The repeated sit-to-stand maneuver is a superior method for cardiac baroreflex assessment: a comparison with the modified Oxford method and Valsalva maneuver

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Horsman HM, Tzeng YC, Galletly DC, Peebles KC. The repeated sit-to-stand maneuver is a superior method for cardiac baroreflex assessment: a comparison with the modified Oxford method and Valsalva maneuver. Am J Physiol Regul Integr Comp Physiol 307: R1345–R1352, 2014. First published October 1, 2014; doi:10.1152/ajpregu.00376.2014.—Baroreflex assessment has diagnostic and prognostic utility in the clinical and research environments, and there is a need for a reliable, simple, noninvasive method of assessment. The repeated sit-to-stand method induces oscillatory changes in blood pressure (BP) at a desired frequency and is suitable for assessing dynamic baroreflex sensitivity (BRS). However, little is known about the reliability of this method and its ability to discern fundamental properties of the baroreflex. In this study we sought to: 1) evaluate the reliability of the sit-to-stand method for assessing BRS and compare its performance against two established methods (Oxford method and Valsalva maneuver), and 2) examine whether the frequency of the sit-to-stand method influences hysteresis. Sixteen healthy participants underwent three trials of each method. For the sit-to-stand method, which was performed at 0.1 and 0.05 Hz, BRS was quantified as an integrated response (BRS\textsubscript{INT}) and in response to falling and rising BP (BRS\textsubscript{DOWN} and BRS\textsubscript{UP}, respectively). Test retest reliability was assessed using the intraclass correlation coefficient (ICC). Irrespective of frequency, the ICC for BRS\textsubscript{INT} during the sit-to-stand method was ≥0.88. The ICC for a rising BP evoked by phenylephrine (PEGAIN) in the Oxford method was 0.78 and ≤0.5 for the remaining measures. During the sit-to-stand method, hysteresis was apparent in all participants at 0.1 Hz but was absent at 0.05 Hz. These findings indicate the sit-to-stand method is a statistically reliable BRS assessment tool and suitable for the examination of baroreflex hysteresis. Using this approach we showed that baroreflex hysteresis is a frequency-dependent phenomenon. We recently used the sit-to-stand method to explore the frequency-dependent characteristics of the baroreflex (17). Participants performed the maneuver at four frequencies (0.03, 0.05, 0.07, and 0.1 Hz) after which the integrated BRS (BRS during both increasing and decreasing BP) was measured. We found that the integrated BRS (BRS\textsubscript{INT}) was highest at 0.05 Hz and lowest at 0.1 Hz supporting the hypothesis that BRS\textsubscript{INT} is frequency dependent across a comprehensive range. These findings were in accord with an earlier study by Zhang et al. (53) who used a similar orthostatic challenge (i.e., the repeated squat-stand method) but narrower frequency range. We concluded that the sit-to-stand method captures important information regarding baroreflex function. However, before any method can be adopted, performance indicators such as reliability must also be determined (7, 42, 49). To our knowledge no studies to date have assessed whether the sit-to-stand method is reliable, although its evoking of uniform, accurately timed, orthostatic changes points in its favor. Another important characteristic of the baroreflex is hysteresis, which typically manifests as a lower BRS during decreasing BP (BRS\textsubscript{DOWN}) than during an increasing BP (BRS\textsubscript{UP}) (13, 46). Evidence suggests that assessing baroreflex hysteresis may give additional insight into baroreflex function (41) and afford a better indicator of baroreflex function than BRS\textsubscript{INT} alone (37, 52). However, by convention BRS is assessed as an integrated response, and directional BRS, i.e., BRS after sitting (BRS\textsubscript{UP}) and standing (BRS\textsubscript{DOWN}), are not separated. However, our previous study led us to consider that baroreflex hysteresis could be frequency dependent, and to our knowledge, no study has assessed hysteresis using the sit-to-stand method. Therefore, the aims of this study were twofold. First, we sought to examine the test retest reliability of the sit-to-stand method compared with that of two established methods of BRS assessment, i.e., the modified Oxford method and Valsalva maneuver (VM) (11, 29). Second, we wanted to determine whether the rate of the sit-to-stand method would differentially affect BRS\textsubscript{DOWN} and BRS\textsubscript{UP}. We hypothesized that the sit-to-stand method would demonstrate good estimates of reliability, and BRS\textsubscript{DOWN} and BRS\textsubscript{UP} would exhibit frequency dependence.

