The cerebro-cardiovascular response to periodic squat-stand maneuvers in healthy subjects: a time-domain analysis

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Abstract

Background: Squat-stand maneuvers (SSMs) have been used to improve the coherence of transfer function analysis (TFA) estimates during the assessment of dynamic cerebral autoregulation (dCA). There is a need to understand the influence of peripheral changes resulting from SSMs on cerebral blood flow, which might confound estimates of dCA.

Methods: 29 healthy subjects underwent recordings at rest (5 min standing) and 15 SSMs (0.05Hz). Heart rate (3-lead ECG), end-tidal CO₂ (capnography), blood pressure (Finometer), cerebral blood velocity (CBV, transcranial Doppler, MCA) and the angle of the thigh (tilt sensor) were measured continuously. The response of CBV to SSMs was decomposed into the relative contributions of mean arterial pressure (MAP), resistance area product (RAP) and critical closing pressure (CrCP).

Results: Upon squatting, a rise in MAP (83.6 ± 21.1 % contribution) is followed by increased CBV. A dCA response can be detected, determined by adjustments in RAP and CrCP (left hemisphere) with peak contributions of 24.8 ± 12.7 % and 27.4 ± 22.8 %, respectively, at different times during SSMs. No interhemispheric differences were detected. During standing, the contributions of MAP, RAP and CrCP change considerably.

Conclusions: The changes of CBV subcomponents during repeated SSMs indicate a complex response of CBV to SSMs that can only be partially explained by myogenic mechanisms. More work is needed to clarify the potential contribution of other co-factors, such as breath-to-breath changes in pCO₂, HR, stroke volume and the neurogenic component of dynamic CA.

New and Noteworthy

We describe the different contributions to the cerebral blood flow response following squat-stand maneuvers, and furthermore we demonstrate the complex interaction of peripheral and cerebral parameters for the first time. Moreover, we show that the CBV response to squatting is likely to include a significant metabolic component.

Keywords

cerebral autoregulation, cerebral blood flow, cerebral haemodynamics, critical closing pressure, transcranial Doppler.

Introduction

Dynamic cerebral autoregulation (dCA) was first proposed by Aaslid, who used thigh-cuff deflation to induce step changes in mean arterial pressure (MAP) (2). Subsequently, it was demonstrated that dCA could also be assessed using the spontaneous fluctuations in blood pressure (BP) that occur naturally at rest, using transfer function analysis (TFA) (26, 28, 50). However, concerns about the low levels of coherence reported in these studies, and the limited reproducibility of estimated parameters led to a search for methods that could induce changes in MAP larger than those occurring spontaneously (50). Proposed approaches included periodic breathing (13), oscillatory lower-body negative pressure (3), the Valsalva manoeuvre (42) and the cold-pressor test (23), with each technique having advantages and disadvantages in terms of feasibility, tolerability, and efficacy.

Recently, repeated squat-stand maneuvers (SSMs) have also been proposed as a suitable approach to assess dCA due to large changes in MAP induced by the manoeuvre (10, 37). SSMs have been used to induce BP oscillations at a variety of frequencies (4, 10, 35-38, 48), and have been verified as a safe, effective and reliable method of altering BP for the assessment of dCA that can improve the reproducibility of autoregulatory indices (37). However, whilst much has been written about the various physiological mechanisms that mediate the physiological response to the SSM (20, 24, 32, 34), the influence of concurrent peripheral and cerebrovascular physiological changes induced by the SSM on estimates of dCA have not been described. In particular, the extent to which the dynamic relationship between MAP and cerebral blood flow (CBF), usually estimated as CBF velocity (CBV) with transcranial Doppler (TCD) (27), truly reflects myogenic regulatory control of CBF has not been investigated. Analysis of SSM recordings is usually performed in the frequency domain, based on TFA (26), which does not allow for the identification of the peripheral and cerebrovascular influences on CBF. Examples of possible sources of interference would be the baroreflex, autonomic nervous system activity, the muscle mechanoreflex, and changes in breathing frequency and corresponding $p\text{CO}_2$ levels.

