Random squat-stand maneuvers: a novel approach for assessment of dynamic cerebral autoregulation?

SC Barnes[1], N Ball[1], RB Panerai[1, 2] TG Robinson[1, 2], VJ Haunton[1, 2]

[1]*Department of Cardiovascular Sciences, University of Leicester, Leicester, UK.*

[2]*National institute for Health Research (NIHR), Biomedical Research Unit in Cardiovascular Sciences, Clinical Sciences Wing, Glenfield Hospital, LE3 9QP, Leicester, UK.*

Corresponding author:

Victoria J. Haunton

Department of Cardiovascular Sciences

Room 210, Robert Kilpatrick Clinical Sciences Building

University of Leicester

PO Box 65

Leicester LE2 7LX

Email: vjh12@le.ac.uk

Phone: 0116 252 5802

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Abstract

Background: Squat-stand maneuvers (SSM) have been used to assess dynamic cerebral autoregulation (dCA), but always at a fixed-frequency (FF). This study aimed to assess the use of random-frequency (RF) SSMs as a stimulus for measuring dCA, and to determine the reproducibility of FF and RFSSMs.

Method: 29 healthy volunteers (19 male, mean age 23.0 [4.9] years) completed the study; 11 returned for a repeat visit (median 45 days). Heart rate, beat-to-beat blood pressure, middle cerebral artery (MCA) blood flow velocity, end-tidal CO_2 and angle of the squat movement were measured. Subjects underwent four recordings: 5 minutes sitting; 5 minutes standing; FFSSMs (0.05Hz); RFSSMs. Subjects were asked to rate the degree of exertion experienced while performing these maneuvers.

Results: 29 subjects completed the protocol; 9 data sets were deemed unsuitable for further analysis. Mean ARI of 6.21 (1.04) while standing was significantly greater than during the SSMs ($p<0.01$); mean (SD) ARI during the FF and RFSSMs being 5.16 (1.43) and 5.37 (1.21), respectively. However, no significant difference was found between the ARI estimates from the two SSMs ($p=0.856$) or for each of the four recordings between the two visits ($p=0.645$). RFSSMs were found to be significantly less tiring than FFSSMs ($p<0.01$).

Conclusion: RFSSMs are an effective and non-invasive method of assessing dCA. There is no difference in the ARI estimates in comparison with FFSSMs. While FFSSMs have been well tolerated previously, RFSSMs are preferred by healthy subjects and thus may be better tolerated by a patient population in a clinical setting.

New and noteworthy

RFSSMs provided comparable estimates of autoregulatory indices to FFSSMs. Instead of point-estimates at the driven frequency, RFSSMs generate a broader power spectrum of changes in arterial blood pressure and cerebral blood flow velocity allowing direct comparison with spontaneous fluctuations through transfer function analysis. Moreover, random-frequency SSMs are preferred by participants. They are a novel tool by which larger blood pressure oscillations can be elicited for the reliable measurement of dynamic cerebral autoregulation.

Keywords

Transcranial Doppler ultrasound, reproducibility, transfer function analysis, cerebral hemodynamics, cerebral blood flow velocity.

Introduction

Cerebral autoregulation (CA) refers to the ability of the cerebrovasculature to maintain a relatively constant cerebral blood flow (CBF) despite fluctuations in blood pressure (BP). It can be described as either static or dynamic. Static cerebral autoregulation (sCA) refers to the control of CBF over long periods of time, without considering the speed at which changes in CBF occur following changes in BP (19). In contrast, dynamic CA (dCA) describes the acute changes in cerebrovascular resistance that occur in response to sudden changes in cerebral perfusion pressure (CPP). CA is known to be frequency dependent, and can be conceptualised as a 'high-pass filter'. BP fluctuations of below 0.20Hz are dampened down by the cerebrovasculature to maintain a constant CBF, but above this frequency CA becomes less effective (8, 9, 34, 35).

Transfer function analysis (TFA) is most commonly used to relate changes in CBF velocity (CBFV, output) to fluctuations in BP (input) (20, 41). Three parameters are yielded from TFA; phase describes the temporal relationship between CBFV and BP waveforms, gain provides a measure of the changes in the amplitude of the CBF waveform after a change in BP, and coherence expresses the fraction of output power that can be linearly explained by the input signal (9, 18, 35, 41). The degree of coherence is of the utmost importance in TFA. Similar to the correlation coefficient, it ranges from zero to one. When coherence is low, the reliability of phase and gain estimates reduces (7), often leading to the rejection of recordings (41). Low coherence could be the result of measurements with low signal-noise ratio, because the system is nonlinear, or because there are multiple inputs for the output variable (41). In order to maximise coherence, it is therefore necessary to improve the signal-noise ratio. This can be done by inducing larger BP fluctuations which create a more distinct relationship between the input (BP) and output (CBFV) variables.

