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Longitudinal study of cerebral blood flow regulation during exercise in pregnancy

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Abstract

Cerebrovascular adaptation to pregnancy is poorly understood. We sought to assess cerebrovascular regulation in response to visual stimulation, hypercapnia and exercise across the three trimesters of pregnancy. Using transcranial Doppler (TCD) ultrasound, middle and posterior cerebral artery mean blood velocities ($MCAv_{mean}$ and $PCAv_{mean}$) were measured continuously at rest and in response to (1) visual stimulation to assess neurovascular coupling (NVC); (2) a modified Duffin hyperoxic CO_2 rebreathe test, and (3) an incremental cycling exercise test to volitional fatigue in non-pregnant ($n = 26$; NP) and pregnant women (first trimester [$n = 13$; TM1], second trimester [$n = 21$; TM2], and third trimester [$n = 20$; TM3]) in total 47 women. At rest, $MCAv_{mean}$ and $P_{ET}CO_2$ were lower in TM2 compared to NP. $PCAv_{mean}$ was lower in TM2 but not TM1 or TM3 compared to NP. Cerebrovascular reactivity in $MCAv_{mean}$ and $PCAv_{mean}$ during the hypercapnic rebreathing test was not different between pregnant and non-pregnant women. $MCAv_{mean}$ continued to increase over the second half of the exercise test in TM2 and TM3, while it decreased in NP due to differences in $\Delta P_{ET}CO_2$ between groups. Pregnant women experienced a delayed decrease in $MCAv_{mean}$ in response to maximal exercise compared to non-pregnant controls which was explained by CO_2 reactivity and $P_{ET}CO_2$ level.

Keywords

Cardiovascular, cerebral blood flow, exercise, physiology, pregnancy

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Introduction

Pregnancy is associated with profound cardiovascular adaptations in order to support the needs of the growing fetus. Maternal blood volume expands by 50% throughout gestation[1] which is accompanied by an increase in heart rate and stroke volume, and a significant drop in systemic vascular resistance (SVR) which reaches a nadir in the second trimester.[2] In non-pregnant populations, cerebral blood flow (CBF) is exquisitely regulated to maintain adequate perfusion to the brain.[3] The increased cardiovascular load and circulating factors that rise over the course of pregnancy pose a unique challenge for the brain, which requires a constant and carefully regulated blood supply. Based on rigorous work conducted in animals, we know that high levels of circulating growth factors and cytokines that promote substantial hemodynamic changes in other vascular beds during pregnancy exhibit a limited influence on cerebral circulation.[4] Remodeling of arteries, which is highly important for vascular function and structure in other organs during pregnancy, is prevented and even reversed in cerebral arteries.[5] Remarkably, blood–brain barrier function is unperturbed in healthy pregnancy, despite a significant increase in circulating vasoactive permeability factors.[6]

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Recent statistics indicate a stroke risk between 4 and 40 per 100,000 in women of childbearing age.[7] Although the risk of stroke remains relatively low during pregnancy (the overall prevalence in the antenatal and postpartum period is estimated at 30 per 100,000),[8] this is higher than the non-pregnant population. In addition, the risk of stroke is four to six times higher in women with hypertensive disorders in pregnancy and in the peripartum period.[9] These findings strongly suggest that the pregnant brain may be vulnerable to physiological stress. Yet the impact of pregnancy on cerebrovascular function in humans remains poorly understood.[10,11]

To date, there have been a handful of investigations examining the cerebrovascular regulation in healthy human pregnancy. While, we recently demonstrated progressively augmented cerebrovascular responsiveness to changes in CO_2 over the course of pregnancy in a longitudinal case report,[27] other data suggest that cerebrovascular $P_{ET}CO_2$ reactivity may be similar between pregnant women in the third trimester compared to non-pregnant controls.[12] Additional data suggest similar cerebral neurovascular coupling responses in third trimester women compared to non-pregnant controls.[13] To date, our case study remains the only data related to cerebrovascular regulation in early and mid-pregnancy and no data exist to our knowledge on the functional cerebral responses to exercise.

Regular physical activity during pregnancy is associated with a 40% decrease in the risk of developing serious pregnancy complications such as gestational hypertension and preeclampsia which are known to impact cerebral circulation.[14] In non-pregnant populations, exercise intensity up to 60% of maximal oxygen uptake results in a continuous increase in CBF.[15] However, higher intensity exercise leads to a decline in CBF towards baseline values due to the influence of hyperventilation-induced cerebral vasoconstriction.[15] To date, the cerebrovascular response to acute exercise during pregnancy is unknown. Therefore, the objective of the current study was to examine the impact of pregnancy on resting CBF and reactivity to (1) visual stimulation (NVC); (2) hypercapnic rebreathe (humoral stimulation); and (3) progressive exercise to volitional fatigue. We hypothesized that resting CBF would be similar or slightly decreased over the three trimesters of pregnancy compared to non-pregnant women. We further hypothesized that the reactivity of the middle and posterior cerebral arteries (MCA and PCA, respectively) to visual stimulation, CO_2 rebreathe, and exercise would be increased from the first to the third trimester compared to non-pregnant controls.

Materials and methods

Ethical approval

This test protocol was approved by the Health Research Ethics Board at the University of Alberta (Approval No. Pro00040722) and conformed to the standards set by the latest revision of the Declaration of Helsinki.