METHODS

This study was approved by the Central Regional Ethics Committee and conformed to the standards set by the Declaration of Helsinki. All participants gave written informed consent.
**Participants**

Sixteen healthy participants (12 male; aged 29 ± 7 yr; body mass index 24 ± 3 kg/m²) were recruited to perform the sit-to-stand, Oxford, and VM methods. All participants were non-smokers, free from respiratory, cardiovascular, neurological, and endocrine disease and were not taking any medication known to alter autonomic function (e.g., β-blockers). Females were not pregnant and were studied within the early follicular phase of their menstrual cycle or in their pill-free days.

To determine test retest reliability, the sample size calculation suggested that 16 participants undergoing 3 replicates for each method would be sufficient for an expected Intraclass Correlation Coefficient (ICC) of 0.8 and a 95% confidence interval (95% CI) of 0.3 (42). The sample size required to examine hysteresis during the sit-to-stand maneuver was based on a previous study that indicated 7 participants would be required.

**Instrumentation**

An 18-g cannula was inserted into an antecubital vein for drug infusions required for the Oxford method. A 3-lead electrocardiogram (ADInstruments, Colorado Springs, CO) was used to measure heart rate (HR). Finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) was used to measure continuous systolic and diastolic arterial BP (SAP and DAP, respectively), and mean arterial pressure (MAP) was calculated from their waveforms. The finometer cuff was referenced to heart level, and finometer BP measurements were verified against oscillometric BP measurements (Seinex Electronics) taken on the contralateral arm finometer BP measurements were verified against oscillometric BP measurements (Seinex Electronics) taken on the contralateral arm throughout the experimental protocol. An infrared CO₂ gas analyzer (model ML206, ADInstruments) measured the partial pressure of end-tidal CO₂ (PETCO₂) sampled from a nasal line. All data signals were recorded continuously at 1 kHz using an analog-to-digital converter (Powerlab/16SP ML795, ADInstruments) and stored for offline analysis.

**Experimental Design**

The studies were performed in morning sessions (commencing at 9 AM) in a temperature-controlled laboratory (~22°C). Participants refrained from strenuous exercise for at least 24 h and caffeine and alcohol for at least 12 h before testing. Each participant was asked to confirm they had consumed a light breakfast. Their hydration status was also checked. After instrumentation, acclimatization, and before each trial, 5 min of baseline data was collected. BRS was then assessed using the sit-to-stand, Oxford, and VM methods. With the order of the methods having been randomized, each method was repeated three consecutive times. Each participant completed the entire protocol within 4 h.

**Sit-to-stand method.** Participants sat in a chair with their feet flat on the floor and their thighs parallel to the ground. A sling was placed on the arm bearing the Finometer to support the hand at heart level and stabilize BP recordings during movement. Repeated sit-to-stand maneuvers were then performed three times at: 1) 0.05 Hz (10 s sit followed by 10 s stand for 15 cycles) and 2) 0.1 Hz (5 s sit followed by 5 s stand for 15 cycles), with at least 5 min separation for rest and recovery between each trial. For this method, the frequency (0.05 Hz vs. 0.1 Hz) of sitting to standing was also randomized. An electronic metronome was used to guide the participant to sit and stand at the correct frequency. Participants were instructed to breathe normally (i.e., avoid inadvertently performing VMs) and minimize excessive forward flexion when standing up. PETCO₂ levels were monitored to ensure that the participants breathing was regular, their breathing frequency was above the sit-to-stand frequency (0.05 Hz or 0.1 Hz), and no exercise-induced hyperventilation occurred. From this method we calculated BRS₀⁻, BRS₀⁺, and BRS₁⁻. BRS₀⁻ was calculated from the composite falling and rising BP changes, whereas directional BRS was calculated either during a falling BP evoked by standing (BRS₀⁻) or a rise in BP evoked by sitting (BRS₀⁺).

