Methodological comparison of active- and passive-driven oscillations in blood pressure; implications for the assessment of cerebral pressure-flow relationships

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Smirl JD, Hoffman K, Tzeng Y-C, Hansen A, Ainslie PN. Methodological comparison of active- and passive-driven oscillations in blood pressure; implications for the assessment of cerebral pressure-flow relationships. J Appl Physiol 119: 487–501, 2015. First published July 16, 2015; doi:10.1152/japplphysiol.00264.2015.—We examined the between-day reproducibility of active (squat-stand maneuvers)- and passive [oscillatory lower-body negative pressure (OLBNP) maneuvers]-driven oscillations in blood pressure. These relationships were examined in both younger (n = 10; 25 ± 3 yr) and older (n = 9; 66 ± 4 yr) adults. Each testing protocol incorporated rest (5 min), followed by driven maneuvers at 0.05 (5 min) and 0.10 (5 min) Hz to increase blood-pressure variability and improve assessment of the pressure-flow dynamics using linear transfer function analysis. Beat-to-beat blood pressure, middle cerebral artery velocity, and end-tidal partial pressure of CO2 were monitored. The pressure-flow relationship was quantified in the very low (0.02–0.07 Hz) and low (0.07–0.20 Hz) frequencies (LF; spontaneous data) and at 0.05 and 0.10 Hz (driven maneuvers point estimates). Although there were no between-age differences, very few spontaneous and OLBNP transfer function metrics met the criteria for acceptable reproducibility, as reflected in a between-day, within-subject coefficient of variation (CoV) of <20%. Combined CoV data consist of LF coherence (15.1 ± 12.2%), LF gain (15.1 ± 12.2%), and LF normalized gain (18.5 ± 10.9%); OLBNP data consist of 0.05 (12.1 ± 15.%) and 0.10 (4.7 ± 7.8%) Hz coherence. In contrast, the squat-stand maneuvers revealed that all metrics (coherence: 0.6 ± 0.5 and 0.3 ± 0.5%; gain: 17.4 ± 12.3 and 12.7 ± 11.0%; normalized gain: 16.7 ± 10.9 and 15.7 ± 11.0%; and phase: 11.6 ± 10.2 and 17.3 ± 10.8%) at 0.05 and 0.10 Hz, respectively, were considered biologically acceptable for reproducibility. These findings have important implications for the reliable assessment and interpretation of cerebral pressure-flow dynamics in humans.

cerebral blood flow; blood pressure; cerebral pressure-flow relationship; transcranial Doppler ultrasound; transfer function analysis

The relationship between arterial blood pressure and cerebral blood flow (CBF) is a frequency-dependent process that functions as a high-pass filter (13, 61, 68). Higher frequency oscillations (>0.20 Hz) in blood pressure are passed along through the cerebrovasculature, relatively unimpeded, whereas slower oscillations (<0.20 Hz) are adjusted and dampened by the cerebral vessels (1, 13, 61, 68). The most common method to assess this dynamic relationship is through the use of the linear mathematical approach of transfer function analysis (TFA) (6, 21, 61, 68).

TFA provides information on metrics, such as the timing of waveforms (phase) (1, 6, 13, 28, 61, 68) and modulation (gain) (6, 13, 21, 61, 68) between the input (blood pressure) and output [cerebral blood velocity (CBV)] signals. The coherence metric gives an indication of the reliability of the TFA and is of utmost importance when interpreting the phase and gain metrics (60). For example, a high coherence (e.g., close to 1.0) indicates that the system is linear, and thus the changes to phase and gain are interpretable, whereas a low coherence (<0.50) could be a result of a multitude of factors (60, 61, 68). Namely, the low coherence could occur, due to a poor signal-to-noise ratio, the fact that the system is nonlinear, or multiple inputs that regulate the output variable, or there is just no relationship present between the input and output variables (68). As such, phase and gain values are typically not reported when coherence is <0.50 (13, 40, 60, 61, 68). Although it has been noted that coherence values >0.20 (for a specific frequency point estimate) during a 5-min sample can provide mathematically interpretable results (22), this still means that >80% of input and output signal power spectra at this point estimate are possibly unrelated, due to noise in the signals or nonlinearities in the relationship between blood pressure and CBV.

To address this concern, numerous methodologies to drive blood pressure (and thus improve coherence by maximizing the signal-to-noise ratio) have been proposed and include deep breathing (16, 40), oscillatory lower-body negative pressure (OLBNP) (11, 24, 54, 62), squat-stand maneuvers (6, 13, 51–53), and passive leg raises (17).

Of these proposed, driven methodologies, the squat-stand maneuvers (6, 13, 51–53) and OLBNP (11, 24, 54, 62) elicited the greatest oscillations in blood pressure within the high-pass filter frequency range (<0.20 Hz) of the cerebrovasculature. These large swings in blood pressure not only could be used to increase the statistical reliability of the phase and gain metrics but also do so in a physiologically relevant manner; i.e., the amplitude of these swings represents challenges that the cerebrovasculature endures on a daily basis, e.g., coughing, posture changes, exercise, defecation, and others.

During the squat-stand maneuvers, the squat phase engages the muscles of the legs, which increases the skeletal muscle pump and results in a large transient increase in venous return and blood pressure within 2–3 s (see Fig. 1 for a typical trace). Upon standing, the muscles of the legs are relaxed, decreasing the pressure applied to the veins, enabling venous pooling to...
increase, and resulting in a subsequent decrease in blood pressure. These large swings in mean arterial pressure (up to 40–45 Torr) (51–53) are performed at frequencies within the high-pass filter range (0.05 and 0.10 Hz) and are transmitted to the cerebrovasculature. The squat-stand maneuvers elicit this response, whether active or passive maneuvers are performed (67). These large oscillations result in greatly increased coherence values at the frequency of interest (e.g., >0.98), which is believed to occur as a result of an improved signal-to-noise ratio (6, 13, 51–53). During the OLBNP maneuvers, a large negative pressure (typically between −30 and −120 Torr, depending on the size of the negative pressure box) (11, 24, 62) is rapidly applied to the lower half of the torso and legs, which increases venous pooling, thus reducing blood pressure. When the negative pressure is cycled off, the pooled venous blood returns to the heart, and blood pressure is increased in a passive manner. Although the swings in blood pressure induced with OLBNP (up to 25 Torr) (11, 62) are not as large as those during the squat-stand maneuvers, they still elicit large increases in coherence values (e.g., >0.90; see Fig. 2 for a typical trace) (11, 62).

To date, no study has directly compared these two driven methods within the same subjects to ensure that the reported changes in TFA metrics are comparable in the broader literature (6, 11, 13, 24, 51–53, 62).

The hypotheses for this study are threefold: 1) squat-stand and OLBNP maneuvers will induce increases in TFA coherence (as compared with spontaneous data); 2) the phase and gain metrics associated with the squat-stand and OLBNP maneuvers will have less day-to-day variability than spontaneous measures; and 3) despite the decrease in CBF within the older adult populations (4, 18, 38, 48), both younger and older adults will have comparable cerebral pressure-flow responses.

METHODS

Ethical Approval

This study was approved by the Clinical Ethical Committee of the University of British Columbia and adhered to the principles of the Declaration of Helsinki. All volunteers provided written, informed consent, and procedures were followed in accordance with institutional guidelines.