Recently, repeated squat-stand maneuvers (SSMs) have been used for this purpose (1, 6, 16, 27-30, 40). SSMs have been verified as a safe, effective and physiologically relevant method of assessing dCA. They lead to significant increases in coherence (1, 6, 27, 29), with resultant

improvement in the reliability of TFA metrics. Upon squatting, the muscles of the lower limbs engage to deplete venous pooling, therefore increasing circulating volume and BP. When the subject stands, a combination of reduced peripheral vascular resistance and sudden lower limb venous pooling leads to a BP reduction (26, 37). When performed repeatedly, large and periodic fluctuations in BP are created at a frequency of the researcher's choosing (6).

Previously, SSMs have been performed at a fixed frequency (FF) of between 0.025-0.10Hz to challenge autoregulatory processes within a particular frequency range (1, 6, 16, 27-30, 40). However, to our knowledge, SSMs at a random frequency (RFSSMs) have not been trialled. They may be more physiologically relevant than FFSSMs, due to the unpredictable nature of the squats performed in day-to-day life, e.g. tying shoelaces or picking up a pen, where the duration of the SSM may vary. Another potential advantage of RFSSM, is that instead of inducing changes in BP and CBF at a fixed frequency, often requiring repetition at another frequency within the dCA active range, it should generate changes that cover a range of frequencies, hence providing a broader spectrum of signal power that could improve estimation of TFA parameters. Furthermore, only one study has explored the reproducibility of the TFA metrics elicited by SSMs (29), and no study has previously quantified the depth of the SSM. Therefore, we aimed to determine the between-visit reproducibility of both FFSSMs and RFSSMs, as well as the depth of the SSM achieved. In summary, this study addressed three main hypotheses: 1) RFSSMs provide similar coherence, gain, phase and autoregulation index (ARI) estimates to FFSSMs; 2) RFSSMs have similar reproducibility as FFSSMs at a repeat visit; and 3) RFSSMs are better tolerated than FFSSMs by healthy volunteers.

Materials and methods

Participants

Twenty-nine healthy volunteers (19 male, mean age 23.0 ± 4.9 years) were recruited from University of Leicester staff and students. Of these, 11 were invited back for a repeat recording.

The study was carried out according to the latest approved protocol, International Conference on Harmonisation-Good Clinical Practice (ICH-GCP), relevant regulations and standard operating procedures as well as in accordance with the Declaration of Helsinki (University of Leicester ethics reference 8442-vjh12-cardiovascularsciences). All participants provided written informed consent.

Instrumentation

Heart rate was measured using three-lead ECG. A tilt-sensor was attached to the subject's right thigh 20cm above the superior border of the patella to measure the angle of the squatting motion. 2MHz Doppler probes (Viasys companion III) were placed over the left and right temporal windows, and were held in a constant position at a fixed angle by a custom-built headset to measure CBFV in the middle cerebral arteries (MCA). Nasal capnography (Salter labs, ref 4000) was used to measure end-tidal CO_2 ($EtCO_2$). Beat-to-beat estimates of BP were obtained through arterial volume-clamping of the digital artery (Finometer, FMS, Amsterdam, Netherlands); this method being shown to accurately reflect intra-arterial BP changes (15, 24). The servo-reset mechanism was disabled throughout the recordings to allow for a continuous BP trace, but enabled between recordings. The right hand was held in position with a sling to minimise movement throughout the recordings, and to keep the finger cuff at heart height. Finally, intermittent brachial BP was measured using a validated electrosphygmomanometer (UA 767 BP monitor) to calibrate the Finometer recordings.

Continuous analogue recordings were digitised at 500 samples/s by a Physiological Data Acquisition System (PHYSIDAS) designed by the Leicester Medical Physics Department for subsequent analysis.

Experimental procedures

Experiments were performed in a well-lit, environmentally controlled laboratory that was free from distraction and kept at a temperature of 20-24^{0}C. Participants were asked to avoid strenuous exercise, caffeine, smoking, large meals and alcohol in the four hours prior to their visit.

Following a 10-min period of rest and stable recordings, four recordings were performed, maintaining minimal background noise and distraction of the subject. The recordings were as follows: a 5-min baseline recording of the patient sitting quietly with their eyes open; a 5-min baseline recording of the patient standing quietly with their eyes open; FFSSMs (15 squats at a frequency of 0.05Hz, preceded and followed by 90s standing); RFSSMs (15 squats of random duration with random periods of standing between them, preceded and followed by 90s standing).

For both FF and RFSSMs, a computer program provided visual cues to guide the timing of the squatting motion. For RFSSMs the sequence of visual cues aimed to achieve the largest degree of randomness possible within the limitations imposed by the time involved in reaching the squat position and then returning to stand. For this purpose, time intervals for either position were limited to the interval 2-20 s. Random gaussian sequences of 30 intervals were generated 1000 times, and the corresponding spectral power of each sequence was calculated with the fast Fourier transform. Given that absolute randomness would correspond to a perfectly flat spectrum, this was assessed by calculating the coefficient of variation (CoV) of the spectrum in the frequency interval 0.01 to 0.20 Hz, which is the relevant bandwidth for dCA. The sequence leading to the flattest spectrum, as indicated by the minimum CoV was then adopted for the RFSSM protocol (Figs. 1.E & 2.E).

