Utilization of the repeated squat-stand model for studying the directional sensitivity of the cerebral 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 MAP acutely decreases. This phenomenon can be evaluated by comparing the change in middle cerebral artery mean blood velocity (MCAv) per change in MAP 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 a novel metric using the greatest MAP oscillations induced by RSS without using an independent baseline value and adjusted for time intervals ($\Delta MCAv_T/\Delta MAP_T$). We also examined whether this metric during each RSS transition were comparable between each other over a 5-min period. $\Delta MCAv_T/\Delta MAP_T$ 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 $\Delta MCAv_T/\Delta MAP_T$ during MAP increases and decreases in 74 healthy subjects [9 women; 32 $\pm$ 13 years]. $\Delta MCAv_T/\Delta MAP_T$ was lower for MAP increases than MAP decreases at 0.10 Hz RSS only (0.91 $\pm$ 0.34 vs. 1.01 $\pm$ 0.44 $cm \cdot s^{-1}/mmHg$; $p = 0.0013$). For both frequency and MAP direction, time during RSS had no effect on $\Delta MCAv_T/\Delta MAP_T$. This novel analytical method supports the use of the RSS model to evaluate the directional sensitivity of the pressure-flow relationship. These results contribute to the importance of considering the direction of MAP changes when evaluating dynamic cerebral autoregulation.

News & Noteworthy

Repeated squat-stand maneuvers are able to examine the directional sensitivity of the cerebral pressure-flow relationship. These maneuvers induce stable physiological cyclic changes where brain blood flow changes with blood pressure increases are buffered more than decreases. These results highlight the importance of considering directional blood pressure changes within cerebral autoregulation.

Key words

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

Introduction

Dynamic cerebral autoregulation (dCA) defines the ability of the cerebrovasculature to react to rapid blood pressure changes. Evidence suggests asymmetrical responses of cerebral blood flow (CBF) to transient changes in mean arterial pressure (MAP). Specifically, the augmentation in CBF is attenuated when MAP acutely increases compared to the decline in CBF when MAP acutely decreases (1, 8, 27, 36). Since the brain is located in the skull, an inflexible structure, this phenomenon is thought to serve as a protective mechanism against blood pressure surges (39). This notion has been previously highlighted as the cerebrovasculature is more efficient at buffering changes in blood pressure associated with the systolic aspect of the cardiac cycle (11, 32).

Initially, the above-mentioned asymmetrical responses of CBF to acute changes in MAP have been reported using cyclic inflation and deflation of thigh cuffs in patients with head injury (1) and pharmacological interventions in healthy participants (36). It is important to ensure the hysteresis-like pattern of the cerebral pressure-flow relationship exists during non-pharmacologically and physiologically induced acute increases and decreases in MAP in health and disease. Accordingly, the development of a valid non-pharmacological model to examine the directional sensitivity of the cerebral pressure-flow relationship is imperative.

Unfortunately, the previously used thigh cuff deflation technique may not represent the best model to investigate the directional sensitivity of the cerebral pressure-flow relationship, considering the poor reproducibility associated with dCA metrics quantified with this technique (reviewed in (29)). 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 (29). 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 at two different repeated squat-stands frequencies (0.05 and 0.10 Hz) with the transfer function analysis, an analytical method which does not consider the direction of MAP (15). These two squatting frequencies are utilized since they are included in the frequency bands where dCA is thought to have the most important influence on the cerebral pressure-flow dynamics (historically, these frequency bands being 0.02-0.07 Hz for the very low frequency and 0.07-0.20 for the low frequency) (15). Also, these two frequencies are most prevalent in the literature (7, 10-12, 14, 18, 21, 22, 25, 28-31). Interestingly, the repeated squat-stand maneuver induces a cyclic physiological stress that can also be utilized to evaluate the CBV-MAP relationship in the time domain (6, 8, 27). 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 sensitivity of the cerebral pressure-flow relationship (8). 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) (27). These results support the usefulness of analyzing MAP oscillations induced by the repeated squat-stand model in the time domain to evaluate the directional sensitivity of the cerebral pressure-flow relationship. Furthermore, the 5-min repeated squat-stand model allows for the average of several induced responses. Thus, providing a more reliable estimate of the physiological response than the utilization of only one transient response for each MAP direction as had previous be performed with the rate-of-regulation (2) and the autoregulatory index (34) measures. Additionally, we need to assume the metric used to characterize the hysteresis-like pattern during each squat-stand repetition is comparable over the 5 min, which is presently unknown.

In our previous analysis, we characterized the directional sensitivity 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)

144 (%ΔMCAv/%ΔMAP) calculated for each squat-stand executed over a period of 5 min at
145 the frequency of interest (0.05 and 0.10 Hz). These relative changes were calculated from
146 a seated baseline obtained prior-to the repeated squat-stand maneuver (8). However, the
147 utilization of a separate resting period as the baseline value has been recently criticized
148 (6). Barnes et al. argued the magnitude of MAP oscillations induced by repeated squat-
149 stands is greater when utilizing resting seated MAP as a reference value compared to a
150 resting standing MAP (6). In fact, they reasoned that the decrease in MAP from a resting
151 seated position to the minimum reached during a stand, is greater and would explain the
152 hysteresis pattern. These authors suggested using a reference value in the standing
153 position immediately before the repeated squat-stand protocol would be more valid.
154
155 While we agree calculating a baseline value from a separate control recording may be
156 misleading, the utilization of a reference value from a prolonged standing position
157 immediately before the beginning of the repeated squat-stand maneuver can also be
158 problematic. In this case, the increase in MAP from a resting quiet-stance position to the
159 maximum reached during a squat would be greater than the MAP decrease and balance
160 the difference between both. In addition, a prolonged period of standing, necessary to
161 reach a steady state of measured variables, has the potential to lead to orthostatic
162 hypotension in some individuals. In light of these issues, we consider there is no real
163 baseline we can employ for this particular protocol as there is not a truly stable
164 physiological reference point we can compare the changes during the squat-stand
165 maneuvers to.
166
167 A notion we did not consider in our previous study was the time intervals when the
168 transition of MCAv and MAP took place, which represents an additional issue with this
169 work (8). There are delays in physiological responses and regulating mechanisms vary
170 whether changes in MCAv and MAP are increasing or decreasing (1). Therefore, we
171 cannot assume repeated squat-stands induce MCAv and MAP changes of comparable
172 time intervals when MAP acutely decreases and increases via repeated squat-stands

