Assessment of cerebral autoregulation: the quandary of quantification

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Cerebral metabolism is critically dependent on the regulation of cerebral blood flow (CBF), with dysfunction having dire consequences (32). An important mechanism thought to play a vital role in maintaining adequate CBF regulation is the active modulation of cerebrovascular resistance/cerebrovascular conductance (CVC) in response to changes in cerebral perfusion pressure, a response termed cerebral autoregulation (CA) (1, 48, 56). In experimental settings, CA is often assessed in both healthy subjects and patients to study the effect of hemodynamic perturbations such as orthostasis (47), central hypovolemia (41), heat stress (54), and exercise (29) on the ability of the cerebral circulation to maintain adequate perfusion. Likewise, there has been significant clinical interest in the role that CA impairment might play in the causation, progression, and risk of debilitating disorders such as ischemic stroke (9), intracranial hemorrhage (37), and traumatic brain injury (13, 14). However, while a wide variety of techniques have been developed and adopted for CA assessment over the last 20 yr (34), there remains no consensus on which approach can be considered the “gold standard.” Consequently, many techniques are presently applied, making it difficult to compare and validate results across studies (32). Given that conclusions from research trials are often drawn from select measures of CA, it is essential to understand to what extent the various measures of CA can be used interchangeably to provide similar clinical information when CA is compared across individuals in a population or within individuals under different conditions (4).

To that end, three methodological approaches that have gained widespread popularity warrant close scrutiny: 1) the rate of regulation (RoR), 2) the autoregulatory index (ARI), and 3) transfer function analysis. The RoR index quantifies the rate of change in beat-to-beat CVC (or resistance) after an acute and transient hypotensive stimulus (e.g., after bilateral thigh-cuff deflation) (1). Tieck’s ARI, on the other hand, is derived by fitting the arterial pressure and CBF velocity data within a 30-s window after a transient hypotensive stimulus to a mathematical model that incorporates both the latency and “gain” of CA (48). Both the RoR and ARI are applied on the basis that lower values represent “slower” and/or “weaker” CA and vice versa. In contrast, transfer function analysis relates dynamic changes in arterial pressure and CBF velocity across a broad range of time scales in the frequency domain. This approach yields three interpretable parameters that describe the magnitude with which CBF changes are driven by arterial pressure (gain) as well as the timing (phase) and linearity (coherence) of the relationship. The current interpretation of transfer function metrics is based on the presumption that pressure fluctuations are more liable to induce linear and pressure-synchronous CBF fluctuations of enhanced magnitude in the absence of CA. Thus, higher values of coherence and gain and lower values of phase are generally taken to reflect weaker CA and vice versa (56).

The objectives of this study were twofold. First, we sought to systematically evaluate the convergent validity of these purported metrics of CA and determine the extent to which the measures may be used interchangeably for CA characterization across individuals. Considering that all of these metrics have been applied in the literature on the implicit assumption that they accurately reflect the integrity of CA, clear proportionality should exist between them to permit their interchangeable...
use. Our second objective was to determine the extent to which transfer function metrics may be used interchangeably for CA characterization in situations where repeat measurements are taken within subjects across varying conditions that are known to alter CA (3). To achieve this objective, we manipulated arterial PCO2 (Paco2) across a wide range of hypercapnia and hypocapnia levels using a novel Paco2 clamping technique (46). Considering that these metrics are based on distinct theoretical and mathematical constructs, we hypothesized that metric convergence would be poor both across and within subjects.

MATERIALS AND METHODS

Subjects

Two retrospective and two prospective data sets from three research centers were analyzed in this study. All research protocols conformed with Declaration of Helsinki standards and were approved by the New Zealand Central Regional Ethics Committee, the University of British Columbia Clinical Ethics Board, and the Institutional Review Board for the Protection of Human Subjects in Research of the University of Texas (San Antonio, TX). All subjects gave written, informed consent and were healthy, normotensive, nonsmoking volunteers without any prior history of cardiovascular, respiratory, or endocrine disease. Subjects were not taking any prescription or nonprescription medications (including herbal) except for contraceptive pills for some female subjects. Female subjects were not pregnant, as confirmed by an over-the-counter pregnancy test. All subjects were advised to abstain from caffeinated beverages and exercise for at least 12 h before the study and to have fasted for at least 2 h before the study. Experiments were conducted between 0800 and 1000 hours or between 1200 and 1500 hours in three temperature-controlled laboratories (22–23°C).

Data set A comprised 6-min baseline recordings from 105 subjects (71 men and 34 women, mean age: 26 ± 7 yr) made in the supine position as part of previous research protocols. This retrospective data set was used to evaluate the relationships between spontaneous transfer function metrics.

Data set B comprised 29 (21 men and 8 women, mean age: 30 ± 10 yr) of the 105 subjects in data set A who had also undergone thigh-cuff deflation tests immediately after the baseline recordings. This subset was used to describe the relationship between spontaneous transfer function metrics and the ARI and RoR.

Data set C comprised prospective data recorded from 29 subjects (23 men and 6 women, mean age: 32 ± 12 yr) who underwent 5 min of baseline recordings in the seated position followed by repeated squat-to-stand maneuvers at 0.05 and 0.10 Hz (5 min each). These data were used to evaluate the relationships between transfer function metrics derived from spontaneous and forced arterial pressure fluctuations at different frequencies.

Data set D comprised prospective data recorded from 16 subjects (11 men and 5 women, mean age: 22 ± 3.2 yr) who underwent stepwise changes in Paco2, indexed by stepwise changes in end-tidal PCO2 (PETCO2). This data set was used to examine the relationships among transfer function coherence, gain, and phase across a wide range of PETCO2 levels.