Subjects

Ten healthy, younger adult [10 men, 24.8 ± 2.7 yr, body mass index (BMI): 23.6 ± 2.4 kg/m²] and 10 healthy, older adult (eight men, 66.4 ± 3.7 yr, BMI: 25.6 ± 2.7 kg/m²) subjects were recruited for this study. All subjects had a clear history of cardiorespiratory and cerebrovascular diseases and were not taking any form of medication. All older adult subjects were screened for any evidence of carotid stenosis; however, after completion of the screening process, one man was excluded from the study, and therefore, the older adult data are based on n = 9. All subjects abstained from exercise, caffeine, and alcoholic beverages for a period of 12 h before the study. Each subject underwent a familiarization of the laboratory and testing protocols before the initiation of the protocols.
Experimental Protocols

The subjects were required to visit the laboratory twice, with a minimum of 48 h to a maximum of 144 h between visits (younger adults, 67.2 ± 27.2 h; older adults, 72.0 ± 31.7 h). Both visitations were performed at the same time of day and involved two hemodynamic challenge protocols (squat-stand and OLBNP). Before collection of the driven oscillatory blood-pressure data, the subjects began with a 5-min seated resting period to obtain spontaneous baseline data. For the squat-stand maneuvers, the subjects began in a standing position; the subjects then squatted down until the back of their legs obtained an ~90° angle. This position was held for a set period of time, after which, they stood back up. The squat-stand cycle was performed at two different frequencies for 5 min each. The first frequency was 0.10 Hz (5 s squatting, followed by 5 s standing). The second frequency was at 0.05 Hz (10 s squatting, followed by 10 s standing). These frequencies were selected, as they are within the range where cerebral autoregulation is thought to have its greatest influence on the cerebral pressure-flow dynamics (68). During the OLBNP maneuvers, the subjects were in a supine position in industrially designed pressure boxes that sealed at their waist and were held in place via a mounted bicycle seat to prevent movement into the OLBNP box. The straps used to seal the participants in the OLBNP box did not interfere with their breathing patterns. Before initiating the OLBNP, a 5-min resting period at 0 Torr was performed to collect baseline data. The pressure in the box was then oscillated with a 50-Torr pressure was then oscillated at a frequency of 0.05 Hz (10 s at −50 Torr, followed by 10 s at 0 Torr). The −50-Torr pressure change occurred within 1.5 s; the −50-Torr pressure was selected to match the pressure generated in the steady-state findings of Formes et al. (19), who tested a similarly aged population (68 ± 1 yr) at a negative pressure of −50 Torr. The results from this study revealed that this level of negative pressure is well tolerated in older individuals. The order of the 0.05- and 0.10-Hz trials was randomly selected for the squat-stand and OLBNP maneuvers.

Instrumentation

Subjects first received a three-lead ECG for measurement of the R-R interval. Blood pressure was measured in the finger by photoplethysmography, with a brachial cuff used to provide the pressure difference between the finger and the arm (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands). This method has been shown to assess reliably the dynamic changes in beat-to-beat blood pressure that correlate well with the intra-arterial recordings and can be used to characterize the dynamic relationship between blood pressure and CBV (34, 43). During the squat-stand maneuvers, the subjects were asked to maintain their arm position throughout the trials to minimize any potential movement artifact in the blood-pressure signal.

Bilateral middle cerebral arteries were insonated by placing 2 MHz Doppler probes (Spencer Technologies, Seattle, WA) to obtain CBV. The middle cerebral arteries were identified and optimized according to their signal depth, waveform, and velocities (2, 66). Once the cerebral arteries were identified, the probes were secured and locked in place with a headband (Spencer Technologies). Cerebrovascular resistance index (CVRi) was calculated from mean arterial pressure/mean CBV. Partial pressure of end-tidal CO2 (PETCO2) was sampled with a mouthpiece and monitored with an online gas analyzer (ML206; ADInstruments, Colorado Springs, CO), which was calibrated with a known gas concentration before each subject. Heart rate was calculated from the ECG. All data were recorded and stored for subsequent analysis using commercially available software (LabChart version 7.1; ADInstruments).

Data Processing

All data were sampled simultaneously at 1,000 Hz via an analog-to-digital converter (PowerLab 16/30 ML880; ADInstruments). Real-
time, beat-to-beat mean values of blood pressure and middle cerebral artery velocity were determined from each R-R interval. All data that were collected were viewed in real time by an experienced investigator to ensure that there were no artifacts. All data were processed and analyzed with custom-designed software in LabVIEW 2011 (National Instruments, Austin, TX).

Power Spectrum and TFA

Beat-to-beat blood pressure and middle cerebral artery velocity signals were spline interpolated and resampled at 4 Hz for spectral and TFA based on the Welch algorithm. Each 5-min recording was first subdivided into five successive windows that overlapped by 50%. Data within each window were linearly detrended and passed through a Hanning window before discrete Fourier transform analysis. For TFA, the cross-spectrum between blood pressure and middle cerebral artery velocity was determined and divided by the mean arterial pressure autospectrum to derive the TFA coherence, gain, and phase.

The TFA coherence, gain, and phase of the driven blood-pressure oscillations were calculated at the point estimate of the driven frequency (0.05 or 0.10 Hz). These point estimates were selected, as they are in the very low (0.02–0.07 Hz) and low (0.07–0.20 Hz)-frequency (VLF and LF, respectively) ranges, where cerebral autoregulation is thought to be operant (24, 61, 68). Phase wraparound was not present at any of the point-estimate values for either OLBNP or squat-stand maneuvers at 0.05 and 0.10 Hz.

Within-Subject Coefficient of Variation

The relationship between arterial blood pressure and CBV is bound to be variable, as there are many other physiological variables that can influence CBV (37). This can confound the interpretation of the relationship between arterial blood pressure and CBV in terms of cerebral autoregulation, as the separation of random error from true physiological variability is difficult (37). As such, this study was not designed to examine the intricacies of cerebral autoregulation per se but is merely designed to determine the level of reproducibility via the within-subject coefficient of variation (CoV) for linear TFA metrics. In other fields, many authors have used the within-subject CoV as an indication of good reproducibility (<10%) and reasonable variability for reproducibility (<20%) (15, 39, 44). These cutoff points were applied to the findings in this study as a way to indicate which method had the least variability between measurement days and thus the highest level of reproducibility within the findings. It has also been shown that the within-subject CoV is a useful index for the reliability of very small sample sizes (n = 5) (58, 59) and for comparisons between metrics with different scales (49).

Statistical Analysis

Statistical analyses were performed using SPSS version 20.0. The within-subject CoV was calculated to determine testing day-to-day reproducibility of the mean arterial pressure, left and right middle cerebral artery velocities, PCr, and heart rate. Additionally, the Fourier transform power spectra for mean arterial pressure and CBV and TFA coherence, phase, gain, and normalized gain metrics CoV were also used to determine day-to-day reproducibility. Bland-Altman plots were constructed to demonstrate the bias in significant differences between squat-stand and OLBNP maneuvers (8). A paired t-test was performed to determine the effect of CoV on modality (upright vs. supine or OLBNP vs. squat-stand). A multivariate two-way ANOVA was conducted to examine the effect of age and day on the cerebral pressure-flow relationship. Bivariate correlations were performed between CVRI and driven TFA metrics. Data are presented as means ± SD. Significance was set a priori at P < 0.05.

RESULTS

Hemodynamic and Cerebrovascular Responses

The older adults had a significantly higher resting heart rate and mean arterial pressure and lower PCr and middle cerebral artery velocity compared with the younger adults (Table 1). There were no significantly different hemodynamic or cerebrovascular responses from day 1 to day 2 in either younger or older adult populations, and all assessed measures revealed good CoV reproducibility (<10%). However, after the posture change from upright to supine, PCr universally rose by ~2 Torr (P < 0.05), which resulted in an increase in middle cerebral artery velocity of ~10% (P < 0.005) and a ~9% reduction in CVRi (P < 0.05).

Fourier and TFA

Power spectrum. Under spontaneous conditions, there were relatively low average power spectrums in the VLF and LF ranges for both the mean arterial pressure (<12 Torr²) and middle cerebral artery velocity (<11 cm²/s²) throughout aging (Tables 2 and 3). These reduced input levels were enhanced dramatically when the oscillations in blood pressure were induced. The power spectrum of mean arterial pressure was increased 100- to 500-fold in the squat-stand maneuvers and 15- to 80-fold during the OLBNP maneuvers (Figs. 3 and 4). These increases in the input signal of the cerebral pressure-flow response are reflected in the output signal (middle cerebral artery power spectrum), with a 100- to 300-fold increase during squat-stand maneuvers and 5- to 35-fold increase with OLBNP (Figs. 3 and 4).

