Utilization of the repeated squat-stand model for studying the directional behavior of the cerebral pressure-flow relationship

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Running title: Hysteresis in the pressure-flow relationship

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Abstract

Hysteresis in the cerebral pressure-flow relationship describes the superior ability of the cerebrovasculature to buffer cerebral blood flow changes when mean arterial pressure (MAP) acutely increases compared to when it decreases. This phenomenon can be evaluated by comparing the relative change in middle cerebral artery mean blood velocity (MCAv) per relative change in MAP (%ΔMCAv/%ΔMAP) during either acute increases or decreases in MAP induced by repeated squat-stands (RSS). However, no real baseline can be employed for this particular protocol as there is no true stable reference point. Herein, we characterized the %ΔMCAv/%ΔMAP metric using the greatest MAP oscillations induced by RSS without using an independent baseline value. We also examined whether %ΔMCAv/%ΔMAP during each RSS transition were comparable between each other over the 5-min period. %ΔMCAv/%ΔMAP was calculated using the minimum to maximum MCAv and MAP for each RSS performed at 0.05 Hz and 0.10 Hz. We compared averaged %ΔMCAv/%ΔMAP during MAP increases and decreases in 74 healthy subjects [9 women; 32 ± 13 years]. %ΔMCAv/%ΔMAP was lower for MAP increases than MAP decreases (0.05 Hz: 1.25 ± 0.22 vs. 1.35 ± 0.27 %/%, $p<0.0001$; 0.10Hz: 1.31 ± 0.24 vs. 1.60 ± 0.50 %/%, $p<0.0001$). For both frequency and MAP direction, time during RSS had no effect on %ΔMCAv/%ΔMAP. This novel analytical method supports the use of the RSS model to evaluate the directional behavior of the pressure-flow relationship. These results contribute to the importance of considering the direction of MAP changes when evaluating dynamic cerebral autoregulation.

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These findings support the use of the repeated squat-stand model to examine the directional behavior of the cerebral pressure-flow relationship. We show this maneuver induces stable physiological cyclic changes and allows for interindividual variability analysis. These results contribute to the importance of considering the direction of mean arterial pressure changes when evaluating dynamic cerebral autoregulation.

Key words

hysteresis, mean arterial pressure, middle cerebral artery blood velocity, squat-stand

Introduction

Dynamic cerebral autoregulation (dCA) defines the ability of the cerebrovasculature to react to rapid blood pressure changes. Asymmetrical responses of cerebral blood flow to transient changes in mean arterial pressure (MAP) have initially been reported using cyclic inflation and deflation of thigh cuffs in patients with head injury (1) and pharmacological interventions in healthy subjects (2). Specifically, the augmentation in cerebral blood flow was shown to be considerably attenuated when MAP increases compared to the decline in cerebral blood flow when MAP decreases (2,3). Since the brain is located in the skull, an inflexible structure, this hysteresis-like pattern is thought to serve as a protective mechanism against blood pressure surges, the cerebrovasculature being more efficient at buffering acute increases than decreases in MAP (4-6). Several prevalent modeling techniques utilized for dCA quantification with spontaneous or driven blood pressure oscillations do not take the directionality of blood pressure changes into consideration. Confirmation of asymmetrical behavior of the cerebral pressure-flow relationship thus has the potential to refine and optimize the current methods of dCA assessment. However, the optimal physiological approach to study this phenomenon remains to be determined.

In the cerebrovascular research community, a repeated squat-stand protocol of 5 min is recognized as a reproducible method for inducing large blood pressure oscillations aimed at improving the linear interpretability of the transfer function metrics (7). This 5-min period of repeated squat-stands has been commonly used to examine the relationship between cerebral blood velocity (CBV) and MAP in the frequency domain with the transfer function analysis, an analytical method which does not consider the direction of MAP (8). Interestingly, this maneuver induces a cyclic physiological stress that can also be utilized to evaluate the CBV-MAP relationship in the time domain (9-11). When comparing the relative change in middle cerebral artery mean blood velocity (MCAv) per relative change in MAP during repeated squat-stands, our team previously demonstrated the

directional behavior of the cerebral pressure-flow relationship (9). Using the autoregulation index (ARI), Panerai et al. demonstrated a better autoregulatory capacity during the squatting phase (transient increase in MAP) than the standing phase (transient decrease in MAP) (11). These results support the usefulness of analyzing MAP oscillations induced by the repeated squat-stand model in the time domain to evaluate the directional behavior of the cerebral pressure-flow relationship. While the 5-min repeated squat-stand model allows for the average of several transient responses, providing a more reliable estimate of the physiological response than the utilization of only one transient response for each MAP direction, we need to assume the metric used to characterize the hysteresis-like pattern during each squat-stand repetition is comparable over the 5 minutes, which is presently unknown.

