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# REDUCED CEREBROVASCULAR REACTIVITY AND VASCULAR ACTIVATION IN POSTMENOPAUSAL WOMEN WITH HISTORIES OF PREECLAMPSIA

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## Abstract

Cerebrovascular reactivity is reduced in patients with cognitive decline. Women with a history of preeclampsia are at increased risk for cognitive decline. This study examined an association between pregnancy history and cerebrovascular reactivity using a subgroup of 40 age- and parity-matched pairs of women having histories of preeclampsia (n=27) or normotensive pregnancy (n=29), and the association of activated blood elements with cerebrovascular reactivity. Middle cerebral artery velocity was measured by Doppler ultrasound before and during hypercapnia to assess cerebrovascular reactivity. Thirty-eight parameters of blood cellular elements, microvesicles and cell-cell interactions measured in venous blood were assessed for association with cerebrovascular reactivity using principal component analysis. Middle cerebral artery velocity was lower in the preeclampsia compared to the normotensive group at baseline (63±4 vs. 73±3 cm/s; P=0.047) and during hypercapnia (P=0.013 to 0.056). Cerebrovascular reactivity was significantly lower in the preeclampsia compared to the normotensive group (2.1±1.3 vs. 2.9±1.1 cm/s/mmHg; P=0.009). Globally, the association of the 7 identified principal components with preeclampsia (P=0.107) and with baseline middle cerebral artery velocity (P=0.067) did not reach statistical significance. The interaction between pregnancy history and principal components with respect to cerebrovascular reactivity (P=0.084), was driven by a nominally significant interaction between preeclampsia and the individual principal component defined by blood elements, platelet aggregation, and interactions of platelets with monocytes and granulocytes (P=0.008). These

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results suggest that having a history of preeclampsia negatively affects the cerebral circulation years beyond the pregnancy and that this effect was associated with activated blood elements.

**Keywords**

Cerebral blood flow; leukocytes; microvesicles; middle cerebral artery; platelets

## Introduction

A history of hypertensive disorders of pregnancy, including preeclampsia (PE), is a sex-specific independent risk factor for hypertension, cardiovascular disease, and stroke later in life [1–4]. Additionally, a history of PE places women at an increased risk of developing brain pathology and cognitive decline immediately post-partum and beyond [5–8]. Mechanisms contributing to elevated risk of developing cognitive decline in postmenopausal women with a history of PE have not been identified.

In healthy adults, blood flow velocity in the middle cerebral artery (MCA) increases by 3–5% per mmHg increase in the partial pressure of $CO_2$ in arterial blood [9]. Cerebrovascular reactivity (CVR) is defined as the changes in cerebral blood flow in response to a stimulus like $CO_2$ and may reflect cerebral microvessel function [10]. In patients with cognitive impairment, CVR is reduced, supporting an association between CVR and cognitive decline [11–13]. Women with lower CVR during the first trimester of pregnancy (before the onset of preeclampsia), are more likely to develop preeclampsia 14 weeks later [14]. In addition, CVR is lower during pregnancy in women with PE compared to women with normotensive pregnancy (NP) [15, 16]. It is unknown, however, if reduced CVR persists in women with a history of PE beyond the immediate post-partum period.

Circulating activated blood elements (platelets and leukocytes including monocytes, granulocytes and neutrophils) and the vascular endothelium release vasoactive and mitogenic substances and cell-derived microvesicles that affect functions of other cells in the vascular compartment and the vascular wall [17–19]. These cellular interactions, in addition to various factors released from the cells, are influenced by hormonal status and co-existing cardiovascular risk factors such as hypertension, hyperlipidemia, and insulin sensitivity [4, 17, 20–22]. The extent to which these cells and cell-derived microvesicles are associated with CVR has not been explored in postmenopausal women.

This study investigated the relationship of history of PE with cerebral blood flow characteristics and CVR in postmenopausal women. In addition, we evaluated specific cellular elements of the blood that may associate with CVR. We hypothesized that women with a history of PE would have lower baseline MCA flow velocity, reduced CVR, and different populations of activated cellular elements compared to women with a history of NP.