Table 1. Hemodynamic and cerebrovascular responses during spontaneous baselines

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Between-Day CoV, %</th>
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<tbody>
<tr>
<td><strong>Upright—younger adults</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure, Torr</td>
<td>85.4 ± 14.1*</td>
<td>82.7 ± 10.1*</td>
<td>5.5 ± 4.4</td>
</tr>
<tr>
<td>Mean left MCAv, cm/s</td>
<td>58.9 ± 2.7</td>
<td>59.4 ± 2.6</td>
<td>1.9 ± 1.6</td>
</tr>
<tr>
<td>Mean right MCAv, cm/s</td>
<td>58.4 ± 5.9</td>
<td>57.4 ± 5.9</td>
<td>3.5 ± 3.1</td>
</tr>
<tr>
<td>Mean heart rate, beats/min</td>
<td>67.4 ± 13.3</td>
<td>67.4 ± 13.1</td>
<td>4.7 ± 3.5</td>
</tr>
<tr>
<td>End-tidal CO₂, Torr</td>
<td>37.9 ± 2.0</td>
<td>38.2 ± 2.3</td>
<td>1.8 ± 1.9</td>
</tr>
<tr>
<td><strong>Supine—younger adults</strong></td>
<td></td>
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<tr>
<td>Mean arterial pressure, Torr</td>
<td>83.9 ± 9.9</td>
<td>82.5 ± 9.9</td>
<td>5.7 ± 4.3</td>
</tr>
<tr>
<td>Mean left MCAv, cm/s</td>
<td>66.9 ± 6.4*</td>
<td>66.6 ± 4.7*</td>
<td>3.3 ± 2.1</td>
</tr>
<tr>
<td>Mean right MCAv, cm/s</td>
<td>64.1 ± 9.2*</td>
<td>63.2 ± 9.2*</td>
<td>4.3 ± 2.6</td>
</tr>
<tr>
<td>Mean heart rate, beats/min</td>
<td>61.5 ± 13.2</td>
<td>59.2 ± 11.5</td>
<td>4.4 ± 5.3</td>
</tr>
<tr>
<td>End-tidal CO₂, Torr</td>
<td>39.7 ± 2.6*</td>
<td>39.5 ± 2.6*</td>
<td>1.4 ± 1.1</td>
</tr>
<tr>
<td><strong>Upright—older adults</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure, Torr</td>
<td>99.5 ± 13.6†</td>
<td>100.7 ± 10.0†</td>
<td>2.6 ± 1.6</td>
</tr>
<tr>
<td>Mean left MCAv, cm/s</td>
<td>50.0 ± 9.9†</td>
<td>48.4 ± 9.3†</td>
<td>6.0 ± 3.3</td>
</tr>
<tr>
<td>Mean right MCAv, cm/s</td>
<td>52.2 ± 6.6†</td>
<td>52.1 ± 6.7†</td>
<td>5.4 ± 3.7</td>
</tr>
<tr>
<td>Mean heart rate, beats/min</td>
<td>70.5 ± 14.0</td>
<td>67.6 ± 12.4</td>
<td>6.4 ± 6.0</td>
</tr>
<tr>
<td>End-tidal CO₂, Torr</td>
<td>34.3 ± 3.1†</td>
<td>35.6 ± 3.0†</td>
<td>3.0 ± 2.4</td>
</tr>
<tr>
<td><strong>Supine—older adults</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean arterial pressure, Torr</td>
<td>107.5 ± 17.1†</td>
<td>106.9 ± 14.6†</td>
<td>4.3 ± 2.4</td>
</tr>
<tr>
<td>Mean left MCAv, cm/s</td>
<td>52.1 ± 8.5†</td>
<td>47.4 ± 8.8†</td>
<td>7.2 ± 5.3</td>
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<tr>
<td>Mean right MCAv, cm/s</td>
<td>55.3 ± 7.6†</td>
<td>56.4 ± 5.7†</td>
<td>4.3 ± 2.4</td>
</tr>
<tr>
<td>Mean heart rate, beats/min</td>
<td>69.1 ± 13.3</td>
<td>73.8 ± 17.1</td>
<td>4.2 ± 4.2</td>
</tr>
<tr>
<td>End-tidal CO₂, Torr</td>
<td>37.1 ± 2.5†</td>
<td>37.0 ± 3.0†</td>
<td>2.1 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. CoV, coefficient of variation; MCAv, middle cerebral artery velocity; Good reproducibility, <10%; acceptable reproducibility, <20% (15, 39, 44). Statistical significance was set at P < 0.05. Note: there were no significant differences from day 1 to day 2. *Different from upright condition; †different from younger adults.
Coherence. During the spontaneous conditions, there were relatively low average coherence values in the VLF (younger: 0.44 ± 0.16; older: 0.45 ± 0.15) and LF (younger: 0.69 ± 0.15; older: 0.65 ± 0.17) ranges (Tables 2 and 3). However, these relatively low coherence values were increased dramatically with the increase in input and output signal strength during driven oscillations in blood pressure. During squat-stand maneuvers, both frequencies resulted in a coherence of 0.99–1.00 regardless of age. The OLBNP maneuvers increased coherence values to ~0.89 (younger) and ~0.94 (older) at 0.05 Hz and >0.95 at 0.10 Hz (regardless of age; Tables 4 and 5). The proportional bias between the two modalities in both frequencies are illustrated in Figs. 5 and 6.

Transfer function phase and gain. Both spontaneous and driven TFA phase and gain metrics are consistent with the high-pass filter model, with phase decreasing and gain increasing as frequency increases from 0.02 to 0.20 Hz. However, there was a relatively large variation in values during spontaneous and driven conditions. Under spontaneous conditions, the absolute gain measures in younger adults showed significant increases when the subject was supine ($P < 0.05$).

During the driven metrics, although all Fourier transform and TFA metrics were significantly different between the OLBNP and squat-stand maneuvers, there were no differences between the younger and older adults (Tables 4 and 5). The OLBNP maneuvers resulted in an average bias in the younger adults of +0.97 radians (phase) and −0.31%/% (normalized gain) at 0.05 Hz and +0.34 radians (phase) and −0.38%/% (normalized gain) at 0.10 Hz ($P < 0.001$; Fig. 3). Similar findings for OLBNP bias were observed in the older adults, +0.48 radians (phase) and −0.47%/% (normalized gain) at 0.05 Hz and +0.25 radians (phase) and −0.61%/% (normalized gain) at 0.10 Hz ($P < 0.001$; Fig. 4).

Comparison of between-Day Reproducibility of TFA Metrics

Spontaneous data. Between-day reproducibility of the spontaneous and supine TFA metrics demonstrated that although the mean data for each measurement were not significantly different from day 1 to day 2, there were few metrics that showed a reasonable level of between-day CoV (<20%; Fig. 7 and Tables 2 and 3). Across the aging spectrum, only the LF coherence (15.1 ± 12.2%), LF absolute gain (15.1 ± 12.2%), and LF normalized gain (18.5 ± 10.9%) values met this criterion.

OLBNP data. The findings from the spontaneous data were reflected in the between-day reproducibility of the OLBNP maneuvers (Tables 4 and 5). The mean data for all metrics were, once again, not significantly different from day 1 to day 2; however, the only metrics that met the reasonable variability value for CoV (<20%) were the 0.05 Hz (12.1 ± 15.0%) and 0.10 Hz coherence (4.7 ± 7.8%) values.