**Modified Oxford method.** The Oxford method was performed as previously described (41). Briefly, a bolus injection (100–200 µg) of sodium nitroprusside (SNP) was administered followed 60 s later by a bolus injection (100–200 µg) of phenylephrine (PE). Drug doses were titrated to evoke at least 15-mmHg decreases and increases in BP, respectively. We allowed 15 min recovery between each trial for drug washout and participants were closely monitored for ECG irregularities. From this method we only calculated directional BRS, as per convention. BRS was calculated during a falling BP evoked by SNP (SNP_GAIN) and during a rising BP evoked by PE (PE_GAIN).

**VM method.** Participants (trained to the maneuver) were seated and asked to exhale forcibly into a mouthpiece connected to a pressure transducer. To prevent the glottis closing, there was a small leak in the mouthpiece. During exhalation, participants were instructed to maintain a pressure of ~40 mmHg for 15 s. This was aided by a visual display of the expiratory pressure and time count. Participants were instructed to resume normal breathing at the end of the exhalation and were allowed at least 5 min for rest and recovery between each VM. The VM and its four distinct phases have previously been described (16, 23, 30, 39). At the beginning of the strain there is a transient rise in BP (phase I) and then a fall in BP due to impaired venous return and reduced stroke volume accompanied by a compensatory increase in HR (early phase II). Upon release of the strain, there is a sudden reduction in intrathoracic pressure, which evokes a transient decrease in BP (phase III). This is followed by an abrupt rise in BP to above baseline accompanied by a baroreflex-induced reduction in HR (phase IV) (54). From this method we only calculated directional BRS, as per convention. In keeping with others (3, 30) BRS was calculated during a falling BP evoked during early phase II (phase II_GAIN) and during a rising BP evoked during phase IV (phase IV_GAIN). These phases are considered to represent the cardiovagal baroreflex response (21, 26, 48).

**Data Analysis**

All data analysis was conducted using custom-written software in LabView 8.2 (National Instruments). Data were checked for artifacts and erroneously detected or missed R waves were corrected by linear interpolation. An 18-g cannula was inserted into an antecubital vein for drug infusions required for the Oxford method. A 3-lead electrocardiogram (ADInstruments, Colorado Springs, CO) was used to measure heart rate (HR). Finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) was used to measure continuous systolic and diastolic arterial BP (SAP and DAP, respectively), and mean arterial pressure (MAP) was calculated from their waveforms. The finometer cuff was referenced to heart level, and finometer BP measurements were verified against oscillometric BP measurements (Seinex Electronics) taken on the contralateral arm finometer BP measurements were verified against oscillometric BP measurements (Seinex Electronics) taken on the contralateral arm throughout the experimental protocol. An infrared CO₂ gas analyzer (model ML206, ADInstruments) measured the partial pressure of end-tidal CO₂ (PETCO₂) sampled from a nasal line. All data signals were recorded continuously at 1 kHz using an analog-to-digital converter (Powerlab/16SP ML795, ADInstruments) and stored for offline analysis.

**Integrated BRS from the sit-to-stand method.** Sit-to-stand BRS_₀ was analyzed as described by others (19, 53). For each 15-cycle epoch, the R-R interval and BP series were filtered, resampled at 4 Hz, and then linearly detrended. These series were then passed through a Hanning window before undergoing Fast Fourier Transformation and spectral analysis. Transfer function gain, phase, and coherence at each sit-to-stand frequency were derived from the cross spectrum of the SAP signal (input) and R-R interval (output). Transfer function gain represents the change in output for a given change in input and reflects BRS (19). Gain was only taken as an estimate of BRS_₀ when the estimated squared coherence was ≥0.6, indicating the input and output signals were related (40). Transfer function phase represents the lag between input and output signals and is representative of the physiological latency of the baroreflex response.

**Directional BRS from the sit-to-stand, Oxford, and VM methods.** Directional BRS for all three methods were calculated using the same approach, also described by others (24, 41). As per convention, for each trial of the Oxford and VM methods, directional BRS was calculated from a single fall and single rise in BP. However, the repetitive nature of the sit-to-stand maneuver allowed BRS_DOWN and BRS_UP to be calculated from 15 BP oscillations. For each BP change the R-R interval was plotted against SAP to visually identify the saturation and threshold regions (28), which were then automatically identified using piecewise linear regression as previously described (46). The changes in SAP were matched to the concurrent heart period, and when the R-R intervals were between 500 and 800 ms a...
one-beat delay was used. To account for respiratory oscillations in R-R interval and SAP readings, both time series were averaged across 3-mmHg bins (20). For all methods, the slope of the relationship between SAP and R-R interval was constructed using at least five data points, and the slope of the line of best fit was determined using least squares linear regression (38, 46). The slope was taken as an estimate of BRS provided the correlation coefficient was ≥0.8 (24).