# Methods

## Participants

Women who had given birth in Olmsted County, MN between the years of 1976 – 1982 were recruited from the Rochester Epidemiology Project [4, 23]. Forty women identified from their medical record as having preeclampsia (PE) were age/parity-matched with 40 women having a normotensive pregnancy (NP). To be included in this prospective study measuring CVR, women had to be non-smokers, non-obese (body mass index, BMI < 35 kg / $m^2$), not taking hormone replacement therapy, and without a history of cardiovascular disease (with the exception of controlled hypertension). Of these 80 women, 56 met inclusion criteria and agreed to participate: 29 women had a history of NP and 27 women had a history of PE. All study procedures were approved by the Institutional Review Board of Mayo Clinic, were performed according to the Declaration of Helsinki, and participants gave written informed consent.

## Experimental Procedures

The tests were conducted in the Clinical Research Unit of the Mayo Clinic in Rochester, MN, USA. Participants were asked to abstain from caffeine, exercise, and alcohol 24 hours prior to the study visit, not to take any over-the-counter medications the day of the study visit, and fast for at least 4 hours before coming into the laboratory. Upon arrival, height and weight were measured using a standard scale. Body mass index (BMI) was calculated as $kg/m^2$. Mean arterial pressure (MAP) was measured after a 10-minute supine resting period from the right arm using a brachial blood pressure cuff. Throughout the study protocol, beat-to-beat arterial blood pressure was monitored using a finger photoplethysmography (Nexfin, Edwards Lifesciences, Irvine, CA), heart rate (HR) was acquired from a standard three-lead electrocardiogram; oxygen saturation was monitored using pulse oximetry ($SpO_2$); and breath-by-breath end-tidal $CO_2$ ($ETCO_2$) was acquired using a nasal cannula.

## Cerebral Blood Flow Velocity

A 2-MHz Doppler probe (Transcranial Doppler, Neurovision System, Multigon, Yonkers, NY) was used to estimate right MCA blood flow velocity (MCAv). The basal portion of the MCA was identified by insonating over the temporal bone just above the zygomatic arch between the frontal process and the front of the ear. Optimal signals were obtained by adjusting the depth of the signal, location, and angle of the Doppler probe. The probe was secured with a headband device throughout the protocol in order to maintain the proper position and angle.

## Cerebrovascular Reactivity

A steady-state open-circuit technique was utilized to assess cerebrovascular responses to hypercapnia [24]. While supine, participants were fitted with a mask attached to a one-way valve to prevent rebreathing (Hans Rudolph, Shawnee, KS). After participants breathed room air for at least 3 minutes, three stepwise elevations of $CO_2$ were applied by adding 2%, 4%, and 6% fractional concentration of inspired $CO_2$ ($FICO_2$). The end tidal $CO_2$ ($ETCO_2$) was elevated for 3 minutes at each level of $FICO_2$. Consistent levels of atmospheric $O_2$

(21%) and balanced nitrogen were maintained throughout the protocol. Beat-to-beat measurements of HR, MAP, $SpO_2$, MCAv and breath-by-breath measurements of $ETCO_2$ were measured continuously.

## Measures of Vascular Cellular Activation

**Blood collection—**Blood was collected in the early morning after overnight fasting from antecubital venipuncture with a 21-gauge needle (with initial 2mL discarded) into an anticoagulant dictated by the requirement of specific assays [20, 21] and processed within 30 minutes of collection [25].

**Blood platelet reactivity assays—**Blood platelets and mean platelet volume were measured by Beckman Coulter® Ac.T diff 2 Hematology Analyzer counter, Division of Hematology Research, Mayo Clinic, Rochester, MN. *Whole blood platelet aggregation* was measured by lumi-aggregometer (Chrono-Log Corporation, Model 700, Havertown, PA). *Platelet dense granular ATP secretion* in diluted platelet rich plasma was measured in real time by bioluminescence at a final platelet concentration of 250–500 platelets/μL as previously described [20, 26]. Measurement of phosphatidylserine (annexin-V binding), P-selectin, and fibrinogen receptor (PAC-1 binding) on platelet surfaces under basal conditions was evaluated using standard flow cytometry [20, 26].