In our previous analysis, we characterized the directional behavior of the cerebral pressure-flow relationship using the change in MCAv in response to transient increases versus decreases in MAP induced by repeated squat-stands. Specifically, we averaged the percent change in MCAv (from baseline) per percent change in MAP (from baseline) (%ΔMCAv/%ΔMAP) calculated for each squat-stand executed over a period of 5 min at the frequency of interest (0.05 and 0.10 Hz). These relative changes were calculated from a seated baseline obtained prior to the repeated squat-stand maneuver (9). However, the utilization of a separate resting period as the baseline value has been recently criticized (10). Barnes et al. argued the magnitude of MAP oscillations induced by repeated squat-stands is greater when utilizing resting seated MAP as a reference value compared to a resting standing MAP (10). In fact, they reasoned that the decrease in MAP from a resting seated position to the minimum reached during a stand, is greater and would explain the hysteresis pattern. These authors suggested using a reference value in the standing position immediately before the repeated squat-stand protocol would be more valid.

While we agree calculating a baseline value from a separate control recording may be misleading, the utilization of a reference value from a prolonged standing position

immediately before the beginning of the repeated squat-stand maneuver can also be problematic. In this case, the increase in MAP from a resting standing position to the maximum reached during a squat would be greater than the MAP decrease and balance the difference between both. In addition, a prolonged period of standing, necessary to reach a steady-state of measured variables, has the potential to lead to orthostatic hypotension in some individuals. In light of these issues, we consider there is no real baseline we can employ for this particular protocol as there is not a truly stable physiological reference point we can compare to.

For these reasons, the primary aim of this study was to analyze the %ΔMCAv/%ΔMAP metric using the largest concurrent MAP oscillations induced by the squat-stand maneuver without using an independent baseline value (either seated or standing). Accordingly, we calculated this metric by using the minimum to maximum MCAv and MAP (in response to squatting) compared to the maximum to minimum MCAv and MAP (in response to standing) for each squat-stand transition. The secondary aim of this study was to examine whether %ΔMCAv/%ΔMAP during each squat-stand transition at both 0.05 and 0.10 Hz were comparable between each other over the 5-min period. We hypothesized the %ΔMCAv/%ΔMAP metric would be lower when MAP acutely increases compared to when MAP acutely decreases, and that this 5-min repeated squat-stand model is an adequate protocol for the characterization of %ΔMCAv/%ΔMAP since this metric will be comparable between all repeated squat-stand transitions.

Material and methods

Participants

Seventy-four subjects were included in this analysis. Participants had previously been involved in research studies at one of two institutions (Université Laval, Canada; University of British Columbia, Canada). All participants provided informed written consent before participating to these studies. The original research studies were

approved by the *Comité d'éthique de la recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec* (CER : 20869 and 21180) and the University of British Columbia Clinical Review Ethical Board (CER: H11-02576, H11-03287) according to the principles established by the Declaration of Helsinki (except for registration in a database). Since the current study included a secondary analysis from previous institutional review board approved studies and the use of de-identified data sharing, this study did not require institutional review board review. All the participants were healthy, free from medical condition and had no history of cardiorespiratory or cerebrovascular disease. Apart from oral contraceptives in some women, subjects were not taking any blood pressure-altering medication. Women included in the study were either taking oral contraceptive continuously for more than a year (n=2), wearing an intrauterine device (n=1) or were tested during menses or the early follicular phase (day 1 to 10) of their menstrual cycle (n=6).

Experimental protocol

The experimental protocol has been described previously (9). Participants arrived fasted at the laboratory around 7AM and had a standardized snack (granola bar and juice). An initial 5-min seated baseline recording was made for all participants for baseline characteristics. Following a 5-min standing rest, the participants performed squat-stands for a period of at least 5 minutes at a frequency of 0.05 Hz (10-s squat, 10-s standing) and 0.10 Hz (5-s squat, 5-s standing). These frequencies were selected as they are within the range where dCA is thought to have its greatest influence on the cerebral pressure-flow dynamics (12). Familiarization with the squat-stand movement was done by mirroring the experimenter before the experimentation. During recording, the experimenter ensured the transition speed and depth was similar throughout the squat-stand repetition protocol for each subject.

Instrumentation

Maximal O_2 consumption (VO_{2max}) was determined during a preliminary visit in 53 subjects. All these subjects performed a graded ramp exercise test up to exhaustion on an electromagnetically braked upright cycling ergometer. After 1 min of unloaded pedaling, the workload increased progressively (20-30 W/min) until volitional exhaustion. A gas exchange analysis system, combining fast responding oxygen and carbon dioxide sensors and a pneumotach (Breezesuite, MedGraphics Corp., MN, USA; Ultima™ CardiO2® Gas Exchange Analysis System, MGC Diagnostics®, MN, USA), continuously recorded expired air to determine VO_2. VO_{2max} was determined as the highest 30-s mean VO_2 coincident with a respiratory exchange ratio equivalent or greater to 1.15.

The day of the study, MAP was measured with a finger photoplethysmograph with a height correcting unit for correction of values (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands or Nexfin, Edwards Lifesciences, Irvine, CA, USA). Middle cerebral artery mean blood velocity (MCAv) was measured with a transcranial Doppler (Spencer Technologies, Seattle, WA or Doppler Box, Compumedics DWL USA, San Juan Capistrano, CA) on left temporal window. The MCA was identified using a standardized protocol (13). Once the MCA was identified, a probe was fixed in place with a headband and adhesive ultrasonic gel.