Measurements

We recorded the electrocardiogram, noninvasive beat-to-beat blood pressure (via finger photoplethysmography, Finometer, Finapres Medical Systems, Amsterdam, The Netherlands), right middle cerebral artery (MCA) blood velocity (MCAv; via 2-MHz pulsed Doppler ultrasound, MultiDop T, DWL Electronics, Siplingen, Germany, and ST3 Power M-mode transcranial Doppler, Spencer Technologies, Seattle, WA), and PETCO2 sampled from a face mask (gas analyzer model ML206, AD Instruments, Colorado Springs, CO, and Infrared CO2 analyzer, Gambro, Enström, Sweden). All data were acquired continuously at 0.5–1 kHz/channel via an analog-to-digital converter (Powerlab/16SP ML795, AD Instruments, and WinDAQ, Dataq Instruments, Akron, OH) interfaced with a computer and stored for subsequent analysis. From the recorded electrocardiogram, blood pressure, and MCAv waveforms, we determined the time of each R wave and beat-to-beat values for mean MCAv (MCAvmean) and mean arterial pressure (MAP) calibrated periodically against brachial-cuff pressure recordings. Data were processed and analyzed with custom-written software in LabView 11 (National Instruments).

Procedures

Thigh-cuff deflation. This procedure involved 3 min of bilateral suprasystolic (220 mmHg) thigh-cuff inflation followed by rapid (<1 s) cuff deflation. From this procedure, the RoR (1) and ARI (48) were derived.

Squat-to-stand maneuver. Participants performed the repeated squat-to-stand maneuver to create forced oscillations in MAP and MCAvmean. This technique involved squatting and standing for 5 min at 0.05 Hz (10-s squat followed by 10-s stand) and 0.1 Hz (5-s squat followed by 5-s stand). Each 5-min session (at either 0.05 or 0.1 Hz) was performed in random order and separated by at least 5 min of recovery. A metronome facilitated timing of the squat-to-stand maneuver. We chose to perform the technique at these frequencies as they incorporated the frequency ranges where CA is thought to be operant [i.e., the very-low-frequency (VLF; 0.02–0.07 Hz) and low-frequency (LF; 0.07–0.20 Hz) ranges]. During the assessment, we instructed participants to breathe normally, to avoid the Valsalva maneuver, and to minimize forward flexion.

PETCO2 clamping. After the administration of local anesthesia (1% lidocaine) to the subjects, a 20-gauge catheter (Arrow Canada, Markham, ON, Canada) was placed into the radial artery and attached to a pressure transducer (AD Instruments) positioned at the level of the right atrium in the midaxillary line for the measurement of arterial blood pressure. After cannulation, subjects rested quietly in the supine position, breathing room air for at least 30 min to allow the setup of monitoring equipment, including calibration of the pressure transducer. An automated gas blender adjusted the composition and flow to a sequential gas delivery mask and breathing circuit (RespirAct, Thornhill Research, Toronto, ON, Canada), as previously described (46). This apparatus enables prospective control of the individual’s PETCO2 and end-tidal PO2 (PETO2) independently of each other and of minute ventilation. Using this approach, we recorded data during spontaneous breathing at baseline, normocapnic baseline (+0 mmHg), and during progressive hypercapnia and hypocapnia in sequential steps that approximated −10, −20, −30, +10, +15, +20, and +25 mmHg relative to the normocapnic baseline. The normocapnic baseline was, on average, 2 mmHg (range: 0.14–3.9 mmHg) above or below the spontaneous baseline PETCO2, and therefore was considered an additional step. Although the exact duration of PETCO2 steps depended on the length of time it took to reach the desired PETCO2 level and subject tolerance to the CO2 level, transfer function analyses were consistently (110 of the 114 steps) performed on the last 5–6 min of each stable recording except in four recordings, which were shorter (range: 3–4 min) due poor subject tolerance. In data sets A–C, the level of PETCO2 was monitored.

CA

Transfer function analysis. Beat-to-beat MAP and MCAvmean from all data sets were spline interpolated and resampled at 4 Hz for spectral and transfer function analyses based on the Welch algorithm. Each 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 fast Fourier transform analysis. For transfer function analysis, the cross-spectrum between

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MAP and MCAv\textsubscript{mean} was determined and divided by the MAP autospectrum to derive the transfer function coherence, gain, and phase. Individual transfer functions were calculated without attempting to unwrap the phase spectrum. Spontaneous MAP and MCAv\textsubscript{mean} spectral power and the mean values of transfer function coherence, gain, and phase were calculated in the LF and VLF ranges (56). The contribution of an individual transfer function estimate toward the band average was weighted according to their individual precision, as indicated by the coherence function (see APPENDIX). Phase and gain estimates were also determined using two alternative coherence criteria (see APPENDIX for expanded methods and results). For squat-to-stand data, we derived the transfer function coherence, phase, and gain at 0.1 and 0.05 Hz given that the frequency of forced oscillations was explicitly known. To correct for interindividual differences in MCA diameter, transfer function gain was also assessed using normalized units (n-gain), defined as beat-to-beat values divided by the mean value relative to changes in beat-to-beat blood pressure (\%/mmHg) (32). In this study, n-gain was considered a separate CA index.

RoR and ARI. For the determination of RoR, the CVC index (CVCi) was calculated by dividing MCAv\textsubscript{mean} by MAP. MAP, MCAv\textsubscript{mean}, and CVCi values were determined relative to control values, defined as the mean of the 4 s immediately before thigh-cuff release (1, 30). The RoR index was taken as follows:

$$\text{RoR} = \frac{\Delta \text{CVCi}/\Delta t}{\Delta \text{MAP}}$$

where \Delta CVCi/\Delta t is the slope of the linear regression between CVCi and time and \Delta MAP is the difference between baseline MAP (MAP\textsubscript{base}) and the average MAP between 1 and 7 s (6-s window) after cuff release. Although this window is longer than the 2.5-s window typically applied for this analysis, the modification is justified because the shorter window often yields regressions that encompass only three data points. Furthermore, the average coefficient of determination using the 6-s window was 0.96 ± 0.040, indicating that CVCi increases linearly after thigh-cuff deflation within this timeframe. According to this construct, RoR is directly proportional to the rate of the CA response after a transient hypotensive stimulus.