**Characterization of intravascular cell-cell interactions—**Cell-cell interactions were measured using antibodies and digital flow cytometry (FACSCanto™, BD Biosciences, San Jose, CA) methods previously validated and published by our group [27].

**Antibodies used to determine interactions of platelets with leukocytes and vascular endothelium:** Platelet (CD42a) - antibody in combination with antibodies for common leukocytes (CD45), granulocytes (CD15), monocytes (CD14), T-lymphocytes (CD3), B-lymphocytes (CD19), and vascular endothelium (CD62E) and / or with fluorophore conjugated recombinant annexin-V (binds to surface phosphatidylserine). Platelets labeled with fluorophore conjugated CD42a antibody were identified by forward and side scatter. Ten thousand gated events (counts) were collected for each sample. The number of platelets positive for antigens for leukocytes and endothelial cells are expressed as percentages of platelets positive from a total 10,000 gated platelet events.

**Antibodies used to determine interactions of leukocytes with platelets and vascular endothelium:** Blood cells were counted using a Beckman Coulter® Ac.T diff 2 Hematology Analyzer. Allophycocyanin (APC) - conjugated common leukocyte (CD45) - antibody in combination with phycoerythrin conjugated antibodies for platelets (CD42a), and vascular endothelium (CD62E), and / or with FITC conjugated annexin-V (binds to surface phosphatidylserine). Leukocytes labeled with APC-conjugated CD45 antibody were identified by forward and side scatter; 5,000 gated leukocyte events were collected for each sample. The number of platelet- and endothelial- antigen positive granulocytes, monocytes, and lymphocytes are expressed as percentages of platelet- and / or endothelial- antigen positive granulocytes, monocytes, and lymphocytes from total granulocytes, monocytes, and lymphocytes of 5,000 gated CD45-positive leukocyte events, respectively.

**Isolation, identification, and characterization of blood-borne microvesicles (MV)—**Detailed standardized methodologies were used for isolation, identification and characterization of blood borne MV [25, 27]. The concentration of blood-borne MV is expressed as MV/μL plasma.

## Data Analysis and Statistics

Cerebrovascular data were acquired at 250 Hz, stored on a computer, and analyzed off-line with signaling processing software. All calculations and analyses were independently confirmed with use of SAS statistical software (SAS Institute). Variables for analysis interest were averaged over the final minute of room air and final minute at each level of hypercapnia.. Cerebrovascular conductance index (CVCi) was calculated as MCAv / MAP. The slopes expressing the linear relationship between $ETCO_2$ and MCAv or CVCi were calculated to estimate CVR during hypercapnia in each participant. CVR was also calculated as the percent change from baseline in MCAv or CVCi relative to the percent change from baseline in $ETCO_2$. Gosling pulsatility index (PI) was calculated by $MCAv_{systolic}$ - $MCAv_{diastolic}$ / $MCAv_{mean}$ and used as an index of cerebrovascular resistance.

A 2-sample t-test or Pearson's $X^2$ test was used to compare participant demographics and cerebral blood flow measures between the PE and NP groups. Analysis of the cerebrovascular and hemodynamic variables included separate comparisons for each of the baseline and stages of hypercapnia. These comparisons were repeated using the corresponding $ETCO_2$ value as a covariate in analysis of covariance models with little effect on the results. A two-stage approach was used to summarize and compare the responses to hypercapnia as a single MCAv or CVCi CVR. In the first stage, linear regression was used to fit a separate model to each participant's 4 measurements (outcomes regressed on the $ETCO_2$ values), from which the least squares slopes was used to estimate MCAv or CVCi CVR. These slope measures, along with their alternate expressions as percent were then compared between groups in the second stage using t-tests. Similar calculations were made to compare the linear and percent change in PI over $ETCO_2$ measurements. Statistical significance was set a priori at $P < 0.05$.

