Cerebrovascular Reactivity and Vascular Activation in Postmenopausal Women With Histories of Preeclampsia

Jill N. Barnes, Ronée E. Harvey, Kathleen B. Miller, Muthuvel Jayachandran, Katherine R. Malterer, Brian D. Lahr, Kent R. Bailey, Michael J. Joyner, Virginia M. Miller

Abstract—Cerebrovascular reactivity (CVR) 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 CVR 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 CVR. Middle cerebral artery velocity was measured by Doppler ultrasound before and during hypercapnia to assess CVR. Thirty-eight parameters of blood cellular elements, microvesicles, and cell–cell interactions measured in venous blood were assessed for association with CVR using principal component analysis. Middle cerebral artery velocity was lower in the preeclampsia compared with the normotensive group at baseline (63±4 versus 73±3 cm/s; P=0.047) and during hypercapnia (P=0.013–0.056). CVR was significantly lower in the preeclampsia compared with the normotensive group (2.1±1.3 versus 2.9±1.1 cm·s·mm Hg; 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 CVR (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 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. **(*Hypertension*. 2018;71:110-117. DOI: 10.1161/HYPERTENSIONAHA.117.10248.)**

● **Online Data Supplement**

Key Words: hypercapnia ■ middle cerebral artery ■ monocytes ■ platelet aggregation ■ pregnancy

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

In healthy adults, blood flow velocity in the middle cerebral artery (MCA) increases by 3% to 5% per mm Hg 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 preeclampsia compared with women with a normotensive pregnancy.[15,16] It is unknown, however, if reduced CVR persists in women with a history of preeclampsia beyond the immediate postpartum 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 coexisting 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.

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From the Bruno Balke Biodynamics Laboratory, Department of Kinesiology, University of Wisconsin-Madison (J.N.B., K.B.M.); and Department of Anesthesiology (J.N.B., K.R.M., M.J.J.), College of Medicine and Science (R.E.H.), Department of Physiology and Biomedical Engineering (M.J., V.M.M.), Heath Science Research, Division of Epidemiology and Biostatistics (B.D.L., K.R.B.), and Department of Surgery (V.M.M.), Mayo Clinic, Rochester, MN.

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Correspondence to Jill N. Barnes, Department of Kinesiology, University of Wisconsin-Madison, 2000 Observatory Dr, Madison, WI 53706. E-mail jnbarnes@wisc.edu

This study investigated the relationship of history of preeclampsia 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 preeclampsia would have lower baseline MCA flow velocity, reduced CVR, and different populations of activated cellular elements compared with women with a history of NP.

Methods

Participants

Women who had given birth in Olmsted County, MN, between the years of 1976 to 1982 were recruited from the Rochester Epidemiology Project.[4,23] Forty women identified from their medical record as having a preeclamptic pregnancy (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 nonsmokers, nonobese (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 at Mayo Clinic in Rochester, MN. Participants were asked to abstain from caffeine, exercise, and alcohol 24 hours before 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. On arrival, height and weight were measured using a standard scale. BMI was calculated as kg/m^2. Mean arterial pressure 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 was acquired from a standard 3-lead ECG; oxygen saturation was monitored using pulse oximetry; 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 to maintain the proper position and angle.

Cerebrovascular Reactivity

A steady-state open circuit technique was used to assess cerebrovascular responses to hypercapnia.[24] Although supine, participants were fitted with a mask attached to a 1-way valve to prevent rebreathing (Hans Rudolph, Shawnee, KS). After participants breathed room air for at least 3 minutes, 3 stepwise elevations of CO_2 were applied by adding 2%, 4%, and 6% fractional concentration of inspired CO_2. The $ETCO_2$ was elevated for 3 minutes at each level of fractional concentration of inspired CO_2. Consistent levels of atmospheric O_2 (21%) and balanced nitrogen were maintained throughout the protocol. Beat-to-beat measurements of heart rate, mean arterial pressure, pulse oximetry, 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 2 mL 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 to 500 platelets/μL as described previously.[20,21] Measurements of phosphatidylserine (annexin-V binding), P-selectin, and fibrinogen receptor (PAC-1 binding) on platelet surfaces under basal conditions were evaluated using standard flow cytometry.[20,21]