173 induced at 0.05 Hz or 0.10 Hz. Thus, upward (from minimum to maximum) and downward
174 (from maximum to minimum) changes in MCAv/MAP of similar amplitudes, but occurring
175 across different time intervals, would indicate vessels are reacting differently according
176 to the direction of the MAP changes. We also believe taking into consideration time
177 intervals when MCAv and MAP changes are taking place would be more appropriate in
178 order to compare our metric between RSS frequencies (0.05 vs. 0.10 Hz).
179
180 The prevalent modeling techniques utilized for dCA quantification with spontaneous or
181 driven blood pressure oscillations do not take the directionality of blood pressure changes
182 into consideration (i.e. autoregulation index, transfer function analysis of spontaneous
183 and forced blood pressure oscillations). In other words, these approaches assume the
184 cerebrovasculature reacts similarly when MAP acutely increases or decreases.
185 Confirmation of asymmetrical sensitivity of the cerebral pressure-flow relationship using
186 the repeated squat-stand model thus has the potential to refine and optimize the current
187 methods of dCA assessment (15). If it becomes clear this hysteresis-like pattern of the
188 cerebral pressure-flow relationship is a common characteristic of the healthy
189 cerebrovasculature, which could be influenced by physiological and pathological
190 conditions, there will be a need for a global change in the assessment of dCA in order to
191 take MAP direction into account. However, the optimal physiological approach to study
192 this phenomenon remains to be elucidated.
193
194 For these reasons, the primary aim of this study was to refine a metric using the largest
195 concurrent MAP oscillations induced by the repeated squat-stand maneuver without
196 using an independent baseline value (either seated or standing). Accordingly, we
197 calculated this metric by using the minimum to maximum MCAv and MAP (in response to
198 squatting) compared to the maximum to minimum MCAv and MAP (in response to
199 standing) for each squat-stand transition taking time intervals when the MCAv and MAP
200 transition took place into account. The secondary aim of this study was to examine
201 whether the novel metric during each squat-stand transition at both 0.05 and 0.10 Hz

202 were comparable between each other over the 5-min period. We hypothesized the metric
203 would be lower when MAP acutely increases compared to when MAP acutely decreases,
204 and this 5-min repeated squat-stand model is an adequate protocol for the
205 characterization of the metric since it will be comparable between all repeated squat-
206 stand transitions.
207
208 **Material and methods**
209
210 *Participants*
211 Seventy-four participants were included in this analysis. Participants had previously been
212 involved in research studies at one of two institutions (Université Laval, Canada;
213 University of British Columbia, Canada). All participants provided informed written
214 consent before participating to these studies. The original research studies were
215 approved by the *Comité d'éthique de la recherche de l'Institut universitaire de cardiologie*
216 *et de pneumologie de Québec* (CER : 20869 and 21180) and the University of British
217 Columbia Clinical Review Ethical Board (CER: H11-02576, H11-03287) according to the
218 principles established by the Declaration of Helsinki (except for registration in a
219 database). Since the current study included a secondary analysis from previous
220 institutional review board approved studies and the use of de-identified data sharing, this
221 study did not require additional institutional review board review. All the participants
222 were healthy, free from medical condition and had no history of cardiorespiratory or
223 cerebrovascular disease. Apart from oral contraceptives in some women, participants
224 were not taking any blood pressure-altering medication. Women included in the study
225 were either taking oral contraceptive continuously for more than a year (n=2), wearing
226 an intrauterine device (n=1) or were tested during menses or the early follicular phase
227 (day 1 to 10) of their menstrual cycle (n=6).
228
229 *Experimental protocol*

230 The experimental protocol has been described previously (8). Participants arrived fasted
231 at the laboratory around 7AM and had a standardized snack (granola bar and juice). An
232 initial 5-min seated baseline recording was made for all participants for baseline
233 characteristics. Following a 5-min standing rest, the participants performed repeated
234 squat-stands for a period of at least 5 min at a frequency of 0.05 Hz (10-s squat, 10-s
235 standing) and 0.10 Hz (5-s squat, 5-s standing). As previously mentioned, these
236 frequencies were selected since they are within the range where dCA is thought to have
237 its greatest influence on the cerebral pressure-flow dynamics (40). Familiarization with
238 the squat-stand movement was done by mirroring the experimenter before the
239 experimentation. During recording, the experimenter ensured the transition speed and
240 depth was similar throughout the squat-stand repetition protocol for each participant.
241
242 *Instrumentation*
243 Maximal oxygen consumption (VO_{2max}) was determined during a preliminary visit in 53
244 participants. All these participants performed a graded ramp exercise test up to
245 exhaustion on an electromagnetically braked upright cycling ergometer. After 1 min of
246 unloaded pedaling, the workload increased progressively (20-30 W/min) until volitional
247 exhaustion. A gas exchange analysis system, combining fast responding oxygen and
248 carbon dioxide sensors and a pneumotach (Breezesuite, MedGraphics Corp., MN, USA;
249 Ultima™ CardiO2® Gas Exchange Analysis System, MGC Diagnostics®, MN, USA),
250 continuously recorded expired air to determine VO_2. VO_{2max} was determined as the
251 highest 30-s mean VO_2 coincident with a respiratory exchange ratio equivalent or greater
252 to 1.15.
253
254 The day of the study, MAP was measured with a finger photoplethysmograph with a
255 height correcting unit for correction of values (Finometer Pro, Finapres Medical Systems,
256 Amsterdam, The Netherlands or Nexfin, Edwards Lifesciences, Irvine, CA, USA). MCAv was
257 measured with a transcranial Doppler ultrasound (Spencer Technologies, Seattle, WA or
258 Doppler Box, Compumedics DWL USA, San Juan Capistrano, CA) on left temporal window.
259 The MCA was identified using a standardized protocol (38). Once the MCA was identified,

260 a probe was fixed in place with a headband and adhesive ultrasonic gel.

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

269 All data were simultaneously sampled at 1000 Hz with an analog-to-digital converter
270 (Powerlab 16/30 ML880, AD Instruments) and saved for later analysis using commercially
271 available software (LabChart version 7.1, AD Instruments). There was no delay in
272 recording variables from either transcranial Doppler ultrasound units. In addition, we did
273 not apply any time shift in LabChart. However, to account for established delays in our
274 blood pressure monitoring devices, we applied a -250- and -1,000-ms time shift to ensure
275 Nexfin and Finometer, respectively, and transcranial Doppler ultrasound data were time
276 aligned. Any delays within the data collection units were negated when the structured
277 time-aligned analysis was performed. $P_{ET}CO_2$ was time-aligned with the other signals.