An exploratory analysis was conducted to examine whether there is any association of measures of blood cellular activation with pregnancy history and CVR. First, an a priori set of 38 individual cellular elements (supplemental Table S2) were subjected to principal components (PC) analysis to reduce this high dimensional data to its most important components. The best combination of features that explain the variability in the data was selected at multiple steps during this process, with the resulting component at each step scored as a linear combination of all 38 cellular variables. The first 7 components identified in the PC analysis were then assessed individually for association with pregnancy history using t tests, whereas their joint significance was tested in a multivariate analysis of variance model with group as a single predictor. Next, the 7 PCs were individually examined for association with 2 cerebrovascular outcomes of interest using Spearman's rank correlation coefficient ($r_S$). All correlations were adjusted for pregnancy group with partial tests for association, except subgroup correlations derived within the two groups. In addition, the joint significance of all 7 PCs was tested in a multiple variable linear regression model, one

for each outcome, with adjustment for pregnancy group. We also examined the possibility of a differential relationship by pregnancy history by adding interaction terms to the model for group and each PC. Their significance was tested as a group, and no attempt was made to interpret individual interactions if this result was not significant.

## Results

Women with a history of PE had significantly higher BMI compared to women with a history of NP (Table 1). Additionally, the percentage of women with a current diagnosis of hypertension was greater in the PE group (Table 1).

There were no group differences in MAP (Table S1) or $ETCO_2$ at baseline or during any stage of hypercapnia. MCAv and CVCi were significantly lower in PE women compared to NP women at baseline and during several stages of stepped hypercapnia (Table S1).

CVR expressed as slopes of MCAv and CVCi was significantly lower in the PE compared to the NP group (Table 2; Figure 1). When expressed as percent change, CVCi reactivity was also lower in the PE group while MCAv reactivity trended toward a significant difference (P=0.087). After accounting for baseline differences in MCAv or CVCi, these measures of CVR showed significantly lower levels in women with histories of PE compared to NP women (Table 2). Differences in MCAv CVR slope were attenuated by adjustment for baseline differences in BMI and hypertension (P=0.095 and 0.096 for slope and percent change measures, respectively), whereas significant differences in CVCi CVR slope persisted after adjustment (P=0.032 and 0.036). Although PI was not significantly different between groups at baseline or during any stage of the stepped hypercapnia protocol (Table S1), the linear and percent change in PI values were significantly lower in women with a history of PE compared with NP (Table 2).

The exploratory analyses of 38 activated blood cell variables using principal components (PC) analysis obtained 7 components, which accounted for 64% of the total variance (Table S3). The first PC representing a composite of basal activation of platelets, granulocytes and monocytes (expression of annexin-V binding phosphotidylserine), and their interactions with each other and the endothelium (cells positive for monocyte or endothelial markers) explained 19% of the variation in the set of variables. Globally, the 7 blood-related PCs did not have a significant association with history of PE, and individually only the first PC showed a significant difference between groups (Table S3).

Global tests showed no statistically significant association between PCs and baseline MCAv in either group, or the combined group (Table 3). Individually, two PC’s specifically had a significant correlation with baseline MCAv: PC #6 representing the collective and contrasting loadings of platelets expressing P-selectin, activation of granulocytes and monocytes, with negative interactions of platelets with lymphocytes (Tables S3 and 3); and PC #7 representing contrasting loadings between platelet volume, ATP secretion from platelets and activated lymphocytes, versus numbers of platelets and monocyte interactions (Tables S3 and 3). When evaluating baseline CVCi, the results were similar to that of MCAv (data not shown).

There was no global association of PCs with MCAv CVR slope in either group, despite evidence of PC#1 as a correlate in women with a history of PE (Table 3). Furthermore, blood-related PCs did not show a global association with MCAv CVR slope in the combined group. A global test for interaction between pregnancy history and PC terms with respect to CVR was not significant (interactions P=0.084, 7 d.f.), despite the individual interaction between pregnancy group and PC#3 (defined by numbers of blood cellular elements, whole blood platelet aggregation, and platelet-monocyte and granulocyte interactions) showing significance (interaction P=0.008, 1 d.f.). When evaluating CVCi CVR slope, results were consistent with MCAv CVR slope (data not shown).