$P_{ET}CO_2$ was recorded beath-by-breath in 48 subjects during all the repeated squat-stand through a gas analyzer with fast responding oxygen and carbon dioxide sensors and a pneumotach (Breezesuite, MedGraphics Corp., MN, USA; Ultima™ CardiO2® Gas Exchange Analysis System, MGC Diagnostics®, MN, USA; ML206, AD Instruments, Colorado Springs, CO) calibrated to known gas concentrations following manufacturer instructions before each evaluation. The analyzer was adjusted for room temperature, atmospheric pressure and percentage of humidity the day of testing.

All data were simultaneously sampled at 1000 Hz with an analog-to-digital converter (Powerlab 16/30 ML880, AD Instruments) and saved for later analysis using commercially

available software (LabChart version 7.1, AD Instruments). There was no delay in recording variables from either transcranial Doppler ultrasound units. In addition, we did not apply any time shift in LabChart. However, to account for established delays in our blood pressure monitoring devices, we applied a -250- and -1,000-ms time shift to ensure Nexfin and Finometer, respectively, and transcranial Doppler ultrasound data were time aligned. Any delays within the data collection units were negated when the structured time-aligned analysis was performed. $P_{ET}CO_2$ was time-aligned with the other signals.

Data analysis

To characterize the change in MCAv in response to transient increases and decreases in MAP, we calculated %ΔMCAv/%ΔMAP for each increase (from minimum to maximum) and decrease (from maximum to minimum) and averaged %ΔMCAv/%ΔMAP for each individual's repeated squat-stands over the 5-min recording and for each frequency.

%ΔMCAv/%ΔMAP during transient decreases in MAP was calculated between a maximum and the following minimum as follows (Figure 1):

$$\frac{\frac{(MCAv_{max} - MCAv_{min})}{MCAv_{max}}}{\frac{(MAP_{max} - MAP_{min})}{MAP_{max}}}$$

%ΔMCAv/%ΔMAP during transient increases in MAP was calculated between a minimum and the following maximum as follows (Figure 1):

$$\frac{\frac{(MCAv_{min} - MCAv_{max})}{MCAv_{min}}}{\frac{(MAP_{min} - MAP_{max})}{MAP_{min}}}$$

Statistical analysis

The normality of distributions was tested using Shapiro-Wilk test. Since distributions were not normally distributed, the Wilcoxon matched pairs signed rank test was used to compare variables between transient increases and decreases for each frequency. To test the influence of time on %ΔMCAv/%ΔMAP over the 5-min recording, a repeated-measured ANOVA, fitting a mixed model, was used. Considering the impact of cardiorespiratory fitness and age on the cerebral pressure-flow relationship (14-17), correlations between %ΔMCAv/%ΔMAP and age or VO_2max were determined using Spearman's rho correlations. P values < 0.05 were considered statistically significant.

Results

A total of 74 subjects were included in this analysis (9 women, 65 men). For the 0.05 Hz analysis, 6 subjects were excluded due to inconsistency of MAP and MCAv oscillations (n=5) and poor recordings of the Nexfin (n=1). For the 0.10 Hz analysis, 3 subjects were excluded because squats were not completed (n=1) and poor recordings of the Nexfin (n=2). Final samples for 0.05 Hz and 0.10 Hz repeated squat-stands were 68 and 71, respectively. VO_2max was determined in 53 participants. Baseline characteristics as well as resting hemodynamic and cerebrovascular seated baseline values are shown in Table 1. Seated baseline MAP and MCAv were missing for 4 subjects.

Absolute changes in MAP during acute increases in MAP were +42 ± 16 mmHg (0.05 Hz) and +38 ± 14 mmHg (0.10 Hz), whereas they were -42 ± 15 mmHg (0.05 Hz) and -38 ± 14 mmHg (0.10 Hz) during acute decreases in MAP. Absolute changes in MCAv during acute increases in MAP were +35 ± 14 cm/s (0.05 Hz) and +32 ± 11 cm/s (0.10 Hz), whereas they were -35 ± 14 cm/s (0.05 Hz) and -32 ± 11 cm/s (0.10 Hz) during acute decreases in MAP. Averaged $P_{ET}CO_2$ during the 5-min squat-stand repetitions were not different between frequency (0.05 Hz: 38.7 ± 3.7 mmHg, 0.10 Hz: 38.2 ± 3.4 mmHg; p = 0.0916) and similar to baseline resting values (Table 1).

For 0.05 Hz repeated squat-stands, the %ΔMCAv/%ΔMAP was attenuated by 7 % during acute increases than acute decreases in MAP [1.25 ± 0.22 vs. 1.35 ± 0.27 %/%; $p < 0.0001$; Figure 2]. For the 0.10 Hz repeated squat-stands, the %ΔMCAv/%ΔMAP was attenuated by 18 % during acute increases than acute decreases in MAP (1.31 ± 0.24 vs. 1.60 ± 0.50 %/%; $p < 0.0001$; Figure 2).

For both frequency and MAP direction, time during repeated squat-stands had no effect on %ΔMCAv/%ΔMAP (all $p > 0.05$; Figure 3). When looking at Figure 3, we noticed a higher variability in the %ΔMCAv/%ΔMAP when MAP acutely decreased during the 0.10 Hz repeated squat-stands. To characterize the variability of %ΔMCAv/%ΔMAP during each 5-min repeated squat-stand for each subject, the coefficient of variation (CoV) was calculated for each MAP direction at each repeated squat-stands frequency. There was an ANOVA MAP direction effect for the CoV ($p < 0.001$). Multiple comparisons revealed significant difference in CoVs, being greater for acute decreases than increases in MAP at both frequencies (all $p < 0.001$) (Figure 4).