Squat-stand data. In contrast to the above-mentioned findings, the squat-stand maneuver reproducibility revealed that all...
Fig. 3. Absolute values of the power spectral density (PSD) for the mean arterial pressure (MAP) and cerebral blood velocity (CBV) in younger adults under spontaneous (gray lines), 0.05 Hz (dashed lines), and 0.10 Hz (black, solid lines) conditions. The PSD during the OLBNP maneuvers is represented on the left (A–D), and squat-stand maneuvers are on the right (E–H). The PSD for the day 1 measures are represented in traces A, C, E, and G, with Day 2 measures represented in traces B, D, F, and H. Note: there were no significant differences between days 1 and 2; for SD values, see Tables 2 and 4.

TFA metrics (gain, phase, and coherence in both 0.05 and 0.10 Hz) were considered, on average, to have reasonable variability. In particular, the 0.05 (0.5 ± 0.5 and 0.6 ± 0.5 %)- and 0.10 (0.1 ± 0.1 and 0.3 ± 0.5 %)-Hz coherence values in the younger and older adult population, respectively, were the most robust of all findings in the study. The reported TFA coherence (P < 0.01 at both 0.05 and 0.10 Hz) and phase (P = 0.01 at 0.05 Hz, and P = 0.03 at 0.10 Hz) during squat-stand maneuvers had generally better reproducibility than that assessed using OLBNP (Fig. 7).

DISCUSSION

With the use of day-to-day repeatability comparisons between squat-stand maneuvers and OLBNP maneuvers, the main findings of the study as they pertain to the linear relationship between arterial blood pressure and CBV were the following: 1) although coherence is improved during both driven protocols, squat-stand maneuvers elicited the highest coherence values; 2) the squat-stand maneuvers resulted in more reproducibility across all TFA metrics; and 3) the cerebral pressure-flow responses during the driven oscillations are comparable in younger and older adults. Collectively, at least in the healthy populations studied, these findings demonstrate that the optimal protocol for creating oscillations in blood pressure to increase the input power and enhance the linear interpretability of the TFA metrics in both younger and older adult populations is through the use of squat-stand maneuvers. These findings have methodological implications for the reliable assessment and interpretation of the linear aspect of the cerebral pressure-flow relationship in humans.

Comparison with Previous Studies

To our knowledge, there have been eight studies to date (3, 13, 36, 51–53, 63, 64) that have used repeated squat-stand or sit-to-stand maneuvers to elicit oscillations in blood pressure to enhance the signal-to-noise ratio and thus interpretability of the TFA metrics. Collectively, these studies have demonstrated significant improvements in coherence in both the VLF [0.53 ± 0.13 (spontaneous) to 0.78 ± 0.07 (driven)] and LF [0.69 ± 0.13 (spontaneous) to 0.88 ± 0.08 (driven)] ranges. However, comparisons among these studies are difficult, as the studies either used a point-estimate approach (51–53) or narrow frequency bands (0.02–0.06 and 0.08–0.14 Hz) (3, 13, 36, 63, 64) to sample the TFA metrics. The use of narrow frequency bands leads to a general reduction in coherence values, as the peak input power
(and thus signal-to-noise ratio) occurs at the driven frequency and is diminished rapidly the further away sampling occurs from this driven point (53). As a result, the point-estimate studies reported coherence in the range of 0.92–1.00 (51–53), whereas the narrow frequency-band studies reported coherence in the range of 0.60–0.90 (3, 13, 36, 63, 64). Contradictory to the current findings (where coherence /H11005 0.99–1.00 at both 0.05 and 0.10 Hz) and previous findings from our research group (51–53) are the reports that the 0.10-Hz-driven frequencies result in minimal increases in mean arterial pressure power, which result in reductions in coherence (3, 36, 63, 64). It was speculated that these reductions were due to a higher signal-to-noise ratio at this frequency and should be avoided, due to their limited reliability (3, 36, 63, 64). However, such speculation contravenes the reproducibility findings by van Beek et al. (64), which showed that the only frequency from sit-to-stand maneuvers that revealed acceptable reproducibility of TFA metrics was 0.10 Hz. Even with the discrepancy in coherence values between these sampling methods, the phase (0.05 Hz: ~0.70 radians; 0.10 Hz: ~0.45 radians) and normalized gain (0.05 Hz: ~0.90%/Torr; 0.10 Hz: ~1.20%/Torr) values are relatively consistent (3, 13, 36, 51–53, 63, 64) and are comparable with the present findings (Tables 4 and 5). It is also noted that the use of a method, such as the squat-stand maneuvers, which results in greater reproducibility of the coherence (and associated phase and gain metrics), does not necessarily distinguish between healthy and pathological cerebral autoregulation. This latter potential difference should be examined in future studies.

The OLBNP methodology has been used with pressure ranges from ~30 to ~120 Torr (11, 24, 25, 54, 55, 62), with larger negative pressures (in excess of ~70 Torr) generating greater increases in coherence values (~30 to ~40 Torr: 0.60–0.80; ~70 to ~120 Torr: 0.90–0.95) (11, 24, 25, 62). Although the ~50-Torr-negative pressure within this study was not as severe as some of the previous literature (~70 and ~120 Torr) (11, 62), these studies were performed in a younger adult population (mean age of 37 ± 12 yr at ~70

Fig. 4. Absolute values of the PSD for the MAP and CBV in older adults under spontaneous (gray lines), 0.05 Hz (dashed lines), and 0.10 Hz (black, solid lines) conditions. The PSD during the OLBNP maneuvers are represented on the left (A–D), and squat-stand maneuvers are on the right (E–H). The PSD for the day 1 measures are represented in traces A, C, E, and G, with day 2 measures represented in traces B, D, F, and H. Note: there were no significant differences between days 1 and 2; for SD values, see Tables 3 and 5.
adults measured on days 1 and 2

is calculated for between-day, within-subject CoV. (19), who tested a similarly aged population (68

pressure generated in the steady-state findings of Formes et al. (19), which revealed that once again, the only reproducible day-to-day variability was in coherence and gain metrics. Consistent with these findings are those presented in the current study. The presented findings for the pressure-flow relationship during spontaneous upright and supine conditions (Tables 2 and 3) reveal a similar outcome as the previous reproducibility studies (Table 6). Whereas the mean values of all spontaneously assessed metrics were not significantly different from day to day, there were very few metrics (only LF coherence and gain) that revealed reasonable between-day reproducibility CoV values (<20%) (15, 39, 44). These findings were also consistent with those reported for the OLBNP maneuvers, which revealed that once again, the only reproducible day-to-day TFA metrics are the coherence measures (Tables 4 and 5). Contrary to the aforementioned findings are those of the findings of the squat-stand maneuvers (Tables 4 and 5). These data revealed consistent and reproducible data for all TFA metrics (coherence, phase, gain, and normalized gain) at both 0.05 and 0.10 Hz (Fig. 7). Squat-stand maneuvers as the “Gold Standard” for Linear TFA Metric Interpretation

As noted by Bland and Altman in their seminal work published in 1986 (8), the examination of the reproducibility of a measurement tool across repeat measurements within the

Torr) (11). Instead, our study was designed to match the pressure generated in the steady-state findings of Formes et al. (19), who tested a similarly aged population (68 ± 1 yr) at a negative pressure of −50 Torr, as this pressure has been shown to be tolerable for both sedentary and active older individuals. The spontaneous LF findings for coherence (0.63 ± 0.06), phase (0.65 ± 0.15 radians), and gain (1.41 ± 0.18 cm·s⁻¹·Torr⁻¹), presented by Formes et al. (19), are comparable with those reported in this study (Table 3). The phase and gain findings in the current study (Tables 4 and 5) are consistent with the previous literature at 0.05 and 0.10 Hz (11, 24, 62).