The CA response to transient hypotension was also assessed using Tiecks’ ARI (48). This involved applying a second-order linear differential equation, defined as follows:

$$\frac{dP_n}{dt} = \frac{\text{MAP} - \text{MAP}_{\text{base}}}{\text{MAP}_{\text{base}} - \text{CCP}} \times \frac{(x_1n - 2D \times x_2n)}{f \times T}$$

$$x_2n = x_2n-1 + \frac{(x_1n - 2D \times x_2n)}{f \times T}$$

$$x_1n = x_1n-1 + \frac{(dP_n - x_2n)}{f \times T}$$

$$mV_n = \text{MCAv}_{\text{base}} \times (1 + dP_n - k \times x_2n)$$

where \(dP_n\) is the normalized change in MAP relative to the control value (MAP\textsubscript{base}) adjusted for the estimated critical closing pressure (CCP; equal to 12 mmHg), \(x_2n\) and \(x_1n\) are state variables (equal to 0 at baseline), \(mV_n\) is modeled mean velocity, MCAv\textsubscript{base} is baseline MCAV, \(f\) is the sampling frequency (10 Hz), and \(n\) is the sample number. \(mV_n\) values generated from 10 predefined combinations of the time constant \(T\), dampening factor \(D\), and autoregulatory gain \(k\) were fitted to the actual MCAV recording within a 30-s window to identify the best-fit model associated with the minimum quadratic error. The unconstrained ARI, ranging between zero (absence of CA) and nine (strongest CA), was derived via polynomial interpolation (6).

Convergent validity and sensitivity analysis. Convergent validity in the context of this study refers to the degree to which one CA metric is similar to, or converges upon, other metrics of CA. To estimate the strength of relationships among metrics, we used simple correlation analysis. Metrics that reflect a common construct should exhibit high intercorrelations. Given that average estimates of transfer function parameters are likely to vary depending on the precise definition of frequency bands, we also performed a sensitivity analysis to determine whether the changes in band definition might alter our results. This was achieved by repeating the convergent validity analysis with the lower-bound frequency set at 0.02 Hz and the upper-bound frequency set at 0.07, 0.10, 0.15, and 0.20 Hz to ensure even coverage of the frequency ranges where CA is thought to be operant (56).

Statistics

Normality was confirmed for all parameters using the Shapiro-Wilk test. Between-subject relationships were examined using scatter plots and Pearson product-moment correlation coefficients on an a priori basis between metrics (i.e., ARI, RoR, and transfer function metrics in the spontaneous LF and VLF ranges and during squat-to-stand maneuvers at 0.05 and 0.10 Hz). Simple linear regression was performed where significant correlations were identified. Within-subject relationships between transfer function parameters and \(P_{\text{ETCO}_2}\) as well as among the four transfer function metrics were assessed using two statistical approaches. First, for the estimation of the average slopes between pairs of variables, we used linear mixed models, accounting for both fixed and random effects (e.g., participants) (23). Whereas conventional regression analysis requires \(X\)-y data to be reduced to summary measures before secondary analysis, linear mixed models allow the analysis of the data in one step without the loss of valuable information concerning the precision of individual slopes, indicated by the SEs of each slope estimate. Thus, rather than assigning equal weights to slopes with unequal precision, the average slope obtained using linear mixed models are weighted for precision and are more robust. To ensure that slopes were estimated using the most parsimonious models, the inclusion of random-effect terms for slopes and/or intercepts was done only if their addition led to an improved model fit, as determined on the basis of the Bayesian information criterion. Differences across conditions were also assessed using linear mixed models and follow-up contrasts. The coefficient of variation (SD/mean × 100) was calculated for all CA metrics as a measure of data dispersion. Statistical significance was set a priori at \(\alpha = 0.05\). Given that the assessment of relationships between different metric types could involve multiple comparisons (e.g., six correlations between each of the four transfer function metrics), we adjusted \(P\) values using Holm’s correction to control for the inflation of the type I error rate (19). Unless otherwise stated, all values are given as means ± SD and were rounded to two significant figures. Statistical analyses were implemented in SPSS 17 (SPSS, Chicago, IL).

RESULTS

Relationships Among Spontaneous Supine Transfer Function Metrics, ARI, and RoR (Data Sets A and B)

Group-averaged baseline cardiovascular, respiratory, spectral, and transfer function parameters are shown in Table 1. All of the CA metrics examined in this study exhibited a degree of normal between-subject variability with coefficients of variation ranging between 22% and 62% (Figs. 1–4).

Although some transfer function metrics in the LF or VLF were statistically correlated, the strength of the correlations among indexes were generally weak to moderate, with absolute \(r\) values ranging between 0.0096 and 0.90 and only gain versus n-gain reaching >0.50 (Fig. 1). LF coherence was positively correlated with LF n-gain (\(r = 0.31, P < 0.01\)) and LF gain (\(r = 0.34, P < 0.01\)) and inversely related to LF phase (\(r = -0.25, P < 0.05\)). LF phase was inversely related to LF gain (\(r = -0.31, P < 0.01\)) but was unrelated to LF n-gain (\(r = -0.13, P = 0.18\)). LF gain was positively related to LF n-gain (\(r = 0.70, P < 0.01\)). Likewise, relationships between transfer function metrics in the VLF were
generally very weak (Fig. 1). The only evident correlation was between VLF gain and n-gain (r = 0.90, P < 0.01; Fig. 1).

Figure 2 shows relationships among spontaneous LF (A) and VLF (B) coherence, phase, gain, and n-gain against the RoR and ARI. In general, the correlations among indexes were weak to moderate (absolute r values ranging between 0.014 and 0.70), with the vast majority falling below 0.5. The RoR was related with LF phase (r = 0.55, P < 0.01) and LF n-gain (r = 0.70, P < 0.01), whereas no correlations were identified between ARI and any transfer function metric (in either the LF or VLF range). Redefining the frequency ranges (see MATERIALS AND METHODS) for the calculation of band average transfer function estimates did not substantially alter the overall pattern of correlations, being typically <0.5 among these metrics (Fig. 3). We found that the ARI (average: 5.1 ± 1.9) and RoR (average: 0.29 ± 0.11 s⁻¹) were modestly correlated (r = 0.58, P < 0.01; Fig. 4).