A period of instruction and practice preceded the third and fourth recordings, during which the SSM was demonstrated. When performing the SSMs, subjects were instructed to squat down as low as they felt able. They were informed that they would need to perform 15 squats, and to take this into account when choosing their depth. Throughout each recording, subjects were asked to breathe through their nose and to avoid a Valsalva-like manoeuvre during the SSM. Subjects were given as much time to recover as they felt necessary between the FFSSMs and RFSSMs. After both SSMs had been performed, subjects were asked which they found to be the most acceptable, and to rate the degree of exertion on a scale of 1 (no exertion) to 10 (exhaustion).

Data processing

The readings from the Finometer were calibrated to the brachial BP recordings. Data were visually inspected; non-physiological spikes in CBFV were removed through linear interpolation. Files that contained any segments of significantly poor TCD signals were excluded from further analysis. Narrow spikes (<100 ms) and artefacts were removed by linear interpolation. Subsequently, all signals were filtered in the forward and reverse direction using an eighth-order Butterworth low-pass filter with a cut-off frequency of 20 Hz. The beginning and the end of each cardiac cycle were detected in the BP signal, and mean values of BP, CBFV and heart rate were obtained for each heartbeat. Beat-to-beat parameters were interpolated with a third-order polynomial and resampled at 5 Hz to generate signals with a uniform time base.

dCA was modelled using transfer function analysis (TFA), using mean BP as input and corresponding changes in CBFV as output as described previously (9, 18, 35, 41). The Welch method was adopted for smoothing spectral estimates obtained with the fast Fourier transform (102.4 s segments, 50% superposition) leading to frequency dependent estimates of coherence, gain, and phase. For FFSSMs, point estimates of coherence, phase and gain were calculated at 0.05Hz (6, 29). For RFSSMs, estimates were averaged for the very-low (VLF, 0.02-0.07 Hz), low (LF, 0.07-0.20 Hz) and high (HF, 0.20-0.50 Hz) frequency ranges (7).

Negative values of phase are indicative of the wrap-around phenomenon and were not included in the calculation of mean phase values in these frequency bands. Using the inverse fast Fourier transform, the CBFV response to a step change in BP was also derived (18). The CBFV step response was compared with 10 template curves proposed by Tiecks (33) and the best fit curve corresponded to the ARI. Values of ARI = 0 indicate absence of CA, whilst ARI = 9 corresponds to the most efficient CA that can be observed (33). A new procedure was adopted using the normalised mean square error for fitting the Tiecks model to the CBFV step response and a minimum threshold for the coherence function (0.15-0.25Hz) to accept or reject estimates of ARI (17) .

Statistical analysis

Data are given as mean ± SD. Student's t-tests were used to compare parameters for the left and right MCAs to determine any hemispheric differences. Comparisons between each of the four recordings, and between the two visits were performed using repeated-measures ANOVA. Point-estimates for FFSSMs were compared against other recordings in the VLF range, although at LF and HF comparison between all four recordings were not possible due to the driven frequency of 0.05Hz lying outside the LF range. Comparisons at LF and HF are therefore restricted to the sitting, standing and RFSSM recordings. Tukey post-hoc tests were employed when ANOVA F-values were significant. The intraclass correlation coefficient (ICC) and within-subject CoV were calculated to assess the reliability of parameter estimates at two different visits to the lab. $P < 0.05$ was adopted as level of significance.

Results

29 subjects completed the protocol. Of these, 9 were rejected due to failure to achieve bilateral MCA waveforms (4/9) or excessive signal disruption during SSM (5/9).

Haemodynamic effects of posture and squat-stand maneuvers

The baseline details of the 20 subjects with sufficient data for further analyses are presented in Table 11. Mean CBFV varied between maneuvers, being significantly higher in the sitting

compared to standing position ($p<0.01$), and in response to FFSSMs compared to RFSSMs (Table 1, $p=0.03$). Mean arterial and diastolic BP were significantly lower when sitting compared to FFSSMs ($p=0.02$). $EtCO_2$ was significantly lower during RFSSMs than during FFSSMs, with mean values of 38.3 ± 3.4 mmHg and 39.3 ± 3.0 mmHg respectively ($p=0.02$). Figures 1 and 2 depict representative temporal changes of the main parameters for FFSSMs and RFSSMs, respectively, showing considerable changes in MAP, CBFV, heart rate and $EtCO_2$, coinciding with the squatting movement recorded with the tilt sensor attached to the thigh (Figs 1.E & 2.E).