278 *Data analysis*

279 To characterize the cerebral pressure-flow relationship in response to transient increases
280 and decreases in MAP, we calculated a time-adjusted ratio between MCAv and MAP
281 changes for each squat-stand transition in each MAP direction. To do so, absolute changes
282 in MCAv ($\Delta MCAv_T$) and MAP (ΔMAP_T) for each increase (from minimum to maximum) and
283 decrease (from maximum to minimum) were calculated and adjusted for time intervals
284 when the MCAv and MAP transition took place. Specifically, we divided the changes by
285 the duration of the transition (i.e. time interval between minimum to maximum or
286 between maximum to minimum). Then, we averaged $\Delta MCAv_T/\Delta MAP_T$ for each

individual's repeated squat-stands over the 5-min recording period and for each frequency.

$\Delta MCAv_T/\Delta MAP_T$ during transient decreases in MAP was calculated between a maximum and the following minimum as follows (Figure 1):

$$\frac{\frac{(MCAv_{min} - MCAv_{max})}{(Time_{MCAvmin} - Time_{MCAvmax})}}{\frac{(MAP_{min} - MAP_{max})}{(Time_{MAPmin} - Time_{MAPmax})}} = \frac{\frac{\Delta \mathrm{MCAv}}{\Delta \mathrm{Time}_{MCAv}}}{\frac{\Delta \mathrm{MAP}}{\Delta \mathrm{Time}_{MAP}}} = \frac{\Delta \mathrm{MCAv}_T}{\Delta \mathrm{MAP}_T}$$

$\Delta MCAv_T/\Delta MAP_T$ during transient increases in MAP was calculated between a minimum and the following maximum as follows (Figure 1):

$$\frac{\frac{(MCAv_{max} - MCAv_{min})}{(Time_{MCAvmax} - Time_{MCAvmin})}}{\frac{(MAP_{max} - MAP_{min})}{(Time_{MAPmax} - Time_{MAPmin})}} = \frac{\frac{\Delta \mathrm{MCAv}}{\Delta \mathrm{Time}_{MCAv}}}{\frac{\Delta \mathrm{MAP}}{\Delta \mathrm{Time}_{MAP}}} = \frac{\Delta \mathrm{MCAv}_T}{\Delta \mathrm{MAP}_T}$$

Statistical analysis

The normality of distributions was tested using Shapiro-Wilk tests. If distributions were normal, a paired t-test was used. If not, 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 $\Delta MCAv_T/\Delta MAP_T$ over the 5-min recording, a nonparametric repeated-measures Friedman test was performed. Since this test does not support missing values, all participants with missing values for one or more transitions were excluded. Considering the impact of cardiorespiratory fitness and age on the cerebral pressure-flow relationship (4, 5, 22, 24), correlations between $\Delta MCAv_T/\Delta MAP_T$ 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 participants were included in this analysis (9 women, 65 men). For the 0.05 Hz analysis, 6 participants 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 participants 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 participants.

Absolute changes in MAP during acute increases in MAP were +43 ± 15 mmHg (0.05 Hz) and +37 ± 14 mmHg (0.10 Hz), whereas they were -43 ± 15 mmHg (0.05 Hz) and -37 ± 14 mmHg (0.10 Hz) during acute decreases in MAP. Absolute changes in MCAv during acute increases in MAP were +36 ± 14 $cm \cdot s^{-1}$ (0.05 Hz) and +32 ± 11 $cm \cdot s^{-1}$ (0.10 Hz), whereas they were -35 ± 14 $cm \cdot s^{-1}$ (0.05 Hz) and -32 ± 11 $cm \cdot s^{-1}$ (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 ± 3.8 Torr, 0.10 Hz: 38.2 ± 3.4 Torr; $p = 0.0916$) and similar to baseline resting values (Table 1).

At 0.05 Hz, averaged time intervals for MAP and MCAv changes were shorter during acute MAP increases than MAP decreases (MAP: 9.0 ± 1.0 vs. 11.0 ± 1.0 sec; $p < 0.0001$; MCAv: 9.3 ± 1.5 vs. 10.7 ± 1.4 $cm \cdot s^{-1}$; $p < 0.0001$). At 0.10 Hz, averaged time intervals for MAP and MCAv changes were shorter during acute increases than decreases for MAP (4.9 ± 0.4 vs. 5.2 ± 0.4 sec; $p = 0.0024$), but not for MCAv (5.1 ± 0.5 vs. 4.9 ± 0.5 sec; $p = 0.1028$).

For 0.05 Hz repeated squat-stands, $\Delta MCAv_T/\Delta MAP_T$ was similar during acute increases and acute decreases in MAP (0.88 ± 0.28 vs. 0.90 ± 0.23 $cm \cdot s^{-1}$/mmHg; $p = 0.2693$; Figure

342 2). For 0.10 Hz repeated squat-stands, $\Delta MCAv_T/\Delta MAP_T$ was attenuated during acute
343 increases when compared to acute decreases in MAP (0.91 ± 0.34 vs. 1.01 ± 0.44 $cm \cdot s^{-1}$/mmHg; p = 0.0013; Figure 2).
344
345
346 For both frequency and MAP direction, time during repeated squat-stands had no effect
347 on $\Delta MCAv_T/\Delta MAP_T$ (all p> 0.05; Figure 3).
348
349 Cardiorespiratory fitness was correlated to $\Delta MCAv_T/\Delta MAP_T$ for 0.05 Hz repeated squat-
350 stands during acute MAP increases (ρ = 0.33, p = 0.0244) but not for acute MAP decreases
351 (ρ = 0.21, p = 0.1566). Cardiorespiratory fitness was correlated to $\Delta MCAv_T/\Delta MAP_T$ for 0.10
352 Hz repeated squat-stands during both acute increases (ρ = 0.49, p = 0.0003) and decreases
353 (ρ = 0.48, p = 0.0005) in MAP (Figure 4). At 0.05 Hz, age was not correlated to
354 $\Delta MCAv_T/\Delta MAP_T$ during acute MAP increases (ρ = -0.19, p = 0.1278) nor acute decreases
355 in MAP (ρ = -0.17, p = 0.1581). At 0.10 Hz, age was not correlated to $\Delta MCAv_T/\Delta MAP_T$
356 during acute MAP increases (ρ = -0.21, p = 0.0803), but was inversely correlated during
357 acute MAP decreases (ρ = -0.25, p = 0.0346) (Figure 5).
358
359 **Discussion**
360
361 This study demonstrates when MAP is acutely increased from minimum to maximum
362 during repeated squat-stands at 0.10 Hz, the change in MCAv is attenuated compared to
363 when MAP acutely decreases, which is in line with our previous results (8). The novel
364 finding of this study resides in the notion that changes were adjusted for time intervals
365 and calculated on actually induced MAP oscillations, between maximums and minimums
366 *during* the maneuvers, instead of being calculated from a seated baseline value obtained
367 *prior-to* the repeated squat-stands. These results reinforce the evidence of hysteresis in
368 the relationship between MCAv and MAP, indicating cerebral vessels are better adapted
369 to buffer acute increases in MAP at 0.10 Hz, highlighting a probable influence of repeated
370 squat-stand frequency. In addition, our findings convincingly demonstrate the repeated