Participants

Participants ($n = 47$) who were >18, non-smokers (minimum one year), were having a singleton pregnancy, were not pregnant in the last six months (non-pregnant women) and free from known neurological, respiratory, or cardiovascular diseases completed testing at one or more time points. The experimental breakdown included assessments grouped by: nonpregnant assessments [$n = 26$; NP], first trimester assessments [$n = 13$; TM1], second trimester assessments [$n = 21$; TM2], or third trimester assessments [$n = 20$; TM3]. A complete breakdown of participant assessments (NP, TM1, TM2, and TM3) is shown in Supplemental Table 1. All participants provided both verbal and written informed consent prior to participation in this study. Gestational age was calculated from the first day of the last menstrual period and confirmed by ultrasound. None of the participants reported a history of gestational diabetes, gestational hypertension, or preeclampsia. Nonpregnant women were tested during the early follicular phase of the menstrual cycle, with the exception of those who were taking hormonal contraceptives where cycling had ceased (Nuva Ring, $n = 1$; Min-Ovral, $n = 1$; Yasmin, $n = 1$; Yaz, $n = 1$); these women were tested at their convenience. Pregnant women received medical clearance from their health care provider prior to exercise testing (PARMed-X 2013).[16] Nonpregnant women completed the Physical Activity Readiness Questionnaire (PAR-Q) to pre-screen for potential contraindications to exercise (Par-Q and You 2002).[17]

Instrumentation

Participants were instrumented with transcranial Doppler ultrasound (TCD; Multigon Industries, TOC 2MD Yonkers, NJ, USA) of the middle and posterior cerebral arteries (MCA and PCA) to measure the cerebral blood velocity (CBV; cm/s), an approximation of CBF (mL/min).[18,19] Mean MCA and PCA flow velocities ($MCAv_{mean}$ and $PCAv_{mean}$, respectively) were calculated on a beat-by-beat basis as the area under the peak velocity waveform. Probe placement and craniofacial landmarks were traced onto transparencies at

participant's initial visit in order to standardize probe placement for subsequent visits.

Participants were instrumented with a respiratory apparatus consisting of a nose clip, a mouthpiece, a disposable bacteriological filter, and a heated (37 °C) respiratory flow head (MLT1000L, ADInstruments, Colorado Springs, Colo., USA). Respiratory flow was measured by the pneumotachometer (MLT3819H-V, Hans Rudolph Inc., Shawnee, Ky., USA) and spirometer amplifier (FE141, ADInstruments). The respiratory flow head was calibrated daily using a 3-L calibration syringe (Hans Rudolph Inc.).

Continuous electrocardiogram (ECG; lead II) was measured throughout testing. Blood pressure was measured continuously using photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands) and was calibrated post-test to baseline blood pressure values obtained using manual mercury sphygmomanometry. Calibrated pressure waveforms were analyzed to determine beat-by-beat mean (MAP), systolic (SBP), and diastolic blood pressure (DBP). Signals were recorded at a sampling frequency of 1000 Hz.

Experimental protocol

Participants arrived at the laboratory following a 12-h fast and having abstained from caffeine, alcohol, and strenuous exercise for 12 h. On arrival, participants were fed a standardized meal. Participant body weight was measured with a standard calibrated scale, and height was measured with a stadiometer. Participants were seated on a recumbent cycle ergometer (45°; Cardio Comfort 837E, Monark, Sweden) in a laboratory maintained at 20 °C, and they rested for a minimum of 20 min before beginning the protocol which consisted of:

1. Visual stimulus.[20] Following a 2-min eyes-closed baseline in a dark room, participants opened their eyes for five trials of 30 s of flashing light (0.10 s light/dark cycles) separated by four periods of 30 s with eyes-closed in a dark room. For the visual stimulus test, seconds −18 to 0 were combined to represent time 0 (baseline). Seconds 2 to 20 of the stimulus portion of the test were presented as an average value of the five stimulus exposure trials.
2. Modified Duffin hyperoxic CO_2 rebreathing test.[21–23] Following 5 min of baseline, participants voluntarily hyperventilated until $P_{ET}CO_2$ was reduced to ~25 Torr for 1 min. After a full inspiration and expiration, participants were switched using a three-way valve to a bag containing 95% O_2 and 5% CO_2 and instructed to inspire one deep breath. Participants then resumed natural breathing until: (1) their $P_{ET}CO_2$ reached 55 Torr; (2) the bag deflated; or (3) they used previously agreed upon hand signals to indicate they wished to terminate the test.
3. Incremental exercise to volitional fatigue. Following a 5-min inactive baseline, participants cycled for 5 min at 25 W, maintaining a pedal rate of 50 r/min. Following the 5-min warm-up, the pedal rate was maintained at 50 r/min, and the work rate was increased by 25 W/min until volitional fatigue. Perceived exertion was recorded every second increment during exercise, using a Borg scale.[24] On reaching volitional fatigue, participants underwent a 5-min active recovery period at 25 W.