To our knowledge, no study has considered the continuous response of both the cerebrovasculature and the cardiovascular system to SSMs, and no study has considered the response of CBV subcomponents to SSMs. The aims of this study were to quantify the influence of the SSM on cerebrovascular parameters, and to determine the extent to which the CBF response to the SSM can be considered as purely myogenic. Using a time domain analysis of the subcomponents that could influence CBV during SSMs, we tested the hypothesis that the MAP-CBV relationship does not represent a purely myogenic response of the cerebral circulation.

Methods and materials

This study was based on a sub-set of data, collected between September 2016 and January 2017 that were part of a wider study. For the purposes of the present study, we have focused on recordings performed in healthy subjects standing and performing SSM at a frequency of 0.05 Hz.

Ethical approval

Ethical approval was obtained from the University of Leicester (Ref: vjh-8442) and all participants provided written consent. Our inclusion criteria were as follows; male or female aged 18 years or over who were able and willing to comply with all study requirements and had given their informed consent. Females who were pregnant or lactating were excluded.

Instrumentation

CBV was recorded bilaterally with TCD (Viasys Companion III) in the middle cerebral artery (MCA), using 2MHz probes held in place using a head-frame. Heart rate (HR) was recorded using 3-lead ECG, end-tidal CO₂ via nasal capnography, (Salter Labs, ref. 4000; Capnocheck Plus) and continuous beat-to-beat blood pressure (BP) using arterial volume-clamping of the digital artery on the left hand (Finometer, Finapres Medical Systems; Amsterdam, the Netherlands). The hand was held in position with a sling to minimise movement throughout the recordings, and the servo-reset mechanism was disabled throughout the recordings to allow for a continuous BP trace, but enabled between recordings. The brachial BP (UA767

BP monitor) of the dominant arm was measured prior to each recording for calibration of the Finometer device. A tilt-sensor (QG-KI-090AI-K, DIS Sensors, Oostergacht, The Netherlands) was attached to the subject's right thigh with a Velcro strap 20cm above the superior border of the patella to measure the angle of the squatting motion.

Data from the above parameters were simultaneously recorded onto the Physiological Data Acquisition System (PHYSIDAS) designed by the Leicester Medical Physics group for subsequent offline analysis.

Experimental procedures

The protocol took place in the Cerebral Haemodynamics in Ageing and Stroke Medicine (CHIASM) laboratory at the Leicester Royal Infirmary, which is of controlled temperature, well-lit, and free from distraction. Participants were asked to avoid strenuous exercise, caffeine, smoking, large meals and alcohol in the four hours prior to their visit.

Once the participant was prepared and satisfactory signals were obtained from all instruments, a 5 min recording was taken with the subject standing quietly with their eyes open. This was followed by 15 repeated SSMs at a frequency of 0.05 Hz, preceded and followed by 90 seconds standing. A computer program provided the participant with visual cues to guide the timing of the maneuvers.

Each recording was made with minimal background distraction to minimise the effects of auditory stimulation on CBV. A period of instruction and practice preceded the second recording, during which the SSM was demonstrated. When performing 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.

Data processing

The readings from the Finometer were calibrated to the brachial BP recordings. Data were visually inspected; non-physiological spikes in CBV 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 end of each cardiac cycle were detected in the BP signal, and mean values of BP, CBV and HR were obtained for each heartbeat. The critical closing pressure (CrCP) and resistance-area product (RAP) were estimated with the first harmonic method (25). Beat-to-beat parameters were interpolated with a third-order polynomial and resampled at 5 Hz to generate signals with a uniform time base.

The moment at which each subject initiated their 15 SSMs was marked manually during data editing, using a custom-designed computer program. Intra-, followed by inter-subject, averaging generated the population coherent average SSM for the cohort, as well as the inter-subject standard deviation (SD) for each instant of time, synchronised by the beginning of the squat manoeuvre. To quantify the CBV response to SSM, we employed sub-component analysis as described below.