Transfer function analyses

Transfer function estimates are summarised in Table 2. ARI was significantly higher in the standing position compared to both FFSSMs ($p<0.01$) and RFSSMs ($p=0.01$), but there was no significant difference between the two SSMs ($p=0.856$). At the driven frequency of 0.05Hz, coherence during FFSSMs was significantly enhanced compared to the other three recordings ($p<0.001$). VLF and LF coherence during RFSSMs was significantly improved compared to subjects sitting and standing ($p<0.001$) (Table 2, Figure 3). VLF gain was significantly higher in the RFSSMs compared to baseline recordings ($p<0.01$ for both), and in FFSSMs compared to subjects sitting ($p=0.02$). VLF phase was significantly higher in the standing baseline recording compared to both FFSSMs and RFSSMs ($p<0.001$), and LF phase was significantly reduced in RFSSMs compared to both baseline recordings ($p<0.001$ for both).

Reproducibility

Eleven subjects repeated the study protocol at a later date (median interval 45 days, range 17 to 127). There was no significant difference between ARI estimates for each of the four recordings between the two visits (Figure 4).

A summary of both ARI results and TFA metrics from the 11 reproducibility subjects are given in Table 3. No significant differences were found in coherence between the two visits across

the four recordings at all frequency bands. Similarly, no difference was noted in phase, though significant differences in gain were noted between visits at LF and HF.

In order to determine the inter-session variation of the TFA metrics elicited from the two SSMs, repeated-measures ANOVA was performed with data from visit 1 compared directly to data from visit 2 for each SSM. No significant differences existed between visits in either of the SSMs.

The ICC values for the ARI and TFA parameters are given in Table 4. Compared to previous studies in the literature, based on spontaneous fluctuations in ABP and CBFV, the ICC values for ARI during both the FF and RF SSMs are relatively high, whilst some values for the TFA parameters are very low, including the occurrence of some values of ICC=0. The interpretation of these results will be discussed below.

CoV values for ARI and TFA parameters were generally better for SSMs than for the sitting or standing rest positions, with the exception of LF phase for RFSSMs (Table 5). Despite small numerical differences, there were no significant differences in CoV between the FF and RFSSMs for most parameters.

Squat angle

Twenty subjects underwent detailed analysis of the data from their tilt-sensor. The data from all 15 SSMs were averaged for each subject, and then inter-subject averaging was used to create a stereotypical SSM for the cohort (Figure 5). Subjects typically began their SSM with their thigh at 82 ± 4 degrees to the horizontal, and squatted down to 26 ± 16 degrees.

Preference data

26/29 subjects found the RFSSMs to be more acceptable than the FFSSMs. Self-reported exertion was 5.5 ± 1.4 in the FFSSMs and 4.5 ± 1.4 in the RFSSMs ($p<0.01$).

Discussion

Main findings

Random SSMs were more acceptable to participants and led to higher values of VLF coherence compared to spontaneous oscillations at rest, an important consideration when using transfer function analysis to obtain estimates of dCA. When compared to the more traditional FFSSM, random squat-stands showed no difference in estimates of gain, phase or the ARI obtained from the CBFV step response. Moreover, the reproducibility of RFSSMs was also broadly similar to that obtained for FFSSMs.

To the best of our knowledge, this is the first time that the depth of the SSM performed during an assessment of dCA has been quantified with continuous recording. In previous studies using FFSSMs, participants were asked to squat down until their thighs were parallel to the floor (29), which correlates well with the average depth of squatting we recorded (Fig. 5). Having the continuous recording of the squatting movement does provide a more objective approach to monitor compliance, mainly during RFSSMs where some phases are of shorter duration than during FFSSMs (Fig. 2). One additional advantage of having the continuous recording of the thigh angle is to use this signal as an input for multivariate modelling (21).

Finally, this is also the first time that the exertion associated with SSMs has been assessed. Of a total of 29 subjects, 26 preferred the RFSSMs to the FFSSMs, and found them to be significantly less tiring ($p<0.01$). Whilst 'exertion' is a subjective measure, as we assessed intra-subject variation, the subjectivity of this estimate across subjects is of lesser importance.

Taken together, these findings strongly suggest that RFSSMs might be a more promising approach to improve the reliability of estimates of dCA across wider populations.

Reliability of estimated parameters

To our knowledge, this is the first study to estimate the ARI in subjects performing SSMs. ARI varied significantly between recordings, being significantly higher when standing compared to the two SSMs. Furthermore, there was no significant difference in ARI between the two SSMs.

Comparing values of coherence with previous studies is difficult, as some used point estimates (27-30) and some used narrow-frequency bands (6, 16, 40) for quantification. Those which

used point-estimates instead of frequency bands, naturally reported higher coherence values, as the peak input power occurs at a particular frequency, and reduces the further sampling occurs from this point (30). As such, point-estimates reported coherence values of 0.92-1.00 (27-30) and those assessing coherence from a frequency range reported lower coherence in the range of 0.69-0.90 (6, 40). The coherence values in the present study fall within the values reported in the existing literature for both point estimates as well as for frequency band values (VLF and LF).