Characterization of Intravascular Cell–Cell Interactions

Cell–cell interactions were measured using antibodies and digital flow cytometry (FACSCanto, BD Biosciences, San Jose, CA) methods validated previously and published by our group.[26]

Antibodies Used to Determine Interactions of Platelets With Leukocytes and Vascular Endothelium

Platelet (CD42a)-antibody were combined with antibodies for common leukocytes (CD45), granulocytes (CD15), monocytes (CD14), T-lymphocytes (CD3), B-lymphocytes (CD19), and vascular endothelium (CD62E) 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-conjugated common leukocyte (CD45) antibody in combination with phycoerythrin conjugated antibodies for platelets (CD42a), and vascular endothelium (CD62E), and with FITC conjugated annexin-V (binds to surface phosphatidylserine). Leukocytes labeled with allophycocyanin-conjugated CD45 antibody were identified by forward and side scatter; 5000 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 endothelial-antigen positive granulocytes, monocytes, and lymphocytes from total granulocytes, monocytes, and lymphocytes of 5000 gated CD45-positive leukocyte events, respectively.

Isolation, Identification, and Characterization of Blood-Borne Microvesicles

Detailed standardized methodologies were used for isolation, identification, and characterization of blood-borne microvesicles.[25,26] The concentration of blood-borne microvesicles is expressed as microvesicles/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/mean arterial pressure. 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 χ^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 ANCOVA models with little effect on the results. A 2-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 were 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 (Table S2 in the online-only Data Supplement) was 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 ANOVA model with group as a single predictor. Next, the 7 PCs were individually examined for association with 2 cerebrovascular outcomes of interest using Spearman rank correlation coefficient (r_S). All correlations were adjusted for pregnancy group with partial tests for association, except subgroup correlations derived within the 2 groups. In addition, the joint significance of all 7 PCs was tested in a multiple variable linear regression model, 1 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 with 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 mean arterial pressure (Table S1) or $ETCO_2$ at baseline or during any stage of hypercapnia. MCAv and CVCi were significantly lower in the PE group compared with the NP group 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 group compared with the NP group (Table 2; Figure). When expressed as percent change, CVCi reactivity was also lower in the PE group, whereas MCAv reactivity trended toward a significant difference (P=0.087).

Table 1. Participant Characteristics

Variable	History of NP (n=29)	History of PE (n=27)	*P* Value
Age at study consent, y	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)	15 (56)	0.003
Systolic blood pressure, mm Hg	129±18	133±19	0.483
Diastolic blood pressure, mm Hg	74±8	78±10	0.095
Mean arterial pressure, mm Hg	93±11	97±12	0.215
Heart rate, bpm	65±11	65±9	0.729

Demographic data are reported as mean±SD. NP indicates normotensive pregnancy; and PE, preeclamptic pregnancy.

After accounting for baseline differences in MCAv or CVCi, these measures of CVR showed significantly lower levels in women with a history of PE compared with women with a history of NP (Table 2). Differences in MCAv CVR slope were attenuated by adjustment for baseline differences in BMI and hypertension (P=0.095 and P=0.096 for slope and percent change measures, respectively), whereas significant differences in CVCi CVR slope persisted after adjustment (P=0.032 and P=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 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 phosphatidylserine) 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, 2 PCs had a significant correlation with baseline MCAv: PC6 representing the collective and contrasting loadings of platelets expressing P-selectin and activation of granulocytes and monocytes, with negative interactions of platelets with lymphocytes (Table S3; Table 3); and PC7 representing contrasting loadings between platelet volume, ATP secretion from platelets, and activated lymphocytes versus numbers of platelets and monocyte interactions (Table S3; Table 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 PC1 as a correlate in women with a history of PE (Table 3). Furthermore, blood-related PCs did not show a global association with

Table 2. Calculated Cerebrovascular Reactivity Variables

Variable	History of NP (n=29)	History of PE (n=27)	Unadjusted *P* Value	Baseline-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. CVCi indicates cerebrovascular conductance index; MCAv, middle cerebral artery velocity; NP, normotensive pregnancy; PE, preeclamptic pregnancy; and PI, pulsatility index.