**Statistical Analysis**

All analyses were conducted using SPSS 20 (SPSS). Significance was defined at an α level of P < 0.05 for all comparisons. Data are presented as means ± SD. All data were tested for normality using the Shapiro-Wilk test. No transformations were performed.

**Analysis of Reliability**

There is no “gold standard” for measuring reliability, hence, it is recommended that a combination of approaches are used (2). In this study reliability was assessed using three approaches. Specifically, the ICC, the SEM, and Pearson’s correlation coefficient, as discussed below. For each of these methods, test retest reliability was assessed three times within a single session. This was because we wanted to assess the reliability of the method independent of any time-dependent variation in a given physiological response, e.g., day-to-day HR variations. This design also enabled us to determine whether the sit-to-stand method is sufficiently reliable for the method to be performed once for meaningful BRS results, or whether two or three trials are required.

The ICC represents relative reliability and indicates consistency and agreement between measurements relative to the total variability (7). There are several versions of the ICC (for review see Refs, 2, 14, 43, and 49). Within SPSS we specified a two-way random-effect model with single measures (i.e., ICC 2,1 according to the Shrout and Fleiss convention) (43). A random-effect model with absolute agreement was chosen because it addresses both random and systematic error. The ICC is reported as an abstract number on a scale of 0 to 1, where 0 indicates no reliability and 1 represents perfect reliability (2, 9, 49).

The SEM represents absolute reliability and is a measure of variability in individual BRS values across all three trials. The SEM is found by (49):

\[
SEM = SD \sqrt{1 - r}
\]

where \( r \) is the reliability coefficient (i.e., the ICC).

The Pearson’s correlation coefficient represents relative reliability and indicates the strength of linear relationships between trials.

Since ICC values are abstract numbers, interpretation is not always intuitive. To illustrate how ICC values relate to BRS values, we calculated the 25th and 75th percentile values from all observations across all three trials for each method, as examples of “observed” BRS values. We then calculated their estimated true values (ETV) as follows (9):

\[
ETV = M + r(X - M)
\]

where \( M \) is the sample mean (i.e., mean of all observations across all three trials for each method), \( r \) is the reliability coefficient (i.e., the ICC), and \( X \) is the observed value at the 25th or 75th percentile. ETVs were presented with 95% CIs.

The ETV is the value that would be obtained if there were no error in the measurement system, and an observed value is one that contains the true value plus the error component. For the purposes of interpretation, the closer the observed value is to the ETV and the tighter the CI, the lower the error component (9).

**Analysis of Directional BRS**

Directional BRS during the sit-to-stand method was examined using a two-way repeated measure ANOVA to investigate a condition effect (\( \text{BRS}_{\text{DOWN}} \times \text{BRS}_{\text{UP}} \)), a group effect (0.05 Hz × 0.1 Hz), and interaction (direction × frequency). When a significant interaction was detected, Bonferroni-corrected paired-sample t-tests were employed to compare the direction of BP change and frequency. Paired t-tests were also used to compare SNPGAIN and PEGAIN during the Oxford method and phase II_GAIN and phase IV_GAIN during the VM method.

Additionally, a pairwise t-test was used to detect a significant difference in transfer function phase between 0.05 Hz and 0.1 Hz during assessment of BRS_INT.

**RESULTS**

Resting baseline data collected in the sitting and supine positions before each method are summarized in Table 1. Resting HR, SAP, DAP, and MAP were higher in the sitting than supine position (\( P < 0.05 \)), most likely due to postural effects. All baseline parameters were within normal resting levels.