Subcomponent analysis

Similar to the lung, coronary and other circulations, the cerebral circulation shows the existence of a CrCP, as the perfusion pressure where blood flow stops (8, 12, 14, 25). To account for the existence of a CrCP>0, instantaneous pressure-flow (or velocity) relationships can be expressed as:

$$V_0 = \frac{MAP_0 - CrCP_0}{RAP_0} \quad [1]$$

where RAP describes the resistance-area product (15) and the subscript '0' indicates the operating point at baseline.

According to equation [1], changes in CBV can be explained by corresponding changes in its subcomponents, that is BP, CrCP or RAP. Assuming that a change in BP will lead to

accompanying changes in CrCP, RAP, and hence CBV, it is possible to demonstrate that the percent change in CBV can be expressed as the corresponding percent changes in its subcomponents (30), that is:

$$V_0 + \Delta v = \frac{(MAP_0 + \Delta p) - (CrCP_0 + \Delta c)}{(RAP_0 + \Delta r)} \quad [2]$$

where Δv , Δp , Δc and Δr represent small changes in CBV, MAP, CrCP and RAP, respectively.

Expanding and simplifying [2] gives:

$$\Delta v = \frac{\Delta p - \Delta c - V_0 \Delta r}{RAP_0 + \Delta r} \quad [3]$$

Dividing through by V_0 , to calculate the relative magnitude of Δv , yields:

$$\frac{\Delta v}{V_0} = \frac{\Delta p - \Delta c - V_0 \Delta r}{V_0 (RAP_0 + \Delta r)} \quad [4]$$

Thus the contribution of each subcomponent to Δv is defined as:

$$V_{MAP} = \frac{\Delta p}{V_0 (RAP_0 + \Delta r)} \quad [5]$$

$$V_{CrCP} = -\frac{\Delta c}{V_0 (RAP_0 + \Delta r)} \quad [6]$$

$$V_{RAP} = -\frac{\Delta r}{(RAP_0 + \Delta r)} \quad [7]$$

Where

$$\Delta v = V_{MAP} + V_{CrCP} + V_{RAP} \quad [8]$$

Values of V_0 and RAP_0 used for normalisation (eq. 1) were extracted at the beginning of each squat manoeuvre, to take into account the dynamic nature of the baseline period (Fig. 1). In most applications, $\Delta r \ll R_0$, thus making V_{MAP} and V_{CrCP} independent of Δr .

For the interpretation of the V_{CrCP} and V_{RAP} terms, it is important to note the negative sign in eqs. [6] & [7] leads to a positive contribution to ΔV when both RAP and CrCP decrease. Conversely, when these parameters increase, they will induce reductions in CBV. Furthermore, previous work suggests that the two subcomponents represent different parts of the dCA response. V_{CrCP} is thought to be representative of the metabolic component of dCA,

and V_{RAP} may reflect the myogenic component (29). These presumed associations will be further addressed in the Discussion.

The utility of subcomponent analysis has been previously demonstrated for physiological studies, as well as for the interpretation of cerebrovascular alterations in patients with clinical conditions including stroke and pre-eclampsia (22, 29, 30, 33, 44).

Statistical analysis

Data are given as means $\pm$ SD. Paired Student's t-tests were used to compare data from the left and right brain hemispheres, and between the two recordings. Significance was set at $p < 0.05$.

Results

Twenty nine healthy volunteers (nineteen male, mean age 23.0 ± 4.9 years) were recruited from University of Leicester staff and students. Of these, 9 were rejected due to poor quality TCD waveforms (4/9) or disruption of signal during SSMs (5/9). The Velcro thigh-cuff was disrupted in four subjects of the remaining twenty subjects towards the end of the SSMs. Without a reliable marker of the beginning of each squat, it was not possible to produce synchronised averaging of the variables under analysis, thus rendering their data unusable and leaving sixteen data sets for subcomponent analysis.