Cardiorespiratory fitness was correlated to %ΔMCAv/%ΔMAP for 0.10 Hz squats repetitions during both acute increases ($\rho = 0.33$, $r^2 = 0.07$, $p = 0.0226$) and decreases ($\rho = 0.44$, $r^2 = 0.22$, $p = 0.0016$) in MAP (Figure 5), but not during 0.05 Hz squats repetitions (MAP increases: $\rho = 0.16$, $p = 0.02680$; MAP decreases: $\rho = 0.01$, $p = 0.9333$). Age was not correlated to %ΔMCAv/%ΔMAP during either acute increases (0.05 Hz: $\rho = -0.03$, $p = 0.7823$; 0.10 Hz: $\rho = -0.11$, $p = 0.3418$) or decreases (0.05 Hz: $\rho = -0.07$, $p = 0.5336$; 0.10 Hz: $\rho = -0.13$, $p = 0.2868$) in MAP for both frequencies.

Discussion

This study demonstrates when MAP is acutely increased from minimum to maximum during repeated squat-stands, the change in MCAv is attenuated compared to when MAP

acutely decreases, which confirms our previous results (9). The novel finding of this study resides in the notion the changes were calculated on actually induced MAP oscillations, between maximums and minimums *during* the maneuvers, instead of being calculated from a seated baseline value obtained *prior to* the repeated squat-stands. These results reinforce the evidence of hysteresis in the relationship between MCAv and MAP, indicating the cerebral vessels are better adapted to buffer acute increases in MAP. In addition, our findings convincingly demonstrate the repeated squat-stand model can provide a reliable estimate of the physiological response by averaging several transient responses, considering the physiological stress imposed by each squat-stand repetition leads to comparable %ΔMCAv/%ΔMAP (i.e. when the metric for each acute increase in MAP is compared) over the 5-min period, at least in the studied population.

In light of the questions regarding our previous analysis (10), it was important to improve our initial analysis in order to ensure the hysteresis-like pattern was not the result of an inadequate characterization of the %ΔMCAv/%ΔMAP metric. To the best of our knowledge, this is the first study to report the presence of a directional behavior of the human MCAv-MAP relationship when changes are calculated between each maximums and minimums reached during the repeated squat-stands protocol. The results of the present analysis clearly demonstrate the use of actual MAP changes, instead of calculating MAP changes from a separate baseline period, still support the presence of directional behavior of the cerebral pressure-flow relationship in healthy participants. Our study also reinforces the use of the 5-min squat-stand protocol to analyze the MCAv-MAP relationship in the time domain. In fact, as demonstrated in Figure 3, the repetition of the same stimulus over 5 minutes produces valid and stable data over time, reducing the potential influence of outliers.

Although the current study did not aim at examining the mechanisms underlying the asymmetrical behavior of the cerebral pressure-flow relationship, multiple hypotheses are discussed in the literature. First, it was shown in an animal model that cerebral

sympathetic nervous activity (SNA) recorded in the superior cervical ganglia increases in response to mechanically or drug-induced increases in arterial pressure, but not during transient reduction in arterial pressure (18). These results would indicate cerebral SNA increases in response to an acute elevation in MAP to protect the brain from overperfusion. However, the impact of cerebral SNA on cerebral blood flow in humans is still controversial for many reasons (reviewed in (19,20)). A second potential mechanism could be a differing intrinsic myogenic activity when MAP increases than when MAP decreases. Using oscillatory lower body negative pressure, it was demonstrated that the utilization of calcium-channel blockade impairs dCA in humans indicating a role for myogenic mechanisms. However, these results indicate only a global response since the directionality of MAP changes were not considered in this study (21). Recently, Barnes et al. were interested in different components of the cerebrovascular response to repeated acute increases and decreases in MAP during the repeated squat-stand protocol. They demonstrated MAP would be the strongest determinant of CBV when squatting down, overwhelming the myogenic and metabolic components of dCA (10). Overall, these results also make the myogenic mechanism a controversial mechanism to explain the presence of a hysteresis-like pattern in the cerebral pressure-flow relationship. Finally, a transient increase in MAP could elevate superior vena cava's resistance, inducing an increase in intracranial pressure and therefore, a reduction of the passive pressure gradient for venous drainage (22,23), which could represent evidence of the Monro-Kellie doctrine, i.e. a mechanism limiting the amount of cerebral blood flow increase that is able to occur within the encapsulated skull (5).

The increased variability of the %ΔMCAv/%ΔMAP in response to the acute decrease in MAP, independent of repeated squat-stand frequency, has never been demonstrated before and is of great interest. However, reasons explaining this higher variability when MAP acutely decreases remain unclear. It was observed in rats that the cerebral autoregulatory curve demonstrates intersubject variability during progressive, steady-state and controlled hypotension (24). The potential presence of such varying

autoregulatory patterns in the human brain could support our observation of heterogeneity during acute decreases in MAP.