Day-to-Day Reproducibility

To date, there have been numerous studies that reported on the reproducibility of the cerebral pressure-flow relationship with a wide assortment of measurement techniques, including the autoregulatory index (ARI) (9, 10, 12, 17, 22, 27, 32, 42), autoregressive moving average model (30), coherent averaging, (37), mean flow index (31, 35, 41), near-infrared spectroscopy (NIRS) (26, 33), projection pursuit regression (53a), static cerebral autoregulation (20), rate of regulation (50), TFA (7, 10, 17, 22, 31, 35, 41, 64), and vector autoregressive model (30). These 20 studies have been summarized in Table 6.

The collective findings from these studies indicate that although the reproducibility of the pressure-flow relationship has been assessed across a wide range of both age (17–80+ yr) (27, 30, 33, 64) and time between measures (minutes to a 10-yr followup) (10, 12, 32), the bulk of these studies has revealed that the majority of the research in this area has used measurement tools that have relatively low reproducibility [as indexed with an intraclass correlation (ICC) < 0.80 and/or a within-subject CoV > 20%]. For example, every study that indexed the pressure-flow relationship with the traditional ARI or NIRS had an ICC < 0.70, regardless of the measurements that occurred within minutes (12, 27, 32), days (23, 33, 42), or years (10). Similar to the findings for ARI and NIRS are those studies that assessed TFA metrics during spontaneous oscillations in blood pressure (22, 31, 35, 64), which revealed low values for the reproducibility of both phase and gain metrics. Consistent with these findings are those presented in the current study. The presented findings for the pressure-flow relationship during spontaneous upright and supine conditions (Tables 2 and 3) reveal a similar outcome as the previous reproducibility studies (Table 6). Whereas the mean values of all spontaneously assessed metrics were not significantly different from day to day, there were very few metrics (only LF coherence and gain) that revealed reasonable between-day reproducibility CoV values (<20%) (15, 39, 44). These findings were also consistent with those reported for the OLBNP maneuvers, which revealed that once again, the only reproducible day-to-day TFA metrics are the coherence measures (Tables 4 and 5). Squat-stand maneuvers as the “Gold Standard” for Linear TFA Metric Interpretation

As noted by Bland and Altman in their seminal work published in 1986 (8), the examination of the reproducibility of a measurement tool across repeat measurements within the

Table 4. Transfer function analysis of driven data between MAP and MCAv under upright and supine conditions in younger adults measured on days 1 and 2

<table>
<thead>
<tr>
<th>Metric</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Between-Day CoV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squat-stand maneuvers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 Hz MAP power, Torr²/Hz</td>
<td>20.252 ± 11.679</td>
<td>17.186 ± 7.995</td>
<td>23.8 ± 21.1</td>
</tr>
<tr>
<td>0.10 Hz MAP power, Torr/Hz</td>
<td>17.036 ± 7.300</td>
<td>13.847 ± 7.520</td>
<td>42.8 ± 32.4</td>
</tr>
<tr>
<td>0.05 Hz MCAv power, cm · s⁻² · Hz⁻¹</td>
<td>12.110 ± 7.954</td>
<td>9.447 ± 4.956</td>
<td>31.8 ± 20.7</td>
</tr>
<tr>
<td>0.10 Hz MCAv power, cm · s⁻² · Hz⁻¹</td>
<td>16.892 ± 7.261</td>
<td>14.691 ± 8.481</td>
<td>33.0 ± 17.7</td>
</tr>
<tr>
<td>0.05 Hz coherence</td>
<td>0.99 ± 0.01</td>
<td>0.99 ± 0.00</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>0.10 Hz coherence</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>0.05 Hz phase, radians</td>
<td>0.62 ± 0.23</td>
<td>0.64 ± 0.24</td>
<td>15.2 ± 12.5</td>
</tr>
<tr>
<td>0.10 Hz phase, radians</td>
<td>0.31 ± 0.14</td>
<td>0.35 ± 0.23</td>
<td>15.3 ± 10.8</td>
</tr>
<tr>
<td>0.05 Hz gain, cm · s⁻¹ · Torr⁻¹</td>
<td>0.78 ± 0.17</td>
<td>0.74 ± 0.12</td>
<td>16.9 ± 9.0</td>
</tr>
<tr>
<td>0.10 Hz gain, cm · s⁻¹ · Torr⁻¹</td>
<td>1.01 ± 0.17</td>
<td>1.01 ± 0.15</td>
<td>14.4 ± 11.8</td>
</tr>
<tr>
<td>0.05 Hz gain, %/%/</td>
<td>1.16 ± 0.22</td>
<td>1.06 ± 0.12</td>
<td>15.1 ± 11.1</td>
</tr>
<tr>
<td>0.10 Hz gain, %/%/</td>
<td>1.49 ± 0.28</td>
<td>1.51 ± 0.38</td>
<td>16.9 ± 12.7</td>
</tr>
<tr>
<td>Oscillatory lower-body negative pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 Hz MAP power, Torr²/Hz</td>
<td>934 ± 616*</td>
<td>944 ± 649*</td>
<td>53.2 ± 41.7</td>
</tr>
<tr>
<td>0.10 Hz MAP power, Torr/Hz</td>
<td>804 ± 486*</td>
<td>727 ± 480*</td>
<td>42.8 ± 32.4</td>
</tr>
<tr>
<td>0.05 Hz MCAv power, cm · s⁻² · Hz⁻¹</td>
<td>412 ± 300*</td>
<td>412 ± 247*</td>
<td>36.3 ± 28.1</td>
</tr>
<tr>
<td>0.10 Hz MCAv power, cm · s⁻² · Hz⁻¹</td>
<td>671 ± 576*</td>
<td>501 ± 332*</td>
<td>33.0 ± 17.7</td>
</tr>
<tr>
<td>0.05 Hz coherence</td>
<td>0.85 ± 0.12*</td>
<td>0.74 ± 0.17*</td>
<td>16.5 ± 18.4*</td>
</tr>
<tr>
<td>0.10 Hz coherence</td>
<td>0.97 ± 0.03*</td>
<td>0.90 ± 0.19*</td>
<td>2.6 ± 2.8*</td>
</tr>
<tr>
<td>0.05 Hz phase, radians</td>
<td>1.54 ± 0.57*</td>
<td>1.66 ± 0.69*</td>
<td>34.1 ± 22.8*</td>
</tr>
<tr>
<td>0.10 Hz phase, radians</td>
<td>0.65 ± 0.34*</td>
<td>0.64 ± 0.43*</td>
<td>36.2 ± 28.3*</td>
</tr>
<tr>
<td>0.05 Hz gain, cm · s⁻¹ · Torr⁻¹</td>
<td>0.67 ± 0.29*</td>
<td>0.58 ± 0.22*</td>
<td>22.7 ± 19.5</td>
</tr>
<tr>
<td>0.10 Hz gain, cm · s⁻¹ · Torr⁻¹</td>
<td>0.87 ± 0.29*</td>
<td>0.82 ± 0.22*</td>
<td>19.5 ± 13.9</td>
</tr>
<tr>
<td>0.05 Hz gain, %/%/</td>
<td>0.90 ± 0.41*</td>
<td>0.70 ± 0.23*</td>
<td>24.6 ± 19.6</td>
</tr>
<tr>
<td>0.10 Hz gain, %/%/</td>
<td>1.13 ± 0.29*</td>
<td>1.10 ± 0.34*</td>
<td>23.1 ± 15.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. Good reproducibility, <10%; acceptable reproducibility, <20% (15, 39, 44). Statistical significance was set at P < 0.05. Note: there were no significant differences from day 1 to day 2 nor between younger and older adults. *Significantly different from squat-stand maneuvers. Reproducibility is calculated for between-day, within-subject CoV.
same subject group is the best way to ensure the repeatability of that measurement tool. There should be a minimal difference (close to zero) in the metrics associated with a measurement tool if it is indeed measuring the same metric over time (8). In the current study, the only method that met this criterion was the squat-stand maneuvers (Fig. 7). When multiple measurement tools are being used during a study (in this case, spontaneous supine and upright oscillation data; OLBNP maneuvers; squat-stand maneuvers), the level of repeatability of the methods will greatly impact the agreement between trials (8). When one measurement tool has poor reproducibility, the level of agreement between methods will likely be reduced, a problem that is exacerbated when both methods used have poor reproducibility (8).