No significant differences were noted in mean, systolic, or diastolic CBV between the two hemispheres. Mean values across the two recordings are detailed in Table 1. With the exception of diastolic BP, all other haemodynamic parameters showed significant differences between the standing and squatting recordings.

Subcomponent analysis

In the population coherent averages, $t=0s$ marked the beginning of the squat manoeuvre, whilst $t=10s$ represented the return to the standing position.

Fig. 1 illustrates the large changes induced by SSM in both peripheral and cerebrovascular parameters. Population coherence averages of peripheral parameters (Fig. 2) show substantial changes in MAP, HR, pulse pressure and end-tidal CO₂ following the squat phase, and return to the original values with standing. HR demonstrated an asymmetrical response. Following a rapid drop in response to the rise in MAP, HR started to return to its original value five seconds into the squat position, but with a much slower rate of rise than observed for the sharp drop at the beginning of the squat. Pulse pressure decreased immediately following the SSM, but then increased before decreasing again while the subject was in the squat position (Fig. 2B). As the subject stood pulse pressure increased sharply for four seconds before reducing down towards baseline.

Bilateral cerebral parameters did not show interhemispheric differences as demonstrated in Fig. 3. For this reason, subsequent graphical results for sub-component analysis will refer only to the changes in the left hemisphere, since the majority of our subjects (14/16) were right handed as assessed by the Edinburgh Handedness Inventory.

With the beginning of the squat manoeuvre (Fig. 4, t=0), both V_{MAP} and ΔV increased rapidly, but whilst V_{MAP} remained approximately stable, ΔV showed a marked trend to return to its original value, thus providing a visual indication of an autoregulatory response. These changes were reversed when subjects returned to the standing position (Fig. 4, t=10 s). Both V_{RAP} and V_{CrCP} contributed towards reducing ΔV following squatting (Fig. 4B & D). Although both sub-components had a delay compared to the V_{MAP} change, V_{CrCP} showed an asymmetrical response when subjects returned to the standing position with a further drop and a much slower return to baseline than V_{RAP} . Table 2 presents mean $\pm$ SD values for the subcomponents every five seconds along the averaged 20 s squat-stand cycle for both hemispheres.

Discussion

SSMs have been employed in multiple studies of dynamic CA, in both healthy subjects and patients with clinical conditions (9, 36, 37). To our knowledge, this is the first study to provide

a time-domain analysis of the manoeuvre, describing the different contributions to the CBV response and their complex interaction with peripheral and cerebral parameters. We have shown that the temporal pattern of the CBV response confirms the presence of active dCA in healthy subjects. This substantiates the more common analytic approach of TFA which, despite providing estimates of gain, phase, and significantly enhanced coherence, could represent spurious values resulting from multiple peripheral and cerebrovascular co-factors varying in synchrony with the frequency of SSM. Moreover, we also demonstrated that the CBV response to squatting is likely to include a significant metabolic component (as measured by V_{CrCP}), in addition to the myogenic activation (as measured by V_{RAP}) in response to the sudden rise in BP when subjects squat (29). This interpretation of the CrCP and RAP changes during SSMs will be discussed below.

The physiology of the SSM has previously been described; as the subject squats, contraction of the leg muscles depletes venous pooling, causing an increase in the effective circulating volume (34). This increase in venous return increases end-diastolic volume and consequently stroke volume, thus increasing BP and CBF (21). The muscle mechanoreflex also contributes to the resultant increase in MAP and CBF (48). Meanwhile, the baroreflex responds to the increased BP by decreasing HR (48). Furthermore, the constriction of vessels by the musculature induces a degree of peripheral ischaemia, predisposing to vasodilation once the subject stands (32, 41). Upon subsequently standing, BP falls. Total peripheral resistance is low because of the removal of the muscle pump, the release of vasodilatory metabolites during the squat and the activation of the cardiopulmonary baroreflex in response to a sudden increase in the volume of blood reaching the heart (40, 45, 46), all of which promote vasodilation. As a result, blood returns to venous pooling. Simultaneously, HR increases due to decreased vagal tone (6, 16, 18, 32). A second, more gradual, increase in HR occurs between five and twelve seconds (46). BP rebounds seven seconds after standing due to sympathetically mediated vasoconstriction, in response to the fall in blood pressure and the subsequent unloading of the baroreceptors (5, 6).

