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The effect of steady-state CO_2 on regional brain blood flow responses to increases in blood pressure via the cold pressor test

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ABSTRACT

The pressure-passive cerebrovasculature is affected by alterations in cerebral perfusion pressure (CPP) and arterial blood gases (e.g., pressure of arterial [Pa]CO_2), where acute changes in either stimulus can influence cerebral blood flow (CBF). The effect of superimposed increases in CPP at different levels of steady-state $PaCO_2$ on regional CBF regulation is unclear. In 17 healthy participants, we simultaneously recorded continuous heart rate (electrocardiogram), blood pressure (finometer), pressure of end-tidal CO_2 ($P_{ET}CO_2$; gas analyzer), and middle (MCA) and posterior (PCA) cerebral artery blood velocity (CBV; transcranial Doppler ultrasound). Three separate CPTs were administered by passive immersion of both feet into 0–1 °C of ice water for 3-min under three randomized and coached steady-state $P_{ET}CO_2$ conditions: normocapnia (room air), hypocapnia (−10 Torr; hyperventilation) and hypercapnia (+9 Torr; 5% inspired CO_2;). CBV responses were calculated as the absolute difference (Δ) between baseline and mean MCAv and PCAv during the 3-min CPT. Both the ΔMCAv and ΔPCAv responses to the CPT were larger under hypercapnic conditions. The absolute ΔMCAv response was larger than the ΔPCAv during the CPT across all three CO_2 trials. Cerebrovascular CO_2 reactivity (CVR) was larger in the MCA than PCA in both CPT and baseline conditions, but there were no differences in CVR between CPT and baseline conditions. Our data indicate that (a) increases in CO_2 increases the CBV responses to a CPT, (b) the anterior cerebrovasculature is more responsive to a CPT-induced increases in MAP, and (c) although unchanged during a CPT, CVR is larger in the anterior cerebral circulation.

1. Introduction

The cerebrovasculature is responsive to changes in cerebral perfusion pressure (CPP) and arterial blood gases (e.g., the partial pressure of arterial carbon dioxide; $PaCO_2$; Ainslie & Bailey, 2013). Acute changes in mean arterial pressure (MAP) can represent changes in CPP if the intracranial pressure remains unchanged. Whether CBF is maintained at near constant levels during changes in CPP is still under debate. For example, Lassen (1959), and Liu et al. (2013) have demonstrated that CBF is maintained during steady-state changes in MAP, whereas Lucas et al. (2010) and Claassen, Levine, and Zhang (2009) suggested that the cerebrovascular is more sensitive to acute changes in perfusion pressure (i.e., pressure-passive).

An acute perturbation in MAP can be experimentally-induced using the cold pressor test (CPT), which elicits an increase in sympathetic nervous system activity in response to a body part (e.g., arm or leg) being immersed in near-freezing water (e.g. 0–1 °C; Liu, Cao, Duan, Yang, & Yuan, 2011). The CPT results in an increase in heart rate (HR; Flück et al., 2017) and total peripheral vascular resistance (TPR; Greene, Boltax, Lustig, & Rogow, 1965; Victor, Leimbach, Seals, Wallin, & Mark, 1987). This increased CO and TPR both contribute to the observed increase in MAP, which has also been demonstrated to increase CBF (Tymko, Kerstens, Wildfong, & Ainslie, 2017; Perry, Bear, Lucas, & Mündel, 2016; Lucas et al., 2010; Claassen et al., 2009).

Experimental reductions in $PaCO_2$ (i.e., hypocapnia) elicits cerebral vasoconstriction, which results in decreases in CBF and washout of metabolically-derived CO_2. In contrast, experimental elevations in $PaCO_2$ (i.e., hypercapnia) elicits vasodilation, resulting in an increase in CBF, likely resulting in increased CO_2 washout from tissue in intact systems (Skow et al., 2013; Ainslie, Ashmead, Ide, Morgan, & Poulin, 2005; Lennox & Gibbs, 1932). These changes in CBF in response to changes in $PaCO_2$ are referred to as cerebrovascular CO_2 reactivity (Duffin et al., 2015). Previous data indicate that there may be a difference in cerebrovascular CO_2 reactivity between the MCA and the

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PCA, with the MCA having greater reactivity (Flück et al., 2017; Skow et al., 2013), however, this is not consistent in all studies (Willie et al., 2012). It is currently unknown to what extent resting $PaCO_2$ impacts the cerebrovascular response to increases in MAP. Since regional differences in CBF regulation have been suggested for both CO_2 reactivity and MAP reactivity, we aimed to determine if regional differences in CBF regulation were present during a CPT (as a means to experimentally increase MAP) and simultaneous changes in resting $PaCO_2$. Therefore, we aimed (1) to characterize the effects of superimposed increases in MAP via the CPT and (2) assess the effects of superimposed alterations in resting $P_{ET}CO_2$ on regional CBF regulation during a CPT. Specifically, we hypothesized that CBF responses to the CPT would be exaggerated under hypercapnia. We also hypothesized that anterior cerebral blood flow responses to the CPT would be greater in magnitude under all CO_2 conditions, compared to posterior cerebral circulations.

2. Methods

2.1. Ethics and participant recruitment

This study abided by the Canadian Government Tri-Council Policy on Research Ethics Policy Statement (TCPS2) and the Declaration of Helsinki, except for registration in a database. Ethical approval was obtained in advance from the Mount Royal University Human Research Ethics Board (Protocol 101,122). Participants were recruited through word of mouth and written and verbal informed consent were obtained in all cases. Participants had no history of respiratory, cardiovascular, cerebrovascular, and metabolic diseases. Additionally, participants were excluded if they smoked or were currently using prescription medications, with the exception of oral contraceptives.