Data and statistical analyses

One-way ANOVA with Tukey post hoc test and Fischer's exact test were used to determine whether descriptive characteristics of the participants varied between groups. Multilevel mixed-effects models were employed to examine the impact of TM1, TM2, and TM3 of pregnancy compared to NP (*reference group*) on physiological outcomes over (1) seconds during the visual stimulus test and (2) watts during the exercise test until volitional fatigue due to the unbalanced nature of measurements per participant. Consecutively, linear, quadratic, cubic, quartic, and quintic time, and the interaction between phase of pregnancy and these time components were considered and all lower order variables were included in the model when higher order variables were significant. Analyses of the hypercapnic rebreathe test were conducted using multilevel modelling of the cardiovascular and cerebrovascular responses to CO_2 Torr during the initial response component of the test (see Online Supplement Figure 1). The cerebral blood flow response to exercise has been shown to be bimodal, with a plateau or drop above anaerobic threshold due to a concomitant reduction in $P_{ET}CO_2$.[15] MCA and PCA resistances were calculated as $\frac{blood\ pressure}{flow}$, while MCA and PCA conductances were calculated as $\frac{flow}{blood\ pressure}$. For the incremental exercise test, corrected $MCAv_{mean}$ and $PCAv_{mean}$ values were adjusted for participants' CO_2 reactivity to the CO_2 rebreathe test and the change in $P_{ET}CO_2$ from baseline during the exercise test ($MCAv_{mean}$ and $PCAv_{mean}$ corrected).

Models were conducted with clustering at the participant and the (nested) participant-by-phase level. Unstructured variance/covariance structure was employed in all mixed-effects models unless covariance was non-significant at an alpha level of 0.05, in which case independent variance/covariance structure was more appropriate. First, second, and third level residuals were graphed to test the assumptions of normality

and homogeneity. All statistical analyses were carried out in consultation with a statistician with formal training in advanced multilevel modelling. Statistical analyses were conducted using Stata MP 13.

Results

Anthropometric and baseline characteristics

In our analysis of 213 assessments from 47 participants (see Supplemental Table 1), gestation (wk) and weight (kg) were significantly different between groups (as expected), while height (cm), non/prepregnant BMI (kg/m^2), parity, and pregnant BMI (kg/m^2) between the three pregnant groups were not different (Table 1). As expected, baseline mean arterial pressure (MAP) was lower, while heart rate was higher across pregnancy compared to NP. $P_{ET}CO_2$ was lower in TM2 and TM3 compared to NP. Tidal volume was significantly higher in TM2 and TM3, while VE was significantly increased in TM3 only. Respiratory rate was not significantly different in pregnancy. $MCAv_{mean}$ was progressively lower in TM2 and TM3 compared to NP (TM2 β: −5.87, 95% CI: −11.18, −0.55;

Table 1. Descriptive and baseline characteristics of the participants.

	NP ($n=26$)	TM1 ($n=13$)	TM2 ($n=21$)	TM3 ($n=20$)
Age (y)	28±6	31±4	31±4	31±4
Gestation (wk)*	N/A	11±1	24±1	33±2
Height (cm)	167±6	167±6	167±7	168±7
Weight (kg)*	65±11	71±20	73±12	**76±11**†
Non/prepregnant BMI (kg/m^2)	23.1±3.4	23.2±1.9	23.1±2.6	23.1±2.7
Pregnant BMI (kg/m^2)	N/A	25.5±7.4	25.8±4.2	26.6±2.3
Parity (count 1/0; % with parity=1)	7/19 (26%)	5/8 (38%)	7/14 (33%)	5/15 (25%)
Ethnicity				
Caucasian	22 (85%)	12 (92%)	19 (90%)	17 (85%)
Asian	2 (8%)	1 (8%)	1 (5%)	2 (10%)
Other	2 (8%)	0	1 (5%)	1 (5%)
MAP (mmHg)*	90.99	**−4.60**†	**−10.31**§	**−7.54**§
	(88.37, 93.62)	**(−9.11, −0.08)**	**(−14.16, −6.47)**	**(−11.44, −3.64)**
Heart rate (bpm)*	71.59	**+8.24**§	**+9.34**§	**+13.89**§
	(68.03, 75.15)	**(3.88, 12.60)**	**(5.49, 13.19)**	**(10.01, 17.78)**
P_{ET} CO_2 (Torr)*	39.54	−2.27	**−2.45**†	**−3.87**‡
	(37.79, 41.28)	(−4.90, 0.36)	**(−4.72, −0.18)**	**(−6.18, −1.56)**
Tidal volume*	0.75	+0.03	**+0.14**†	**+0.18**‡
	(0.65, 0.85)	(−0.10, 0.15)	**(0.03, 0.25)**	**(0.08, 0.29)**
Respiratory rate	19.13	−1.10	−1.36	−0.82
	(17.83, 20.43)	(−3.13, 0.94)	(−3.14, 0.41)	(−2.61, 0.97)
VE*	14.07	+0.09	+2.49	**+3.97**†
	(10.34, 17.79)	(−3.75, 3.94)	(−0.95, 5.92)	**(0.63, 7.31)**
$MCAv_{mean}$*	63.93	−2.63	**−5.87**†	**−6.39**†
	(60.11, 67.74)	(−8.88, 3.62)	**(−11.18, −0.55)**	**(−11.83, −0.95)**
$PCAv_{mean}$*	42.28	−3.00	**−8.17**‡	−4.62
	(38.38, 46.18)	(−9.21, 3.22)	**(−13.51, −2.84)**	(−10.15, 0.91)
MCA resistance	1.48	−0.09	−0.04	−0.03
	(1.37, 1.59)	(−0.29, 0.11)	(−0.20, 0.13)	(−0.20, 0.14)
PCA resistance	2.39	−0.07	+0.16	−0.05
	(2.05, 2.73)	(−0.63, 0.49)	(−0.32, 0.64)	(−0.55, 0.44)
MCA conductance	0.69	−0.02	+0.0006	−0.008
	(0.63, 0.76)	(−0.13, 0.10)	(−0.09, 0.10)	(−0.10, 0.09)
PCA conductance	0.49	+0.05	−0.04	−0.02
	(0.44, 0.54)	(−0.04, 0.14)	(−0.12, 0.04)	(−0.10, 0.06)

Note: Data are presented as means ± SD or constant term (95% CI) and β coef (95% CI). Significant values are in bold.
*Significant difference between groups ($p < 0.05$).
†Significant difference vs. NP ($p < 0.05$).
‡Significant difference vs. NP ($p < 0.01$).
§Significant difference vs. NP ($p < 0.001$).
p-values adjusted for clustering by individual.
BMI: body mass index; NP: nonpregnant; TM1: first trimester; TM2: second trimester; TM3: third trimester.