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 degree of freedom), despite the individual interaction between pregnancy group and PC3 (defined by numbers of blood cellular elements, whole blood platelet aggregation, and platelet-monocyte and granulocyte interactions) showing significance (interaction P=0.008, 1 degree of freedom). When evaluating CVCi CVR slope, results were consistent with MCAv CVR slope (data not shown).

Discussion

Postmenopausal women with a history of preeclampsia demonstrated a lower baseline MCAv, baseline CVCi, and CVR slopes compared with women with a history of normotensive pregnancy 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 coexisting current hypertension in the setting of preeclampsia remains unclear as alterations in CVR was not affected by mild hypertension during pregnancy[27]; or (3) a difference in vascular phenotype that existed before pregnancy but was not exposed until pregnancy occurred.

Elevated perfusion pressure from preeclampsia manifests in humans as an abnormal augmentation in cerebral blood flow velocity[28] and decreased cerebral PI (both measured using transcranial Doppler), ultimately causing unwanted hyperperfusion.[29–33] In addition, women with preeclampsia experience elevated systemic vasoconstriction and increased cerebral perfusion pressure during pregnancy.[28,29,34] Animal models of preeclampsia suggest that preeclampsia not only induces hyperperfusion, but also results in lasting damage to the cerebral vasculature and blood–brain barrier.[35,36] Collectively, studies in both humans and animal models suggest that cerebral hemodynamic dysfunction during preeclampsia may subsequently contribute to lasting damage of the cerebral circulation and brain structure.

In the present study, postmenopausal women with a history of PE demonstrated lower MCAv and CVCi compared