The reproducibility of FFSSMs has been previously evaluated by Smirl et al (29), using the CoV or SEM as a measure of absolute reliability. Their conclusion was that TFA metrics were reproducible at 0.05Hz and 0.10Hz, as CoV values for phase, gain and coherence during FFSSMs at 0.05Hz were reported as below 20%, which has been suggested as the threshold value for acceptable reproducibility (25). When sampled at the driven frequency of 0.05Hz, the reproducibility of TFA metrics in the current study was comparable to that reported previously (29).

The ICC coefficient is usually regarded as the best metric to assess the reliability of physiological measurements in the absence of a (‘gold’) standard reference. However, an important limitation of the ICC is that when calculated for a homogeneous population, as is the case in our healthy group of subjects, it can provide distorted values given the narrow range of parameter values as compared to the much larger scatter that would be expected in the presence of pathological conditions. With subjects in the supine position, Brodie found ARI ICC values similar to those reported in the present study under seated and standing conditions (4). The noticeable increase in ICC, with either the FF or RFSSMs, demonstrates the improved reliability of these maneuvers for assessing ARI and VLF phase, although no previous data are available for comparison.

For TFA parameters, ICC values need to be interpreted taking into consideration the type of parameter and the frequency region in each case. First of all, values in the HF region are not relevant since dCA is not active in this frequency band, although the recent CARNet White

Paper recommends that values for HF should always be reported (7). Secondly, for coherence, reproducibility is not relevant, as long at its values are above the 95% confidence limit, which is clearly the case in our study (7). Thirdly, for the gain parameter, limited reproducibility was already demonstrated by the ANOVA results, hence the low values of ICC are not surprising and seem to confirm the poor reliability of gain as a metric of dCA (7). Finally, and somewhat surprisingly, some of the phase ICC values are fairly low. To our knowledge, only one previous study reported ICC values for TFA parameters (10). The values of VLF phase ICC reported in the present study are in good agreement with the estimates of Gommer et al for healthy subjects resting supine, although at LF our values exceed those reported in the previous study (10); the discrepancy may be due to the different frequency bands used in the two studies. Noteworthy, with either SSM, ICC for VLF phase increases from 'moderate' to 'good', similar to that found with ARI.

Due to the difference between point estimates at 0.05Hz versus frequency band estimates (VLF and LF), parameters like coherence, CoV and ICC will normally demonstrate a tendency towards better reliability in the former, as we have found for FFSSMs in comparison with RFSSMs (Tables 2-5). Importantly, interpretation of these parameters usually takes into account a threshold for acceptability. As an example, for coherence, the 95% confidence limit usually adopted is 0.5 or less, depending on the degrees of freedom of TFA estimates (7). In dCA assessments based on spontaneous fluctuations of BP and CBFV, estimates of gain, phase or ARI are often rejected due to low values of coherence (7, 10, 14, 17, 19, 35, 41). In practice, the higher values of coherence obtained with FFSSMs (point estimates) as compared to the VLF and LF values obtained for the RFSSMs frequency bands, do not represent a palpable advantage as both are substantially higher than the 95% confidence limit for coherence. To some extent, similar considerations apply to CoV and ICC figures.

In summary, ARI and phase demonstrate superior reliability as markers of dCA during SSMs as measured by both the ICC and CoV.

Physiological perspectives

One key question in the use of either FF or RFSSMs is the extent to which other physiological co-variates can distort estimates of dCA by modulating CBF independently of the more direct influence of BP (21). On one hand, the presence of these exogenous influences would be expected to reduce the univariate coherence between BP and CBFV. On the other hand , if these influences have a temporal pattern similar to BP and CBFV oscillations (Figs 1 & 2), one would expect an increase, rather than a reduction in coherence, thus implying that coherence per se cannot answer the key question above.

As the subject squats, muscular contractions decrease venous pooling to increase the effective circulating volume (26). This increase in venous return increases end-diastolic volume and consequently stroke volume due to the Frank-Starling relationship (12). The muscle mechanoreflex also contributes to the increase in BP and CBF that follow (40). Meanwhile, the constriction of the lower limb vasculature by the musculature induces a degree of peripheral ischaemia, predisposing to vasodilation once the subject stands (23, 32). Upon standing, BP falls. Total peripheral resistance (TPR) is low because of the removal of the muscle pump, the release of vasodilatory metabolites and the activation of the cardiopulmonary baroreflex (31, 36, 37), all of which promote vasodilation. As a result, blood returns to venous pooling. BP rebounds seven seconds after standing due to sympathetically mediated vasoconstriction, in response to the BP fall and the subsequent unloading of the baroreceptors (2, 3). Despite the change in HR, stroke volume remains relatively constant (37), leading to a substantial increase in cardiac output (32). From the interplay of these peripheral regulatory mechanisms one would expect that oscillations in autonomic nervous system activity, cardiac output and pulse BP would also contribute to the changes in CBFV, independently of the main influence of mean BP, which could distort estimates of CA, in comparison with other physiological maneuvers, or the use of spontaneous fluctuations at rest.