Relationships Among Spontaneous Transfer Function Metrics With Forced Oscillations Induced by Squat-Stand Maneuvers (Data Set C)

Group-averaged baseline cardiovascular, respiratory, spectral, and transfer function parameters in the sitting position are shown in Table 1. Repeated squat-to-stand maneuvers augmented coherence at both 0.10 Hz (0.99 ± 0.020) and 0.05 Hz (0.97 ± 0.040). The average phase, gain, and n-gain at 0.05 Hz were 0.69 ± 0.26 rad, 0.64 ± 0.21 cm·s⁻¹·mmHg⁻¹, and 1.2 ± 0.27%/mmHg, respectively. The corresponding values at 0.10 Hz were 0.46 ± 0.14 rad, 0.88 ± 0.23 cm·s⁻¹·mmHg⁻¹, and 1.6 ± 0.23%/mmHg. Correlations between spontaneous and squat-to-stand transfer function indexes were generally weak, with absolute r values ranging between 0.0029 and 0.77, with only LF gain and 0.10-Hz gain reaching >0.5 (r = 0.77, P < 0.01; Table 2).

Within-Subject Relationships: Step Changes in PETCO₂ (Data Set D)

The influences of PETCO₂-mediated changes in transfer function parameters were examined in 16 subjects to determine the nature and extent of within-subject relations between metrics. For this analysis, all subjects contributed data to the spontaneous baseline, normocapnic baseline, and each of the +10- and −10-mmHg PETCO₂ steps. However, due to reduced tolerance at more extreme CO₂ levels, only subsets of individuals completed the +15 (n = 14)-, +20 (n = 8)-, +25 (n = 4)-, −20 (n = 15)-, and −30 (n = 9)-mmHg steps. Baseline cardiovascular and respiratory parameters during each PETCO₂ step are shown in Table 3. Hypercapnia steps were associated with increases in PETCO₂, MCAVmean, MAP, and heart rate relative to
baseline, whereas hypocapnia steps were associated with reductions in PETCO2 and MCAVmean and increases in heart rate. The relationship between PETCO2 and transfer function coherence, phase, gain, and n-gain in the LF and VLF range are shown in Fig. 5. In the LF range, PETCO2 was positively related to coherence \( (\beta = 0.0065 \text{ arbitrary units (AU)/mmHg}, P < 0.01) \) and gain \( (\beta = 0.0070 \text{ cm·s}^{-1}·\text{mmHg}^{-2}, P < 0.01) \) and inversely related to phase \( (\beta = -0.026 \text{ rad/mmHg}, P < 0.01) \) and n-gain \( (\beta = -0.042\%/	ext{mmHg}^2, P < 0.01) \). Similar relationships were observed in the VLF range (Fig. 5). Therelationship between spontaneous LF and VLF transfer function metrics across the full range of PETCO2 values studied are shown in Figs. 6 and 7, respectively. In the LF range, average phase was positively related to n-gain \( (\beta = 1.3\%·\text{mmHg}^{-1}·\text{rad}^{-1}, P < 0.01) \) but inversely related to gain \( (\beta = -0.29 \text{ cm·s}^{-1}·\text{mmHg}^{-1}·\text{rad}^{-1}, P < 0.01) \). Coherence was inversely related to n-gain \( (\beta = -2.0\%·\text{mmHg}^{-1}·\text{AU}^{-1}, P < 0.01) \) and phase \( (\beta = -1.7 \text{ rad/} \text{AU}, P < 0.01) \) but was positively related to gain \( (\beta = 0.94 \text{ cm·s}^{-1}·\text{mmHg}^{-1}·\text{AU}^{-1}, P < 0.01) \). A similar pattern of relationships was also observed in the VLF range with the exception that phase was unrelated to gain and coherence did not correlate with n-gain (Fig. 7).

Group-averaged transfer function coherence, phase, gain, and n-gain as a function of frequency at baseline (+0 mmHg) and during steady-state hypocapnia and hypercapnia are shown in Fig. 8. At baseline, the group-averaged coherence increased as a function of frequency, crossing above 0.5 at \( \sim 0.05 \text{ Hz} \). Phase increased between 0 and \( \sim 0.40 \text{ Hz} \) and then fell gradually between 0.04 and 0.30 Hz, whereas both gain and n-gain both fell between 0 and \( \sim 0.04 \text{ Hz} \) before increasing as a function of frequency. These qualitative patterns of rising coherence, falling phase, and rising gain with increasing frequency were seen across all PETCO2 levels but with significant quantitative differences (Fig. 9). In general, the coherence and phase responses with changes in PETCO2 occurred within the VLF and LF bands; however, the precise response ranges differed between hypocapnia and hypercapnia. Reductions in coherence with hypocapnia were seen between 0.02 and 0.07 Hz at \( \sim 10 \text{ and } \sim 20 \text{ mmHg} \) and also between 0.15 and 0.22 Hz at \( \sim 20 \text{ mmHg} \) (Fig. 9). Increases in coherence with hypercapnia were evident between 0.02 and 0.15 Hz at both \( +10 \text{ and } +15 \text{ mmHg} \). Hypocapnia was associated with phase increases between 0.07 and 0.15 Hz at \( \sim 10 \text{ mmHg} \) and between 0.07 and 0.22 Hz at \( \sim 20 \text{ mmHg} \), whereas phase reductions occurred...
between a slightly more extended range of 0.04–0.20 Hz with hypercapnia at both +10 and +15 mmHg. There were no consistent coherence or phase changes above 0.20 Hz.

Gain responses varied depending on whether units were normalized, and both absolute gain and n-gain differed markedly compared with the change in coherence and phase. For absolute gain, clear reductions with hypercapnia occurred between −0.02 and 0.10 Hz at −10 mmHg and between −0.2 and 0.13 Hz at −20 mmHg. Absolute gain appeared insensitive to hypercapnia between 0.02 and 0.20 Hz but decreased at frequencies above 0.20 Hz. For n-gain, the most noticeable change was the clear reduction between 0.02 and 0.30 Hz with hypercapnia. For hypercapnia, n-gain decreased at −0.05 Hz but increased between 0.10 and 0.20 Hz at −10 mmHg and between 0.15 and 0.20 at −20 mmHg.