Subcomponent analysis

Subcomponent analysis allows for the identification of the relative contribution of the different influences on CBV throughout SSMs. Previously, subcomponent analysis has been used to assess changes in CBV in response to other stimuli including mental activation (30) and breath holding (44). Previous work has led to a hypothetical association between changes in RAP as representing the myogenic response, whilst CrCP would express metabolically driven changes in CBF (22, 25, 29, 30, 33, 44). Confirmation of the validity and generalizability of these associations will require more fundamental experimental work. For this reason, caution must be exercised when interpreting our results and our simplistic use of these presumed associations throughout this paper.

Our results demonstrate that V_{MAP} increases significantly as the subject squats. This is to be expected, as the change in CBV associated with the SSM is thought to be caused by the large BP oscillations elicited (10). RAP increases throughout the squat as the cerebrovasculature attempts to protect the brain from the increased flow by increasing resistance, and therefore V_{RAP} decreases. Similarly, V_{CrCP} decreases when the squat manoeuvre is performed, following the rise in CrCP in real terms during this time. This suggests that the strongest influence of the change in CBV during the SSM is MAP, which overwhelms the metabolic (as measured by CrCP) and myogenic (as measured by RAP) autoregulatory processes to be the driving force behind the increased CBV (29). If RAP represents the myogenic response, and if the response of CBV to the SSM is purely myogenic, RAP should explain the drop in ΔV starting three seconds into the squat phase (Fig. 4). As shown in Fig. 5 though, the contribution of V_{RAP} is not enough to explain the reduction in ΔV and according to Eq. [8], the difference should be ascribed to the contribution of V_{CrCP} .

Previous studies employing healthy controls have found the magnitude of V_{MAP} to be significantly reduced compared to the current study (30). These studies employed cognitive paradigms, motor paradigms (33) and breath holding (44) to alter CBV. In these studies, V_{RAP} and V_{CrCP} are of a similar magnitude to V_{MAP} , if not greater. However, in the current study the

magnitude of V_{MAP} was much greater than in the previous studies, and much greater than the magnitude of V_{RAP} and V_{CrCP} . Whilst this is not surprising in the context of repeated SSMs when the underlying physiology is considered, it is nonetheless a novel finding in the field of subcomponent analysis.

Fig. 4 demonstrates the response of CBV and its subcomponents to the SSM. By comparing Figs. 4A and 4C, we see that CBV returns towards baseline faster than MAP. This may simply illustrate dynamic CA, although it is possible that there are external influences on the response of CBV. End-tidal CO_2 , which climbs during the performance of the squat (Fig. 2D) is known to reduce CrCP, thus increasing V_{CrCP} (25). This is just one example of physiological parameters interfering with the subcomponent response; it is possible that other mechanisms, such as activation of the sympathetic nervous system, cause vasoconstriction to decrease V_{CrCP} during the performance of the squat. Furthermore, the asymmetry of V_{CrCP} (Fig. 4D) when the subject stands suggests the existence of other external influences on the CBV response. Without these external influences, which may include the muscle mechanoreflex and the activation of the sympathetic nervous system amongst others, it is possible that we may see CBV return towards baseline faster or slower than seen currently. Further work is required to verify this, which may involve autonomic blockade to eradicate the influence of the autonomic nervous system on the CBV response. Previous studies employing autonomic blockade have shown that this impairs the autoregulatory response (49, 51). We might therefore expect a reduction in the magnitude of V_{CrCP} and V_{RAP} and an increase in the magnitude of ΔV in such subjects. To our knowledge, the subcomponent response to autonomic blockade has yet to be ascertained; this provides an exciting opportunity for future work.