The two different SSMs may elicit slightly different physiological responses. The time intervals, either squatting or standing in the RFSSMs, are as short as 2s or as long as 17s. Over intervals of 2s, the baroreflex has only just begun to activate, as it acts after a delay of 1s (22). Also, as

BP is known to recover after 7s in the standing position (2, 3), shorter intervals between squats will prevent it from doing so prior to the next maneuver. Furthermore, it is possible that longer durations in the squatting position elicit greater increases in BP; a study that asked participants to squat for 2 minutes found that MAP climbed constantly during this maneuver (11). Together, these differences may place a challenge on the cerebrovasculature that is unique to RF maneuvers.

The variety of duration in the RFSSMs also limits the potential for 'entrainment', a known phenomenon by which periodic stimuli elicit a periodic response, of the same frequency, in a particular physiological parameter (13, 38). By varying the duration of the SSMs we restrict this phenomenon, and the likelihood of exogenous influences contributing to the CBFV variations with the same frequency as in the case of FFSSMs.

Clinical implications

Of considerable relevance, is the feasibility of using SSMs in patients with different degrees of disability. Our population was young and fit, with a mean age of 23.0 ± 4.9 years. In an older cohort, Oudegeest-Sander (16), attempted to use a single SSM to assess dCA and cerebrovascular CO_2 reactivity. Only nine of their eighteen subjects in the very elderly group (78 ± 3 years) were able to perform the maneuver due to various co-morbidities. However, all 20 of their older age group (66 ± 1 years) were able to complete the maneuver (16). In another study, all 8 elderly subjects (66 ± 6 years) were able to complete the maneuvers (40). FFSSMs have also been performed successfully in cohorts of heart transplant patients (28), in subjects with Alzheimer's disease (5), during pharmacological interventions (30) and in subjects at high altitude (27). A potential way of limiting the physical demands on the patient was successfully trialled by Zhang (40), who used a pulley system attached to a hoist to passively move subjects from squatting to standing. These passive maneuvers were associated with a reduction in the magnitude of BP oscillations compared to active SSMs, but the estimates of transfer function between the passive and active SSMs did not change (40). Future work is needed to assess the feasibility of RFSSMs, either active or passive, in a patient population.

Study limitations

TCD is used to provide a surrogate measure of CBF, under the assumption that the diameter of the MCA is constant. As CBFV for a given flow is inversely proportional to the cross-sectional area of a vessel, a significant change in diameter will change CBFV regardless of any true change in CBF. Traditional studies considering the impact of $PaCO_2$ on the diameter of the MCA have generally shown no significant effects when arterial gases are in normal ranges (39). In this present study, mean end-tidal CO_2 values for the four recordings were within 3.3mmHg of each other and did not reach the levels where changes in MCA diameter would be expected. Due to the variation in the duration of the SSM during RFSSMs, subjects were placed under varying exertion throughout the recordings, which led to variable $EtCO_2$ during these maneuvers in some subjects. Despite this variation, $EtCO_2$ values for the FFSSMs and RFSSMs were within 1mmHg of each other. It is therefore unlikely that $EtCO_2$ contributed to physiological differences between FF and RFSSMs.

SSMs constitute aerobic exercise, and it is known that brain activation increases during exercise in order to plan and execute motor skills (14). The variety in RFSSMs may require more focus on the part of the participant, so it is possible that this could have an impact, albeit small, on regional CBF.

T-tests were performed on TFA metrics to determine whether any differences were present between the two hemispheres. No significant differences were noted except for VLF gain in the standing recording ($p=0.007$), LF gain in the sitting recording ($p=0.04$) and LF phase during RFSSMs ($p=0.04$). In our analysis the TFA metrics from the two hemispheres were averaged irrespective of these findings, in the context of overall agreement between the two sides. It is unlikely that this has any significant bearing on the results.

Conclusion

RFSSMs were found to be less physically demanding by healthy volunteers, in comparison with SSM at a fixed 0.05 Hz frequency, whilst maintaining the improvements in signal quality and parameter reliability of FFSSMs. RFSSMs produce broader spectral changes in the induced changes in BP and CBFV, allowing TFA estimates of gain and phase that can be compared with the approach adopted in the analysis of dCA estimates from spontaneous fluctuations at rest. We therefore propose that RFSSMs could be a convenient alternative to FFSSMs as a tool to assess dCA. Further work is needed to validate this approach in older subjects, as well as in patients with different cerebrovascular conditions.

Disclosure/Conflict of Interest

The authors declare no conflict of interest.