**DISCUSSION**

This study examined the relationships between commonly used metrics that have been applied, either independently or in combination, as indexes of CA. We found that the metrics studied were generally statistically unrelated or showed only weak to moderate correlations. While some metric combinations were strongly correlated, these were invariably between mathematically coupled parameters that share common denominators (e.g., LF gain vs. n-gain) or between metrics with identical mathematical constructs that overlapped in frequency (i.e., 0.10-Hz gain vs. LF gain). Furthermore, within-subjects analysis of the coherence, phase, and gain response to PETCO2 manipulation revealed that metric convergence was poor, with the potential for some metric combinations to yield discrepant conclusions. Thus, consistent with our hypothesis, our data indicate that the metrics studied have poor convergent validity and that only selected metrics can be used interchangeably for the comparison of CA across or within individuals. These findings prompt discussion on the validity of the measures, potential explanations for the observations, and the implications for their clinical application and interpretation.

**Convergent Validity**

The absence of strong metric correlations raises concerns over the convergent validity of these measures. In the context of this study, convergent validity refers to the degree to which metrics of CA (which should theoretically be related to one another) are indeed related. By convention, the evaluation of metric convergence is typically done through correlation analysis, which may involve comparisons against a gold standard (21). However, such comparisons are not possible in the present study given the lack of an accepted gold standard index of CA. Therefore, we examined the relationships between all metrics without a priori assumptions of a gold standard. Cor-

### Table 2. Relationships between spontaneous and repeated squat-to-stand transfer function metrics

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<th>VLF vs. 0.05 Hz</th>
<th>LF vs. 0.10 Hz</th>
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<td>Phase</td>
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<tr>
<td><strong>Phase</strong></td>
<td>0.073</td>
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<tr>
<td><strong>Gain</strong></td>
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<tr>
<td><strong>n-Gain</strong></td>
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Values are Pearson’s correlation coefficients between phase (in rad), gain (in cm·s⁻¹·mmHg⁻¹), and n-gain (in %/mmHg). VLF. *P < 0.05.
relation analysis revealed a general lack of convergence in metrics, even between parameters where strong relations might be expected. For example, despite the frequent application of LF transfer function gain and phase as independent measures of CA (10, 52), phase was unrelated to n-gain and was only weakly related to gain ($r = -0.31$). Likewise, although ARI was positively related to RoR, the coefficient of determination relating the two measures was only 0.34, indicating that linear predictions of one variable cannot be reliably achieved with knowledge of the other given that 66% of the regression variance remains unexplained. Although the absence of strong correlations may reflect nonlinear relations between metrics,
the generally unstructured appearance of the scatterplots sug-

gests that this is unlikely.

The lack of metric convergence was also evident in our data

collected under graded changes in PETCO₂. We found that

transfer function metric correlations within subjects were

highly inconsistent and that some metrics could yield contra-
dictory conclusions. A previous study (1) has indicated that

hypocapnia (~22 mmHg) can double the RoR index, indicat-
ing an improvement in CA. Conversely, hypercapnia (~47

mmHg via 5% CO₂ inhalation) can cause the RoR to decrease

up to 45%, reflecting a proposed impairment of CA (1).

However, these findings are not universally supported by all

studies using different CA metrics. For example, one study

(11) showed that LF phase and n-gain were the only transfer

function metrics that differed across a limited range of PETCO₂

levels (~5 to +5 mmHg) in healthy volunteers. In keeping

with these findings, we found that progressive elevations in

PETCO₂ across an extended range (up to +25 mmHg) lead to

Fig. 6. Relationships between repeated measurements of LF transfer function metrics across the different PETCO₂ levels. Data for each subject are represented by different symbols.

Fig. 7. Relationships between repeated measurements of VLF transfer function metrics across the different PETCO₂ levels. Data for each subject are represented by different symbols.
highly consistent reductions in phase in all subjects, whereas the expected increases in gain were less consistent across individuals (see Fig. 5). Likewise, the expected decrease in gain was less consistent across subjects despite clear increases in phase with reductions in PETCO₂. We also found that n-gain decreased with increasing PETCO₂ such that, on average, phase was inversely related to gain but positively related to n-gain (Fig. 6), at least in the LF range.

These relationships reveal an important inconsistency in the way that these CA metrics are commonly interpreted. Increases in coherence and reductions in phase are consistent with greater passive transmission of blood pressure into CBF fluc-

Fig. 8. Group-averaged transfer function coherence, phase, gain, and n-gain for all subjects at baseline (+0 mmHg) and during steady-state hypocapnia (+10 and +15 mmHg). Data for the +20-, +25-, and −30-mmHg steps are not shown.

Fig. 9. Group-averaged differences in transfer function coherence, phase, gain, and n-gain for all subjects between baseline and steady-state hypocapnia (+10 and −20 mmHg) and hypercapnia (+10 and +15 mmHg). The thick solid lines show averaged estimates; the dashed lines show 95% confidence bands. Vertical dashed lines mark the VLF (0.002–0.07 Hz) and LF (0.07–0.20 Hz) bands. Regions where the 95% confidence limits were consistently above or below zero are marked to indicate the frequency ranges that were most sensitive to hypercapnia and hypocapnia. Data for the +20-, +25-, and −30-mmHg steps not shown.
tations during hypercapnia, presumably due to impairment of CA (3, 35, 56). However, according to conventional interpretation, CA impairment should also yield increased transfer function gain. Our data indicate that this is only manifest if the gain is expressed in absolute units; in keeping with previous studies (2, 16), we found that in normalized units, gain decreased with hypercapnia, suggesting that CA might be paradoxically improved. These observations are important in at least two ways. First, they confirm that discordant conclusions can be drawn depending on whether the gain was calculated in absolute or normalized units (11). Second, the discrepancy highlights an important limitation of the transcranial Doppler, which is that MCAv only reflects changes in volumetric blood flow provided that the MCA diameter remains constant within subjects. Because this requirement has been demonstrated during mild to moderate hypocapnia and hypercapnia (45), the use of MCAv in absolute units may be justified under some conditions. Nevertheless, the inability to account for MCA diameter has promulgated data normalization procedures that supposedly corrects for between-individual differences in MCA caliber (32). While the present study was not designed to test the validity of data normalization, our results suggest that a distinction must be drawn given that n-gain did not match the relationships observed with gain, as had been expected, particularly in light of the relatively strong linear relationship (r = 0.70) between gain and n-gain.