The quantification and interpretation of the linear aspect of the relationship between arterial blood pressure and CBV in the current study were assessed via TFA. A common test of the linearity of this relationship is the coherence metric, with a value close to 1.00 indicating that the system is linear and thus acceptable to be assessed with TFA (68). In the current study, it was determined that the squat-stand maneuvers resulted in coherences of 0.99–1.00 for both age groups (Tables 4 and 5). The most robust between-day CoV data reported are associated with the coherence values at 0.05 (0.5–0.7%) and 0.10 (0.1–0.5%) Hz for the younger and older adults, respectively, during the squat-stand maneuvers. This method (squat-stand maneuvers) also resulted in significantly larger increases to the power spectra for both mean arterial pressure and middle cerebral artery velocity (Figs. 3 and 4), which contributed to the enhanced linearity of the system and significantly higher coherence. Since TFA uses a linear mathematical approach to the quantification and interpretation of the cerebral pressure-flow relationship, the gold standard for the quantification of this relationship with this assessment tool should be the method that results in coherence values that are ~1.00. The only method, to date, that has been proposed that meets this criterion is the squat-stand maneuvers; as such, we suggest that this method should be considered the gold standard for physiological studies in healthy adults. With this notion in mind, we performed comparisons between the two driven methods (OLBNP and squat-stand maneuvers) to determine the bias present between these measures at 0.05 and 0.10 Hz (Figs. 5 and 6). It was observed that the OLBNP maneuvers resulted in a general overestimation (i.e., positive bias) of phase (0.05 Hz: −0.50–1.00 radians; 0.10 Hz: −0.25–0.35 radians) and an underestimation (i.e., negative bias) of normalized gain (0.05 Hz: 0.30–0.50%/%; 0.10 Hz: 0.35–0.60%/%; Figs. 5 and 6). Collectively, the reproducibility (Fig. 7) and Bland-Altman data from this study reveal that the squat-stand and OLBNP maneuvers have major differences in the associated TFA outcome measures and likely cannot be used interchangeably. As the coherence decreases from 1.00, there is a greater amount of noise present in the phase and gain metrics, which likely is the cause for the proportional bias present in the Bland-Altman plots (Figs. 5 and 6). These differences are highlighted with the augmented level of reproducibility present in both age groups for the squat-stand maneuvers for all TFA metrics at all frequencies (Fig. 7). This notion indicates that even a small amount of noise in the signal (such as during the spontaneous oscillations and OLBNP maneuvers; Tables 2–6) can result in less reproducible TFA metrics (Figs. 5 and 6).

Table 5. Transfer function analysis of driven data between MAP and MCAv under upright and supine conditions in older adults measured on days 1 and 2

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Between-Day CoV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squat-stand maneuvers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 Hz MAP power, Torr^2/Hz</td>
<td>16,801 ± 13,821</td>
<td>13,329 ± 12,044</td>
<td>35.6 ± 22.3</td>
</tr>
<tr>
<td>0.10 Hz MAP power, Torr^2/Hz</td>
<td>5,173 ± 3,225</td>
<td>8,067 ± 6,983</td>
<td>32.8 ± 36.2</td>
</tr>
<tr>
<td>0.05 Hz MCAv power, cm · s^−2 · Hz^−1</td>
<td>5,572 ± 4,813</td>
<td>8,029 ± 7,354</td>
<td>50.0 ± 30.1</td>
</tr>
<tr>
<td>0.10 Hz MCAv power, cm · s^−2 · Hz^−1</td>
<td>3,549 ± 1,898</td>
<td>6,547 ± 4,899</td>
<td>37.2 ± 38.4</td>
</tr>
<tr>
<td>0.05 Hz coherence</td>
<td>0.99 ± 0.01</td>
<td>0.99 ± 0.00</td>
<td>0.7 ± 0.6</td>
</tr>
<tr>
<td>0.10 Hz coherence</td>
<td>0.99 ± 0.01</td>
<td>1.00 ± 0.00</td>
<td>0.5 ± 0.6</td>
</tr>
<tr>
<td>0.05 Hz phase, radians</td>
<td>0.75 ± 0.21</td>
<td>0.70 ± 0.13</td>
<td>7.5 ± 4.9</td>
</tr>
<tr>
<td>0.10 Hz phase, radians</td>
<td>0.39 ± 0.17</td>
<td>0.35 ± 0.12</td>
<td>19.4 ± 11.0</td>
</tr>
<tr>
<td>0.05 Hz gain, cm · s^−1 · Torr^−1</td>
<td>0.69 ± 0.22</td>
<td>0.85 ± 0.23</td>
<td>17.9 ± 15.7</td>
</tr>
<tr>
<td>0.10 Hz gain, cm · s^−1 · Torr^−1</td>
<td>0.87 ± 0.24</td>
<td>0.98 ± 0.23</td>
<td>10.0 ± 10.9</td>
</tr>
<tr>
<td>0.05 Hz gain, %/%</td>
<td>1.28 ± 0.38</td>
<td>1.67 ± 0.48</td>
<td>18.4 ± 10.8</td>
</tr>
<tr>
<td>0.10 Hz gain, %/%</td>
<td>1.69 ± 0.38</td>
<td>2.00 ± 0.47</td>
<td>14.4 ± 9.2</td>
</tr>
<tr>
<td>Oscillatory lower-body negative pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 Hz MAP power, Torr^2/Hz</td>
<td>1,567 ± 2,289*</td>
<td>1,845 ± 2,178*</td>
<td>31.5 ± 28.3</td>
</tr>
<tr>
<td>0.10 Hz MAP power, Torr^2/Hz</td>
<td>661 ± 492*</td>
<td>1,311 ± 2,087*</td>
<td>49.2 ± 45.2</td>
</tr>
<tr>
<td>0.05 Hz MCAv power, cm · s^−2 · Hz^−1</td>
<td>403 ± 409*</td>
<td>722 ± 1,070*</td>
<td>45.5 ± 35.3</td>
</tr>
<tr>
<td>0.10 Hz MCAv power, cm · s^−2 · Hz^−1</td>
<td>209 ± 240*</td>
<td>786 ± 1,370*</td>
<td>62.9 ± 40.4</td>
</tr>
<tr>
<td>0.05 Hz coherence</td>
<td>0.89 ± 0.11*</td>
<td>0.90 ± 0.10*</td>
<td>7.1 ± 8.5*</td>
</tr>
<tr>
<td>0.10 Hz coherence</td>
<td>0.90 ± 0.10*</td>
<td>0.89 ± 0.13*</td>
<td>7.1 ± 10.8*</td>
</tr>
<tr>
<td>0.05 Hz phase, radians</td>
<td>1.31 ± 0.41*</td>
<td>1.09 ± 0.50*</td>
<td>28.1 ± 15.1*</td>
</tr>
<tr>
<td>0.10 Hz phase, radians</td>
<td>0.57 ± 0.41*</td>
<td>0.65 ± 0.41*</td>
<td>47.6 ± 38.7*</td>
</tr>
<tr>
<td>0.05 Hz gain, cm · s^−1 · Torr^−1</td>
<td>0.61 ± 0.15*</td>
<td>0.61 ± 0.15*</td>
<td>22.8 ± 19.0</td>
</tr>
<tr>
<td>0.10 Hz gain, cm · s^−1 · Torr^−1</td>
<td>0.66 ± 0.33*</td>
<td>0.76 ± 0.45*</td>
<td>51.3 ± 28.4*</td>
</tr>
<tr>
<td>0.05 Hz gain, %/%</td>
<td>0.93 ± 0.29*</td>
<td>1.07 ± 0.32*</td>
<td>22.0 ± 26.2</td>
</tr>
<tr>
<td>0.10 Hz gain, %/%</td>
<td>1.14 ± 0.55*</td>
<td>1.32 ± 0.75*</td>
<td>48.7 ± 34.8*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Good reproducibility, <10%; acceptable reproducibility, <20% (15, 39, 44). Statistical significance was set at P < 0.05. Note: there were no differences in between-day results nor between younger and older adults. *Significantly different from squat-stand maneuvers. Reproducibility is calculated for between-day, within-subject CoV.
However, when the signal-to-noise ratio is maximized (as noted in the 100- to 300-plus-fold increase in the autospec-
tra of the squat-stand maneuvers; Figs. 3 and 4), there is a
significant decrease in the CoV for all TFA metrics and age
groups (Fig. 7). This finding highlights the fact that the
signal-to-noise ratio does indeed play a role in the linear
interpretation of TFA metrics, as demonstrated by the higher
CoV values associated with the metrics that had lower
coherence values.