## Discussion

Postmenopausal women with a history of PE demonstrated a lower baseline MCAv, baseline CVCi, and CVR slopes compared to women with a history of NP 35 years after pregnancy. The observed differences in the cerebral circulation may be: 1) a direct consequence of the incident pregnancy that alters in structure of the cerebral circulation and its responsiveness to circulating vasoactive factors; 2) an indirect effect of increased cardiometabolic risk as a woman ages; however, the group difference persisted after adjustment for BMI and current hypertension diagnosis. In addition, the contribution of co-existing current hypertension in the setting of preeclampsia remains unclear as alterations in CVR was not affected by mild hypertension during pregnancy [28]; or 3) a difference in vascular phenotype that existed prior to pregnancy, but was not exposed until pregnancy occurred.

Using non-invasive imaging techniques to measure cerebral hemodynamics, elevated perfusion pressure from PE manifests in humans as an abnormal augmentation in cerebral blood flow velocity [29] and decreased cerebral pulsatility index, ultimately causing unwanted hyperperfusion [30–34]. In addition, women with PE experience elevated systemic vasoconstriction and increased cerebral perfusion pressure [29, 30, 35]. Animal models of PE suggest that PE not only induces hyperperfusion, but also results in lasting damage to the cerebral vasculature and blood brain barrier [36, 37]. Ultimately, studies in both humans and animal models suggest that cerebral hemodynamic dysfunction during PE pregnancy may subsequently contribute to lasting damage of the cerebral circulation and the brain.

In the present study, postmenopausal women with a history of PE demonstrated lower MCAv and CVCi compared to age- and parity-matched women with a history of NP indicating impaired cerebral blood flow regulation, a finding consistent with the hypothesis that problems with cerebral perfusion during PE pregnancy may extend into the postmenopausal years. This finding persisted after adjustment for BMI and hypertension. Together, these data suggest that women who experience PE may have a unique vascular phenotype prior to pregnancy such that endogenous stressors (i.e. pregnancy) unveil vascular dysregulation [38]. This hypothesis is based on studies reporting differences in pre-pregnancy peripheral vascular function in women who develop PE compared to women who have a normal pregnancy [39]. Activated vascular cellular elements measured in this study may also reflect a phenotype that contributes to responses to vascular stressors as numbers of several cellular components comprising PC #1 differed between the groups. Activated platelets and thrombogenic MVs associate with development of white matter hyperintensities and

vascular remodeling in the brain [40, 41]. It is possible that changes in the activation of these cellular elements that might occur during PE persist to affect cerebrovascular structure as a woman ages. Alternatively, consequences of PE (regardless of maternal or fetal origin) such as increased risk for insulin resistance and hypertension may accelerate changes in cerebrovascular function. Furthermore, our study does not rule out the possibility that the ischemic placenta may be altering circulating factors that alter cerebrovascular regulation and disrupt the blood brain barrier [42, 43]. These hypotheses need to be tested by longitudinal assessment of these variables from the time of the incident pregnancy thru to menopause.

The reactivity of the cerebral vasculature to oxygen supply is important to match blood flow with neuronal activity. During pregnancy, women with lower CVR to hypercapnia during the first trimester, were more likely to develop PE 14 weeks later [14] and CVR was reduced in PE women compared to NP women [15, 16]. These results suggest that impaired CVR occurs early in pregnancy prior to the development of PE, and continues throughout the pregnancy. The results of the present study extend these observations and indicate that differences in CVR extend to up to 35 years after the PE event. It remains to be determine if there are differences in CVR among women prior to pregnancy that represent a preexisting and predisposing phenotype for PE.

Mechanisms contributing to the sustained decline in CVR in women with a history of PE could not be assessed directly. However, it is possible that activation of circulating blood elements, with concomitant release of vasoactive and mitogenic factors, interact with the vascular wall influencing the reactivity of cerebral vessels to vasodilatory stimuli [21, 22, 40]. In this exploratory analysis, release of vasoactive substances from blood collected was not measured during the actual $CO_2$ perturbation. However, numbers of blood elements, platelet aggregation and platelet-interactions within the endothelium and other blood cells (PC#3) measured at baseline showed a nominally significant interaction with pregnancy history in its association with CVR. These results suggest that differences in vasoactive factors in plasma might affect reactivity of the cerebral vasculature. Although, this hypothesis requires further testing, the observations that myogenic reactivity of isolated cerebral arteries from non-pregnant rats increased when perfused with plasma from pregnant women [44], a response related to decreased release of endothelium-derived hyperpolarizing factor, adds some support to the speculation that factors in the blood affect cerebrovascular reactivity to exogenous stimuli such as $CO_2$.