Strong correlations were observed between very few metric combinations. As mentioned, spontaneous transfer function gain and its normalized equivalent were strongly related in both the LF (r = 0.70) and VLF (r = 0.90) range. However, these strong relations likely reflect the fact that the metrics are mathematically coupled. Spurious correlations due to mathematical coupling, which is a statistical artifact that occurs when one variable is inherent in the calculation of another variable, can be as high as 0.7 (50). Strong correlations were also seen between spontaneous LF gain and gain during forced oscillations at 0.10 Hz (r = 0.77), possibly because the metrics share the same mathematical construct and 0.10 Hz falls within the LF range (0.07–0.20 Hz). However, for reasons that remain unclear, no correlation was observed between 0.05-Hz gain and VLF gain, and no associations were found between VLF and 0.05 Hz or LF and 0.10 Hz n-gain (Table 2).

Potential Explanations for the Lack of Metric Convergence

There are several potential explanations for the lack of metric convergence. One possibility relates to the marked differences in the nature and magnitude of the blood pressure stimulus. Whereas the RoR and ARI are derived from pressure-flow relations during relatively large step reductions in cerebral perfusion pressure, spontaneous transfer function analysis characterizes the relationship between spontaneously occurring pressure and flow fluctuations without a clear exogenous perturbation. Data from animal (20) and human (17) studies have indicated that such differences can influence the nature of cerebrovascular responses. For example, in anesthetized cats with exposed cranial windows for the direct observation of pial arteries, abrupt reductions in blood pressure achieved with sudden arterial occlusion elicited cerebral vasodilatation promptly within 2–3 s, whereas latencies up to 10 s were observed in response to slow oscillatory blood pressure fluctuations (20). It was also observed that vasodilatation tended to occur only if a certain threshold of hypotension was achieved (20). In the present study, even comparison of the same transfer function metrics assessed from spontaneous versus induced oscillations generally yielded only moderate correlations. Collectively, these observations support the notion that the magnitude of blood pressure perturbation influences the nature of the consequent cerebrovascular response.

It is also important to recognize that some of the metrics differ markedly in terms of their basic analytic construct and therefore may not reflect the same aspect of the underlying physiological response. For example, because the RoR (s⁻¹) equates to the rate of CVCi change (usually normalized to baseline blood pressure) in the period immediately after thigh cuff deflation, the metric only reflects the “pace” of CA; it does not explicitly convey information on the extent to which the elicited CA response was able to restore cerebral perfusion. This limitation also applies to the coherence and phase metrics as they describe, respectively, the linearity and timing relationships between cerebral perfusion pressure and flow, but not the extent that pressure is transmitted to flow, which should be reflected in the gain. These limitations may be circumvented with the use of “integral” measures that consider both the temporal and amplitudinal relations between cerebral perfusion pressure and flow, such as the ARI (48), or the impulse response function derived from the inverse Fourier transform of the estimated transfer function (56). Furthermore, the transfer function between MAP and MCAv essentially describes the steady-state admittance properties of the cerebral circulation in the frequency domain, whereas the RoR is based on the calculation of beat-to-beat changes in vascular conductance. Although conductance and admittance are related (conductance is theoretically admittance at 0 Hz), they do nevertheless reflect different aspects of the cerebrovascular system.

In addition to CA, cerebral hemodynamics are also influenced by a myriad of interacting factors, including fluctuations in Paco₂, tension (36), cardiac output (31), steady-state CVC (51), sympathetic activity (18), and regional metabolic activity (5). Evidently, none of these factors can be explicitly accounted for when only pressure and flow are considered in their derivation. The failure to account for these effects can influence the reproducibility and internal consistency of the metrics, which may contribute to the general lack of metric convergence. Indeed, previous studies of ARI (26) and transfer function reproducibility (15) have indicated that these metrics do show considerable intrasubject variation. Although we are not aware of studies on RoR reproducibility, it is likely that the RoR would also exhibit a degree of inherent variation.

Influence of Redefining the Frequency Bands

It is important to consider the possibility that the lack of metric convergence may relate to the parameterization of transfer function analysis, which can be done in a myriad of ways. The upper limit for the VLF band adopted in this study was first defined by Zhang et al. (56), based on the observation that at ~0.07 Hz, the coherence crossed above 0.5, indicating 50% shared variance between MAP and MCAv at frequencies > 0.07 Hz. Despite little experimental validation, many researchers have adopted this arbitrary “threshold” as the upper limit that demarcates the effective operating range of human CA (i.e., 0.05 Hz or LF and 0.10 Hz n-gain (Table 2).
Indicate that responses to PETCO2 manipulation occur within otherwise remained relatively weak. This observation could show that, on average, the strength of metric correlations (i.e., VLF), to 0.10, 0.15, and 0.20 Hz (Fig. 3). This analysis showed that, on average, the strength of metric correlations improved only slightly as the upper frequency increased but otherwise remained relatively weak. This observation could be explained, in part, by the results shown in Fig. 9, which indicate that responses to PETCO2 manipulation occur within different frequency ranges for each metric and that the response ranges frequently overlap the boundaries of the VLF and LF bands. These data indicate that metric correlations can be expected to be stronger and more consistent for indexes that share overlapping response ranges, such as phase and n-gain, but less consistent for metrics with distinct response ranges, such as phase and gain.