2.2. Equipment and measurements

Each participant was fitted with (a) electrocardiogram electrodes (lead II configuration) to determine HR (ECG electrodes in lead II configuration and ADI bioamp ML132), (b) a finometer to non-invasively measure beat-by-beat blood pressure (Finometer Pro, Finapres Medical Systems, Amsterdam, NL; calibrated for every participant), and (c) a personal mouthpiece/nose clip connected to a pneumotachometer to measure inspired tidal volume, respiratory rate, and minute ventilation (800 L flow head and spirometer amplifier; Hans Rudolph and ADI ML141), (d) a gas analyzer connected to the participants' mouthpiece for the continuous measurement of $P_{ET}CO_2$ (ADI ML206; calibrated daily), and (e) a transcranial Doppler ultrasound (TCD) with headpiece and bilateral probes for the measurement of cerebral blood velocity (CBV) in the MCA and PCA (Spencer Technologies, PMD150B; Redmond, WA, USA). To ensure that these vessels were accurately located, previously described verification techniques were used (Willie et al., 2011). Mean CBV was calculated as the mean from the envelope of the peak velocity tracing using LabChart. Participants kept their eyes closed and listened to white noise via ear buds to minimize distraction throughout the baseline period.

Breath-by-breath $P_{ET}CO_2$ was displayed on a screen for participant visual feedback in order to maintain the three different levels of $P_{ET}CO_2$ conditions: hypocapnia, normocapnia, and hypercapnia. Each steady-state level of resting $P_{ET}CO_2$ was achieved through coached breathing. The participant sat in the upright position with their outstretched feet resting on a chair in front of them. A large cooler filled with a slurry of water and ice between 0 and 1 °C (confirmed via digital thermometer prior to each CPT) was placed in front of the participant. The participants were instructed to refrain from moving their feet during the protocol, thus avoiding the skeletal muscle pump and associated cardiovascular changes (Goodman, Freeman, & Goodman, 2007).

Table 1
Baseline variables under baseline conditions and coached CO_2 interventions, prior to cold pressor tests. BL1, initial baseline, prior to coached CO_2 interventions. $BL2_{HYPO}$, baseline variables during coached voluntary hyperventilation. $BL2_{NORMO}$, baseline variables breathing room air. $BL2_{HYPER}$, baseline variables breathing from a Douglas bag containing 5% inspired CO_2 in normoxia.

Variable	BL1	$BL2_{HYPO}$	$BL2_{NORMO}$	$BL2_{HYPER}$
HR (min^{-1})	76.4 ± 9.7	83.8 ± 11.1*	74.5 ± 9.8	75.0 ± 9.1
MAP (mm Hg)	97.3 ± 12.9	104.1 ± 12.9*	104.5 ± 15.9*	105.9 ± 9.1*
MCAv (cm/s)	51.3 ± 12.3	38.4 ± 7.1*	53.4 ± 11.9	68.6 ± 17.3*
PCAv (cm/s)	39.5 ± 9.2	30.4 ± 6.2*	41.1 ± 9.5	52.1 ± 11.5*
$P_{ET}CO_2$ (Torr)	33.5 ± 3.4	24.0 ± 2.2*	34.2 ± 2.8	42.9 ± 2.2*
$\dot{V}_I$ (L/min)	15.8 ± 3.0	28.2 ± 6.6*	15.6 ± 2.8	21.5 ± 4.2*

HR, heart rate. MAP, mean arterial pressure. MCAv, middle cerebral artery velocity. PCAv, posterior cerebral artery velocity. $P_{ET}CO_2$, pressure of end-tidal CO_2 (BTPS). $\dot{V}_I$, minute ventilation.
* Significantly different from BL1 ($P < 0.05$).

2.3. Experimental protocol

We chose to use a CPT to elicit SNS activation for our study. In contrast to drug infusions or exercise stressors, we reasoned that the CPT would provide a more homogenous stressor between trials (e.g., not dependent upon participant volition), and the CPT is repeatable and consistent (see Fig. 2C and D), and has a short washout period. In addition, we did not want changes in vascular resistance resulting from exercise stressor (e.g, sympatholysis) to confound the changes in MAP we sought to elicit. In addition, we chose to apply consistent and steady-state CO_2 challenges, in contrast to studies utilizing a cold water immersion-induced hyperventilation and resulting hypocapnia, which could be different in magnitude between individuals (see Tymko et al., 2017). Instead, we sought to induce large and consistent CO_2 challenges via coached hyperventilation (~ − 10 Torr $P_{ET}CO_2$) or increases in steady-state CO_2 from a standard 5% normoxic challenge, which was approximately + 9 Torr $P_{ET}CO_2$. In this way, we established relatively equal CO_2 steps, within-individuals (see Table 1).

The experimental protocol schematic is found in Fig. 1. Participants began with a 10-minute initial baseline (BL1), after which they were randomly subjected to the hypercapnia, hypocapnia or normocapnia trials. Each trial consisted of a three-minute baseline (BL2), a three-minute CPT, a one-minute washout period (at that level of CO_2), and a 10-minute recovery period breathing room air. Steady-state $P_{ET}CO_2$ levels were maintained across the entire trial through visual feedback and respiratory coaching, where we aimed to maintain the $P_{ET}CO_2$ at ± 1 Torr of BL2 values.

2.4. Data and statistical analysis

All data was collected using ADInstruments LabChart Pro v8.0 Software, and processed in Microsoft Excel. Statistical significance was assumed when $P < 0.05$ (SigmaPlot v14, Systat). In the case of ANOVAs and *t*-tests (below), when tests of normality and/or equal variance failed, the appropriate non-parametric tests were performed With respect to t-tests, they were always two-tailed. With respect to ANOVAs, when significant F-ratios were detected, a Student-Newman-Keuls post hoc test was performed for pair-wide comparisons.

3. Results

17 participants completed the experimental protocol and were included in the data analysis (8 females at various stages in ovarian cycle; age 21.3 ± 1.65 yrs.; BMI 22.6 ± 3.36 kg/m^2).