Thus the collective findings from the current study indi-
cate that when assessing TFA metrics associated with the
cerebral pressure-flow relationship in otherwise healthy
populations, squat-stand maneuvers provide the most repro-
ducible outcome measures. However, under conditions
where squat-stand maneuvers are not feasible (e.g., people
with mobility issues, incapacitated individuals, during cy-
cling interventions), the application of the OLBNP maneu-
vers [or possibly even deep breathing (22, 41) or passive leg
raises (17)] would be appropriate to elicit augmented coher-
ence, provided there was a sufficient magnitude of negative
pressure applied (7, 11, 62). The critical next point to
establish is to examine the ability of these squat-stand
maneuvers to distinguish between healthy and pathological
autoregulation. However, it should also be noted that within
the confines of the current study, the presented findings
focus on the reproducibility (and linear interpretation) of
the TFA metrics, as they pertain solely to the relationship
between mean arterial pressure and middle cerebral artery
velocity and are not necessarily reflective of cerebral auto-
regulation as a whole entity.

Effects of Posture Changes on CBF Regulation

An important consideration is that we have compared the
pressure-flow responses in the supine and upright positions.
Movement from a supine to standing position can result in a
reduction of ~1.5 liters of central blood volume (45, 47). First
(within the first 15 s), there is an initial increase in cardiac
output (+30%) and a reduction in mean arterial pressure
(~45%) before baroreflex adjustments occur (57). Initially,
when a subject moves from the supine to upright position, there
is reduced ventilation/perfusion matching within the lung, and
consequently, partial pressure of arterial CO₂ can decrease by
~1–4 Torr, which results in a decrease in CBV of ~10% (29, 46).

Fig. 5. Bland-Altman plots of the significant differences between squat-stand and OLBNP maneuvers in younger adults. A–C, left and right: differences at 0.05 and 0.10 Hz, respectively. The mean differences were obtained by subtracting the OLBNP data from the squat-stand maneuver data. A: proportional and mean bias for coherence; B: proportional and mean bias in radians for phase; and C: proportional and mean bias in percent/percent for normalized gain (N-Gain). The
dashed, middle horizontal lines denote the mean difference between the 2 modalities; the dotted lines represent the upper and lower 95% limits of agreement;
and the solid lines represent the regression line, indicating the proportional bias.
Consistent with these changes, the results from this study showed that after the posture change from upright to supine, PETCO₂ universally rose by ~2 Torr, which resulted in an increase in CBV of ~10% (Table 1).

The change in posture and PETCO₂ could be a contributing factor to the TFA metrics alterations during driven oscillations in blood pressure (Tables 4 and 5 and Figs. 5 and 6). Part of the alterations to the TFA metrics may also be explained by the acute ~9% decrease in CVRᵢ, as this was positively correlated to the normalized gain in both the younger (r = 0.24 and 0.38, $P < 0.05$) and older (r = 0.47 and 0.31, $P < 0.03$) adults at 0.05 and 0.10 Hz, respectively. The acutely reduced CVRᵢ was also negatively correlated to phase in the older adults at both 0.05 (r = −0.42, $P = 0.005$) and 0.10 Hz (r = −0.48, $P = 0.002$). These findings confirm those presented in our previous study, which induced a 40% increase in CVRᵢ via both pharmacological (Indomethacin) and physiological (hypocapnia) interventions (53). The acute increase in CVRᵢ in the Indomethacin and hypocapnia trials of the previous study was positively correlated with increases in phase and negatively correlated with decreases in gain (53). Thus collectively, the findings from these two studies indicate that changes in CVRᵢ can impact the cerebral pressure-flow response, and acute changes to CVRᵢ should be considered when interpretations of TFA metrics are made.

**Implications for the Assessment and Interpretation of Pressure-Flow Relationships**

The current findings for the TFA metrics match well with the previous literature for both driven methods: squat-stand maneuvers (3, 13, 36, 51–53, 63, 64) and OLBNP maneuvers (11, 24, 54, 62). The reproducibility metrics for spontaneous and OLBNP maneuvers also correspond well to the previous literature in the area (Table 6). However, a surprising limitation of the previous literature is that no study has reported on the day-to-day reproducibility of TFA metrics either during squat-stand maneuvers or in reference to younger and older adult populations within the same study. As indicated by the previous findings on the ARI and TFA metrics during spontaneous blood-pressure oscillations, even though findings from a study can be compared with previous literature in the field, without the knowledge of the reproducibility metrics of the methodology used, caution needs to be applied to the interpretation of single punctual measurements.
This study was designed to be the first study to present reproducibility metrics for both younger and older adults, during spontaneous and driven oscillations in blood pressure. Collectively, the findings reported in the current study emphasize the notion that comparable findings in mean data between days do not necessarily tell the entire story. Although there were no between-day differences for either the younger or older adults under any of the conditions (spontaneous upright, spontaneous supine, OLBNP, squat-stand maneuvers), there were vast differences in the underlying reproducibility and SDs of the between-day measures. Consequently, the findings presented in this study have important implications on the design of future studies where between-day comparisons are needed. Whereas the spontaneous and OLBNP data compare well with the previous literature in the area, there are few metrics with biologically acceptable between-day reproducibility (only the coherence and gain in the LF/0.10-Hz range), which indicates that caution should be applied to single-day measurements with these methods. The implications of the level of variability associated with these methods are likely important for ensuring the planning of effective sample sizes for interventional and clinical orientated studies. In contrast, all of the TFA metrics associated with the squat-stand maneuvers revealed reasonable levels of between-day CoV and had lower between-day variation in coherence and thus more repeatable system linearity (Tables 4 and 5). These findings indicate that the OLBNP and squat-stand maneuvers likely are not interchangeable. They also highlight the notion that when a small sample size is used for a single-day assessment of the relationship between mean arterial pressure and CBF via linear TFA, a method should be used that has a minimal CoV (higher reproducibility) and thus enables a more accurate assessment of the linear TFA metrics.