371 squat-stand model can provide a reliable estimate of the physiological response by
372 averaging several transient responses, considering the physiological stress imposed by
373 each squat-stand repetition leads to comparable $\Delta MCAv_T/\Delta MAP_T$ (i.e. when the metric
374 for each acute increase in MAP is compared) over the 5-min period, at least in the studied
375 population.
376
377 In light of the questions regarding our previous analysis (6), it was important to improve
378 our initial analysis in order to address different concerns, i.e. to avoid the utilization of a
379 separate resting period and take time intervals of MCAv and MAP changes into
380 consideration. To the best of our knowledge, this is the first study to report the presence
381 of a directional sensitivity of the human MCAv-MAP relationship when changes are
382 adjusted for time intervals as well as calculated between each maximums and minimums
383 reached during 0.10 Hz, but not 0.05 Hz, for repeated squat-stands. The results of the
384 present analysis demonstrate the use of actual MAP changes, instead of calculating MAP
385 changes from a separate baseline period, support the presence of a frequency-dependent
386 directional sensitivity of the cerebral pressure-flow relationship in healthy participants.
387 Our study also reinforces the use of the 5-min squat-stand protocol to analyze the MCAv-
388 MAP relationship in the time domain. In fact, as demonstrated in Figure 3, the repetition
389 of the same stimulus over 5 minutes produces valid and stable data over time, reducing
390 the potential influence of outliers.
391
392 Although the current study did not aim at examining the mechanisms underlying the
393 asymmetrical sensitivity of the cerebral pressure-flow relationship, multiple hypotheses
394 are discussed in the literature. First, it was shown in an animal model that cerebral
395 sympathetic nervous activity (SNA) recorded in the superior cervical ganglia increases in
396 response to mechanically or drug-induced increases in arterial pressure, but not during
397 transient reduction in arterial pressure (13). These results would indicate cerebral SNA
398 increases in response to an acute elevation in MAP to protect the brain from
399 overperfusion. Interestingly, it is proposed that 0.10 Hz repeated squat-stand frequency

400 might reveal the influence of SNA on human cerebrovascular regulation (20, 40).
401 Therefore, the lower $\Delta MCAv_T/\Delta MAP_T$ during acute increases in MAP at 0.10 Hz repeated
402 squat-stands could be explained by SNA activation when MAP acutely increases, in order
403 to protect cerebral vessels. However, the impact of cerebral SNA on CBF in humans is still
404 controversial for many reasons (reviewed in (3, 9)). Below frequencies of 0.07 Hz,
405 cerebrovascular regulation is thought to have a myogenic influence (20, 40). Another
406 potential mechanism could therefore be a differing intrinsic myogenic activity when MAP
407 increases than when MAP decreases. Using oscillatory lower body negative pressure and
408 a non-linear approach to quantify dCA, it was demonstrated the utilization of calcium-
409 channel blockade impairs dCA in humans, indicating a role for myogenic mechanisms at
410 frequencies between 0.03 and 0.08 Hz (33). However, these results showed only a global
411 dCA response since the directionality of MAP changes were not considered (33). As MAP
412 direction did not influence $\Delta MCAv_T/\Delta MAP_T$ at 0.05 Hz repeated squat-stands in the
413 present study, it could be argued the myogenic influence on CBF is similar whether MAP
414 acutely increases or decreases, or that our linear approach masks differences. Recently,
415 Barnes et al. were interested in different components of the cerebrovascular response to
416 repeated acute increases and decreases in MAP during the repeated squat-stand
417 protocol. They demonstrated MAP would be the strongest determinant of CBV when
418 squatting down, overwhelming the myogenic and metabolic components of dCA (6).
419 Overall, these results also make the myogenic mechanism a controversial mechanism to
420 explain the presence of a hysteresis-like pattern in the cerebral pressure-flow relationship
421 (33). The absence of directional sensitivity at 0.05 Hz repeated squat-stands could also be
422 explained by the baroreflex latency (19, 36). In fact, during these slower oscillations,
423 baroreceptors have time to sense pressure loading or unloading and induce appropriate
424 cardiovascular adjustments. This could mitigate the impact of MAP on MCAv over a 10-
425 sec time interval. Finally, a transient increase in MAP could elevate superior vena cava's
426 resistance, inducing an increase in intracranial pressure and therefore, a reduction of the
427 passive pressure gradient for venous drainage (16, 17), which could represent evidence

428 of the Monro-Kellie doctrine, i.e. a mechanism limiting the amount of CBF increase that
429 is able to occur within the encapsulated skull (39).
430
431 The consistency of the $\Delta MCAv_T/\Delta MAP_T$ over the 5-min repeated squat-stand at both
432 frequencies is indicative the physiological stress induced is stable for both direction and
433 magnitude of acute changes in MAP. The absence of a drift in the values demonstrates
434 the participants performing repeated squat-stands do not get fatigued from the
435 maneuvers and the addition of squat-stand transitions has no influence on the metric, at
436 least in healthy participants.
437
438 The relationships between $\Delta MCAv_T/\Delta MAP_T$ and cardiorespiratory fitness during acute
439 increases and decreases in MAP at 0.10 Hz, and only during acute increases at 0.05 Hz
440 repeated squat-stand protocol, are also interesting observations. These findings suggest
441 a diminished ability to cope with higher frequency MAP oscillations with increasing
442 cardiorespiratory fitness. We have previously demonstrated an association between
443 cardiorespiratory fitness and TFA gain, normalized gain and phase during 0.10 Hz
444 repeated squat-stands in young and healthy fit men (22). TFA gain metrics represent
445 changes in MCAv for a given change in MAP, which makes logical that $\Delta MCAv_T/\Delta MAP_T$ is
446 also correlated to cardiorespiratory fitness in the current analysis. However, the fact that
447 the current analysis using the $\Delta MCAv_T/\Delta MAP_T$ metric takes the directionality of MAP
448 changes into account is notable. The reason for an augmented $\Delta MCAv_T/\Delta MAP_T$ during
449 acute increases and decreases in MAP, (which could be interpreted as a diminished ability
450 to buffer MAP changes in both directions, i.e. a lowered dCA), with increasing
451 cardiorespiratory fitness and whether this is deleterious or beneficial for the brain, is still
452 unknown. Further studies are needed to evaluate the impact of improving
453 cardiorespiratory fitness on the maintenance of the hysteresis-like pattern in the MCAv-
454 MAP relationship.
455