TM3 β: −6.39, 95% CI: −11.83, −0.95; NP constant term: 63.93). $PCAv_{mean}$ reached a nadir that was lower in TM2 but not TM1 or TM3 compared to NP (β: −8.17, 95% CI: −13.51, −2.84; NP Constant term: 42.28). Taking into account concurrent changes in blood pressure, MCA and PCA resistance and conductance were not different in TM1, TM2, or TM3 compared to NP at rest.

Neurovascular coupling

In response to visual stimulation (increased local metabolism), maximum absolute and relative values of $PCAv_{mean}$ were not different between pregnant and non-pregnant groups (Figure 1). However, the rate of response to stimulation was faster in TM2, (shorter time to peak velocity) compared to NP (β: −2.10 s, 95% CI: −4.37, 0.17; $p = 0.069$). $MCAv_{mean}$ and MCA/PCA conductance responses were not different between groups (see Table 2).

Modified Duffin hyperoxic rebreathing test

There was no difference in response to CO_2 between the NP and TM1, TM2, or TM3 groups during the CO_2 rebreathe test in MAP, heart rate, $MCAv_{mean}$, $PCAv_{mean}$, MCA resistance, PCA resistance, tidal volume, or frequency of breathing (Table 3). While the hypercapnic ventilatory response (HCVR) was significantly predicted by phase of pregnancy, no one phase on its own was significantly different from NP.

Incremental exercise test to volitional fatigue

A similar proportion of participants in each group completed the maximal stage of the exercise test (W = 175): NP – 48%; TM1 – 50%; TM2 – 22%; TM3 – 37%; Fischer's exact test p-value = 0.296. HR increased during the test in all four groups, with HR starting at a lower point and ending at a higher point in NP compared to TM1, TM2, and TM3.