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Figure captions

Figure 1: Representative changes in end-tidal CO_2 (A), heart rate (B), mean arterial pressure (C), cerebral blood flow velocity (D) and the angle of the thigh to the horizontal (E) in response to a fixed-frequency squat-stand maneuver in a 22-year-old male.

Figure 2: Representative changes in end-tidal CO_2 (A), heart rate (B), mean arterial pressure (C), cerebral blood flow velocity (D) and the angle of the thigh to the horizontal (E) in response to a random-frequency squat-stand maneuver in a 22-year-old male.

Figure 3: Population mean ± SD coherence across four recordings. VLF coherence is represented for sitting, standing and RFSSMs. For FFSSMs coherence was calculated as point estimates at 0.05Hz.

Figure 4: Estimates of autoregulation index across two visits for each of the four recordings. Sitting (open circle), standing (open square), fixed-frequency squat-stand maneuver (block circle), random-frequency squat-stand maneuver (block triangle). Data on the left represent mean values from the first visit, data on the right represent mean values from the second visit. Error bars give 95% confidence interval.

Figure 5: Population average squat angle in relation to horizontal for 20 subjects. In each case all 15 squat-stand changes were averaged within subjects, prior to calculation of the inter-subject mean (continuous line) and standard deviation (dashed line). Squat started at t = 19.5 s.

Tables

Table 1: Baseline values according to posture.

Protocol	Sitting	Standing	P-value
Right MCA CBFV ($cm.s^{-1}$)	57.8 ± 12.7	52.6 ± 12.4	< 0.01
Left MCA CBFV ($cm.s^{-1}$)	55.9 ± 12.6	51.7 ± 12.1	<0.01
Mean arterial pressure (mmHg)	88.3 ± 10.0	91.2 ± 9.9	0.19
Systolic blood pressure (mmHg)	123.1 ± 17.4	121.0 ± 12.2	0.51
Diastolic blood pressure (mmHg)	75.1 ± 9.3	79.8 ± 10.7	0.07
Heart rate (bpm)	73.8 ± 8.9	88.3 ± 12.0	<0.01
End-tidal CO_2 (mmHg)	38.7 ± 3.0	36.0 ± 3.0	<0.01

Values are mean ± SD. MCA, middle cerebral artery; CBFV, cerebral blood flow velocity. P value for paired t-tests.

Table 2: Transfer function analysis parameters and autoregulation index

Parameter	Sitting	Standing	FFSSMs	RFSSMs	P-value
ARI	5.6 ± 1.1	6.2 ± 1.0	5.2 ± 1.4†	5.4 ± 1.2†	0.002
Coherence VLF	0.38 ± 0.18$^{\ddagger, \S}$	0.33 ± 0.09$^{\ddagger, \S}$	0.96 ± 0.02	0.86 ± 0.05‡	<0.001
Coherence LF	0.75 ± 0.16§	0.79 ± 0.13§	-	0.89 ± 0.06	<0.001
Coherence HF	0.70 ± 0.16	0.64 ± 0.15§	-	0.76 ± 0.11	0.015
Gain VLF ($cm.s^{-1}.mmHg$)	0.60 ± 0.29$^{\ddagger, \S}$	0.69 ± 0.18§	0.96 ± 0.52	1.11 ± 0.55	<0.001
Gain LF ($cm.s^{-1}.mmHg$)	1.21 ± 0.44§	1.15 ± 0.48§	-	1.53 ± 0.66	0.009
Gain HF ($cm.s^{-1}.mmHg$)	1.42 ± 0.50	1.25 ± 0.50	-	1.44 ± 0.69	0.296
Phase VLF (radians)	0.97 ± 0.40†	1.18 ± 0.39	0.71 ± 0.22*,†	0.72 ± 0.21*,†	<0.001
Phase LF (radians)	0.59 ± 0.16	0.59 ± 0.18	-	0.43 ± 0.18*,†	<0.001
Phase HF (radians)	0.08 ± 0.14	0.01 ± 0.16 §	-	0.12 ± 0.16	0.048

Values are mean ± SD. ARI, autoregulation index; SSM, squat-stand manoeuvre; FF, fixed frequency; RF, random frequency; VLF: very low frequency; LF: low frequency; HF: high frequency. P value from ANOVA F-test. Post-hoc Tukey tests used to further compare between recordings. At VLF, FFSSMs were assessed at the driven frequency of 0.05Hz. All other recordings were assessed from values elicited from the VLF band. *= Reduced compared to sitting, $p<0.05$. † = Reduced compared to standing, $p<0.05$. ‡ = Reduced compared to FFSSMs, $p<0.05$. § = Reduced compared to RFSSMs, $p<0.05$

Table 3: Reproducibility of transfer function analysis parameters and autoregulation index across two visits