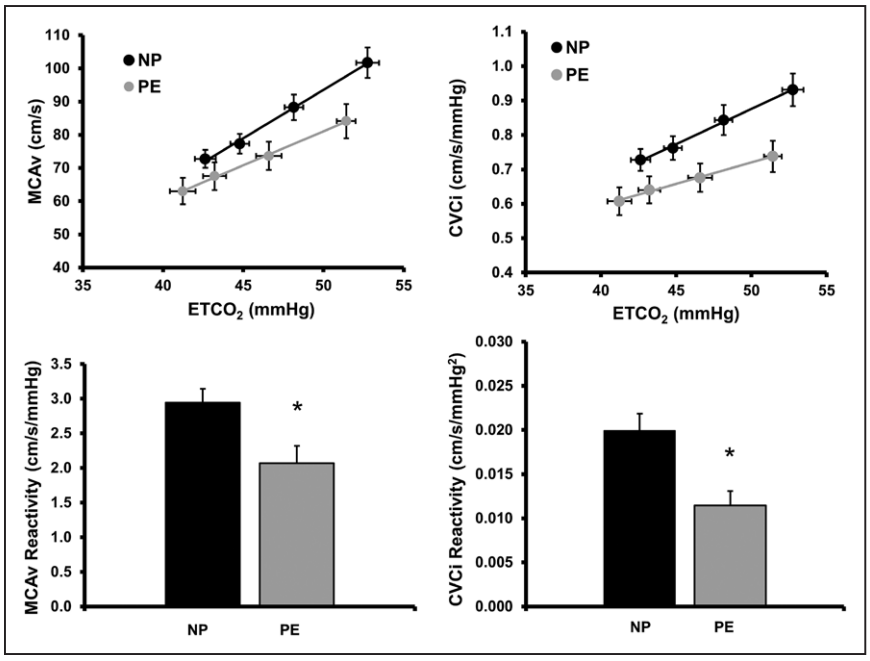

Figure. Middle cerebral artery velocity (MCAv) and cerebrovascular conductance index (CVCi) during hypercapnia. Data are mean±SEM. **Left**, MCAv vs end-tidal CO_2 ($ETCO_2$) during the stepped hypercapnia protocol (**top**) and the calculated MCAv reactivity slope (**bottom**). **Right**, CVCi vs $ETCO_2$ during the stepped hypercapnia protocol (**top**) and the calculated CVCi reactivity slope (**bottom**). Women with normotensive pregnancy (NP) are shown in black and women with preeclamptic pregnancy (PE) are shown in grey. *P<0.05 compared with NP.

Table 3. Association of Vascular Cellular Activation Principal Components With Pregnancy History and CVR

Analysis on PCs	Global Test*	PC1	PC2	PC3	PC4	PC5	PC6	PC7
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	−0.296	−0.128	−0.116	−0.213	0.203	−0.345	0.245
History of PE group	*P*=0.380	−0.038	−0.003	0.115	0.126	−0.070	−0.264	0.388‡
Pooled groups	*P*=0.067	−0.143	−0.099	−0.022	−0.075	0.008	−0.296‡	0.305‡
Test of interaction	*P*=0.844							
Correlation with CVR, Spearman ρ§								
History of NP group	*P*=0.259	−0.160	−0.009	0.341	0.142	0.295	−0.068	0.287
History of PE group	*P*=0.365	0.440‡	0.039	−0.239	0.093	−0.058	−0.034	0.002
Pooled groups	*P*=0.822	0.089	−0.015	0.095	0.116	0.041	−0.043	0.133
Test of interaction	*P*=0.084			‡				

BL indicates baseline; CVR, cerebrovascular reactivity; MCAv, middle cerebral artery velocity; NP, normotensive pregnancy; PC, principal component; PE, preeclamptic pregnancy; and STD, standardized.

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

†Standardized difference is the mean group difference relative to the average SD, with positive values reflecting higher PC scores in the PE group and negative values indicating higher scores in the NP group.

‡Statistically significant result ($P<0.05$).

§Spearman ρ 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).

with 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 preeclampsia may extend into the postmenopausal years. This finding persisted after adjustment for BMI and hypertension. Together, these data suggest that women who experience preeclampsia may have a unique vascular phenotype before pregnancy such that endogenous stressors (ie, pregnancy) unveil vascular dysregulation.[37] This hypothesis is based on studies reporting differences in prepregnancy peripheral vascular function in women who develop preeclampsia compared with women who have a normal pregnancy.[38] 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 PC1 differed between the groups. Activated platelets and thrombogenic microvesicles associate with development of white matter hyperintensities and vascular remodeling in the brain.[39,40] It is possible that changes in the activation of these cellular elements that might occur during preeclamptic pregnancy persist to affect cerebrovascular structure as a woman ages. Alternatively, consequences of preeclampsia (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.[41,42] These hypotheses need to be tested by longitudinal assessment of these variables from the time of the incident pregnancy through 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 preeclampsia 14 weeks later,[14] and CVR was reduced in women with preeclampsia compared with women with a normal pregnancy.[15,16] These results suggest that impaired CVR occurs early in pregnancy before the development of preeclampsia 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 preeclampsia event. It remains to be determined if there are differences in CVR among women before pregnancy that represent a preexisting and predisposing phenotype for preeclampsia.