Implications

An implication of our findings is that conclusions of research trials where CA is compared as the primary outcome measure across individuals or across conditions within individuals may be metric dependent. This lack of metric consistency is clinically problematic because inappropriate treatment strategies may be pursued depending on the metric chosen for diagnosis. For example, in the context of risk stratification, an individual classified as having deficient CA, and therefore assigned a higher risk score for adverse cerebrovascular events on the basis of one metric, may be classified differently on the basis of another. In terms of quantifying treatment responses, our findings corroborate with previous work showing that different, or even contradictory, conclusions may be reached depending on the specific metrics chosen (32, 51). Thus, our findings clearly question whether it remains appropriate to continue the assumption that the metrics in this study are wholly and interchangeably reflective of CA. The absence of convergent validity does not necessarily invalidate previous research or preclude the future application of these metrics. Surrogate markers may predict outcome on a population level but provide nonspecific physiological information on an individual subject/patient basis. For example, analysis of blood pressure and heart rate variability is predictive of cardiovascular outcomes in large-scale clinical trials (22, 42), but their physiological basis is poorly understood (43, 44). Likewise, the surrogate markers examined in this study may not wholly reflect CA but still retain prognostic utility within a population. However, our findings do point to a need for better understanding of the physiological information that each metric may or may not convey.

With respect to transfer function analysis, our results suggest that the classical VLF and LF bands do result in data fragmentation. The conventional banding definition did not fully encompass the precise frequency ranges that demonstrate sensitivity to PETCO2, because they varied between metrics and between hypercapnia and hypocapnia. Our findings suggest that the 0.07-Hz limit may be unnecessary under some circumstances, and that metric-specific bands may enhance the sensitivity of transfer function analysis. For example, as shown in Figs. 8 and 9, hypercapnia was associated with reductions in phase between –0.04 and 0.20 Hz. This indicates that a single 0.04–0.20-Hz band may be suitable for detecting phase changes resulting from impaired CA. In contrast, hypercapnia was associated with increases in phase between –0.07 and 0.15 Hz, suggesting that the narrower frequency band may be more suitable for detecting phase changes associated with enhanced CA. Our results support the latter contention in at least two ways. First, coherence reductions were observed between 0.02 and 0.07 Hz during hypercapnia, which means that transfer function parameters in this range may be estimated with diminished precision. Second, increases in phase associated with improvements in CA increases the likelihood of phase wrap around. We found that phase wrap around did occur frequently during hypercapnia around 0.05 Hz (see Appendix). Therefore, the use of a narrower frequency band that does not encompass 0.05 Hz may yield more reliable phase estimates, although this needs to be verified using alternative experimental approaches. The physiological mechanisms underpinning the complex transfer function response patterns with hypercapnia and hypocapnia clearly warrant further investigation.

Challenges and Future Directions

The lack of convergent validity points to a need to revise our current conception of CA. Several areas warrant further research. First, unproven assumptions or assumptions based on limited evidence need to be closely scrutinized. For example, one major concept in vascular physiology is that cerebral vessels are stiffer compared with their systemic counterparts (27). This idea has lead to the general dismissal of stiffness-related properties when inferences are drawn from cerebral pressure-flow recordings even though the evidence is sparse. Recent studies (7, 51, 55) using surrogate markers of vascular compliance and mathematic models have suggested that cerebral arteries are more compliant than previously thought and that transfer function metrics may partly reflect vascular compliance. Second, there is a need for further experimental validation of current CA metrics. This could be achieved, for example, by evaluating the influence of myogenic blockade on the various CA metrics using Ca2+ channel antagonists. Since CA is mediated in part through myogenic mechanisms, Ca2+ channel blockade should reveal the metrics that reflect this control. These efforts should also be coupled with ongoing analytic innovations that enable the quantification of both linear and nonlinear properties of the cerebral circulation. Third, there is a need for better standardization of terminology. Although the term “cerebral autoregulation” is commonly used, it is not always clear whether the reference is used to describe the same physiological construct (25). Our findings indicate that it may not be possible to reduce CA, a nonlinear and complex process, into a single all-encompassing index. Thus, CA evaluation should take into account the full range of information associated with any given technique rather than narrowly focusing on isolated metrics. Finally, characterization of physiological systems may not necessarily yield more in-
formation than simply measuring the endpoint parameters that the system purportedly controls. A comparative example is the relationship between blood pressure and cardiovascular risk. While many studies have sought to establish surrogates of autonomic function (e.g., baroreflex sensitivity) as potential markers of cardiovascular risk (22), few have proven utility above 24-h blood pressure or blood pressure variability. Thus, new insights may be gained from research that focuses on CBF as the primary dependent variable. Considering that diseases such as ischemic and hemorrhagic stroke are fundamentally due to cerebral perfusion mismatch, it is surprising that no studies have investigated the predictive utility of short- and long-term CBF variability on outcome.

Methodological Considerations

The results of this study need to be taken in context of several methodological considerations. The metrics examined do not represent an exhaustive list of all available techniques. Considering the large number of techniques available, it was not feasible to compare all possible methods. However, while our findings may not apply to methods that were omitted from analysis, this study should have broad external validity given that the metrics studied are very widely applied in the field. An additional issue is that we tested only healthy subjects. We acknowledge that further studies are needed to confirm our findings in different clinical populations.

Conclusions

In summary, the CA indexes examined in this study were generally unrelated to one another or showed only weak to moderate correlations. Strong correlations were only identified between mathematically coupled metrics that share common components and between some metrics with similar mathematical constructs that overlap in frequency. The general lack of metric convergence suggests that most indexes are not directly comparable and may provide inconsistent clinical and physiological information. Our findings highlight the need for further research to clarify the physiological interpretation of these metrics and to ascertain which metric or combination of metrics can be considered to represent a valid standard for CA assessment. Until such time, physiological interpretation of past and future research using these measures should be done with caution.

