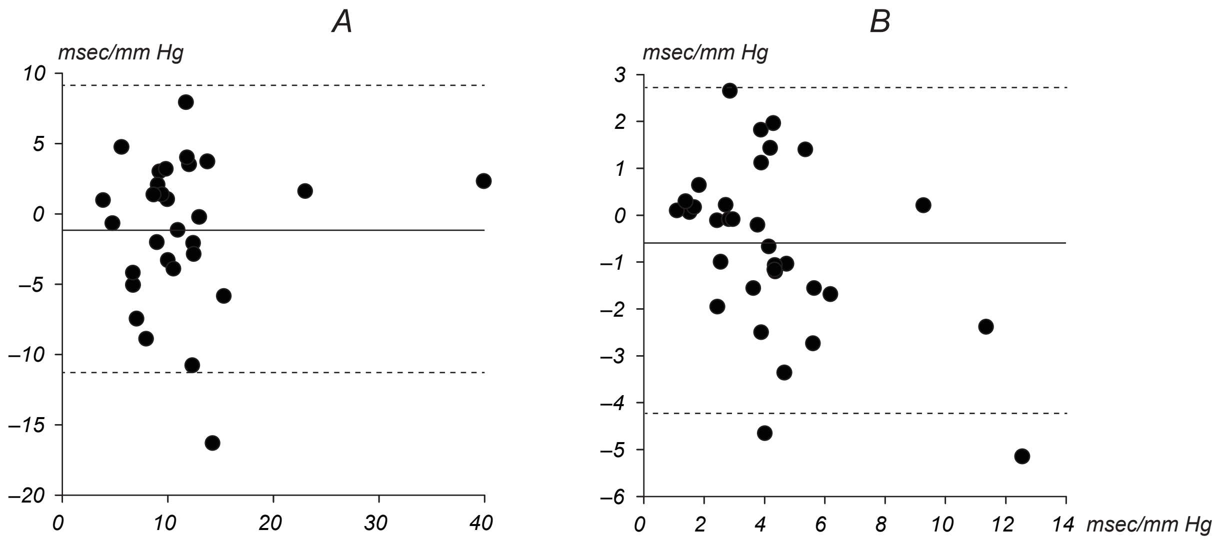
Footnote. Data are means ± s.d.; BRS is baroreflex sensitivity (ms/mm Hg).*P < 0.05 vs. squat-to-stand maneuvers at 3-min intervals.

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



F i g. 2. Least-square regression of baroreflex sensitivities determined in duplicate from standing to squatting (*A*) and from squatting to standing (*B*) maneuvers at 3-min intervals. In each panel, broken line indicates the line of equality, and solid line indicates the regression line.

Р и с. 2. Лінія регресії, визначена для барорефлекторної чутливості, у подвійних змінах пози від положення стоячи до присідання (*A*) та зворотних змінах (*B*), які реалізовувалися з інтервалами 3 хв.



F i g. 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 (ordinate) *vs.* means of the two measurements (abscissa). Solid line indicates the mean difference (bias), and broken lines indicate limits of agreements (mean ± 1.96 s.d.) of the two maneuvers. Note that no extreme outlier exists in our series.

Р и с. 3. Ступінь відтворюваності значень барорефлекторної чутливості при змінах пози від положення стоячи до присідання та зворотних змінах, реалізованих з інтервалами 3 хв.

DISCUSSION

A main finding of our study is that the degree of correlation between the duplicate BRS measurements in the ST depends on the testing interval, as well as on the type of maneuvers. More importantly, the correlation coefficient becomes consistently smaller 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 sec (Table 1). These results indicate that the testing interval should be at least 1 min long but, ideally, longer than or equal to 3 min, when BRS is determined using the ST. Our results are also in agreement with the recent study where frequency-dependent characteristics of the cardiac baroreflex gain derived from the ST between 0.03 and 0.1 sec^{-1} were shown [13], although it was not our intention to determine the mechanism underlying the frequency dependence of cardiac BRS.

Whether or not an approximately 30% difference in duplicate BRS measurements by this method represents true physiological phenomenon remains unclear. A within-subject variation of 27% has been reported for BRS by the phenylephrine pressor test measured one to several months apart under similar conditions [14]. A similar extent of intra-individual variability on three different days has been reported for drug-induced methods using phenylephrine and nitroprusside and also 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 rule out the possibility that a varying degree of background sympathetic activity and central influences (presumably varying within subjects over time) might affect the central baroreflex control or beat-to-beat vagal control of the heart rate over the course of repeated strenuous maneuvers [16].