Mechanisms contributing to the sustained decline in CVR in women with a history of preeclampsia 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.[21,22,39] 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 (PC3) 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 nonpregnant rats increased when perfused with plasma from pregnant women,[43] a response related to decreased release of endothelium-derived hyperpolarizing factor, adds some support to the speculation that factors in the blood affect CVR 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.[44] 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 preeclampsia.[5–7] Although scores on cognitive testing for these women were in the normative range for this age group, these postmenopausal women with a history of 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 preeclampsia that extend beyond the immediate postpartum period. Although 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 subanalysis 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.[45] Importantly, we excluded women with uncontrolled hypertension because of the potential effect on the cerebral circulation.

Because of the high flow volume, importance in supplying to the frontal cortex, and anatomic location allowing for noninvasive imaging,[46] 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 preeclampsia in the cerebral circulation have used transcranial Doppler to evaluate MCAv.[14–16,27,29–33,47] We also used transcranial Doppler, which is considered a reliable methodology to estimate blood flow when the diameter of the vessel remains constant.[48] 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 pressure 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 (eg, magnetic resonance imaging). Although 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.[49] Thus, the differences in CVR in women with a history of PE compared with NP likely reflect differences in cerebral microvascular function. Importantly, if the MCA did vasodilate more in 1 group versus another, the reported group differences would underestimate the effect of preeclampsia on CVR in postmenopausal women. Future studies could use 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 (ie, preeclampsia) 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 before 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 after adverse pregnancy events, and future risk of cognitive decline in women.

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Disclosures

None.

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Novelty and Significance

What Is New?

- A novel aspect of this study is the evaluation of women 35 years postpregnancy 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.

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

SUPPLEMENTAL FILES

Jill N. Barnes[1, 2], Ronee E. Harvey[3], Kathleen B. Miller[1], Muthuvel Jayachandran[4], Katherine R. Malterer[2], Brian D. Lahr[5], Kent R. Bailey[5], Michael J. Joyner[2], and Virginia M. Miller[4,6].

[1]Bruno Balke Biodynamics Laboratory, University of Wisconsin-Madison, Madison, WI
[2]Department of Anesthesiology, Mayo Clinic, Rochester, MN
[3] College of Medicine and Science, Mayo Clinic, Rochester, MN
[4]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN
[5]Heath Science Research, Division of Epidemiology and Biostatistics, Mayo Clinic, Rochester, MN
[6]Department of Surgery, Mayo Clinic, Rochester, MN

Table S1. Cerebrovascular and hemodynamic variables during hypercapnia

Variable	History of NP (n=29)	History of PE (n=27)	P-value
MAP (mmHg)			
. Baseline	101±11	105±10	0.186
. 2% $FICO_2$	103±11*	106±9*	0.246
. 4% $FICO_2$	106±13*	110±10*	0.250
. 6% $FICO_2$	111±14*	115±12*	0.204
MCAv (cm/s)			
. Baseline	73±15	63±21	0.047
. 2% $FICO_2$	77±16*	68±21*	0.056
. 4% $FICO_2$	88±21*	74±22*	0.013
. 6% $FICO_2$	102±24*	84±26*	0.013
CVCi (cm/s/mmHg)			
. Baseline	0.73±0.17	0.61±0.21	0.023
. 2% $FICO_2$	0.76±0.19*	0.64±0.20*	0.024
. 4% $FICO_2$	0.84±0.24*	0.68±0.21*	0.007
. 6% $FICO_2$	0.93±0.25*	0.74±0.23*	0.005
Pulsatility Index			
. Baseline	0.82±0.15	0.82±0.11	0.956
. 2% $FICO_2$	0.80±0.15*	0.81±0.10	0.755
. 4% $FICO_2$	0.76±0.14*	0.79±0.11*	0.332
. 6% $FICO_2$	0.71±0.14*	0.77±0.14*	0.112

Data are mean ± SD. CVCi, cerebrovascular conductance index; $FICO_2$, concentration of inspired carbon dioxide; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity; NP, normotensive pregnancy; PE, preeclamptic pregnancy. *$P<0.05$ vs. baseline value.