In addition, postmenopausal women with a history of PE had ~30% lower MCAv CVR slope and ~40% less CVCi CVR slope when accounting for baseline differences and changes in perfusion pressure. It might be speculated that these differences in conjunction with higher cerebral PI during the vasodilatory stimulus suggest greater cerebrovascular resistance [45]. Furthermore, these differences may compound with widespread microvascular dysfunction in the brain, challenging the ability of the system to regulate cerebral perfusion perhaps contributing to unwanted brain pathologies and the increased risk of cognitive decline in women with a history of PE [5–7]. Although scores on cognitive testing for these women were in the normative range for this age group, these postmenopausal women with PE had lower cognitive scores compared with age-matched women in the NP group [8]. Additional studies

are needed to measure neurovascular coupling and longitudinal studies are needed to determine whether early reductions in CVR predict cognitive decline.

A novel aspect of this study is the evaluation of women 35+ years post pregnancy because there are few follow-up studies on cerebrovascular health in PE that extend beyond the immediate postpartum period. While we are unable to control for cumulative lifestyle factors that may increase variability in our measurements, participants were matched for parity Additionally, preeclampsia was confirmed by review of the medical records according to established definitions [4]. The use of antihypertensive medications in the women included in this study may affect our results. Because few women in the NP group were using antihypertensive medication, it was not possible to perform a sub-analysis of CVR in women with controlled hypertension relative to pregnancy history. However, in a previous study of hypertensive patients undergoing blood pressure reduction treatment, CVR was not altered by antihypertensive medication, thus the use of antihypertensive medications does not likely explain the group differences in our study [46]. Importantly, we excluded women with uncontrolled hypertension because of the potential effect on the cerebral circulation.

Due to the high flow volume, importance in supplying to the frontal cortex, and anatomical location allowing for noninvasive imaging [47], MCA flow and flow velocity is used as a surrogate, rather than direct, measure of global cerebral blood flow. The aforementioned studies evaluating the effect of PE in the cerebral circulation have employed transcranial Doppler to evaluate MCAv [14–16, 28, 30–34, 48]. We also used transcranial Doppler, which is considered a reliable methodology to estimate blood flow when the diameter of the vessel remains constant [49]. We optimized the use of transcranial Doppler by determining transient beat-to-beat changes in MCAv and corresponding changes in blood pressure in women who likely have variable blood pressures responses to vasoactive stimuli based on pregnancy history. Our technique extends previous studies by including conductance measurements that account for these transient changes in blood pressure. Additionally, the information gathered in this study would not be possible using other imaging modalities (e.g. MRI). While there is recent evidence that the MCA vasodilates during acute bouts of hypercapnia in young adults, these results were highly variable in adults over 59 years of age [50]. Thus, the differences in CVR in women with history of PE compared with NP likely reflect differences in cerebral microvascular function. Importantly, if the MCA did vasodilate more in one group vs. another, the reported group differences would underestimate the effect of PE on CVR in postmenopausal women. Future studies could employ multiple imaging modalities in this population to systematically address the possibility of different magnitudes of MCA vasodilation in response to $CO_2$.

## Perspectives

This study demonstrates that an adverse event in pregnancy (i.e. PE) is associated with differences in cerebral blood flow velocity, CVR, and an association between CVR and vascular activation decades later. Future studies should examine CVR in women prior to and during pregnancy in relationship to adverse pregnancy outcomes. Additional work is needed to examine the relationships (whether causal or indirect) among activation of blood cellular

elements, reduced CVR during and following adverse pregnancy events, and future risk of cognitive decline in women.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**What Is New?**

- A novel aspect of this study is the evaluation of women 35 years post-pregnancy to evaluate the long-term effects of preeclampsia on brain blood vessel health.
- Postmenopausal women with a history of preeclampsia have lower brain blood flow velocity and less vasodilatory response in the brain compared with postmenopausal women with a history of normal pregnancy.
- The underlying mechanism is unclear but may involve the activation of blood elements in women with a history of preeclampsia.