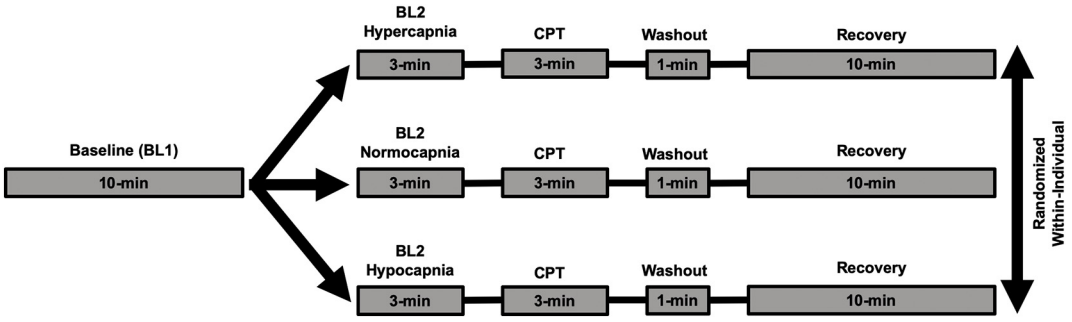

Fig. 1. Schematic representation of protocol. Participants began with a 10-minute baseline following instrumentation. Another 3-minute baseline followed the CO_2 intervention to reach steady-state, after which a 3-minute cold pressor test (CPT) took place. Following a 1-minute washout period, participants had approximately 10-min to recover between trials, while breathing room air.

3.1. Baseline 1

Following instrumentation, the participant breathed normally while remaining seated for 10 min. A baseline measurement was taken as a one-minute average near the end of the 10 min once variables reached a steady-state (see Table 1).

3.2. Steady-state CO_2 and cold pressor test

Following the initial baseline period, the CPT was performed under the three separate and randomized CO_2 conditions. For the normocapnic trial, the participants inspired room air and maintained their $P_{ET}CO_2$ at their BL1 level. For the hypocapnic trial, the participant hyperventilated to maintain a reduction in $P_{ET}CO_2$ of -10 Torr from their baseline normocapnic level. For the hypercapnic trial, participants breathed from a 200 L Douglas bag attached to the inspire port of a two-way valve containing 5% CO_2 in normoxia (i.e., 21%), elevating their $P_{ET}CO_2$ by approximately $\approx +9$ Torr at atmospheric pressure $\approx$665 Torr, 1045 m above sea level (see Table 1).

For all CPT trials, an investigator lowered both the participant's feet and lower legs (up to the lower end of the gastrocnemius) into a bath of ice water held at a temperature between 0 and 1 °C (e.g., Tymko et al., 2017). The duration of the CPT was 3 min, during which the participant maintained the predetermined $P_{ET}CO_2$ condition through visual feedback and coached breathing. This served to reduce the effects of cold-induced hyperventilation during the CPT. After the three-minute period, the participant's legs were raised out of the water and they maintained their $P_{ET}CO_2$ for one more minute CPT 'washout', after which the participant underwent a 10-minute recovery period breathing room air.

3.3. Recovery between trials

After the 10-minute washout period during each trial, the participants were able to place their feet and legs into a warm water bath for about 3 min, held at a comfortably warm temperature (between 25 and 30 °C). This aided in shortening the recovery period between trials by accelerating the return of skin temperature back to normal. Following the warm water bath, the feet and legs were dried off and left to acclimate to room air for 5 min and until blood pressure returned back to baseline levels. Total recovery time was ~10 min between trials.

3.4. Baseline variables

Baseline data was obtained as a one-minute bin at the end of BL1 and each subsequent BL2 (Table 1), expressed as mean ± standard deviation (SD). All variables between BL1 and the subsequent randomized BL2 (i.e., hypo-, normo-, and hypercapnia trials) were compared using a one-factor (1F) repeated-measures analysis of variance (RM ANOVA; Table 1).

3.5. Was there a CPT response?

All cardiovascular and cerebrovascular measurements during the CPT responses were analyzed as the mean of the entire three-minute period. Comparison of cardiovascular and cerebrovascular variables between BL2 and CPT were analyzed using paired *t*-tests (Fig. 2A and C, Fig. 3A and C).

3.6. Was the CPT response different between CO_2 trials?

Changes (i.e., delta BL2 to CPT) in cardiovascular and cerebrovascular variables were compared between CO_2 trials using one-factor (1F) repeated-measures (RM) analysis of variance (ANOVAs) for comparisons between the three CO_2 trials (i.e., Fig. 2B and D, Fig. 3B and D).

3.7. Was there a difference between anterior and posterior CBV responses?

For comparisons between CBV responses (delta and % change) in anterior and posterior cerebrovasculature at each level of CO_2, paired t-tests were utilized (Fig. 4).

3.8. Was there a difference between anterior and posterior CVR with CPT?

Cerebrovascular reactivity (CVR) was calculated as the slope in CBV responses across changes in $P_{ET}CO_2$, within-individuals, where slopes in anterior and posterior, and during baseline and CPT conditions, were compared using paired t-tests (Figs. 5 and 6).

3.9. Was there a sex or pain difference between CO_2 trials?

Sex differences in CVR were assessed using a Student t-test. Pain perception analysis was performed using 1F RM ANOVA on ranks.

3.10. Baseline values

Heart rate was elevated at rest during the hypocapnia trial due to voluntary hyperventilation (Table 1). Mean arterial pressure did not change significantly with alterations in $P_{ET}CO_2$. Hypocapnia ($BL2_{HYPO}$) decreased velocity in both the MCA and the PCA by -4.7 cm/s (-10.0%) and -3.1 cm/s (-9.1%) respectively. Hypercapnia ($BL2_{HYPER}$) increased velocity in both the MCA and the PCA by $+9.36$ cm/s ($+12.3\%$) and $+5.06$ cm/s ($+8.9\%$), respectively. There was no significant difference between BL1 measurements and BL2 values for normocapnia ($BL2_{NORMO}$), except MAP, which was mildly but significantly elevated at for $BL2_{NORMO}$. However, all BL2 MAP values were not different.