One input into the complex CrCP response may be a partial Valsalva manoeuvre, which was noted in some participants upon visual inspection of the data. Dawson et al. found CrCP decreased sharply (hence V_{CrCP} increased) during phase IV of the Valsalva manoeuvre. This occurs after the strain has been released (11), which may parallel the release of strain after a prolonged squat. At this point, V_{CrCP} would be expected to increase in response, but as shown

in Fig. 4D, V_{CrCP} transiently decreases as the subject stands. This transient 'dip' was observed in 14 of the 16 subjects. The reason for this is unclear, but it might have been caused by the complex interaction of multiple co-factors, including the transient increase in $EtCO_2$ (Fig. 2D) and cerebral vasoconstriction due to sympathetic activity.

Oscillations in BP and CBV

Previous studies employing the SSM have focussed more on TFA and the signal power of the BP-CBV relationship, rather than the relevance and interpretation of the physiological response (9, 10, 35, 37, 38). Only one study has reported both the change in MAP and CBV during the performance of periodic SSMs (7). Brassard et al reported a change in CBV during the SSM (0.05Hz) as $14 \pm 9 \text{ cm.s}^{-1}$ during squatting, and $-17 \pm 9 \text{ cm.s}^{-1}$ upon standing. In our study, the mean changes in CBV were $+27.9 \text{ cm.s}^{-1}$ and -20.3 cm.s^{-1} , respectively. The change noted during hypotension is comparable, but in our subjects CBV increased to a much greater degree during the performance of the squat, suggesting that autoregulatory mechanisms during hypotension are more effective than during hypertension. This directly contradicts the findings of Brassard and the findings of other studies assessing the phenomenon of 'hysteresis', which have all found dCA to be more effective in hypertension than hypotension (1, 7, 43).

Brassard found MAP to fluctuate by $21 \pm 12 \text{ mmHg}$ and $-20 \pm 11 \text{ mmHg}$ during repeated maneuvers at 0.05Hz (7) while Querido reported a maximum change of $+15 \text{ mmHg}$ and -13 mmHg during the squat and stand, respectively, but did not report an average oscillation (31). In our study, MAP increased by 35.0 mmHg during the performance of the squat, and decreased by 36.4 mmHg when the subject stood; we also found a relatively symmetrical response to the SSM, but of larger magnitude.

The discrepancy in the direction of hysteresis is most likely due to the reference values used to calculate the magnitude of the oscillations. Brassard used mean values from a separate resting recording of subjects sitting as the reference from which oscillation magnitudes were

calculated (7). However, we believe that using a separate resting period as the reference point is misleading. Prior to the subject standing ($t=10$), BP is greatly elevated compared to mean BP at rest. Therefore, when the subject stands, the true size of the oscillation induced is much greater than that calculated when using resting BP as a reference point. This leads us to question which reference values are more reliable to represent the physiological changes taking place during SSMs. In the case of SSMs, we believe using values immediately prior to the maneuvers is more valid than using a separate control recording as they represent the challenge to the cerebrovasculature at that very moment.

Responses of cardiovascular parameters

The temporal response of MAP to the SSM closely mimics that described by the literature. Classically, MAP is described as decreasing sharply upon standing, and then increasing seven seconds later as the sympathetic nervous system initiates vasoconstriction (32, 46). This pattern correlated very closely with the findings of our study.

The observed overall decrease in HR following the performance of the squat is most likely mediated through the baroreflex (24). Shortly following the performance of the SSM, HR increases slightly despite MAP increasing simultaneously, a finding also noted in the literature (24). This may be due to an 'exercise effect', whereby contraction of the leg muscles creates abrupt vagal inhibition, leading to an increase in HR (5). In the literature this exercise reflex is considered when the subject stands up from a single prolonged squat, but it is known that the muscular effort of changing posture elicits the exercise reflex (5, 18), and therefore this mechanism is applicable to our repeated maneuvers. An anticipatory mechanism may also be involved; anticipation of exercise, in this case the performance of a prolonged squat, is known to increase both HR and MAP (39). After this initial increase in HR, the initiation of the baroreflex in response to a substantial increase in MAP overrides the exercise reflex, leading to the overall reduction in HR that we see during the initial portion of the squat.