The relationship between %ΔMCAv/%ΔMAP and cardiorespiratory fitness during acute increases and decreases in MAP, but only during the 0.10 Hz repeated squat-stand protocol, is also an interesting finding. Of note, the slope relating VO_2max and %ΔMCAv/%ΔMAP is greater for acute decreases in MAP. These findings suggest a diminished ability to cope with higher frequency MAP oscillations, particularly during transient MAP reductions, with increasing cardiorespiratory fitness. We have previously demonstrated a relation between cardiorespiratory fitness with TFA gain, normalized gain and phase during 0.10 Hz repeated squat-stands in young and healthy fit men (14). TFA gain metrics represent changes in MCAv for a given change in MAP, which makes logical that %ΔMCAv/%ΔMAP is also correlated to cardiorespiratory fitness during this specific frequency. However, the fact that the current analysis using the %ΔMCAv/%ΔMAP metric takes the directionality of MAP changes into account is notable. The reason for an augmented %ΔMCAv/%ΔMAP during acute increases and decreases in MAP, (which could be interpreted as a diminished ability to buffer MAP changes in both directions, i.e. a lowered dCA), with increasing cardiorespiratory fitness and whether this is deleterious or beneficial for the brain, is still unknown. Further studies are needed to evaluate the impact of improving cardiorespiratory fitness on the maintenance of the hysteresis-like pattern in the MCAv-MAP relationship.

In the current analysis, age did not correlate with %ΔMCAv/%ΔMAP when MAP increases or decreases. These findings partially contradict results from our previous work (9). Indeed, when calculating relative changes from a seated baseline obtained prior to the repeated squat-stand protocol, age was positively correlated with %ΔMCAv/%ΔMAP when MAP acutely increased for 0.05-Hz repeated squat-stand only. As in our previous study, the correlational analysis included here is exploratory in nature. Statistical power may have been insufficient to address that question. In addition, while we have included

a group of participants with a large rage in age, only a small number of subjects were above 40 years old, which considerably reduce the strength of this analysis. Further well-designed research aimed at specifically examining the influence of age on the directional behavior of the cerebral pressure-flow relationship is thus necessary to address that specific question.

Perspectives

Overall, our updated analysis for repeated squat-stands is adequate to study the directional behavior of the cerebral pressure-flow relationship and optimize the assessment of dCA. It supports the need of a global change in dCA assessment in order to take the direction of MAP into account. In addition, since we showed repeated squat-stands induce a comparable change in %ΔMCAv/%ΔMAP at each squat-stand transition, it eliminates the questioning as to whether the addition of squat-stands (exercise) would have an impact *per se* on this metric. Still, further research is needed to examine the possibility of using less repetitions to calculate the %ΔMCAv/%ΔMAP metric. Indeed, a diminished number of squat-stand repetitions would be an important methodological advantage to examine for the presence of hysteresis in clinical populations for whom a 5-min period of repeated squat-stands would be difficult to complete.

A next important step of major interest would be to evaluate the reproducibility of this metric over time. It would help determine whether the %ΔMCAv/%ΔMAP is reliable over various time frames and if it remains stable over time. While further research is needed to describe the directional behavior of the cerebral pressure-flow relationship in other populations and clinical conditions, this observation is highly relevant for various physiological (rapid eye movement sleep, exercise), and pathological clinical situations (uncontrolled hypertension) associated with MAP surges. Importantly for the clinician, this directional sensitivity of brain vessels to changes in MAP should also be integrated in the framework to guide its management in the operating room. Finally, considering the

link found with cardiorespiratory fitness in the present study, it would be interesting to evaluate the impact of exercise training on the maintenance of the hysteresis-like pattern.

Limitations

There are some limitations to this study that need to be further discussed. Only healthy men and women were included in this analysis and the results cannot be generalized to clinical populations. Moreover, MCAv was evaluated through transcranial Doppler ultrasound, which is known to be representative of flow only if the diameter of the artery is assumed to remain stable. Changes in $P_{ET}CO_2$ have been associated with MCA's diameter variations (25,26). Although $P_{ET}CO_2$ was not available for a part of the male subjects, there was no difference between $P_{ET}CO_2$ during baseline and repeated squat-stand for the subjects in whom it was measured. Moreover, it was previously observed that there was no change in $P_{ET}CO_2$ between squat and stand phases (11). Therefore, $P_{ET}CO_2$ had most likely a minor effect on our results. Only a small part of the group was composed of women either taking oral contraceptives, wearing an intrauterine device or being in the days 1-10 of their menstrual cycle. We are not able to determine whether it had an influence on the results and further research is needed to address that issue. Since days 0-10 represents a large time period, it should be shortened in future studies in order to better control for menstrual cycle. Of note, all participants (n=58) included in our previous work (9) were also included in the current analysis. The two analytical methods were not directly compared as our main goal was to examine whether the hysteresis-like pattern in the cerebral pressure-flow relationship was present with the updated analytical method. A direct comparison would not provide additional meaningful information. We consider the updated analytical method proposed in the current work to be the most valid option to examine the directional behavior of the cerebral pressure-flow relationship with the repeated squat-stand model since our calculated metric cannot be influenced by a separate resting steady-state baseline.