**Reliability of the Sit-To-Stand Method and Comparison to the Oxford and VM Methods**

Table 2 shows mean BRS values for each consecutive trial from which ICC, SEM, and Pearson’s correlation coefficient were derived. Irrespective of frequency or direction of BP change, the ICC values for BRS_INT, BRS_DOWN, and BRS_UP during the sit-to-stand method were close to 1, the SEM ≤ 2.1 ms/mmHg, and significant correlations between trials were found. Although the ICC and correlation for PEGAIN were similar to that observed during the sit-to-stand method, the SEM was ≥4.0 ms/mmHg. Moreover, the ICC and correlation coefficient differed according to the direction of BP change, since both were poorer for SNPGAIN than PEGAIN (see Table 2). Of the three methods the sit-to-stand method showed the greatest reliability (Table 2).

Table 3 illustrates the impact of each ICC estimate on BRS values. For the sit-to-stand method, the observed BRS values at the 25th and 75th percentile were close to their ETV and the 95% CI of the ETV were narrow. In contrast, the observed BRS values for the Oxford method, at each percentile, were further away from their respective ETV and their 95% CIs were broader. Even though the ICC for PEGAIN was 0.78 (see Table 2), the observed BRS values at each percentile were some distance from their ETV and the 95% CI were wide. The VM method showed mixed reliability estimates. Despite the phase II_GAIN having a relatively narrow 95% CI, the distances between the observed values and their respective ETV were large. By contrast the phase IV_GAIN had

| Table 1. Baseline cardiovascular parameters before commencement of each method |
|-------------------------------|------------------|-----------------|-------------------|
|                               | Pre-Sit-to-Stand Method (Sitting) | Pre-Oxford Method (Supine) | Pre-VM Method (Sitting) |
| HR, beats/min                 | 73 ± 7.9         | 63 ± 8.5*       | 69 ± 11           |
| SAP, mmHg                     | 127 ± 18         | 114 ± 13*       | 123 ± 14          |
| DAP, mmHg                     | 66 ± 12          | 59 ± 9.8*       | 65 ± 11           |
| MAP, mmHg                     | 83 ± 13          | 70 ± 19*        | 82 ± 11           |

Values are means ± SD. HR, heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; VM, Valsalva maneuver. *P < 0.05 vs. sit-to-stand method.
For comparison, directional BRS during the Oxford and VM methods are displayed in the inset of Fig. 1. As shown there was a difference between phase II\textsubscript{GAIN} and phase IV\textsubscript{GAIN} during the VM, which is indicative of hysteresis. Although the mean SNP\textsubscript{GAIN} and PE\textsubscript{GAIN} were no different during the Oxford method, 10 of 15 individuals exhibited hysteresis.

### Sit-To-Stand Latency

The transfer function phase for BRS\textsubscript{INT} was less negative at 0.05 Hz than at 0.1 Hz (−0.60 ± 0.26 rads vs. −0.81 ± 0.44 rads at 0.05 Hz and 0.1 Hz, respectively, \(P < 0.01\)). Converted into the time domain, this indicates that the latency was significantly longer at 0.05 Hz than at 0.1 Hz (i.e., 1.9 ± 0.8 s vs. 1.3 ± 0.7 s, respectively).

### DISCUSSION

We systematically compared the sit-to-stand method with the Oxford and VM methods and report two novel findings. First, that the sit-to-stand method, performed at two physio-