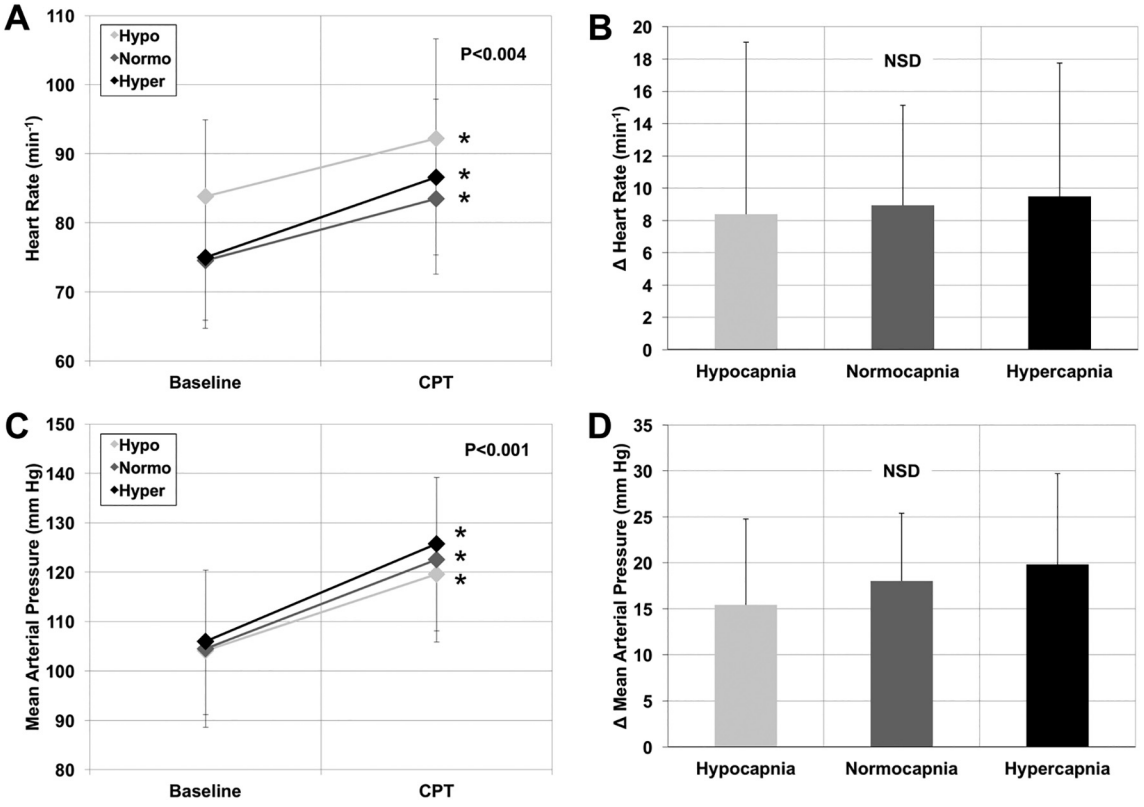

Fig. 2. Cardiovascular responses to the cold pressor test. Changes in heart rate and mean arterial pressure in response to a cold pressor test (Panel A & C) and absolute changes from baseline (panel B & D), for three CO_2 conditions. n = 17. CPT, cold pressor test. * denotes significantly different from BL1 ($P < 0.05$); NSD indicates no significant difference. Fig. 2B NSD: P = 0.1. Fig. 2D NSD: P = 0.1.

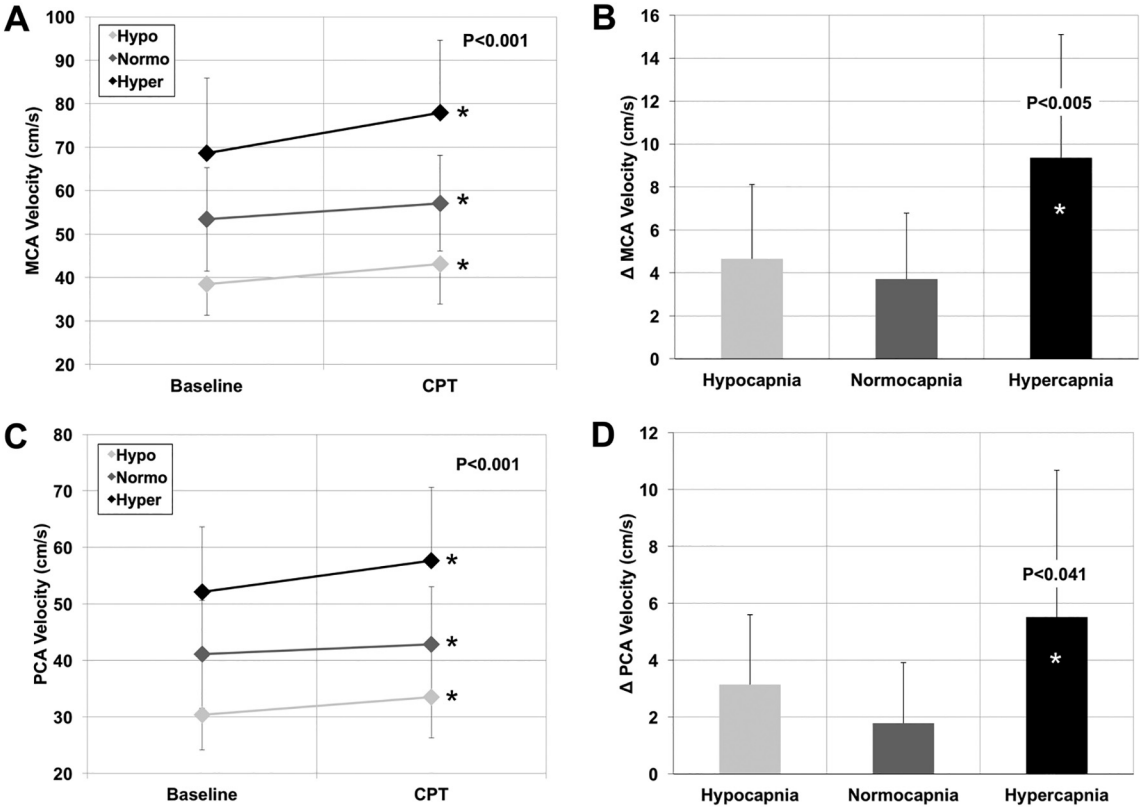

Fig. 3. Cerebrovascular responses to the cold pressor test. Changes in MCAv (Panel A & B) and PCAv (Panel C & D) in response to the CPT for three CO_2 conditions. n = 17 for panel A & B; n = 16 for panel C & D. MCA, middle cerebral artery, PCA, posterior cerebral artery, CPT, cold pressor test. * denotes significantly different from BL1 ($P < 0.05$).