**What Is Relevant?**

- Our study shows that problems that occur during pregnancy may have a long-term impact on the blood vessels in the brain.
- This may explain the greater risk of stroke and cognitive decline in women with a history of preeclampsia.

**Summary**

- A history of preeclampsia negatively affects blood flow in the brain 35 years after the pregnancy.

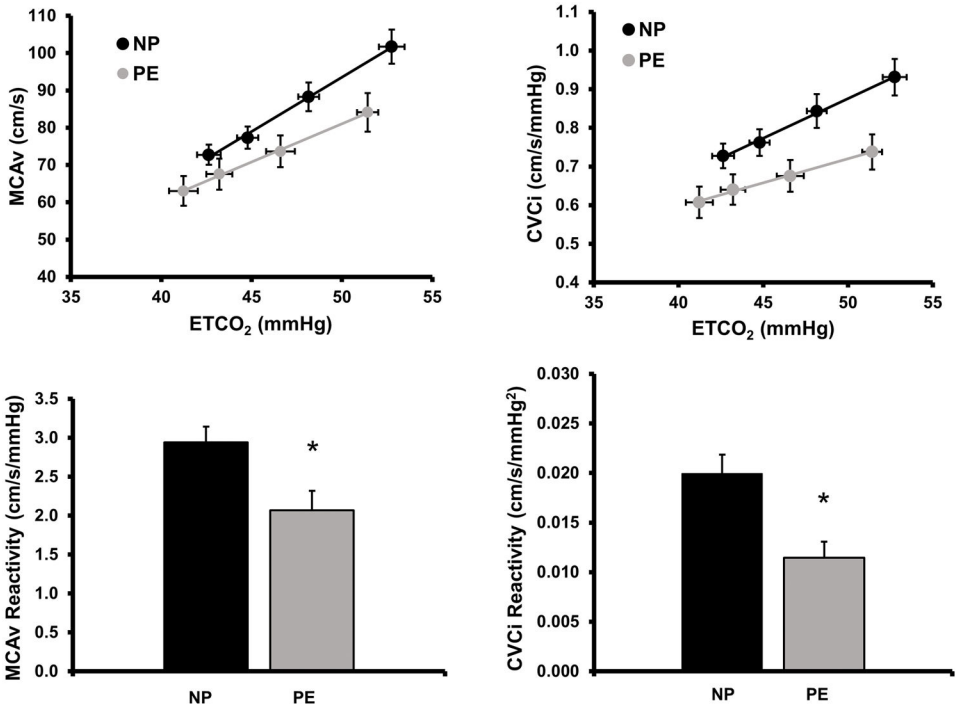


**Figure 1. Middle cerebral artery velocity (MCAv) and cerebrovascular conductance index (CVCi) during hypercapnia**

Data are mean ± SEM. Left: MCAv vs. end tidal carbon dioxide ($ETCO_2$) during the stepped hypercapnia protocol (top panel) and the calculated MCAv reactivity slope (bottom panel). Right: CVCi vs. $ETCO_2$ during the stepped hypercapnia protocol (top panel) and the calculated CVCi reactivity slope (bottom panel). Women with normotensive pregnancy (NP) are shown in black and women with preeclampsia (PE) are shown in grey *P < 0.05 compared to NP.

## Table 1

Participant characteristics

| Variable | History of NP (n=29) | History of PE (n=27) | P-value |
|---|---|---|---|
| Age at Study Consent (years) | 59±5 | 59±5 | 0.917 |
| Years Since PE Pregnancy | 35±3 | 35±4 | 0.878 |
| Body Mass Index (kg/m$^2$) | 26±4 | 29±5 | 0.008 |
| Hypertension, n (%) | 5 (17.2%) | 15 (55.6%) | 0.003 |
| Systolic Blood Pressure (mmHg) | 129±18 | 133±19 | 0.483 |
| Diastolic Blood Pressure (mmHg) | 74±8 | 78±10 | 0.095 |
| Mean Arterial Pressure (mmHg) | 93±11 | 97±12 | 0.215 |
| Heart Rate (bpm) | 65±11 | 65±9 | 0.729 |

Demographic data are reported as mean ± SD. NP, normotensive pregnancy; PE, preeclampsia; bpm, beats per minute.