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

Absolute BRS values in our series are comparable to those reported previously using the ST [9, 12], but these values are considerably smaller than those determined by pharmacological and spontaneous-sequence methods. Calculated BRS values may differ from each other depending on the methods used, sites of baroreceptors stimulated, and rate and extent of blood pressure alterations. The 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]. BRS determined by the ST and the modified Oxford method showed poor concordance [13]. In addition, increasing and decreasing preload/central blood volumes produced by squatting and standing maneuvers, respectively, may exert complex effects on the baroreflex-mediated cardiac responses from cardiopulmonary receptors [22, 23]. These considerations together with previous reports suggest that BRS determined using different approaches 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. (i) Whether the ST can replace the conventional methods remains to be estimated. In other words, correlations between BRS determined by the ST and those determined by other methods need to be validated. It should be mentioned that BRS determined by the ST has been reported to possess some of the characteristics typical of baroreflex responses, such as the inhibitory effect of aging [9, 12]. (ii) Only young healthy individuals were assigned in our study, while involving a variety of subjects with various degrees of autonomic impairment or those with disorders known to affect the autonomic nervous system might have led to better insights into autonomic disorders detected by the ST. (iii) BRS could not be determined by this method in approximately 10% of the subjects due to inadequate correlation between reflex changes in the PI and SBP. Moreover, this method may not be suitable for very old or disabled subjects who have difficulties in performing repeated stand-squat maneuvers. (iv) We did not test intervals longer than 3 min. Whether longer intervals would show better reproducibility in duplicate measurements remains unclear. However, correlation coefficients between duplicate BRS determined at 3-min-long intervals were considered clinically sufficient, and within-subjects variations in our series were similar to those reported earlier [3, 15]. Finally, (v) although both cardiac and sympathetic efferents play important roles in controlling the arterial blood pressure, both arms of the baroreflex function do not correlate within groups of healthy normotensive humans [24].

In conclusion, BRS was measured in duplicate by repeated stand-squat maneuvers at 30-sec, 1-min, and 3-min intervals in healthy volunteers free of cardiovascular or autonomic nervous system disorders. It was found that two measurements of BRS during stand-to-squat and squat-to-stand maneuvers demonstrated significant correlations at both 1-min and 3-min intervals without extreme outlier by the Bland-Altman plot, while the correlation coefficients became consistently greater in each maneuver as the measurement interval was prolonged from 30 sec to 3 min. These results suggest that the intertest interval should be not shorter than 1 min but ideally longer than or equal to 3 min when BRS is determined using the ST.

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

On behalf of all authors, S. Ishitsuka, N. Kusuyama, and M. Tanaka, the corresponding author states that there is no conflict of interest among them.

С. Іщіцука[1]*, Н. Кусуяма*[1]*, М. Танака*[1]

ОПТИМАЛЬНІ ІНТЕРВАЛИ ТЕСТУВАННЯ ПРИ ОЦІНЦІ БАРОРЕФЛЕКТОРНОЇ ЧУТЛИВОСТІ З ВИКОРИСТАННЯМ ТЕСТУ ПРИСІДАННЯ

[1] Університет лікарні Цукуба, Цукуба (Японія).

Р е з ю м е

У нещодавно запропонованому «тесті присідання» (ТП) використовується проста зміна пози для індукції зрушення кров'яного тиску, що дозволяє визначити барорефлекторну чутливість (БРЧ). Ми оцінювали ступінь відтворюваності та оптимальні інтервали між ТП, що реалізовувалися здоровими випробуваними. Групу з 34 тестованих, які не мали будь-яких серцево-судинних розладів та не приймали якихось ліків, інструктували виконувати повторні подвійні ТП у випадковій послідовності з інтервалами 30 с, 1 та 3 хв; при цьому вимірювали систолічний кров'яний тиск (СКТ) та кардіоінтервали. Рефлекторні підвищення та зниження СКТ і послідовні кардіоінтервали під час рухів присідання та повернення у вертикальну позу представляли графічно. Залежність між повторними визначеннями БРЧ при кожному інтервалі між тестами аналізували, встановлюючи коефіцієнти кореляції Пірсона. Виміри БРЧ під час рухів присідання та підйому демонстрували істотну кореляцію при інтервалах 1 та 3 хв, а при інтервалах 30 с кореляція була слабшою. Коефіцієнти кореляції ставали помітно значнішими з кожним рухом і збільшенням інтервалів між вимірами від 30 с до 3 хв. Наші результати вказують на те, що адекватна оцінка барорефлексу може бути забезпечена при інтервалах між ТП не менше 1 хв (бажано 3 хв або більше).

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)," *Lancet*, **351,** 478-484 (1998).
2. T. G. Robinson, S. L. Dawson, P. J. Eames, et al., "Cardiac baroreceptor sensitivity predicts long-term outcome after acute ischemic 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, 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, 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,** 595-598 (1989).
7. D. L. Eckberg, V. A. Convertino, J. M. Fritsch, and D. F. Doerr, "Reproducibility of human vagal carotid baroreceptor-cardiac reflex responses," *Am. J. Physiol.*, **263**, R215-R220 (1992).
8. D. L. Eckberg and J. M. Fritsch, "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. Regulat. Integr. Comp. Physiol.*, **297,** R116-R123 (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**, 638-644 (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**, 1013-1017 (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**, 1389-1395 (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).