Conclusion

Our findings indicate that this novel analytical method supports the use of the repeated squat-stand model to examine the directional behavior of the cerebral pressure-flow relationship, during actual changes in MAP and cerebral blood velocity, without the need for a separate resting baseline. Moreover, it shows this maneuver induces stable physiological cyclic changes and allows for interindividual variability analysis. Importantly, the results contribute to the importance of considering the direction of MAP changes when evaluating dCA.

Acknowledgments

We would like to thank Myriam Paquette, Olivier Le Blanc, Kevan Rahimaly, Sarah Imhoff, Simon Malenfant and Audrey Drapeau for their assistance in data collection.

Grants

This study has been supported by the Ministère de l'Éducation, du Loisir et du Sport du Québec and the Foundation of the Institut universitaire de cardiologie et de pneumologie de Québec. L.L. is supported by a doctoral training scholarship from the Canadian Institutes of Health Research.

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Figures caption

Figure 1. Schematic tracings of mean arterial pressure (MAP) and middle cerebral artery mean blood velocity (MCAv) during a repeated squat-stands performed at 0.10 Hz. Blue color indicates variables used to calculate %ΔMCAv/%ΔMAP during acute decreases in MAP. Red color indicates variables used to calculate %ΔMCAv/%ΔMAP during acute increases in MAP. Purple color indicates minimum values used for the calculation of %ΔMCAv/%ΔMAP during acute decreases and increases in MAP.

Figure 2. Comparison of the %ΔMCAv/%ΔMAP during transient increases (INC) and decreases (DEC) in mean arterial pressure during 0.05 and 0.10 Hz repeated squat-stands. n = 68 for 0.05 Hz, n = 71 for 0.10 Hz. Differences assessed via Wilcoxon matched pairs signed rank test.

Figure 3. Individual %ΔMCAv/%ΔMAP for each squat repetition during acute increases (INC) and decreased (DEC) for 0.05 and 0.10 Hz repeated squat-stands. Numbers on the x-axis represent squat-stand repetitions. For each squat-stand repetition, n = 68 for 0.05 Hz and n = 71 for 0.10 Hz. The effect of time was tested using a repeated-measured mixed model ANOVA.

Figure 4. Individual mean coefficient of variation (CoV) during acute increases (INC) and decreased (DEC) for 0.05 and 0.10 Hz repeated squat-stands. n = 68 for 0.05 Hz, n = 71 for 0.10 Hz. Main effects (direction of mean arterial pressure, frequency of repeated squat-stands) was evaluated via a 2-way ANOVA.

Figure 5. Relationship between VO_2max and %ΔMCAv/%ΔMAP for acute increases (INC) and decreases (DEC) in MAP during repeated squat-stands at 0.05 Hz and 0.10 Hz. n = 47 for 0.05 Hz, n = 50 for 0.10 Hz. Relationships between %ΔMCAv/%ΔMAP and cardiorespiratory fitness (VO_2max) were determined using Spearman's rho correlations.

Table 1. Baseline characteristics and resting seated values

Baseline characteristics	
n	74
Age (years)	32 ± 13 (20 – 74)
Height (m)	1.75 ± 0.09 (1.55 – 1.94)
Weight (kg)	75.5 ± 12.8 (115.0 – 53.0)
Body mass index (kg/m^2)	24.5 ± 3.2 (19.0 – 36.7)
Maximal O_2 consumption ($ml \cdot kg^{-1} \cdot min^{-1}$)	46.9 ± 9.6 (23.5 – 65.4) *
Resting values	
Mean arterial pressure (mmHg)	90 ± 13 (60 – 122) §
Middle cerebral artery mean blood velocity ($cm \cdot s^{-1}$)	63 ± 13 (34 – 100) §
End-tidal carbon dioxide partial pressure (mmHg)	38 ± 4 (29 – 47) †

Data are mean ± SD (ranges).
* n = 53, § n = 70, † n = 48

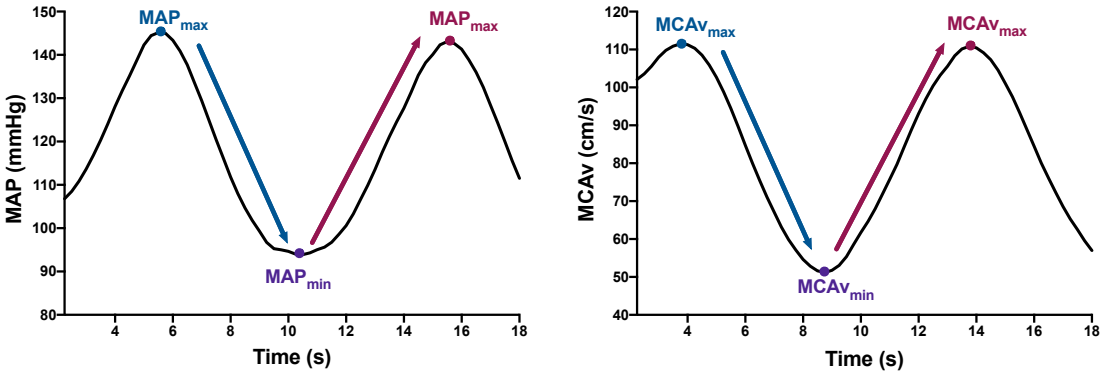
MAP (mmHg)
150
140
130
120
110
100
90
80
MAPmax
MAPmax
MAPmin
4
6
8
10
12
14
16
18
Time (s)
MCAv (cm/s)
120
110
100
90
80
70
60
50
40
MCAvmax
MCAvmax
MCAvmin
4
6
8
10
12
14
16
18
Time (s)

Figure 1.