456 In the current analysis, age inversely correlates with $\Delta MCAv_T/\Delta MAP_T$ when MAP acutely
457 decreases during 0.10 Hz repeated squat-stands. These findings contradict results from
458 our previous work (8). Indeed, when calculating relative changes from a seated baseline
459 obtained prior to the repeated squat-stand protocol, age was positively correlated with
460 %ΔMCAv/%ΔMAP when MAP acutely increased for 0.05-Hz repeated squat-stand only.
461 As in our previous study, the correlational analysis included here is exploratory in nature.
462 Statistical power may have been insufficient to address that question. In addition, while
463 we have included a group of participants with a large range in age, only a small number
464 of subjects were above 40 years old, which considerably reduce the strength of this
465 analysis. Further well-designed research aimed at specifically examining the influence of
466 age on the directional sensitivity of the cerebral pressure-flow relationship is thus
467 necessary to address that specific question.
468
469 *Perspectives*
470
471 Overall, our updated analysis for repeated squat-stands is adequate to study the
472 directional sensitivity of the cerebral pressure-flow relationship and optimize the
473 assessment of dCA. It supports the need of a global change in dCA assessment in order to
474 take the direction of MAP into account. It also reinforces the importance of using the
475 repeated squat-stands, at different frequencies, to provide a broader characterization of
476 the cerebral pressure-flow relationship. In addition, since we showed repeated squat-
477 stands induce a comparable change in $\Delta MCAv_T/\Delta MAP_T$ at each squat-stand transition, it
478 eliminates the questioning as to whether the addition of squat-stands (exercise) would
479 have an impact *per se* on this metric. A next important step of major interest would be to
480 evaluate the reproducibility of this metric over time. It would help determine whether
481 the $\Delta MCAv_T/\Delta MAP_T$ is reliable over various time frames and if it remains stable over time.
482 Still, further research is needed to examine the possibility of using less repetitions to
483 calculate the $\Delta MCAv_T/\Delta MAP_T$ metric. Indeed, a diminished number of squat-stand
484 repetitions would be an important methodological advantage to examine for the

485 presence of hysteresis in clinical populations for whom a 5-min period of repeated squat-
486 stands could potentially be difficult to complete.
487
488 The directional sensitivity in the cerebral pressure-flow relationship is highly relevant for
489 various physiological (rapid eye movement sleep, exercise), and pathological clinical
490 situations associated with MAP surges. Nonetheless, further research is needed to
491 describe this phenomenon in other populations and clinical conditions. We still do not
492 know whether this hysteresis-like pattern remains present in different physiological and
493 pathological states (arterial/carotid stiffness, uncontrolled systemic hypertension,
494 autonomic dysreflexia, etc.). For example, findings from a recent study using a mouse
495 model of arterial stiffness suggest mice with calcified carotid artery have a disrupted
496 cerebral autoregulation in response to steady-state MAP increases compared to healthy
497 animals (26). The next logical step would therefore be to examine whether there is
498 evidence for hysteresis in the cerebral pressure-flow relationship in human clinical
499 populations and the present study serves as a fundamental basis to further explore the
500 phenomenon. Importantly for the clinician, this directional sensitivity of brain vessels to
501 changes in MAP should also be integrated in the framework to guide its management in
502 the operating room. Finally, considering the link found with cardiorespiratory fitness in
503 the present study, it would be interesting to evaluate the impact of exercise training on
504 the maintenance of the hysteresis-like pattern.
505
506 *Methodological considerations*
507
508 There are some limitations to this study that need to be further discussed. Only healthy
509 men and women were included in this analysis and the results cannot be generalized to
510 clinical populations. Moreover, MCAv was evaluated through transcranial Doppler
511 ultrasound, which is known to be representative of flow only if the diameter of the artery
512 is assumed to remain stable. Changes in $P_{ET}CO_2$ have been associated with MCA's
513 diameter variations (23, 37). Although $P_{ET}CO_2$ was not available for a part of the male

514 participants, there was no difference between $P_{ET}CO_2$ during baseline and repeated
515 squat-stand for the participants in whom it was measured. In addition, it was previously
516 observed that there was no change in $P_{ET}CO_2$ between squat and stand phases (27).
517 Therefore, $P_{ET}CO_2$ had most likely a minor effect on our results. Only a small part of the
518 group was composed of women either taking oral contraceptives, wearing an intrauterine
519 device or being in the days 1-10 of their menstrual cycle. We are not able to determine
520 whether it had an influence on the results and further research is needed to address that
521 issue. Since days 0-10 represents a large time period, it should be shortened in future
522 studies in order to better control for menstrual cycle. Of note, all participants (n=58)
523 included in our previous work (8) were also included in the current analysis. From an
524 analytical standpoint, the two approaches are comparable, as both methods are
525 calculating changes in MCAv and MAP in response to repeated squat-stands. One
526 difference resides in the fact the previous method calculated relative changes from a
527 seated baseline, whereas the revised method calculated actual absolute changes adjusted
528 for time intervals. This being acknowledged, the main difference resides in the *baseline*
529 value used to calculate these changes in MCAv and MAP. In the original approach, we
530 have calculated a *baseline* including averaged MCAv and MAP during a steady-state rest.
531 However, this *baseline* with participants in a seated position was measured during a
532 distinct recording period prior to the start of the series of repeated squat-stands.
533 Accordingly, from a physiological standpoint, it makes definitely more sense to calculate
534 changes in MCAv and MAP on actually induced MAP oscillations, between maximums and
535 minimums during the repeated squat-stands, rather than being calculated from a seated
536 baseline value obtained during a distinct period prior to the repeated squat-stands. We
537 consider the updated analytical method proposed in the current work to be the most valid
538 option to examine the directional sensitivity of the cerebral pressure-flow relationship
539 with the repeated squat-stand model since our calculated metric cannot be influenced by
540 a separate resting steady-state baseline. However, the two analytical methods were not
541 directly compared, as our main goal of the current investigation was to examine whether
542 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.

Conclusion

Our findings indicate that this novel analytical method supports the use of the repeated squat-stand model to examine the directional sensitivity 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 and robust 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 and builds upon the prior rate-of-regulation and autoregulatory index measures.

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Figures caption

Figure 1. Panel A represents raw tracings of blood pressure (BP), middle cerebral artery blood velocity (MCAv) and partial pressure of carbon dioxide (PCO_2) at 0.05 and 0.10 Hz repeated squat-stand. Panel B depicts schematic tracings of mean arterial pressure (MAP) and middle cerebral artery mean blood velocity (MCAv) during a repeated squat-stands performed at 0.05 Hz. Enlarged regions depict how the variables were used to calculate $\Delta MCAv_T/\Delta MAP_T$. Blue color indicates variables used to calculate $\Delta MCAv_T/\Delta MAP_T$ during acute decreases in MAP. Red color indicates variables used to calculate $\Delta MCAv_T/\Delta MAP_T$ during acute increases in MAP.