### Table 3. Illustration showing how ICC estimates relate to baroreflex sensitivity (BRS) values

<table>
<thead>
<tr>
<th>Method</th>
<th>Illustration at the BRS 25th Percentile, ms/mmHg</th>
<th>Illustration at the BRS 75th Percentile, ms/mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed Value</td>
<td>ETV (95% CI)</td>
</tr>
<tr>
<td>Sit-to-stand (0.05 Hz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRS\textsubscript{INT}</td>
<td>7.0</td>
<td>7.4 (4.6–10.1)</td>
</tr>
<tr>
<td>BRS\textsubscript{DOWN}</td>
<td>6.4</td>
<td>7.1 (3.7–10.5)</td>
</tr>
<tr>
<td>BRS\textsubscript{SUP}</td>
<td>6.4</td>
<td>7.1 (3.5–10.7)</td>
</tr>
<tr>
<td>Sit-to-stand (0.1 Hz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRS\textsubscript{INT}</td>
<td>4.1</td>
<td>4.3 (2.4–6.3)</td>
</tr>
<tr>
<td>BRS\textsubscript{DOWN}</td>
<td>3.1</td>
<td>3.3 (1.6–5.1)</td>
</tr>
<tr>
<td>BRS\textsubscript{SUP}</td>
<td>5.7</td>
<td>6.0 (3.1–8.8)</td>
</tr>
<tr>
<td>Oxford</td>
<td></td>
<td></td>
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<tr>
<td>SNP\textsubscript{GAIN}</td>
<td>5.6</td>
<td>8.1 (1.9–14.3)</td>
</tr>
<tr>
<td>PE\textsubscript{GAIN}</td>
<td>9.8</td>
<td>10.8 (3.7–17.8)</td>
</tr>
<tr>
<td>VM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase II\textsubscript{GAIN}</td>
<td>4.5</td>
<td>6.1 (3.2–8.9)</td>
</tr>
<tr>
<td>Phase IV\textsubscript{GAIN}</td>
<td>6.5</td>
<td>11.3 (5.1–21.2)</td>
</tr>
</tbody>
</table>

Values are means ± SD. BRS\textsubscript{INT}, Oxford, and VM method \(n = 16\). BRS\textsubscript{DOWN} and BRS\textsubscript{SUP} \(n = 15\). ICC, Intraclass correlation coefficient; ETV, estimated true value; CI, confidence intervals; BRS\textsubscript{DOWN}, BRS during falling BP; BRS\textsubscript{SUP}, BRS during rising BP; SNP\textsubscript{GAIN}, BRS during falling BP evoked by sodium nitroprusside; PE\textsubscript{GAIN}, BRS during rising BP evoked by phenylephrine; VM, Valsalva maneuver; Phase II\textsubscript{GAIN}, BRS during falling BP evoked by the VM; Phase IV\textsubscript{GAIN}, BRS during rising BP evoked by the VM.
logically relevant frequencies [0.05 Hz (period of 20 s) and 0.1 Hz (period of 10 s)] is a reliable method of assessing integrated BRS and its directional components (BRSDOWN and BRSUP). Furthermore, the reliability of the directional components exceeds those of the Oxford and VM methods, thereby challenging the Oxford methods “gold-standard” status. Second, in line with our hypothesis BRSDOWN and BRSUP differed with sit-to-stand frequency. Specifically, at 0.1 Hz, BRSDOWN was lower than BRSUP, which conforms to the pattern of hysteresis; at 0.05 Hz BRSDOWN and BRSUP were similar. These findings demonstrate that the sit-to-stand method is a superior method of assessing BRS and one that is capable of capturing fundamental properties of baroreflex function in healthy humans. This supports its inclusion in the armamentarium of BRS assessment tools.

Reliability

Reliability estimates must be interpreted in the context of their proposed use; however, this is complicated by the lack of clear guidelines for defining “acceptable” reliability. In the case of the ICC some guidelines have been provided. For instance, Nunnaly et al. (34) recommended using an ICC of ≥0.7 in the research environment and an ICC of ≥0.9 in the clinical setting. Using these guidelines, the ICC for BRSDOWN, BRSDOWN, and BRSUP (at 0.05 Hz and 0.1 Hz) reached the minimum criteria for use in the research environment and largely fulfilled the criteria for use in the clinical setting at 0.1 Hz (see Table 2 for details). However, none of the remaining methods fulfilled Nunnaly and coworkers criteria for use in the clinical setting, and in the case of the Oxford method, only the PEGAIN was sufficiently reliable for use in research. In the absence of similar criteria for the SEM and Pearson’s correlation coefficient, we accepted the general consensus that the lower the SEM and the higher the correlation, the greater the reliability. Thus the low SEM and high correlation for the sit-to-stand method reinforce the reliability of the method, whereas the higher SEM and lower correlation for the Oxford and VM methods, highlight their poor reliability. Collectively, these findings support our hypothesis that the sit-to-stand method demonstrates good reliability. Also, its reliability exceeded that of established methods.