## Table 2

Calculated cerebrovascular reactivity variables

| Variable | History of NP (n=29) | History of PE (n=27) | Unadjusted P-value | BL-adjusted P-value |
|---|---|---|---|---|
| MCAv Reactivity Slope | | | | |
| . Slope (linear change) | 2.94±1.10 | 2.07±1.29 | 0.009 | 0.024 |
| . Slope (percent change) | 1.78±0.79 | 1.43±0.73 | 0.087 | 0.024 |
| CVCi Reactivity Slope | | | | |
| . Slope (linear change) | 0.020±0.010 | 0.011±0.008 | 0.001 | 0.003 |
| . Slope (percent change) | 1.24±0.71 | 0.84±0.62 | 0.030 | 0.004 |
| PI Reactivity Slope | | | | |
| . Slope (linear change) | −0.013±0.018 | −0.005±0.009 | 0.035 | 0.028 |
| . Slope (percent change) | −0.62±0.86 | −0.25±0.48 | 0.052 | 0.048 |

Values are mean ± SD, along with unadjusted and baseline adjusted P-values for between-group comparisons. BL, baseline; CVCi, cerebrovascular conductance index; MCAv, middle cerebral artery velocity; NP, normotensive pregnancy; PE, preeclampsia; PI, pulsatility index.

**Table 3**

Association of vascular cellular activation principal components with pregnancy history and CVR

| Analysis on PCs | *Global Test*[*] | PC#1 | PC#2 | PC#3 | PC#4 | PC#5 | PC#6 | PC#7 |
|---|---|---|---|---|---|---|---|---|
| Group Comparisons | | | | | *Mean±SD* | | | |
| History of NP (n=29) | - | 0.81±2.58 | −0.19±1.66 | 0.22±2.03 | −0.24±1.46 | −0.05±1.45 | −0.33±1.52 | 0.02±1.36 |
| History of PE (n=27) | - | −0.87±2.54 | 0.21±2.63 | −0.24±1.69 | 0.26±1.80 | 0.06±1.56 | 0.35±1.23 | −0.02±1.40 |
| STD Difference[†] | *P=0.107* | −0.657[§] | 0.182 | −0.250 | 0.308 | 0.071 | 0.492 | −0.030 |
| Correlation with BL MCAv | | | | | *Spearman ρ*[‡] | | | |
| History of NP group | *P=0.332* | −.296 | −.128 | −.116 | −.213 | 0.203 | −.345 | 0.245 |
| History of PE group | *P=0.380* | −.038 | −.003 | 0.115 | 0.126 | −.070 | −.264 | 0.388[§] |
| Pooled groups | *P=0.067* | −.143 | −.099 | −.022 | −.075 | 0.008 | −.296[§] | 0.305[§] |
| Test of interaction | *P=0.844* | | | | | | | |
| Correlation with CVR | | | | | *Spearman ρ*[‡] | | | |
| History of NP group | *P=0.259* | −.160 | −.009 | 0.341 | 0.142 | 0.295 | −.068 | 0.287 |
| History of PE group | *P=0.365* | 0.440[§] | 0.039 | −.239 | 0.093 | −.058 | −.034 | 0.002 |
| Pooled groups | *P=0.822* | 0.089 | −.015 | 0.095 | 0.116 | 0.041 | −.043 | 0.133 |
| Test of interaction | *P=0.084* | | | [§] | | | | |

[*] Global test for any association among the 7 PCs with cerebrovascular outcome was performed with joint modeling of all PCs simultaneously as predictor variables (i.e., 7 degree-of-freedom joint test of all PC predictor terms).

[†] Standardized difference is the mean group difference relative to the average standard deviation, with positive values reflecting higher PC scores in the preeclampsia (PE) group and negative values indicating higher scores in the normotensive pregnancy (NP) group.

[‡] Spearman $\rho$ correlation coefficients express the strength of association of each PC with BL MCAv and MCAv CVR (similar results were apparent for baseline CVCi and CVCi CVR).

[§] Statistically significant result ($P < 0.05$). STD, standardized; BL, baseline; MCAv, middle cerebral artery velocity; CVR, cerebrovascular reactivity; PC, Principal Component.

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