Figure 2. Comparison of the $\Delta MCAv_T/\Delta MAP_T$ 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 $\Delta MCAv_T/\Delta MAP_T$ 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 = 64 during INC and n = 56 during DEC for 0.05 Hz, n = 58 during INC and n = 59 during DEC for 0.10 Hz. The effect of time was tested using a nonparametric repeated-measures Friedman test.

Figure 4. Relationship between VO_2max and $\Delta MCAv_T/\Delta MAP_T$ 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 $\Delta MCAv_T/\Delta MAP_T$ and cardiorespiratory fitness (VO_2max) were determined using Spearman's rho correlations.

Figure 5. Relationship between age and $\Delta MCAv_T/\Delta MAP_T$ for acute increases (INC) and decreases (DEC) in MAP during repeated squat-stands at 0.05 Hz and 0.10 Hz. n = 68 for 0.05 Hz, n = 71 for 0.10 Hz. Relationships between $\Delta MCAv_T/\Delta MAP_T$ and age were determined using Spearman's rho correlations.

Table 1. Baseline characteristics and resting seated values

751

Baseline characteristics	
n	74
Age (years)	32 ± 13 (20 – 74)
Height (m)	1.75 ± 0.09 (1.55 – 1.94)
Body weight (kg)	75.5 ± 12.8 (53.0 – 115.0)
Body mass index (kg/m^2)	24.5 ± 3.2 (19.0 – 36.7)
Maximal oxygen 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 ($cm \cdot s^{-1}$)	62 ± 13 (34 – 100) §
End-tidal carbon dioxide partial pressure (Torr)	38 ± 4 (29 – 47) †

752 Data are mean ± SD (ranges).
753 * n = 53, § n = 70, † n = 48
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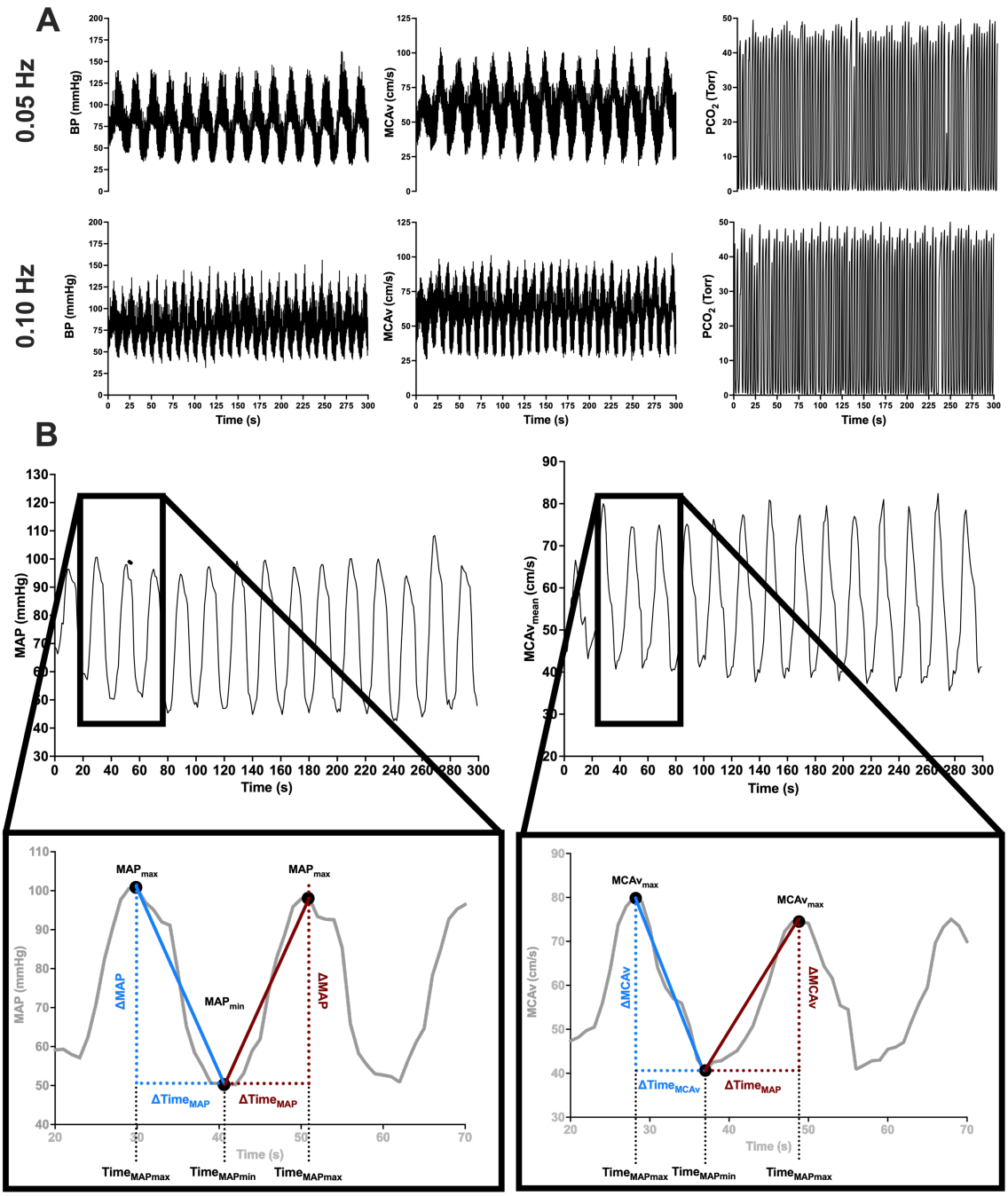
A
0.05 Hz
0.10 Hz
BP (mmHg)
MCAv (cm/s)
PCO2 (Torr)
Time (s)
B
MAP (mmHg)
MCAvmean (cm/s)
MAPmax
MAPmin
ΔMAP
ΔTimeMAP
TimeMAPmax
TimeMAPmin
MCAvmax
ΔMCAv
ΔTimeMCAv

Figure 1.