We consider that there are two main methodological factors that contribute to the greater reliability of the sit-to-stand method. First, the sit-to-stand maneuver provides a relatively uniform stimulus in terms of the rate and magnitude of BP change. The rate is controlled because the frequency of the maneuver was electronically timed, and the magnitude of BP change is controlled because the participant moves through a constant hydrostatic column. Second, all BRS values for the sit-to-stand method measures are calculated over 15 oscillations, thereby minimizing the effect of any spurious cardiovascular changes. By contrast, the Oxford and VM methods afford little control over rate and magnitude of BP change by nature of the fact that infusing drugs (Oxford method) and exerting physical effort (VM) are either investigator or participant dependent. Also, neither method is amenable to multiple repetitions in quick succession.

Baroreflex Hysteresis Assessed by the Sit-To-Stand Method

Our results show that BRSDOWN at 0.1 Hz was depressed in all individuals. However, the frequency of sit-to-stand maneuver did not affect BRSUP at 0.1 Hz or BRSDOWN and BRSUP at 0.05 Hz. Before we discuss our findings further, the physiological changes that occur during the sit to stand maneuver need to be considered.

Active standing evokes immediate increases in HR and cardiac output and a reduction in total peripheral resistance (TPR) (44, 45, 50). There is some controversy as to the cause of the immediate increase in HR, which peaks/~3 s after standing. This immediate increase in HR has been attributed to the activation of muscle mechanoreceptors and/or central command pathways, whereas the more gradual increase in HR occurring after ~3 s is considered to be baroreflex mediated (6, 44). In the absence of any alteration in stroke volume, this increase in HR causes a net increase in cardiac output. Nevertheless, BP falls due to a pronounced reduction in TPR, which lasts ~6–8 s. The latter is evoked by stimulation of cardiopulmonary receptors secondary to an increase in right atrial pressure. Arterial BP begins to normalize after ~7 s as the prevailing hypotension unloads the baroreceptors and sympathetic vasoconstriction restores TPR (15, 50). This is facilitated
by a slower baroreflex-mediated increase in HR, which peaks at \( \approx 12 \) s (6, 44, 50).

During the sit-to-stand method at 0.05 Hz and 0.1 Hz, changes in BP and R-R interval are assessed during 10-s and 5-s periods, respectively. Given the timeframe of hemodynamic changes that occur on standing, we questioned to what extent the changes in R-R interval at 0.1 Hz reflected baroreflex feedback control, activation of mechanoreceptors, and/or central command. Based on the following evidence we believe the baroreflex is the primary contributor. Previous studies have shown that the baroreflex response occurs well within the 5-s time frame (12, 22) and any influence of the mechanoreceptors and central command to R-R changes is minor (53). By implication, changes in R-R interval at 0.05 Hz (within a 10-s window) also reflect baroreflex function.

In consideration of the above, we suggest that differences in BRS upon sitting and standing at 0.1 Hz reflect differences in BRS during the two maneuvers. In other words, the fact that BRS\(_{\text{DOWN}}\) is lower than BRS\(_{\text{UP}}\) at 0.1 Hz suggests the baroreflex is relatively depressed upon standing (falling BP). Indeed, this pattern of response was not unexpected and conforms to that of hysteresis. On the other hand, the lack of any differential changes in BRS at 0.05 Hz implies that hysteresis is not present at this frequency. These findings support our hypothesis that BRS\(_{\text{DOWN}}\) and BRS\(_{\text{UP}}\) exhibit frequency dependence.

Interestingly, frequency dependence only resided in the down response, since BRS\(_{\text{UP}}\) was similar at both frequencies. Our study was not designed to probe why this was the case. However, it is known that the cardiopulmonary baroreflex is activated upon standing (50), and it modulates the arterial baroreflex response during changes in posture and exercise (15, 36). It is possible that the relative contributions from the low- or high-pressure baroreceptors are frequency dependent. However, without further investigations this remains speculative.

It has been suggested that hysteresis is derived from an interaction between the mechanical and neural components of the baroreflex (46, 47, 51). Our study was not designed to isolate these components, their interactions, or relative contributions to hysteresis, but others have demonstrated the mechanical and neural components exhibit frequency dependence.