APPENDIX

Derivation of Transfer Function Gain and Phase

Transfer function analysis has gained acceptance as a method for characterizing the relationship between the input and output of cardiovascular control systems. Because a transfer function represents the input and output relationship of a linear time-invariant system, it is commonly acknowledged that the assumption of linearity needs to be satisfied. To achieve this, most studies have considered phase and gain estimates within predefined frequency bands only where the coherence exceeds a critical threshold (commonly defined as 0.5). However, there is no consensus over how this coherence criterion should be implemented, with some studies even challenging the need to consider coherence (39). Furthermore, although the coherence threshold is commonly set at 0.5, indicating at least 50% shared variance, others have determined the coherence threshold based on the total degrees of freedom ($V_{\text{total}}$), calculated as follows:

$$\gamma^2_{\min} = 1 - \alpha V_{\text{total}}$$

where $\gamma^2_{\min}$ is the coherence threshold and $V_{\text{total}}$ is the product of the degrees of freedom associated with spectral averaging and smoothing. Thus, a number of approaches can be applied, including the following methods.

Method 1: averaging only points associated with a coherence that is greater than a predefined threshold. In this study, the threshold was set at 0.5 (by convention) and statistically at 0.6 based on the calculated degrees of freedom associated with spectral processing. The degrees of freedom associated with spectral averaging component is proportional to $NL$, where $N$ is the total number of samples in the recording and $L$ is the number of samples per data segment. The spectral smoothing component for the Hanning window is constant at 2.67. Thus, $V_{\text{total}} = 2.67NL = 8$, giving $\gamma^2_{\min}$ = 0.6.

Method 2: averaging all data within a band without considering the coherence. This approach is equivalent to setting the threshold at zero. This approach produces phase and gain estimates that are comparable with those obtained under conditions of high coherence (e.g., during cyclic squat-to-stand exercise) (8, 53), presumably because averaging filters out the random variability of the function around its true value (38).

Method 3: averaging all data points according to the statistical precision of each phase and gain value (49). This approach does not dichotomize data points based on arbitrary cutoffs but considers their

Table A1. Relationships between LF and VLF transfer function metrics

<table>
<thead>
<tr>
<th>Coherence threshold = 0.6</th>
<th>VLF</th>
<th>Coherence</th>
<th>Phase</th>
<th>Gain</th>
<th>n-Gain</th>
<th>LF</th>
<th>Coherence</th>
<th>Phase</th>
<th>Gain</th>
<th>n-Gain</th>
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<tr>
<td>Coherence</td>
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<td>Phase</td>
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<tr>
<td>Gain</td>
<td>0.099</td>
<td>-0.348†</td>
<td>1</td>
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<tr>
<td>n-Gain</td>
<td>0.014</td>
<td>-0.303*</td>
<td>0.882†</td>
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<td>Coherence</td>
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<tr>
<td>Phase</td>
<td>-0.132</td>
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<tr>
<td>Gain</td>
<td>0.100</td>
<td>-0.133</td>
<td>1</td>
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<tr>
<td>n-Gain</td>
<td>0.048</td>
<td>-0.141</td>
<td>0.884‡</td>
<td>1</td>
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<td>Coherence threshold = 0</td>
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<td>Coherence</td>
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<tr>
<td>Phase</td>
<td>0.137</td>
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<tr>
<td>Gain</td>
<td>0.363†</td>
<td>-0.048</td>
<td>1</td>
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<tr>
<td>n-Gain</td>
<td>0.306†</td>
<td>-0.036</td>
<td>0.902‡</td>
<td>1</td>
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Values are Pearson’s correlation coefficients. *$P < 0.05$ and †$P < 0.01$. 

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individual precision as indicated by the coherence and appropriately weighting down the influence of imprecise values.

To ensure that our study findings were not confounded by the choice of methodology, we implemented cerebral transfer function analysis using all three methods. For method 1, setting the threshold to 0.5 eliminated 11 cases in the VLF range. At a coherence threshold of 0.6, 32 cases were eliminated in the VLF range and 3 cases in the LF range. No eliminations occurred with methods 2 and 3. The results for method 3 are presented in the main text. The results for methods 1 and 2 are shown in Table A1.

The results showed that irrespective of methodology, transfer function metrics were either unrelated or showed only weak to moderate correlations. Strong correlations were found only between gain and n-gain. These are mathematically coupled parameters that share common components.

**Phase Wrap Around**

Wrap around of transfer function phase estimates often occur near 0.05 Hz or at lower frequencies. The occurrence of wrap around adds large negative values of phase that can distort estimates of mean phase within a given frequency band (33). There are currently no clear guidelines on how data exhibiting phase wrap around should be processed. Previous studies have eliminated data affected by phase wrapping (24), applied simple corrections such as adding 2π to the phase wrapped point (28), or used more complex procedures (e.g., circular statistics) (12). The vast majority of studies using cerebral transfer function analysis have not reported how phase wrapping was managed. Our analysis indicates that the importance of phase wrapping varies depending on the analysis approach. For example, of the 105 subjects in data set A, we identified 27 cases of phase wrapping. Of these cases, the wrapped data point was < 0.02 Hz in six cases. In the remaining 21 cases, the wrapped data points were associated with very low coherence (average coherence = 0.13 ± 0.14), with only one case where the coherence of a wrapped point was > 0.5. Thus, in keeping with one study (40), we did not implement any special phase wrapping algorithms as phase wrap points had either no impact on the calculation of band averages (if the 0.5 or 0.6 coherence criterion were applied) or their influence were heavily weighed down. However, our results indicate that values calculated using the coherence = 0 approach would be affected as all data points are assigned equal weights. For data set D, we observed no cases of phase wrapping during normocapnia or hypercapnia. Phase wrapping was seen in 7 of 9 subjects, 7 of 15 subjects, and 13 of 16 subjects who were able to complete the ~30– ~20–, and ~10-mmHg hypercapnics steps, respectively. The average frequency at which phase wrapping occurred was 0.047 ± 0.034 Hz, suggesting that phase wrapping is an important additional source of phase variance within the VLF range during hypocapnia. This may partly explain why hypocapnia did not consistently alter phase estimates at frequencies < 0.07 Hz (Figs. 8 and 9).

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