Before the squat portion of the manoeuvre is complete, HR begins to increase again (Fig. 2). This occurs prior to MAP decreasing towards baseline, which suggests that the deactivation of baroreflex is not responsible for this initial increase. The reason for this is unclear; it is possible that the exercise effect begins to override the baroreflex as the subject starts to struggle with the exertion of the SSM. One study reported a continuously low HR following the performance of a sustained squat (20), another reported a recovery of HR after 60 s of squatting (17), but no literature could be found that described an increase in HR prior to a reduction in BP during the SSM.

However, it is known that BP decreases and HR increases prior to the completion of the Valsalva manoeuvre, due to increased intrathoracic pressure limiting cardiac filling, and therefore compromising cardiac output (42). A similar pattern was seen during the latter part of the squat, which may be due to a partial Valsalva manoeuvre when participants exerted themselves, as described above.

The fluctuating response of pulse pressure during the performance of the squat may be due to concurrent changes in HR and MAP (Fig. 2). The increase in pulse pressure from $t=2-5$ s occurs as HR falls simultaneously. This fall HR allows for increased cardiac filling, and consequently increased stroke volume, to generate a greater pulse pressure. As the subject stands, the initial increase in pulse pressure is most likely caused by decreased afterload,(32) as demonstrated by the simultaneous fall in MAP (Fig. 2). Better understanding of the peripheral changes contributing to the cerebrovascular response to SSM might be gained in future studies by estimating the continuous temporal changes in peripheral resistance or conductance, although this would represent a considerable technical challenge using state-of-the-art techniques for accurate continuous measurement of stroke volume.

The asymmetrical response of end-tidal CO_2 can perhaps be explained by considering the underlying physiology. During the SSM, the contracting muscles of the leg create a closed system, restricting blood flow to the muscle fibres and thus creating a relative anaerobic environment that strongly favours the production of lactic acid (19). This lactic acid is then

converted into CO₂ and exhaled through the respiratory system. As a result, the rate of CO₂ production, and therefore exhalation, during the active portion of the SSM should be greater than during the passive portion, as was found in our study. Another possible explanation is a degree of breath-holding by participants when performing the squat, as noted above. Breath-holding will lead to the accumulation of CO₂, which is released once the subject exhales. The degree to which this contributes to the asymmetry of the CO₂ curve is uncertain, but it is likely to play a role.

Limitations

We used TCD to measure CBV in the MCAs. Using CBV as a surrogate measure for CBF relies on the assumption that the diameter of the MCA remains relatively constant. If the diameter of the MCA were to change, it would be possible for a change in flow to be overestimated in the case of vasoconstriction or underestimated in the case of vasodilation. Traditional studies considering the influence of pCO₂ on the diameter of the MCA have generally shown there to be no significant effect when arterial gases are in normal ranges (47). In this present study, mean end-tidal CO₂ values for the four recordings were within 3.3 mmHg of each other.

In our female volunteers, we have not controlled for the stage of their menstrual cycle and this could potentially be a confounder in the cerebrovascular response to SSM.

Conclusions

In summary, the time-domain analysis of the SSM we performed has confirmed that the CBV response to the manoeuvre reflects activation of dynamic CA following substantial changes in BP as the subject squats and then stands. Both myogenic and metabolic components of the dynamic CA response contribute significantly to the CBV changes observed, but more work is needed to clarify the potential contribution of other co-factors, such as breath-to-breath changes in pCO₂, HR, stroke volume and the neurogenic component of dynamic CA.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1: Representative recordings for a 22-year-old male demonstrating changes in CBV (A), mean arterial pressure (B), heart rate (C), end-tidal CO₂ (D), resistance area product (E) and critical closing pressure (F) during 15 SSMs at 0.05Hz.