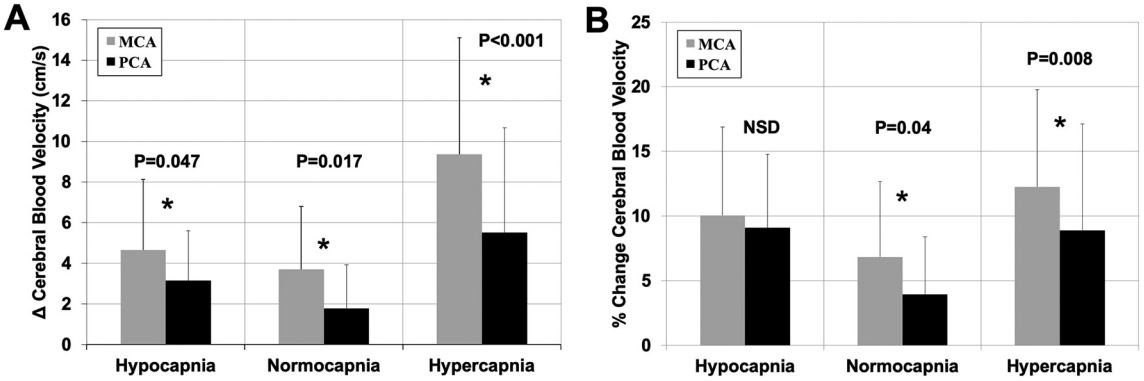

Fig. 4. Anterior vs. posterior response comparisons to the cold pressor test. MCA and PCA velocity responses to CPT expressed as absolute differences from baseline and % change, for three separate CO_2 conditions. n = 16 for all normocapnia and hypercapnia trials, n = 15 for all hypocapnia trials. MCA, middle cerebral artery, PCA, posterior cerebral artery. * indicates significant difference between the MCA and PCA responses for the specific CO_2 conditions ($P < 0.05$); NSD indicates no significant difference. Fig. 4B NSD: P = 0.5.

3.11. $P_{ET}CO_2$ during CPT trials

Although the cold water temperature was maintained at 0–1 °C throughout the 3-min CPT intervention, we also aimed to maintain the relative $P_{ET}CO_2$ values for each CO_2 trials at BL2 levels (i.e., hypo-, normo- and hypercapnia). We calculated an average of $P_{ET}CO_2$ across all three CPT trials, and compared them to the mean BL2 values for each trials using paired *t*-tests (see Table 1 for BL2 values). For the hypocapnic CPT trial, mean $P_{ET}CO_2$ was 24.0 ± 2.2. (compared to 24.0 ± 2.2 at BL2; P = 0.85). For the normocapnic CPT trial, mean $P_{ET}CO_2$ was 33.8 ± 2.6 (compared to 34.2 ± 2.8 at BL2; P = 0.25). For the hypercapnic CPT trial, mean $P_{ET}CO_2$ was 43.6 ± 2.6 (compared to 42.9 ± 2.2 at BL2; P = 0.02).

3.12. Cardiovascular responses to CPT

Our data shows the CPT was successful in eliciting a stress response, characterized by an increase in HR and MAP across all three CO_2 trials ($P < 0.001$; Fig. 2A & C). These cardiovascular increases (i.e., difference between respective BL2 and the CPT) were not different in magnitude across the three trials (Fig. 2B & D).