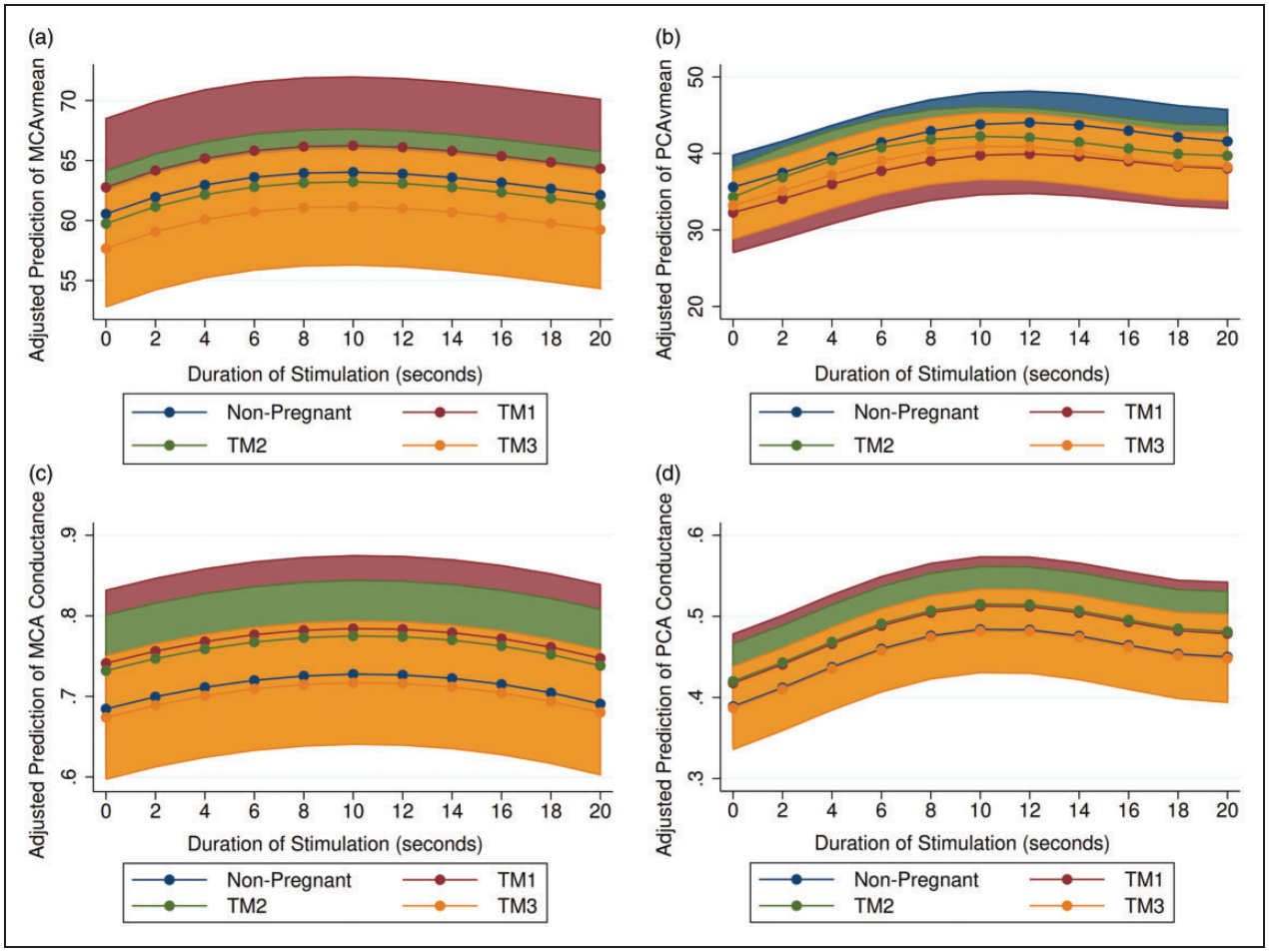

Figure 1. The predicted response to NVC challenge in: (a) $MCAv_{mean}$; (b) $PCAv_{mean}$; (c) MCA conductance; and (d) PCA conductance over light stimulation duration in seconds. Light stimulation began at time 1 s and ceased at time 30 s. All models adjusted for within-participant variability. Shaded area represents 95% Confidence Interval. Blue – nonpregnant; red – TM1; green – TM2; yellow – TM3. BL: baseline; MCA: middle cerebral artery; PCA: posterior cerebral artery; TM1: trimester 1; TM2: trimester 2; TM3: trimester 3.

Table 2. CBF and hemodynamic results from mixed-effects MLM across time (seconds) during the NVC light stimulus test.

Variable	**NP** constant term (95% CI)	**TM1** β Coef. (95% CI)	**TM2** β Coef. (95% CI)	**TM3** β Coef. (95% CI)	**Significant interaction with time**
MAP(mm Hg)	88.64 (84.60, 92.69)	**−6.65**† **(−12.52, −0.78)**	**−7.46**† **(−12.60, −2.31)**	−2.52 (−7.92, 2.89)	
Heart rate (bpm)	67.85 (63.83, 71.88)	**+8.93**† **(3.02, 14.84)**	**+11.15**‡ **(5.99, 16.31)**	**+15.86**‡ **(10.44, 21.29)**	
$MCAv_{mean}$ (cm/s)	60.56 (55.92, 65.19)	+2.21 (−5.06, 9.48)	−0.81 (−7.05, 5.43)	−2.88 (−9.47, 3.71)	
$PCAv_{mean}$ (cm/s)	35.58 (31.87, 39.30)	−3.39 (−9.30, 2.51)	−1.23 (−6.29, 3.83)	−2.35 (−7.69, 3.00)	**TM2**
Time to peak $PCAv_{mean}$	12.00 (10.35, 13.65)	+0.33 (−2.27, 2.94)	**−2.10**‡ **(−4.37, 0.17)**	−0.24 (−2.60, 2.13)	
MCA conductance	0.68 (0.61, 0.76)	+0.06 (−0.06, 0.17)	+0.05 (−0.05, 0.15)	−0.01 (−0.11, 0.09)	
PCA conductance	0.389 (0.34, 0.44)	+0.029 (−0.05, 0.11)	+0.031 (−0.03, 0.10)	−0.002 (−0.07, 0.07)	

Note: Data are presented as adjusted means (for reference term), β coefficients, and 95% Confidence Intervals. Analyses were adjusted for clustering by participant and participant × phase.
Significant values in bold. †Significant difference vs. NP ($p < 0.05$).
‡Significant difference vs. NP ($p < 0.01$).
BP: blood pressure; MCA: middle cerebral artery; NP: nonpregnant; TM1: first trimester; TM2: second trimester; TM3: third trimester; PCA: posterior cerebral artery.

Table 3. Cardiovascular, cerebrovascular and respiratory responses during the initial response phase of the modified Duffin CO_2 rebreathe test.

	NP Constant term (95% CI)	**TM1** β Coef. (95% CI)	**TM2** β Coef. (95% CI)	**TM3** β Coef. (95% CI)
MAP (mmHg)	0.69 (0.21, 1.16)	+0.06 (−0.57, 0.69)	−0.08 (−0.87, 0.72)	−0.14 (−0.68, 0.40)
Heart rate (bpm)	−0.90 (−1.47, −0.32)	−0.53 (−1.49, 0.42)	+0.22 (−0.66, 1.11)	+0.37 (−0.42, 1.16)
$MCAv_{mean}$	2.86 (2.28, 3.45)	+0.79 (−0.26, 1.84)	+0.42 (−0.32, 1.17)	+0.35 (−0.42, 1.12)
$PCAv_{mean}$	1.61 (1.19, 2.03)	+0.42 (−0.36, 1.20)	+0.30 (−0.31, 0.92)	+0.25 (−0.32, 0.82)
MCA resistance	−0.067 (−0.09, −0.05)	−0.002 (−0.03, 0.03)	−0.012 (−0.05, 0.03)	+0.003 (−0.02, 0.03)
PCA resistance	−0.09 (−0.12, −0.06)	−0.031 (−0.09, 0.03)	−0.040 (−0.10, 0.02)	−0.004 (−0.05, 0.04)
Tidal volume	0.02 (−0.04, 0.08)	−0.02 (−0.11, 0.07)	−0.01 (−0.09, 0.06)	+0.03 (−0.04, 0.10)
Frequency of breathing	0.03 (−0.22, 0.28)	−0.45 (−1.05, 0.15)	+0.09 (−0.33, 0.50)	+0.06 (−0.50, 0.63)
VE*	0.21 (−0.37, 0.80)	−0.46 (−1.40, 0.47)	−0.07 (−0.86, 0.73)	+0.62 (−0.24, 1.48)

Note: Data are presented as adjusted means (for reference term), β coefficients, and 95% confidence intervals. Analyses were adjusted for clustering by participant.
*Pregnancy phase significantly predicted values according to linear regression adjusted for clustering by participant ($p < 0.05$).
BP: blood pressure; MCA: middle cerebral artery; NP: nonpregnant; TM1: first trimester; TM2: second trimester; TM3: third trimester; PCA: posterior cerebral artery.