The concept of mechanical frequency dependence is supported by studies showing the arterial wall (mechanical component) becomes stiffer when it is stretched quickly, which lowers BRS\(_{\text{INT}}\) (5, 27, 35). The concept of neural frequency dependence is supported by studies showing that the recruitment of baroreceptor afferents (Type A and C) is influenced by the rate of stretch (4, 8, 46). Hence, we suspect that the appearance of hysteresis at 0.1 Hz, but not at 0.05 Hz, is due to the fact that any mechanical and neural contributions are frequency dependent. Further investigation is required to examine how different frequencies of BP oscillation affect the neural and mechanical components of the baroreflex and how this affects their relative contributions to baroreflex hysteresis.

Some individuals showed hysteresis during the Oxford method, but significance was not reached. It is also important to point out that by convention the rate of BP change is not controlled or identified during the Oxford method, hence frequency dependence is not considered. Most individuals showed hysteresis during the VM method. However, the importance of these findings is uncertain due to the very poor reliability of the method. Collectively these results support our suggestion that the sit-to-stand method is the best of these methods to examine hysteresis.

**Significance of Baroreflex Hysteresis**

Because of the complexity of the underpinning mechanisms, it remains unclear whether hysteresis enhances or degrades baroreflex function. In engineering systems, hysteresis is used to stabilize negative feedback control systems by preventing the rapid switching from one state to another (1). It is therefore plausible that baroreflex stability may also benefit from hysteresis during rapid changes in BP. Baroreflex latency is also an important component of baroreflex stability (18, 22, 31, 32), although its role in baroreflex function is highly complex. Interestingly, the baroreflex latency was shorter at 0.1 Hz, i.e., when hysteresis was present, than at 0.05 Hz. However, the significance of these findings requires further investigation.

**Methodological Considerations**

As with any analytical method, reliability will degrade if environmental conditions and testing procedures are not standardized. To ensure that this study produced a valid representation of method reliability and to maximize internal validity, care was taken to standardize conditions (e.g., chair height, starting positions, timing of each sit-to-stand). Participants were also trained before each maneuver (e.g., the VM) to minimize any learning effect.

During analysis of directional BRS, it is acknowledged that the visual detection of the saturation and threshold regions of the baroreflex curve is a source of variability. To reduce this, all directional BRS data (i.e., BRS\(_{\text{DOWN}}\), BRS\(_{\text{UP}}\), SNPGAIN, PEGAIN, phase II\(_{\text{GAIN}}\) and phase IV\(_{\text{GAIN}}\)) were analyzed using the piecewise linear regression process proposed by Studinger et al. (46). As the BRS\(_{\text{DOWN}}\) and BRS\(_{\text{UP}}\) results demonstrated good reliability, we concluded that the higher variability observed in the Oxford and VM methods was not due to variability incurred during data analysis.

Finally, it is acknowledged that the current analyses only compared the reliability of methods for assessing BRS in a healthy population. Hence, further studies are required to ascertain whether the findings transfer to patient cohorts, e.g., patients with cardiovascular disease.

**Perspectives and Significance**

The findings of this study confirm the sit-to-stand method is a reliable method of assessing integrated BRS, and a direct comparison between the sit-to-stand method and other traditional techniques revealed that the sit-to-stand method is the most reliable. We believe these findings may be of interest to researchers and clinicians who require simple, noninvasive, and physiologically or functionally relevant measures of BRS to facilitate clinical risk stratification. Our findings also indicate that the sit-to-stand method is sufficiently reliable for BRS to be assessed in a single trial, thereby negating the need for multiple trials, which are time consuming. Additionally, we demonstrated that the sit-to-stand method could be used to assess hysteresis, which may enhance our understanding of baroreflex function. Important in our results was the finding that hysteresis was only observed at 0.1 Hz. This challenges the notion that hysteresis is an innate property of the baroreflex and...
reinforces the importance of controlling the rate of BP oscillation in studies examining BRS. Further mechanistic studies are required to determine precisely how frequency impacts on the neural and mechanical components of the baroreflex. Based on these collective findings we believe the sit-to-stand method should play a more prominent role in BRS assessment in the research and clinical settings.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: H.M.H., Y.T., D.C.G., and K.C.P. conception and design of research; H.M.H. and K.C.P. performed experiments; H.M.H. analyzed data; H.M.H., Y.T., and K.C.P. interpreted results of experiments; H.M.H. and K.C.P. drafted manuscript; H.M.H., Y.T., D.C.G., and K.C.P. critical review of methods and results.

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