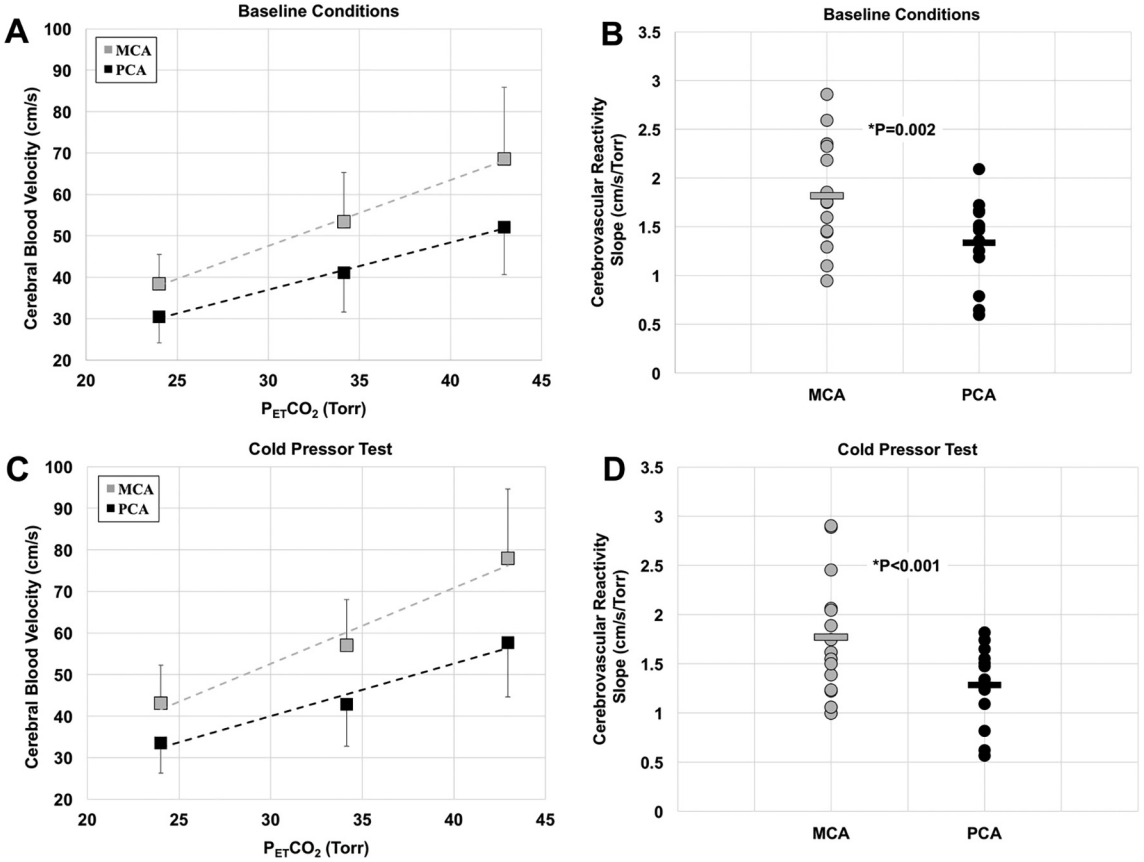

Fig. 5. Anterior vs. posterior CO_2 cerebrovascular reactivity comparisons. CVR was plotted as the slope of MCAv or PCAv over each $P_{ET}CO_2$ for each participant. The grey points represent each individual's slope, while the black points are the mean. n = 15. $P_{ET}CO_2$, end-tidal CO_2, MCA, middle cerebral artery, PCA, posterior cerebral artery. * indicates significant difference between MCA and PCA responses ($P < 0.05$).

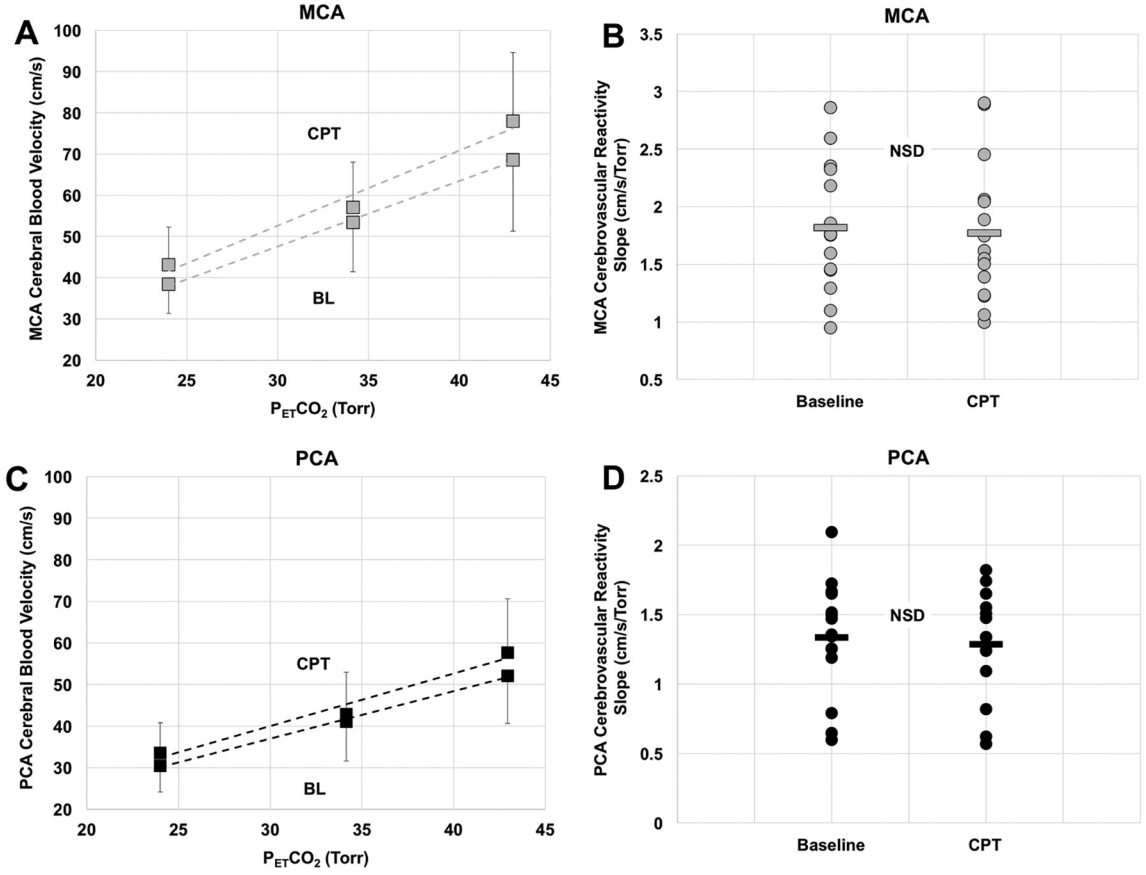

Fig. 6. Baseline vs. cold pressor test cerebrovascular reactivity comparisons. Panel A & C depict CVR during BL and the CPT. Panel B and D quantify the slope differences between BL and the CPT for both the MCA and the PCA. n = 15. $P_{ET}CO_2$, end-tidal CO_2, MCA, middle cerebral artery, PCA, posterior cerebral artery. NSD indicates no significant difference ($P > 0.05$). Fig. 6B NSD: P = 0.3. Fig. 6D NSD: P = 0.2.

3.13. Cerebrovascular responses to CPT

The CPT also evoked a significant increase from baseline in both MCAv and PCAv during all three CO_2 trials ($P < 0.001$; Fig. 3A & C). This CBV response was elevated in the hypercapnic trial in both the MCA and the PCA (both $P < 0.05$) compared to normocapnia and hypocapnia.

3.14. Comparing anterior and posterior CPT responses

When comparing the absolute responses of the MCA to the PCA, the MCA had a greater absolute increase in velocity across all CO_2 states (hypocapnic, P = 0.047; normocapnic, P = 0.017; hypercapnic, P < 0001; Fig. 4A). A similar pattern was observed in normocapnia (P = 0.040) and hypercapnia (P = 0.008) when percent changes in velocity responses were compared, however the percent change increase in velocity was not significantly different between vessels in the hypocapnic trial (P = 0.502; n = 15; Fig. 4B).

3.15. Anterior and posterior cerebrovascular CO_2 reactivity and CPT

The MCA was more reactive to CO_2 changes during both the baseline (P = 0.002) and CPT ($P < 0.001$), when compared to the PCA (Fig. 5). However, there was no difference in CO_2 CVR when comparing the baseline to CPT, for both the MCA (P = 0.318) and PCA (P = 0.149; Fig. 6).