MAP increased linearly and equivalently in all four groups during the course of the test but remained lower in TM2 and TM3 compared to NP. Prior to exercise, $MCAv_{mean}$ was significantly lower in TM2 and TM3 compared to NP, and progressively decreased over gestation as pregnancy progressed (see Table 4). As expected, $MCAv_{mean}$ decreased from 100 to 175 W (test end) in NP (Figure 2). However, this drop in $MCAv_{mean}$ did not occur in TM2, and $MCAv_{mean}$ actually continued to rise until volitional fatigue in TM3. Following correction for individual CO_2 reactivity from the Duffin hypercapnic rebreathe test and change in $P_{ET}CO_2$ during the exercise test ($MCAv_{mean}$ Corrected), estimated change in $MCAv_{mean}$ in response to exercise was no longer different in TM2 and TM3 compared to NP. The response in $PCAv_{mean}$ during the exercise test was similar between pregnant and non-pregnant participants (see Online Supplement Figure 2). Correction for individual CO_2 reactivity from the Duffin hypercapnic rebreathe test and change in $P_{ET}CO_2$ during the exercise test ($PCAv_{mean}$ Corrected) did not explain the response in $PCAv_{mean}$ to exercise in pregnancy. The response in MCA conductance during exercise was also different between pregnant and non-pregnant participants. MCA conductance decreased from 50 to 175 W (test end) in NP but did not decrease in TM1, TM2, or TM3 until 150 to 175 W. As a result, predicted MCA conductance appeared to be lower at 175 W in NP compared to TM1, TM2, and TM3. Similarly, the response of PCA conductance was significantly different in TM2. PCA conductance slowly decreased from 50 to 175 W in NP, but not TM2. Changes in O_2 and CO_2 in response to exercise can be seen in Online Supplement Figure 3.

Discussion

Our study demonstrates several novel observations of cerebral blood flow regulation in pregnancy. Foremost, $MCAv_{mean}$ was progressively lower at rest in TM2 and TM3. $PCAv_{mean}$, on the other hand, was significantly lower at rest in TM2, but returned to non-pregnant values in TM3. Second, the time to peak $PCAv_{mean}$ value during the neurovascular coupling (NVC) test occurred earlier in TM2 than NP ($p = 0.07$). Third, the change in CBF in response to progressive hypercapnic exposure (modified Duffin rebreathe test) was not different during pregnancy. Finally, $MCAv_{mean}$ did not decrease during maximal exercise in TM2 and TM3 like it did in NP.

Influence of pregnancy on resting $MCAv_{mean}$ and $PCAv_{mean}$

Our findings suggest that $MCAv_{mean}$ and $PCAv_{mean}$ are decreased during mid-pregnancy at rest. However, while $MCAv_{mean}$ decreased further in late pregnancy, $PCAv_{mean}$ returned to non-pregnant values in the third trimester, which suggests that blood flow in these two arteries is controlled by divergent processes.

Table 4. CBF results from mixed-effects MLM across watts (0(25)200) during incremental exercise to volitional fatigue.

Variable	**NP (*ref*)** Constant term (95% CI)	**TM1** β Coef. (95% CI)	**TM2** β Coef. (95% CI)	**TM3** β Coef. (95% CI)	**Significant interaction with time**
$MCAv_{mean}$	63.64 (59.73, 67.54)	−1.54 (−8.05, 4.96)	**−5.64*** **(−11.15, −0.12)**	**−6.23*** **(−11.88, −0.57)**	**TM2 TM3**
$MCAv_{mean}$ corrected	63.37 (58.60, 68.14)	−1.00 (−9.25, 7.24)	−4.86 (−11.86, 2.15)	−3.33 (−10.45, 3.79)	
$PCAv_{mean}$	42.30 (38.29, 46.32)	−2.17 (−8.54, 4.20)	**−8.59†** **(−14.05, −3.12)**	−4.82 (−10.49, 0.85)	
$PCAv_{mean}$ corrected	42.17 (36.00, 48.33)	+1.23 (−9.21, 11.67)	−2.35 (−11.27, 6.57)	−1.37 (−10.45, 7.71)	**TM1 TM3**
MCA conductance	0.69 (0.63, 0.76)	−0.012 (−0.13, 0.10)	+0.001 (−0.10, 0.10)	−0.010 (−0.11, 0.09)	**TM1 TM2 TM3**
PCA conductance	0.49 (0.44, 0.54)	+0.06 (−0.03, 0.15)	−0.04 (−0.12, 0.04)	−0.02 (−0.10, 0.06)	**TM2**

Note: Multilevel model adjusted for random intercept at the participant and (nested) participant x phase of pregnancy levels. Data are presented as adjusted means (for reference term), β coefficients, and 95% confidence intervals. Analyses were adjusted for clustering by participant.

*Significant difference vs. NP ($p < 0.05$).

†Significant difference vs. NP ($p < 0.01$).

BP: blood pressure; MCA: middle cerebral artery; NP: nonpregnant; TM1: first trimester; TM2: second trimester; TM3: third trimester; PCA: posterior cerebral artery.