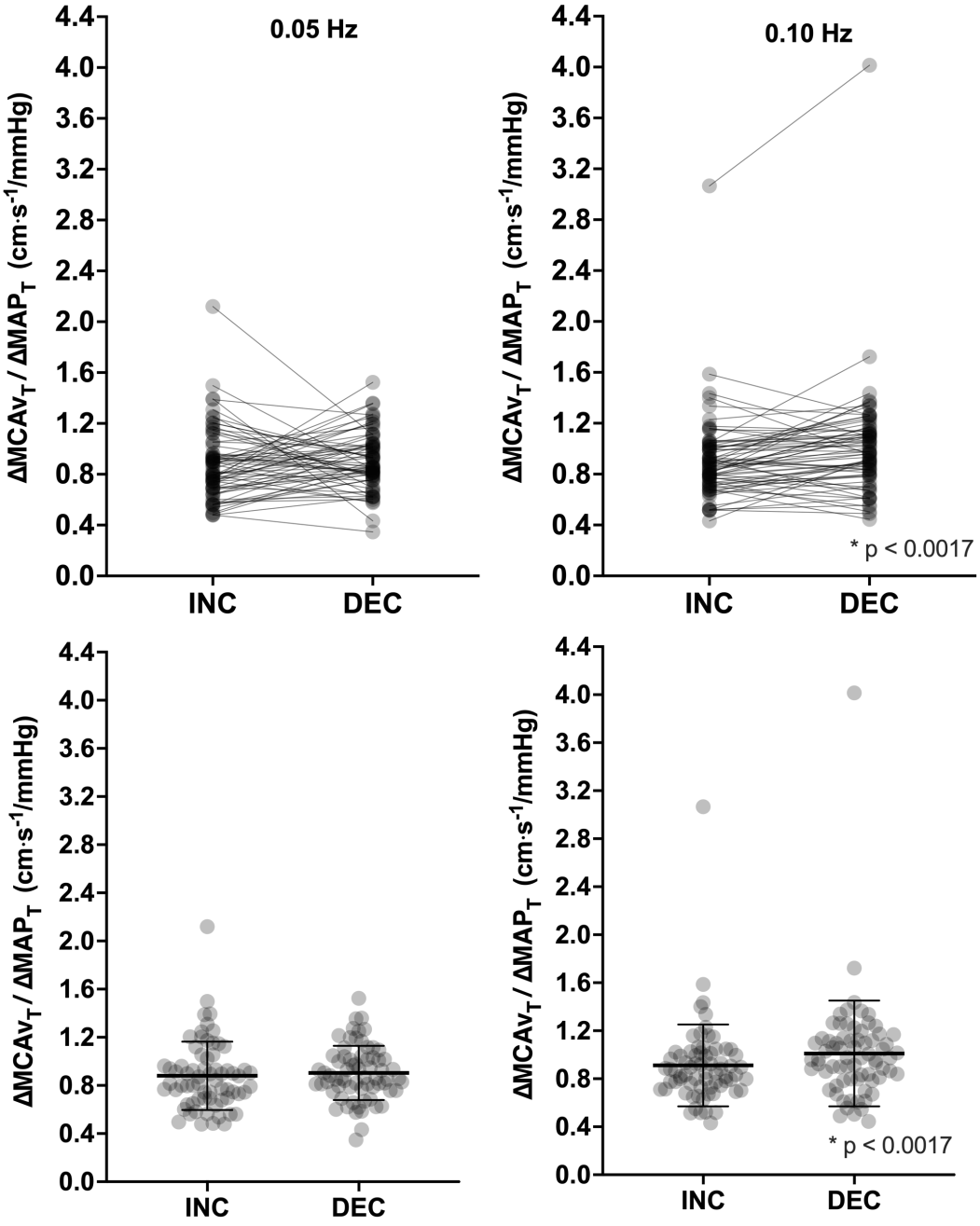
0.05 Hz
0.10 Hz
ΔMCAv$_T$ / ΔMAP$_T$ (cm·s^{-1}/mmHg)
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
INC
DEC
* p < 0.0017

Figure 2.

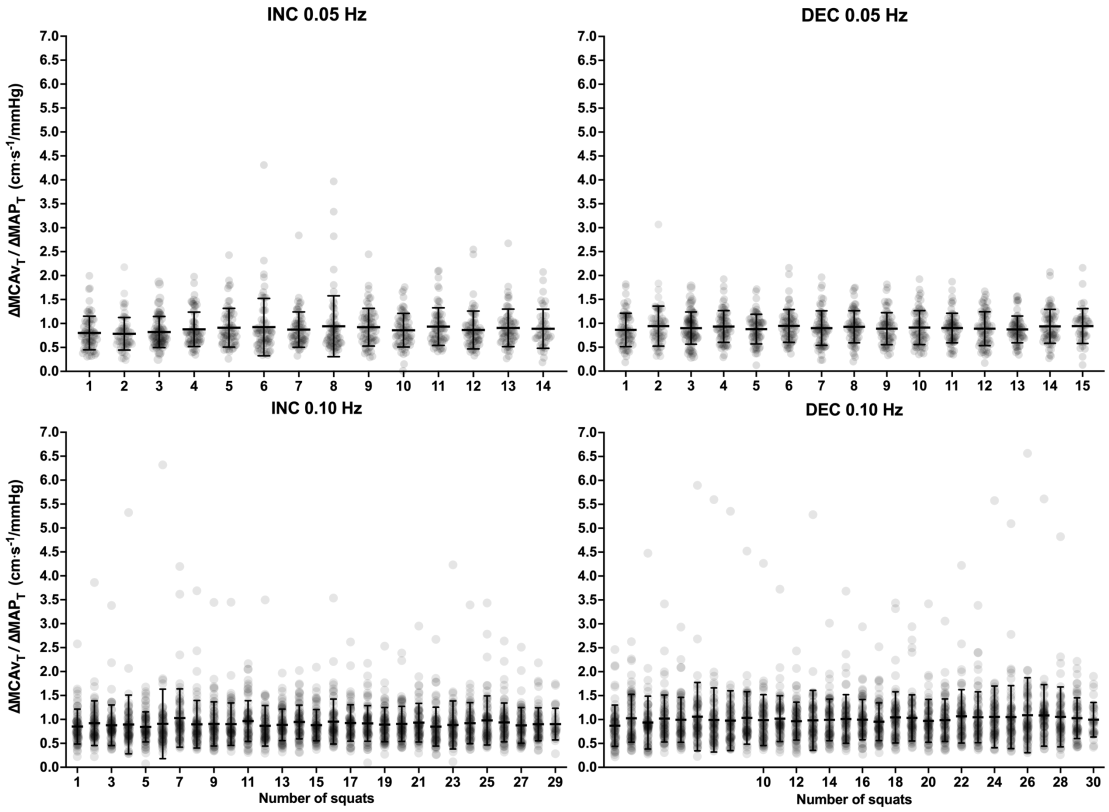
INC 0.05 Hz
DEC 0.05 Hz
INC 0.10 Hz
DEC 0.10 Hz
ΔMCAv_T / ΔMAP_T (cm·s⁻¹/mmHg)
Number of squats

Figure 3.