3.16. Sex differences in CPT responses

Although not our primary research aim, we compared the MCA and PCA responses between males (n = 9) and females (n = 8) for each CO_2 state. The male and female MAP response to the CPT was not different across all three CO_2 trials ($P > 0.32$). During the normocapnic CPT, male MCAv response was elevated compared to females (P = 0.037), but this was not observed during hypo- nor hypercapnia CPT trials. There were no sex differences in the PCAv between trials ($P > 0.14$), or CVR of either vessel for any of the CO_2 trials ($P > 0.15$). The present study found no significant differences in the self-reported pain responses to the CPT between trials ($P > 0.74$) nor between sexes ($P > 0.44$). Given the low n for each sex, we would suggest that these results are interpreted with caution.

4. Discussion

We aimed to quantify the cerebrovascular responses to the CPT during three different levels of steady-state $P_{ET}CO_2$ (i.e., relative hypo-, normo- and hypercapnia). The principal findings of this study were that (a) cardiovascular responses to CPT (HR and MAP) were not different across the three CO_2 conditions, (b) increases in MCAv and PCAv during CPT were markedly elevated in the hypercapnia CPT trial compared to the normo- and hypocapnic trial, (c) absolute increases in MCAv were consistently greater than those of PCAv during all three CO_2 states and (d) the MCA demonstrated greater CVR to CO_2 changes than the PCA and (e) that CVR was unchanged between baseline conditions and the CPT.

4.1. Cardiovascular responses to the CPT

Both HR and MAP increased consistently from respective BL2 levels during the CPT, which suggests that neither response was affected by

changes in $P_{ET}CO_2$. Xie et al., (2001) and Steinback, Salzer, Medeiros, Kowalchuk, and Shoemaker (2008) showed that hypercapnia and hypoxia result in sympathetic nervous system activation, similar to sympathetic responses observed during the CPT. However, Xie, Skatrud, Puleo, and Morgan (2001) found that sympathetic activation lasted longer in hypoxia compared to hypercapnia, suggesting there may be varying levels of sympathetic activation with alterations in blood gasses. A logical follow-up study would be to measure SNA responses during hypo- normo- and hypercapnia. Unfortunately, in this study we do not have any direct or surrogate metrics regarding SNS activation aside from the increases in HR and MAP. Regardless, our cardiovascular responses were similar between CO_2 trials (both baseline and delta responses, see Table 1 and Fig. 2), suggesting that our CPT tests elicited similar magnitude stress responses between CO_2 trials, allowing for meaningful comparisons.

It has been previously shown that there may be varying degrees of pain associated with the CPT with alterations in blood gasses. Stokes III, Chapman, and Smith (1948) found that the threshold for pain was higher in hypercapnia compared to hypoxia. Mitchell, MacDonald, and Brodie (2004) also found that the pain response to the CPT was higher in males than in females. Because the present study found no significant differences in the self-reported pain responses to the CPT between trials, this uniformity in pain perception may account for the relatively uniform MAP and HR response to the CPT.

4.2. Cerebrovascular responses to the CPT

The CPT led to an increase in CBV compared to BL2 for all three CO_2 trials, but unlike the HR and MAP responses, the magnitude of increase in CBV was significantly greater during the hypercapnia CPT (see Fig. 3). It is well-understood that hypercapnia induces cerebral arteriolar vasodilation, reducing vascular resistance, and thereby increasing blood flow (Ainslie et al., 2005; Lennox & Gibbs, 1932; Skow et al., 2013; Verbree et al., 2014). When superimposed with CPT-mediated increases in MAP, the hypercapnia-mediated lowered resistance of the cerebral vasculature likely accounts for this permissive hyperemia. This is in accordance with previous studies on that have demonstrated that hypercapnia impairs the ability of the cerebrovasculature to regulate blood flow (i.e. cerebral autoregulation; Ainslie et al., 2005; Panerai, Deverson, Mahony, Hayes, & Evans, 1999; Tzeng, MacRae, Ainslie, & Chan, 2014). While the main determinant of CBV is likely changes in cerebral perfusion pressure (Panerai et al., 1999), when $P_{ET}CO_2$ is elevated by inspiring 5% CO_2, the increase in CBV may plateau due to the arterioles reaching their maximum dilatory capacity. It is currently unknown if there is a point where the vessels' hypercapnic dilatory capacity becomes saturated, or if the impairment of cerebral autoregulation is dose-dependent. Another logical follow-up study would be to test cerebral autoregulation at varying degrees of hypercapnia.

One possible explanation for not observing a decrease in hypocapnic CBV during the CPT (compared to normocapnia), is that there may have been some changes in the diameter of the conduit vessel being measured. However, there is conflicting evidence whether this occurs in the conduit arteries of the brain during blood gas challenges (Coverdale, Gati, Opalevych, Perrotta, & Shoemaker, 2014; Giller, Bowman, Dyer, Mootz, & Krippner, 1993; Laan, Dijk, Elting, Staal, & Absalom, 2013; Verbree et al., 2014; Verbree et al., 2017; Wilson et al., 2011; Hoiland & Ainslie, 2016, argue that artery diameter does change; Brothers & Zhang, 2016; Serrador, Picot, Rutt, Shoemaker, & Bondar, 2000, argue that they do not). If the MCA and PCA diameter did decrease during hypocapnia, then the effective blood velocity through that conduit likely increased, thus overestimating the absolute increase in CBF demonstrated in Fig. 3.

4.3. Anterior vs. posterior comparisons

The MCA demonstrated a larger absolute response than the PCA across all CO_2 states, consistent with our hypothesis and other studies (Flück et al., 2017; Skow et al., 2013). This is consistent with the possibility that the relative volume of downstream vasoactive vasculature is greater in areas perfused by the MCA vs. PCA (Willie et al., 2011; Willie, Tzeng, Fisher, & Ainslie, 2014). However, when analyzing %-change increase in velocity, the MCA and PCA response were not different in hypocapnia. The variable reactivity measured in anterior vs posterior conduits can partially be attributed to different baseline values (MacKay et al., 2016; Skow et al., 2013), regional differences in adrenergic innervation (e.g., Kondo, Miyazaki, Fujiwara, Yano, & Tabei, 1991), or regional differences in the bioavailability of nitric oxide, which is known to increase arterial compliance (Maeda et al., 2005; Sugawara et al., 2009). Although controversial, there is emerging evidence of the sympathetic nervous system affecting CBF regulation (e.g., Brassard, Tymko, & Ainslie, 2017; Cassaglia, Griffiths, & Walker, 2008). The potential differences between anterior and posterior cerebrovasculature may alter the response to a powerful sympathetic stimulator like a CPT, potentially increasing the degree of sympathetically-mediated vasoconstriction in the PCA (or downstream vessels) to dampen the CBV response compared to the MCA.