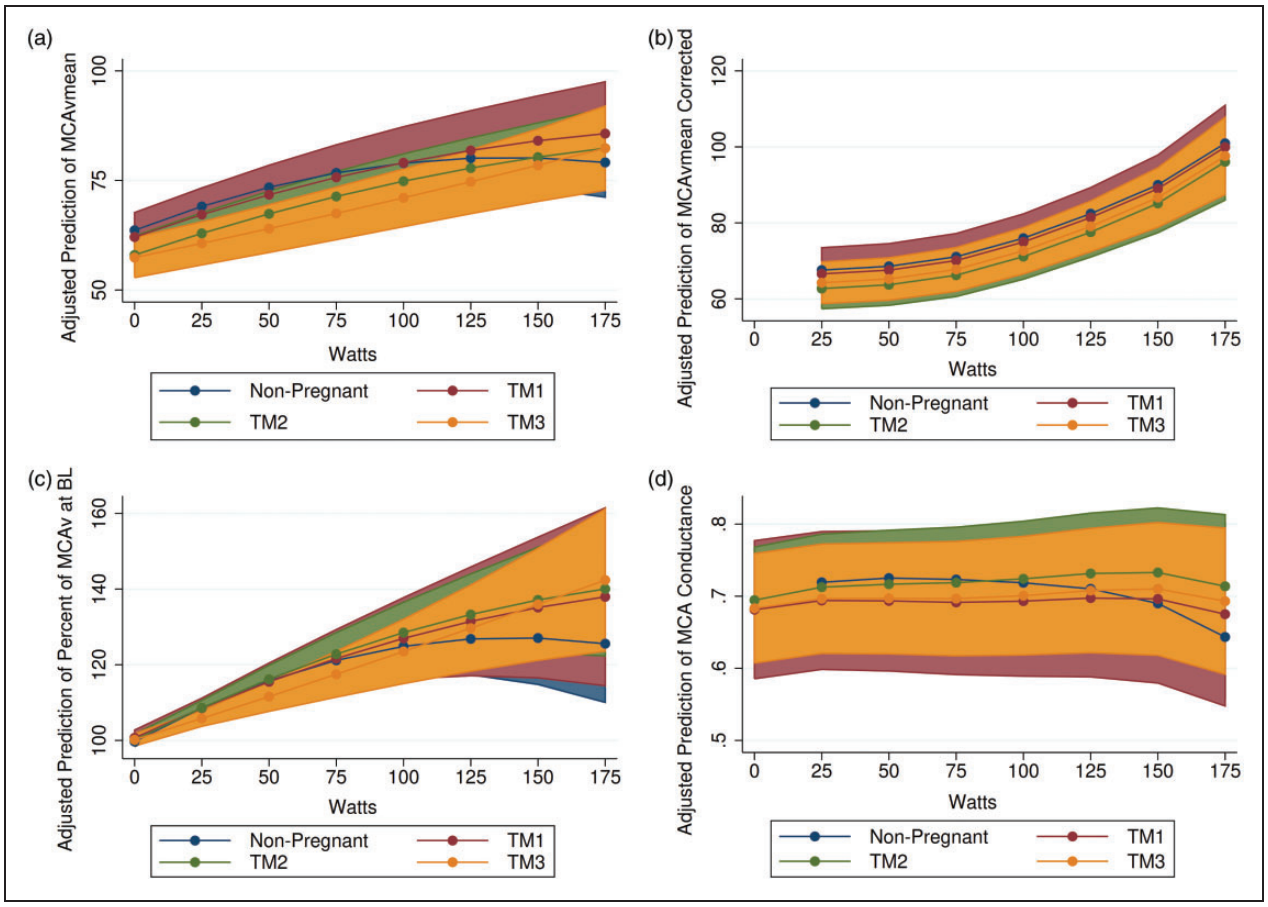

Figure 2. The predicted response to incremental exercise to volitional fatigue in (a) $MCAv_{mean}$; (b) $MCAv_{mean}$ corrected for CO_2 reactivity; (c) MCAv at baseline (BL); and (d) MCA conductance adjusted for within-participant variability. Exercise was conducted on a recumbent ergometer. Following a 5-min warm-up, the pedal rate was maintained at 50 r/min, and the work rate was increased by 25 W/min until volitional fatigue. Shaded area represents 95% Confidence Interval. Blue – nonpregnant; red – TM1; green – TM2; yellow – TM3. MCA: middle cerebral artery; TM1: trimester 1; TM2: trimester 2; TM3: trimester 3.

Cerebrovascular function response to stimuli

Neurovascular coupling. Neural activation elicits local increases in CBF to support neural metabolism.[25] Functional hyperemia in response to stimulation maintains blood flow to maintain the supply of oxygen and nutrients to active areas of the brain.[25] One published study examining NVC during late pregnancy demonstrated similar responses to visual stimulation between third trimester pregnant versus non-pregnant women.[13] However, latency of visually evoked potentials have also been found to be shorter in pregnancy compared to the postpartum period.[26] Our data confirm a similar NVC response in late pregnancy but extend the findings to demonstrate that time to peak response may be reached sooner for $PCAv_{mean}$ in TM2 compared to NP. Reduced pH buffering capacity in response to increased CO_2 may explain this association.[27] As pregnancy is associated with a dynamic changes in cardiovascular function (e.g. curvilinear response to BP across gestation), longitudinal assessments of cerebrovascular function are needed to fully understand the dynamics of adaptation of the pregnant brain. In comparison to normotensive pregnancy, severe preeclampsia has been associated with a prolonged latency of visually evoked potentials.[26] Interestingly, this delay in response resolved in the postpartum period.[26]