Table S2. Measures of blood elements and cell-derived microvesicles in women based on their pregnancy history

Variable	Normotensive (n=29)	Preeclampsia (n=27)	Unadjusted *P*-value	Adjusted *P*-value*
Blood platelet count (10^3/µL)	273.1±52.94	274.8±46.97	0.901	0.901
White blood cell count (10^3/µL)	5.14±1.35	5.44±1.03	0.365	0.683
Granulocyte count (10^3/µL)	3.31±1.18	3.55±0.71	0.375	0.683
Lymphocyte count (10^3/µL)	1.48±0.40	1.57±0.42	0.374	0.683
Monocyte count (10^3/µL)	0.34±0.08	0.32±0.11	0.449	0.688
Mean platelet volume (MPV, fL)	7.99±0.73	7.82±0.76	0.403	0.683
Whole blood platelet aggregation (amplitude)	21.28±4.30	21.74±4.13	0.682	0.838
ATP Secretion (amoles/platelets)	16.62±10.16	15.19±6.63	0.537	0.723
Basal expression of Annexin-V (%)	7.49±3.23	5.23±2.55	0.006	0.066
Basal expression of P-Selectin (%)	1.67±1.15	1.45±0.75	0.395	0.683
Basal expression of PAC-1 (%)	0.95±0.90	0.90±0.81	0.834	0.874
Platelet-derived (CD42a) positive (MV/µL)	851.5±510.4	900.7±495.2	0.716	0.846
Leucocyte-derived (CD45) positive (MV/ µL)	14.19±5.15	18.36±13.28	0.123	0.398
Erythrocyte-derived (CD235a) positive (MV/uL)	29.03±16.54	45.03±41.59	0.061	0.296
Endothelium-derived (CD62E) positive (MV/ µL)	7.41±4.10	8.45±13.16	0.688	0.838
Smooth muscle cell-derived (SM22alpha) pos (MV/ µL)	1.57±1.32	2.83±3.83	0.100	0.353
Stem/Progenitor cell (CD117) positive (MV/uL)	4.72±2.91	11.89±12.92	0.005	0.066
Pref-1 FITC (MV/µL)	8.46±6.44	12.99±15.54	0.154	0.463
P16-set PE (MV/µL)	1.16±1.50	1.70±2.09	0.273	0.683
Inter-Cellular Adhesion Mol-1 (ICAM-1) pos (MV/ µL)	4.18±2.41	5.12±6.63	0.476	0.688
Vascular Cell Adhesion Mol-1 (VCAM-1) pos (MV/ µL)	2.00±2.20	2.38±3.03	0.588	0.764
Phosphatidylserine (annexin-V) positive (MV/ µL)	983.8±556.4	1102.3±666.3	0.472	0.688
Tissue factor (TF) positive (MV/ µL)	11.99±5.61	27.05±34.08	0.023	0.146
Platelets pos. for leukocyte (CD45) (%)	3.66±1.08	2.97±1.36	0.039	0.219
Platelets pos. for granulocyte (CD15) (%)	3.24±2.30	2.06±1.10	0.019	0.144
Platelets pos. for monocyte (CD14) (%)	4.07±1.73	2.89±1.35	0.007	0.066
Platelets pos. for T-lymphocyte (CD3) (%)	2.18±1.27	1.88±0.56	0.256	0.683
Platelets pos. for B-lymphocyte (CD19) (%)	1.82±1.13	1.78±0.62	0.851	0.874
Platelets pos. for endothelial (CD62E) (%)	5.16±2.73	3.42±1.27	0.004	0.066
Granulocytes pos. for Annexin-V (%)	30.72±20.05	29.13±20.17	0.768	0.874
Granulocytes pos. for CD42a (%)	20.52±10.14	16.06±8.14	0.076	0.331
Granulocytes pos. for CD62E (%)	30.04±20.29	28.75±20.26	0.813	0.874
Monocytes pos. for Annexin-V(%)	24.22±10.59	22.01±10.01	0.427	0.688
Monocytes pos. for CD42a (%)	23.72±10.57	20.94±11.96	0.360	0.683
Monocytes pos. for CD62E (%)	31.90±16.82	27.19±15.83	0.286	0.683