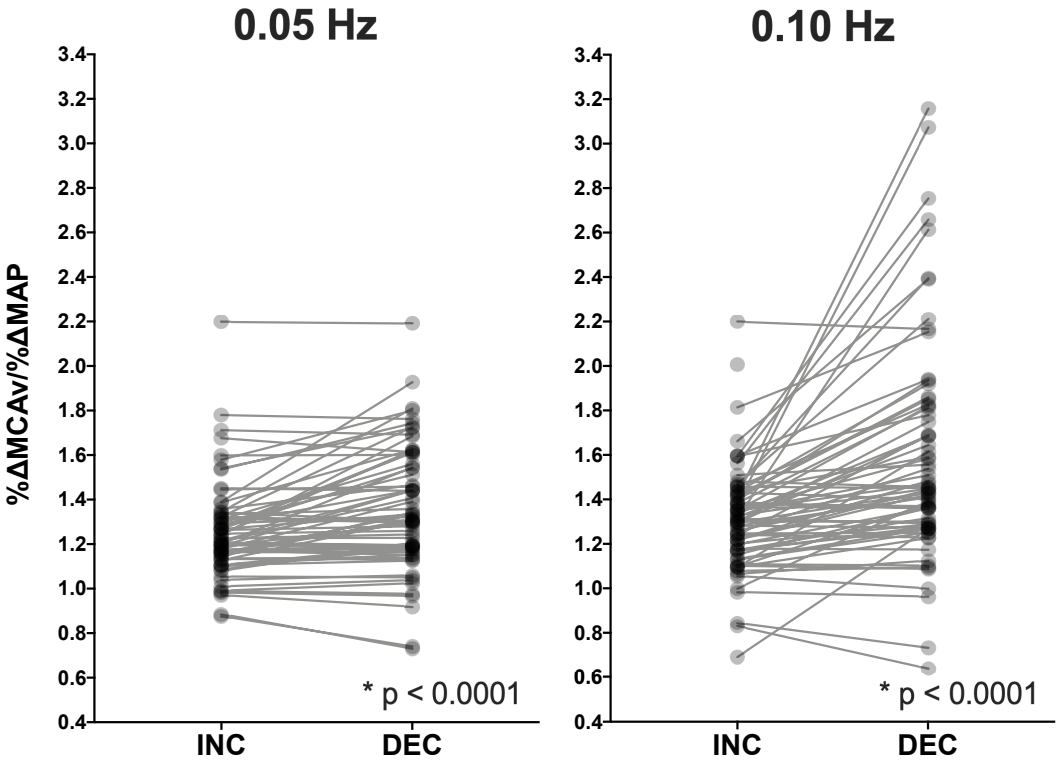
0.05 Hz
0.10 Hz
%ΔMCAv/%ΔMAP
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
* p < 0.0001
* p < 0.0001
INC
DEC
INC
DEC

Figure 2.

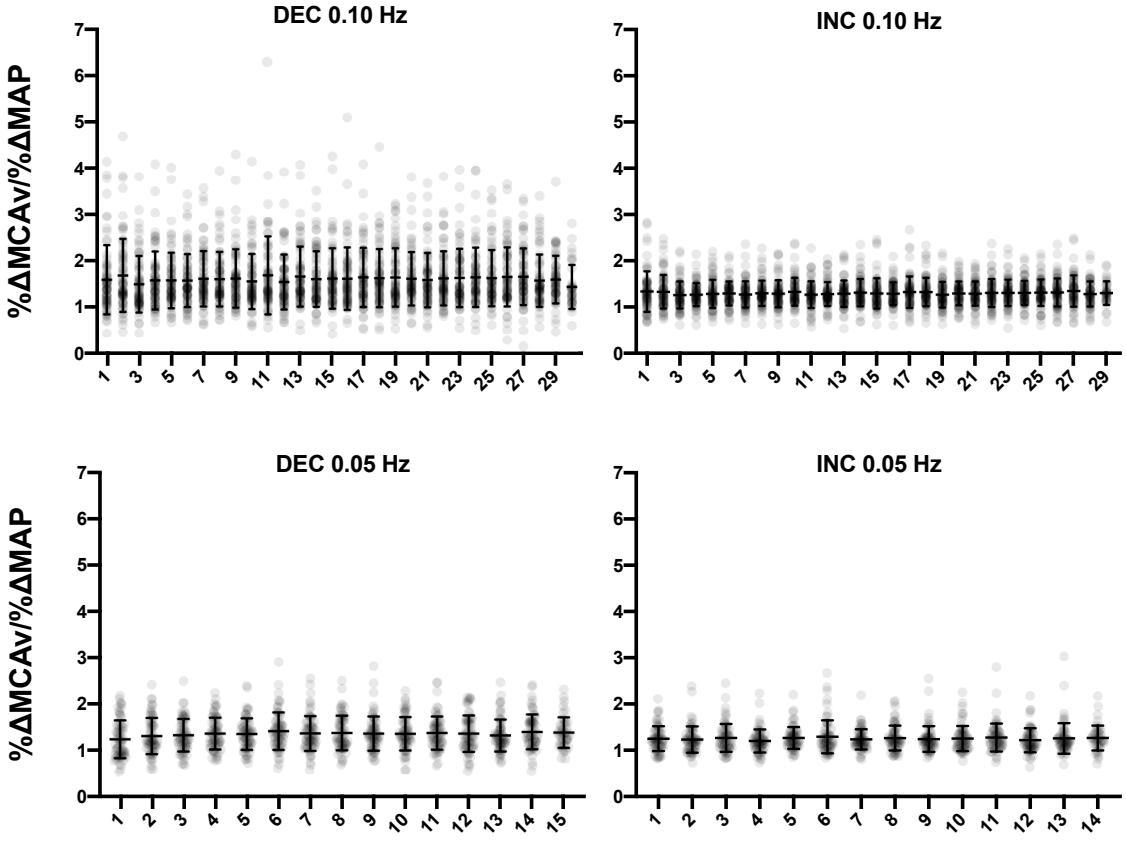
DEC 0.10 Hz
INC 0.10 Hz
DEC 0.05 Hz
INC 0.05 Hz
%ΔMCAv/%ΔMAP
%ΔMCAv/%ΔMAP