Methodological Considerations

Cerebral autoregulation. The interpretation of the presented findings focused on the reproducibility of the TFA metrics as they pertain solely to the relationship between mean arterial pressure and middle cerebral artery velocity and is not necessarily reflective of cerebral autoregulation as a whole entity.
Table 6. Summary of previous literature assessing the reproducibility of the cerebral pressure-flow relationship

<table>
<thead>
<tr>
<th>Authors</th>
<th>n (Cohort)</th>
<th>Age, yr</th>
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| Birch et al. (7) | 5 (Healthy)| 40 ± 5  | TFA at 0.083 Hz               | 8 Sessions spread over 3 mo | With OLBNP, there was a high degree of reproducibility in the TFA phase, and reproducibility improved with increased negative pressure.
| Brodie et al. (9) | 10 (Healthy) | 38 ± 9 | ARI under spontaneous oscillators | 4 Measures with 3–4 days between each | ARI measures had low ICC in the right (0.51) and left (0.43) hemispheres across the measures.
| Houtman et al. (10) | 10 (Healthy) | 35 ± 9 | ARI and spontaneous TFA at 0.035 Hz | 10 yr | ARI fell, on average, by 16% in the 10-yr followup; TFA phase and gain metrics were unchanged.
| Chacon et al. (11) | 16 (Healthy) | 32 ± 9 | Traditional and model-free ARI | 6 Thigh-cuff maneuvers separated by 8 min | Traditional ARI had a much higher within-subject CoV (39 ± 42%) compared with the model-free ARI (16 ± 8%), indicating that the model-free ARI was a superior measure.
| Elting et al. (12) | 16 (Healthy) | 33 ± 10 | ARI and TFA sampled at 0.10 Hz | 2 Leg-raise measures separated by 5 min | ICC was low in both hemispheres for ARI (0.65) and 0.10 Hz phase (0.45) but was better for 0.10 Hz normalized gain (0.75).
| Georgiadiis et al. (13) | 5 (Moderate hypothermia with acute stroke) | 58 ± 11 | Static CA | 2 h | The 5 subjects had a within-subject CoV of 11 ± 7%.
| Gommer et al. (14) | 19 (Healthy) | Range 18–53 | TFA in the 0.04- to 0.16-Hz range and ARI | 1 Morning session and 1 afternoon session, 7 days apart | Under spontaneous conditions, mean ICC values for TFA metrics and ARI were low in morning (0.36–0.61) and afternoon (0.37–0.55). Similar results were shown for paced breathing (JApp Physiol: 0.34–0.73; afternoon: 0.17–0.59).
| Houtman et al. (15) | 10 (Healthy) | Range 23–51 | NIRS | Time between measures not reported | Circulatory measures (stroke volume, heart rate, and blood pressure) had low variance between days; cerebral oxygenation values, however, had a high level of random error and were not reproducible.
| Hu et al. (16) | 30 (Brain injury) | Range 17–69 | Phase shift and ARI | 4 Times within a 5-min recording | ICC was 0.58 for phase shift and was 0.08 for ARI, indicating that both methods have poor reproducibility.
| Jachan et al. (17) | 44 (Severe carotid stenosis) | 71 ± 10 | Transfer function estimate, ARMAX and VAR models | 2.5–6 mo | Kendall’s rank correlations revealed significant between-measure correlations for all 3 pressure-flow estimates, despite a high level of variability in the data.
| Lorenz et al. (18) | 30 (Poor temporal windows) | 68 ± 10 | TFA phase and Mx | Measures repeated within 30 min | Although the phase difference and Mx reproducibility values were not shown, the authors stated that this was done because they were "poor".
| Mahony et al. (19) | 16 (Healthy) | 32 ± 9 | ARI | 6 Thigh-cuff maneuvers separated by 8 min | Within-subject CoV was 42 ± 48%, and within-subject ARI score ranges were 3.3 ± 2.1, indicating poor reproducibility. The authors concluded that a minimum of 3 ARI scores should be averaged for a physiologically relevant value.
| Mehagnoul-Schipper et al. (20) | 27 (Healthy) | 75 ± 8 | NIRS | Minimum of 2 days | Although no between-day mean differences were noted for NIRS measures, all NIRS measures showed a low reliability coefficient (0.14–0.43).
| Ortega-Guiterrez et al. (21) | 19 (Healthy) | Range 21–74 | TFA phase and gain and Mx | 17 Days, range 5–27 | All measures were performed under spontaneous conditions. The ICC for TFA phase was 0.69, TFA gain was 0.59, and Mx was 0.43.
| Panzeri et al. (22) | 39 (Healthy) | 40 ± 15 | Coherent averages | 8–16 Blood-pressure transients/subject over 10 min | Subjects (33 of the 39) reported weak, moderate, and strong coherent averages in the right MCA and 29 of 39, in the left MCA, indicating a large amount of within-subject variability.
| Reinhard et al. (23) | 34 (Healthy) | 65 ± 8 | TFA phase and Dx | 2 ± 1 mo | With deep breathing at 0.10 Hz, the ICC for TFA phase was 0.790 and for Dx was 0.377.
| Saeed et al. (24) | 11 (Healthy) | 37 ± 7 | ARI | 6 Days, range 5–9 | The quality of the CBV signals was determined with TFA coherence cutoff of 0.50. When CBV signals met this criterion, they were passed back into the time domain for ARI analysis. The ICC for ARI was 0.39 with frame-held TCD probes and 0.48 for hand-held TCD probes.
| Smielewski et al. (25) | 11 (Healthy) | Range 20–30 | Carotid artery compression and RoR | All tests performed on same day | RoR and compression were performed twice under hypo-, normo-, and hypercapnia. The CoV for RoR was dependent on CO2 levels: ~47% for hypo-, ~28% for normo-, and ~63% for hypercapnia. The compression test CoV results were ~13% under all conditions.
| Tan (26) | 5 (Healthy) | Range 21–40 | Projection pursuit regression | 2 Separate experimental days | Autoregulatory gain at 0.03 and 0.08 Hz did not change across days based on Lin’s concordance correlation coefficient (0.90 and 0.98, respectively).
| van Beek et al. (27) | 11 (Healthy) | 77 ± 5 | TFA | 3–4 mo | Spontaneous and sit-to-stand maneuvers at 0.05 and 0.10 Hz. The CoV for spontaneous VLF phase (70%) and gain (38%) was higher than LF phase (23%) and gain (17%). A similar trend for CoV was observed in the sit-to-stand maneuvers: 0.05 Hz phase (85%) and gain (93%) were higher than the 0.10-Hz phase (14%) and gain (22%). The authors concluded that the LF and 0.10-Hz ranges were the only ones with acceptable reproducibility.

Values are means ± SD. TFA, transfer function analysis; OLBNP, oscillatory lower-body negative pressure; ARI, autoregulatory index; ICC, intraclass correlation; CA, cerebral autoregulation; NIRS, near-infrared spectroscopy; ARMAX, autoregressive moving average model; VAR, vector autoregressive model; Mx, mean flow index; MCA, middle cerebral artery; Dx, dynamic correlation index; CBV, cerebral blood velocity; TCD, transcranial Doppler ultrasound; RoR, rate of regulation; VLF = 0.02–0.07 Hz; LF = 0.07–0.20 Hz.

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This was performed, as there has yet to be a defined gold-standard assessment tool for cerebral autoregulation quantification (61). The complexities associated with cerebral autoregulation are likely not entirely linear in nature. However, as the presented findings clearly demonstrate, the relationship underlying arterial blood pressure and CBV can be approximated in a linear fashion (coherence 0.99–1.00) if the appropriate method is applied (squat-stand maneuvers) that enhances the reproducibility and interpretability of the associated linear TFA metrics.

Flow vs. velocity. Transcranial Doppler is used to provide an index of CBF, under the assumption that velocity approximates flow values when the diameter of a vessel is constant. Recent high-resolution MRI studies (14, 65) have revealed that when \( P_{\text{ETCO}_2} \) values are held within 8 Torr of eucapnia [reviewed in Ainslie and Hooland (5)], the diameter of the middle cerebral artery is relatively constant. In the current study, \( P_{\text{ETCO}_2} = \pm 2 \) Torr of eucapnia, which indicates that the velocity data are likely representative of CBF and can be used to assess the cerebral pressure-flow relationship.

Conclusion

The use of squat-stand maneuvers provides a reproducible and physiologically relevant protocol for creating oscillations in blood pressure to increase the input power and enhance the linear interpretability of the TFA metrics in both younger and older adult populations. These findings have methodological implications for the assessment and interpretation of the linear aspect of the relationship between arterial blood pressure and CBV in humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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