Variable	Normotensive (n=29)	Preeclampsia (n=27)	Unadjusted *P*-value	Adjusted *P*-value*
Lymphocytes pos. for Annexin-V (%)	6.55±17.18	3.13±1.78	0.308	0.683
Lymphocytes pos. for CD42a (%)	4.08±3.49	4.44±6.75	0.800	0.874
Lymphocytes pos. for CD62E (%)	14.96±10.28	12.74±14.82	0.516	0.718
TF/TFPI Ratio	10.24±10.00	31.11±64.70	0.092	0.353

**P*-values are adjusted for multiple comparisons by controlling for the false discovery rate, as described by Benjamini and Hochberg.

Table S3. Influential loadings of 38 individual vascular activation on first 7 principal components

Variable	PC#1	PC#2	PC#3	PC#4	PC#5	PC#6	PC#7
Blood cells							
Blood platelets			0.21				-0.38
White blood cells			0.40	0.24			
Granulocytes			0.38				
Lymphocytes				0.38			
Monocytes			0.27				
Platelet characteristics							
Mean platelet volume				-0.22			0.49
Whole blood platelet aggregation			0.35				
ATP Secretion					0.39		0.35
Basal expression of Annexin-V	0.27						
Basal expression of P-Selectin					0.26	0.25	
Basal expression of PAC-1	-0.20						
Microvesicles MV (MV/µL)							
Platelet-derived (CD42a)		0.27		-0.29			
Leukocyte-derived (CD45)					0.21		
Erythrocyte-derived (CD235a)							
Endothelium-derived (CD62E)		0.27					
Smooth muscle cell-derived (SM22alpha)							
Stem/Progenitor cell (CD117)				0.30	0.24		
Adipocyte-derived (Pref-1)		0.29		0.21			
Senescent cell-derived (P16-set)		0.29					
Inter-Cellular Adhesion Mol-1 (ICAM-1)		0.25		0.27			
Vascular Cell Adhesion Mol-1 (VCAM-1)		0.34					
Phosphatidylserine (annexin-V) positive		0.28		-0.30			
Tissue factor (TF)		0.28		0.24			
Interactions among cellular elements							
Platelets pos. for leukocyte (CD45)							
Platelets pos. for granulocyte (CD15)						-0.21	
Platelets pos. for monocyte (CD14)	0.31						
Platelets pos. for T-lymphocyte (CD3)					0.27	-0.36	
Platelets pos. for B-lymphocyte (CD19)					0.35	-0.27	
Platelets pos. for endothelial (CD62E)	0.30						
Granulocytes pos. for Annexin-V	0.23					0.35	
Granulocytes pos. for CD42a			0.22		0.21		
Granulocytes pos. for CD62E	0.24					0.32	
Monocytes pos. for Annexin-V	0.29					0.24	
Monocytes pos. for CD42a			0.23		0.34		-0.33
Monocytes pos. for CD62E	0.30						
Lymphocytes pos. for Annexin-V							0.20
Lymphocytes pos. for CD42a							
Lymphocytes pos. for CD62E	0.25						

Variable	PC#1	PC#2	PC#3	PC#4	PC#5	PC#6	PC#7
Percentage of Variability Explained:							
Individual	*18.8%*	*12.4%*	*9.2%*	*7.1%*	*5.8%*	*5.3%*	*4.9%*
Cumulative	*18.8%*	*31.3%*	*40.5%*	*47.6%*	*53.4%*	*58.7%*	*63.6%*

Although each PC is scored as a linear combination of all 38 cellular variables, we report only the most influential loadings for readability sake. To summarize the individual and cumulative contributions, we report percentages of the total variability explained by the PCs at the bottom of the table. PC, Principal Component.