Larger MCA velocity responses may also result from preferential blood distribution to anterior structures in the brain during SNS stimulation. For example, Di Piero et al. (2001) showed an increase in blood flow to numerous structures supplied by the MCA during a CPT, but the thalamus was the only structure supplied by the PCA that was reported to receive an increase in blood flow during a CPT. Lewis et al. (2015), found the vertebral artery (upstream to the PCA) to be more sensitive to hypocapnia than the internal carotid artery (upstream of the MCA), which may be a protective mechanism to maintain consciousness during hypocapnia by favoring more posterior structures. This may partly account for why regional cerebral velocity %-changes were different during hypercapnia and normocapnia, but were not different in the hypocapnic trial.

4.4. Differential cerebrovascular reactivity to CO_2

Cerebrovascular CO_2 reactivity (CVR) in both baseline and CPT conditions was larger in the anterior compared to posterior vessels, consistent with previous reports using a rebreathing test (Reinhard, Waldkircher, Timmer, Weiller, & Hetzel, 2008; Skow et al., 2013). Skow et al. (2013) attribute this finding to a greater increase in cross-sectional area of downstream vascular beds in anterior structures. Whether the $PaCO_2$ sensitivity between anterior and posterior circulations observed in this study is a product of the preferential distribution of flow to anterior structures during CPT as discussed above, (e.g., Di Piero et al., 2001), or due to increased sensitivity of cerebrovasculature in the regions perfused by the MCA (e.g., Reinhard et al., 2008), or a cumulative result of these two factors, remains to be quantified.

CVR did not change between baseline and during CPT in either artery (Fig. 6), suggesting that sympathetic nervous system activation does not alter CVR. This is also consistent with a previous study showing that MCA CVR is unchanged in response to exercise-induced sympathetic activation (Ainslie et al., 2005).

Studies investigating CBF often only insonate anterior vessels such as the MCA, ICA and CCA to give an index of global CBF, as these account for the majority of blood flow to the brain (Ainslie et al., 2005; Claassen et al., 2009; Perry et al., 2016; Tymko et al., 2017). The present study contributes to a body of emerging evidence that demonstrates heterogeneous reactivity of MCAv and PCAv to various stimuli, challenging this approach when assessing global cerebral vascular function and health (Flück et al., 2017; Skow et al., 2013). Differential responses between the two conduits may indicate regional differences in neural and intracellular pathways that are activated under sympathetic stimulation and/or variable $PaCO_2$, potentially distributing CBF unevenly throughout the cerebrovasculature.

4.5. Sex differences in response to the cold pressor test

The comparisons of male and female responses to simultaneous CO_2 alterations and the CPT was not a primary objective of this study, however previous studies have shown that there are differences between males and females during the CPT (e.g., Tymko et al., 2017; Stone, Ainslie, Kerstens, Wildfong, & Tymko, 2019; Joyner, Wallin, & Charkoudian, 2016). Consistent with Tymko et al. (2017), our data indicate that males are more responsive to the CPT during normocapnia than females. Although some studies have shown a larger MAP response in males vs. females (Stone et al., 2019), no changes in MAP were observed between sexes in our study, so this cannot account for the different responses observed. Kilgour and Carvalho (1994) demonstrated that males maintain CPT-induced sympathetic activity longer than females under otherwise equivalent conditions. The same study also showed that males have a larger increase in systemic vascular resistance than females. These findings may not necessarily extend to our own, as the CPT stimulus used in the present study was more severe, which likely evoked a stronger pain response (Mitchell et al., 2004). Studies investigating CBF differences in males and females show conflicting results (Lewis et al., 2014 showed no difference; Wang, Chao, Chung, Huang, & Hu, 2010 showed regional response differences in females to orthostatic stress). Our small number of participants (n = 8 females; n = 9 males) warrants caution when assessing sex differences in our study.

4.6. Limitations

A main limitation of our study was the use of CBV as an index of CBF, which cannot address potential changes in conduit artery diameter. We also used a CPT to invoke the activation of the sympathetic nervous system, however we did not have a method for measuring sympathetic activity more directly (e.g., MSNA, steady-state HRV metrics). In addition, although cerebral autoregulation is in part responsible for dampening the CBV responses during CPT, we did not assess cerebral autoregulation via standard mathematical methods (e.g., transfer function analysis), given the transient nature of our intervention (i.e., not steady-state). Our low sample size made it difficult to draw accurate conclusions from sex comparisons. Furthermore, as sex comparisons was not a primary research aim of this study, and the phase of ovarian cycle was not controlled. Future investigations of this topic should track sex as a variable for understanding cerebral autoregulation in humans, as there is a still debate regarding the existence and relevance of sex differences in cerebrovascular regulation.

5. Conclusion

We tested the potential synergistic effects of a CPT and alterations in CO_2 on anterior and posterior cerebral circulations. Hypercapnia elevated the MCAv and PCAv responses to the CPT, whereas hypocapnia did not diminish the MCAv and PCAv response compared to normocapnia. The MCAv had consistently greater absolute responses to the CPT than the PCAv, independent of CO_2 state. The CVR to CO_2 was not different between baseline and CPT, however, the MCA demonstrated greater reactivity than the PCA during both baseline and CPT. These results indicate that there is a differential regional response to sympathetic stressors throughout the brain. These data add to the growing body of work suggesting (a) the pressure-passive nature of the cerebral circulations, (b) the effects of increased CO_2 on cerebral autoregulation, (c) regional differences in cerebrovascular reactivity (CVR), and suggest a lack of effect of sympathetic stimulation on CO_2 CVR magnitude.

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