Figure 3.

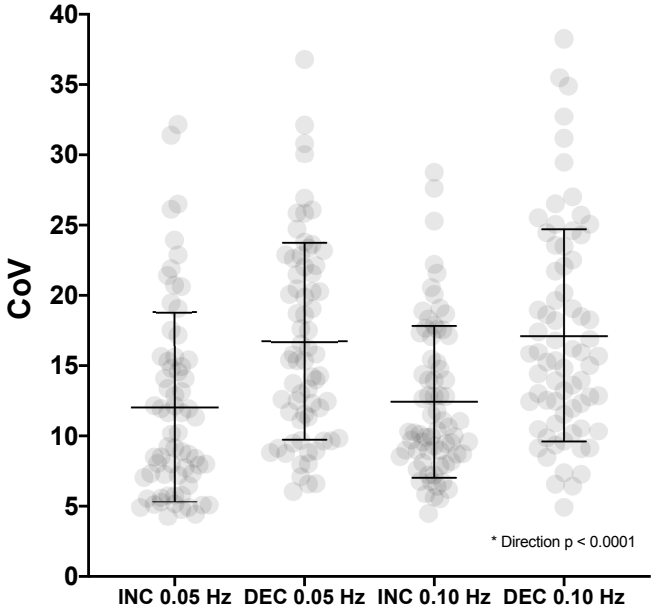
CoV
40
35
30
25
20
15
10
5
0
* Direction p < 0.0001
INC 0.05 Hz
DEC 0.05 Hz
INC 0.10 Hz
DEC 0.10 Hz

Figure 4.

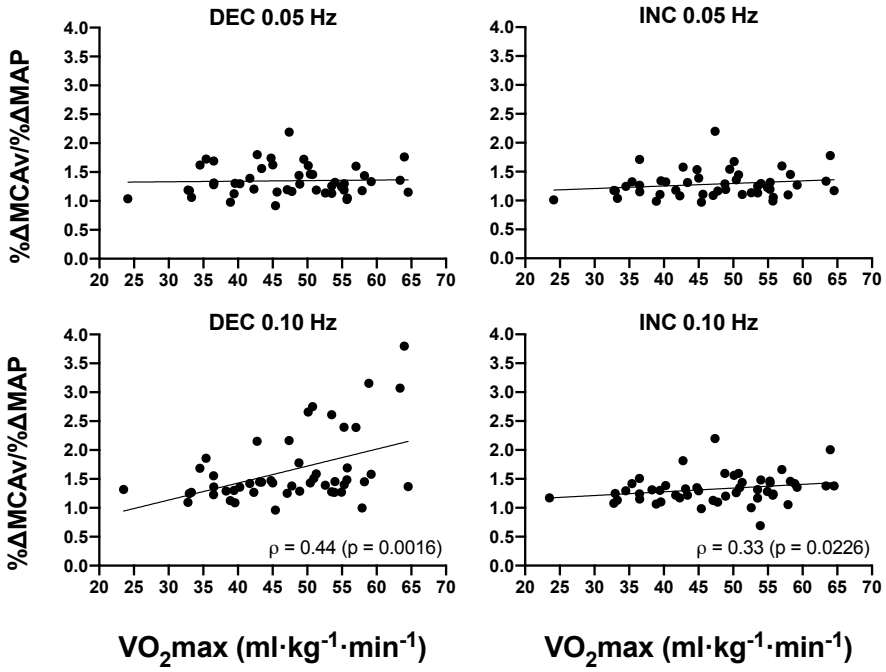
DEC 0.05 Hz
INC 0.05 Hz
DEC 0.10 Hz
INC 0.10 Hz
%ΔMCAv/%ΔMAP
ρ = 0.44 (p = 0.0016)
ρ = 0.33 (p = 0.0226)
VO2max (ml·kg-1·min-1)

Figure 5.