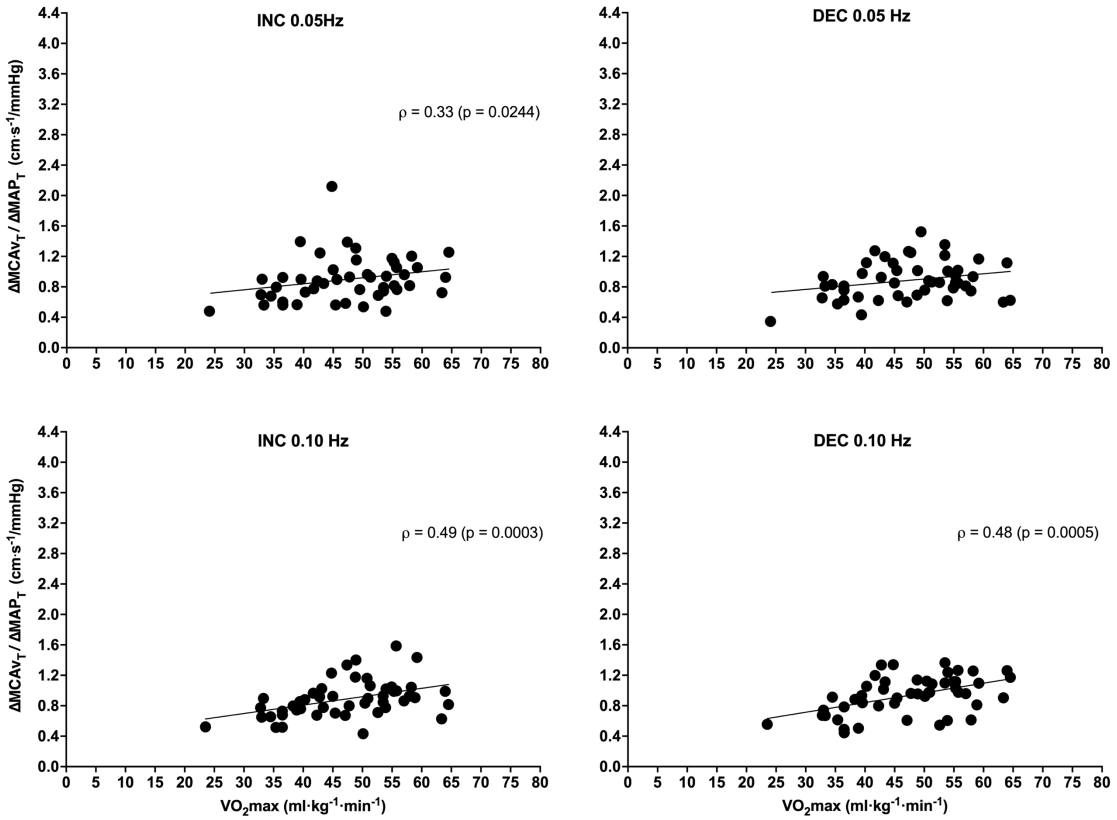
INC 0.05Hz
ρ = 0.33 (p = 0.0244)
DEC 0.05 Hz
INC 0.10 Hz
ρ = 0.49 (p = 0.0003)
DEC 0.10 Hz
ρ = 0.48 (p = 0.0005)
ΔMCAv$_T$ / ΔMAP$_T$ (cm·s^{-1}/mmHg)
VO$_2$max (ml·kg^{-1}·min^{-1})

Figure 4.

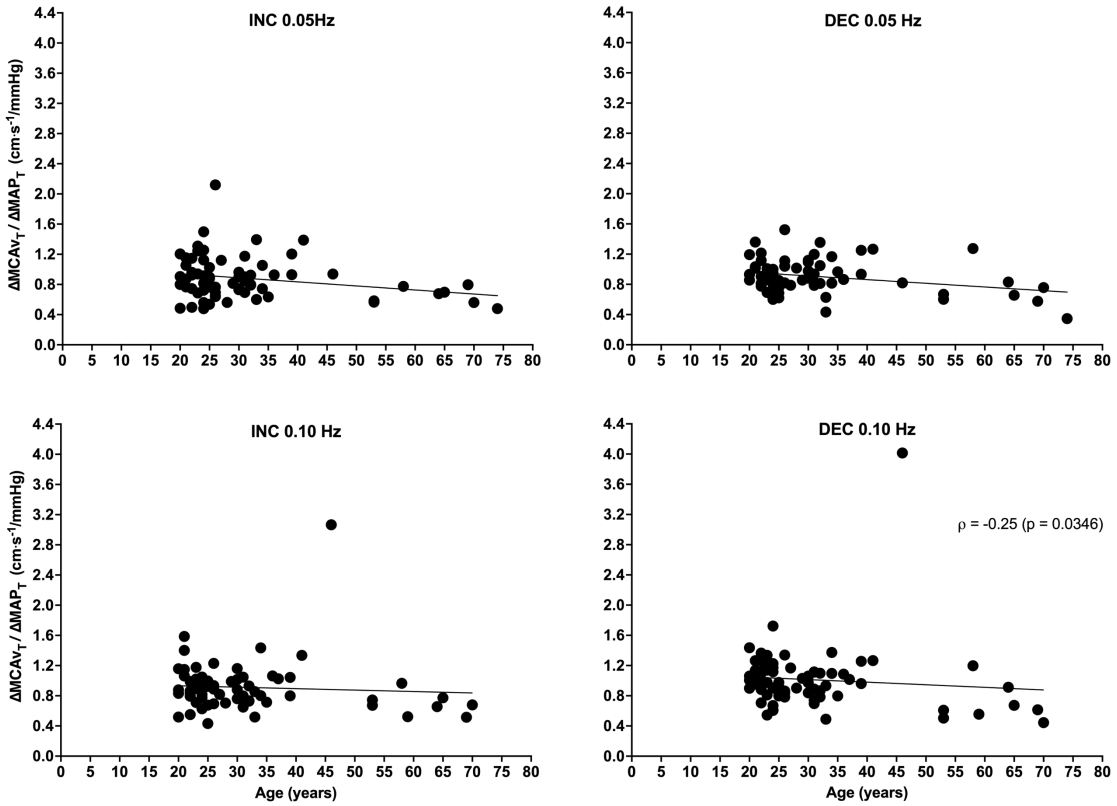
INC 0.05Hz
DEC 0.05 Hz
INC 0.10 Hz
DEC 0.10 Hz
ΔMCAv$_T$ / ΔMAP$_T$ (cm·s^{-1}/mmHg)
Age (years)
ρ = -0.25 (p = 0.0346)

Figure 5.