Hypercapnia. Vasoactive factors act directly on downstream arterioles leading to altered cerebrovascular resistance which ultimately influences CBF.[28] Increasing $PaCO_2$ is commonly used to assess cerebral vascular smooth muscle relaxation via NO-mediated endothelial vasodilation.[29–31] During pregnancy, $MCAv_{mean}$ reactivity to hypercapnia between pregnant and non-pregnant women has been reported to be similar between groups.[13] The Duffin hyperoxic rebreathe method, which involves increasing CO_2 by rebreathing exhaled gases, allows the examination of

cerebrovascular changes to dynamic changes in CO_2 while maintaining elevated O_2 levels. Our previous case study observed an apparent progressive increase in cerebrovascular reactivity to CO_2 across pregnancy.[27] This occurred in the presence of a progressive reduction in basal $P_{ET}CO_2$ (associated with mild alkalosis) and reduced bicarbonate.[27] Therefore, we hypothesized that this previously observed augmentation in reactivity was due to a steeper gain in the hypocapnic range and a reduced capacity to buffer changes in pH owing to a reduced bicarbonate. The present study showed that in response to increasing hypercapnia, cerebrovascular reactivity across pregnancy was not changed, despite lower basal $P_{ET}CO_2$ and CBF values in the second and third trimester. We were unable to collect blood samples in the current study to determine the influence these factors may have had on our current results. Nonetheless, the confidence intervals associated with our measurement of cerebral reactivity to CO_2 do suggest some individuals with augmented reactivity in later pregnancy (see Table 3). It is likely that normal variation in reactivity is dependent on prevailing arterial blood gases and the ability to buffer changes in pH during subsequent alterations in CO_2. This is yet to be confirmed.

Exercise. In non-pregnant populations, CBF rises with increasing exercise intensity until ~60% of maximal oxygen uptake, after which there is a decline in CBF towards baseline values due to the influence of hyperventilation-induced cerebral vasoconstriction.[15] We also found that CBF increased and then began to decline around 100–125 W of output in healthy non-pregnant women. However, we found that women in TM2 and TM3 demonstrate significantly different CBF responses during moderate to high intensity exercise. While $MCAv_{mean}$ was significantly lower at baseline in TM2 and TM3 participants, $MCAv_{mean}$ remained constant in TM2 participants and increased in TM3 participants during the second half of the exercise test, respectively. In non-pregnant individuals, decreasing CO_2 due to hyperventilation drives down CBF. We found that incorporating individual cerebral responsiveness to changes in CO_2 observed in the rebreathing protocol explained this difference. As a result, brain blood flow appeared to be protected during the second and third trimester of pregnancy during moderate to vigorous intensity exercise. Additionally, these results suggest that pregnant women may be protected against syncope during vigorous exercise due to a lesser drop in $P_{ET}CO_2$ values, rather than changes in cerebrovascular reactivity to changes in CO_2 per se.

The impact of chronic exercise on cerebrovascular blood flow and reactivity in pregnancy is unknown. Compared to sedentary individuals, non-pregnant subjects with elevated cardiorespiratory fitness have higher resting intracranial blood velocity in the MCA[32,33] and PCA.[34] However, these increases in resting CBF are accompanied by a reduced reactivity in response to changes in CO_2 in physically fit individuals.[34] Conversely, long-term aerobic exercise training can increase cerebrovascular CO_2 reactivity in previously sedentary non-pregnant populations,[35] and may have a similar influence on pregnant individuals; this has yet to be examined. Additional work is also required to determine whether cerebrovascular reactivity during exercise is altered by pregnancy-related diseases such as pre-eclampsia and whether measures of autoregulation (e.g. transfer function analysis) correlate to clinical outcomes in healthy and complex pregnancy.

Limitations

Despite its usefulness as a measurement technique, the limitations of TCD must be highlighted. Foremost, TCD results in a measure of global, rather than local perfusion.[36] The use of TCD to measure cerebrovascular blood flow makes a key assumption that vessel diameter remains constant throughout testing. Previous studies have demonstrated a decrease in MCA diameter during rhythmic handgrip exercise in humans and during changes in CO_2.[37,38] However, this was not measured in the current study. Nonetheless, our modelling determined that cerebrovascular reactivity to changes in $P_{ET}CO_2$ explained the differences in $MCAv_{mean}$ in pregnant and non-pregnant individuals in response to exercise. Thus, we believe that changes in vessel diameter in response to exercise are likely to be similar in healthy pregnant and non-pregnant populations.

Conclusions

Pregnant women exhibit cerebrovascular adaptations over the course of pregnancy compared to non-pregnant women due to changes in cerebrovascular reactivity. We provide the first evidence that this may decrease the time to peak in PCA blood velocity in response to NVC in the second trimester. Although the change in CBF in response to progressive hypercapnia is similar in pregnant and non-pregnant populations, we have shown that CO_2 level and cerebrovascular reactivity explain the altered response of MCA velocity during moderate to vigorous exercise in the second and third trimester.

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Authors' contributions

MHD, CDS and CM created the study concept and design. RJS, MJ, BAM and PW collected and analyzed the data. BAM conducted the statistical analyses and interpretation. BAM, MHD and CDS prepared the manuscript. All authors contributed to the critical revision of the manuscript.

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Supplemental material

Supplemental material for this article is available online.

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