	Visit 1				Visit 2				
Parameter	Sitting	Standing	Fixed	Random	Sitting	Standing	Fixed	Random	P-value
ARI	5.6 ± 1.2	5.9 ± 1.0	5.1 ± 1.4	5.1 ± 1.1	6.0 ± 1.2	5.8 ± 1.4	5.0 ± 1.2	5.0 ± 1.1	0.645
Coherence VLF	0.38 ± 0.18	0.32 ± 0.10	0.96 ± 0.03	0.84 ± 0.04	0.32 ± 0.19	0.32 ± 0.11	0.97 ± 0.01	0.86 ± 0.06	0.431
Coherence LF	0.76 ± 0.11	0.80 ± 0.08	-	0.89 ± 0.05	0.68 ± 0.12	0.80 ± 0.11	-	0.88 ± 0.08	0.233
Coherence HF	0.72 ± 0.11	0.67 ± 0.14	-	0.77 ± 0.09	0.60 ± 0.17	0.65 ± 0.12	-	0.78 ± 0.13	0.201
Gain VLF ($cm.s^{-1}.mmHg$)	0.59 ± 0.23	0.61 ± 0.34	0.93 ± 0.35	1.01 ± 0.46	0.59 ± 0.31	1.02 ± 0.56	1.00 ± 0.37	1.21 ± 0.46	0.084
Gain LF ($cm.s^{-1}.mmHg$)	1.16 ± 0.31	1.22 ± 0.54	-	1.42 ± 0.47	1.25 ± 0.71	2.06 ± 1.36	-	1.77 ± 0.73*	0.033
Gain HF ($cm.s^{-1}.mmHg$)	1.40 ± 0.34	1.30 ± 0.52	-	1.32 ± 0.42	1.37 ± 0.70	2.03 ± 1.25	-	1.63 ± 0.71*	0.043
Phase VLF (radians)	1.01 ± 0.45	1.14 ± 0.35	0.69 ± 0.24	0.69 ± 0.23	1.27 ± 0.54	1.13 ± 0.48	0.68 ± 0.21	0.67 ± 0.18	0.233
Phase LF (radians)	0.63 ± 0.17	0.59 ± 0.16	-	0.37 ± 0.14	0.61 ± 0.15	0.58 ± 0.18	-	0.40 ± 0.12	0.506
Phase HF (radians)	0.09 ± 0.07	-0.02 ± 0.10	-	0.09 ± 0.11	-0.02 ± 0.22	-0.03 ± 0.11	-	-0.02 ± 0.17	0.403

Data are given as mean ± SD. ARI: autoregulation index; VLF: very low frequency; LF: low frequency; HF: high frequency.

P value from ANOVA F-test. Values in the VLF column are sampled at 0.05Hz for FFSSMs only. At VLF, FFSSMs were assessed at the driven frequency of 0.05Hz. All other recordings were assessed from values elicited from the VLF band.

Table 4: Intraclass correlation coefficients for transfer function analysis parameters and autoregulation index*

	Sitting	Standing	FFSSMs	RFSSMs
ARI	0.50	0.39	0.83	0.75
VLF coherence	0.55	0.27	0.30	0.57
LF coherence	0.45	0.47	-	0.19
VLF gain	0.23	0	0	0.25
LF gain	0	0.02	-	0.10
VLF phase	0.40	0.37	0.93	0.80
LF phase	0.85	0.86	-	0.81

ARI, autoregulation index; VLF, very low frequency; LF, low frequency; HF, high frequency; SSM, squat-stand manoeuvre; FF, fixed frequency; RF, random frequency. At VLF, FFSSMs were assessed at the driven frequency of 0.05Hz. All other recordings were assessed from values elicited from the VLF band.

*Values for HF are not reported, as the HF band does not lie in the autoregulatory range.

Table 5: Coefficients of variation (%) for transfer function analysis parameters and autoregulation index*

	Sitting	Standing	FFSSMs	RFSSMs
ARI	4.5 ± 6.0	5.7 ± 7.3	3.0 ± 3.5	4.7 ± 3.1
VLF coherence	17.5 ± 13.8	13.7 ± 8.5	0.6 ± 0.8	1.7 ± 1.2
LF coherence	4.5 ± 4.7	3.3 ± 3.2	-	2.2 ± 2.5
VLF gain	17.3 ± 12.4	22.3 ± 13.9	14.1 ± 12.1	11.2 ± 13.1
LF gain	13.1 ± 10.4	17.5 ± 13.5	-	11.6 ± 10.9
VLF phase	13.4 ± 9.2	12.1 ± 9.4	3.6 ± 2.4	6.2 ± 4.0
LF phase	4.0 ± 2.7	5.4 ± 4.5	-	6.7 ± 5.0

ARI, autoregulation index; VLF, very low frequency; LF, low frequency; HF, high frequency; SSM, squat-stand manoeuvre; FF, fixed frequency; RF, random frequency. At VLF, FFSSMs were assessed at the driven frequency of 0.05Hz. All other recordings were assessed from values elicited from the VLF band.

*Values for HF are not reported, as the HF band does not lie in the autoregulatory range.