Figure 2: Response of peripheral parameters to repeated squatting (t=0 s), followed by standing (t=10 s); A) MAP, B) Pulse pressure, C) Heart rate and D) End-tidal CO₂, The squat angle relative to horizontal (right axis) is represented as dashed line. Error bars are largest ± 1 SE at the point of occurrence.

Figure 3: Population mean change in cerebral blood flow velocity (CBV, A), resistance area product (RAP, B) and critical closing pressure (CrCP, C) in the left (dashed line) and right (continuous line) brain hemispheres during the performance of the SSM. Squatting started at t=0 s, followed by standing at t=10 s.

Figure 4: Response of the CBV subcomponents in the left hemisphere to the SSM. A) ΔV , Cerebral blood flow velocity change from beginning of squat (t=0 s), followed by standing (t=10 s); B) V_{RAP} , % contribution of resistance area product to ΔCBV ; C) V_{MAP} , % contribution of mean arterial pressure to ΔCBV ; D) V_{CrCP} , % contribution of critical closing pressure to ΔCBV . Error bars correspond to the largest ± 1 SE at the point of occurrence.

Figure 5: Population mean change in ΔV (left axis, solid line) to squatting (t=0) followed by standing (t=10 s), compared with the average contributions of $V_{MAP}+V_{RAP}$ (right axis, dashed line). See text for interpretation and discussion.

Tables

Table 1: Main physiological parameters during standing and squat-stand maneuvers.

Protocol	Standing	SSMs	P-value
Right MCA CBV (cm.s ⁻¹)	52.6 ± 12.4	58.4 ± 12.3	<0.01
Left MCA CBV (cm.s ⁻¹)	51.7 ± 12.1	55.8 ± 11.2	<0.01
Mean arterial pressure (mmHg)	91.2 ± 9.9	97.1 ± 11.7	0.04
Systolic blood pressure (mmHg)	121.0 ± 12.2	131.6 ± 16.8	0.02
Diastolic blood pressure (mmHg)	79.8 ± 10.7	83.8 ± 9.3	0.16
Heart rate (bpm)	88.3 ± 12.0	98.1 ± 11.5	<0.01
End-tidal CO ₂ (mmHg)	36.0 ± 3.0	39.3 ± 3.4	<0.01

Values are mean ± SD (n=16) for the entire five min recording. MCA, middle cerebral artery; CBV, cerebral blood velocity; SSM, squat-stand maneuver. P value, paired Student's t-test.

Table 2: CBV change and its components at selected time intervals after activation (t=0)

Time (s)	Right MCA			Left MCA			Blood pressure
	ΔV	V_{RAP}	V_{CrCP}	ΔV	V_{RAP}	V_{CrCP}	V_{MAP}
0	2.3 ± 1.9	-0.4 ± 1.8	0.2 ± 2.4	2.4 ± 2.1	-0.2 ± 2.1	0.0 ± 2.7	2.5 ± 2.3
5	35.7 ± 21.8	-23.6 ± 10.9	-24.6 ± 31.9	36.4 ± 22.1	-20.3 ± 12.6	-26.9 ± 28.0	83.6 ± 21.1
10	12.0 ± 15.7	-27.3 ± 12.8	-26.7 ± 25.8	13.3 ± 14.7	-24.8 ± 12.7	-27.4 ± 22.8	66.2 ± 39.9
15	-20.4 ± 5.1	0.0 ± 9.5	10.1 ± 8.7	-20.8 ± 5.4	0.3 ± 10.4	-10.6 ± 10.3	-10.1 ± 10.2
20	1.3 ± 1.8	2.0 ± 3.3	1.3 ± 3.8	1.6 ± 1.6	2.6 ± 3.3	0.9 ± 2.9	-2.3 ± 5.7

Values are mean $\pm$ SD. CBV, cerebral blood flow velocity. ΔV , change in CBV (%) normalised to absolute values at beginning of squatting.

V_{RAP} , V_{CrCP} , V_{MAP} : % relative contributions of resistance-area product (RAP), critical closing pressure (CrCP) and mean arterial blood pressure (MAP), respectively, to the overall ΔV change.