Increased Angiotensin II Sensitivity Contributes to Microvascular Dysfunction in Women Who Have Had Preeclampsia

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See Editorial Commentary, pp 245–246

Abstract—Women who have had preeclampsia have increased cardiovascular disease risk; however, the mechanism(s) responsible for this association remain unclear. Microvascular damage sustained during a preeclamptic pregnancy may persist postpartum. The putative mechanisms mediating this dysfunction include a reduction in NO-dependent dilation and an increased sensitivity to angiotensin II. In this study, we evaluated endothelium-dependent dilation, angiotensin II sensitivity, and the therapeutic effect of angiotensin II receptor blockade (losartan) on endothelium-dependent dilation in vivo in the microvasculature of women with a history of preeclampsia (n=12) and control women who had a healthy pregnancy (n=12). We hypothesized that preeclampsia would have (1) reduced endothelium-dependent dilation, (2) reduced NO-mediated dilation, and (3) increased sensitivity to angiotensin II. We further hypothesized that localized losartan would increase endothelium-dependent vasodilation in preeclampsia. We assessed microvascular endothelium-dependent vasodilator function by measurement of cutaneous vascular conductance responses to graded infusion of acetylcholine (acetylcholine; 10^{-7}–102 mmol/L) and a standardized local heating protocol in control sites and sites treated with 15 mmol/L L-NAME (N^G-nitro-L-arginine methyl ester; NO-synthase inhibitor) or 43 µmol/L losartan. Further, we assessed microvascular vasoconstrictor sensitivity to angiotensin II (10^{-20}–10^{-4} mol/L). Preeclampsia had significantly reduced endothelium-dependent dilation (−0.3±0.5 versus −1.0±0.4 $\log_{EC50}$; $P<0.001$) and NO-dependent dilation (16±3% versus 39±6%; $P=0.006$). Preeclampsia also had augmented vasoconstrictor sensitivity to angiotensin II (−10.2±1.3 versus −8.3±0.5; $P=0.006$). Angiotensin II type I receptor inhibition augmented endothelium-dependent vasodilation and NO-dependent dilation in preeclampsia but had no effect in healthy pregnancy. These data suggest that women who have had preeclampsia have persistent microvascular dysfunction postpartum, mediated, in part, by increased sensitivity to angiotensin II. **(*Hypertension*. 2017;70:382-389. DOI: 10.1161/HYPERTENSIONAHA.117.09386.)**

Key Words: angiotensin II ■ endothelium ■ losartan ■ microvessels ■ preeclampsia

Cardiovascular disease (CVD) is the leading cause of death for women in the United States,[1] and as such, CVD risk in women is an important public health concern. In addition to the sex-independent classical risk factors for the development of CVD observed in both men and women, history of preeclampsia during pregnancy has recently emerged as a women-specific risk factor for the development of CVD. Preeclampsia is a hypertensive disorder of pregnancy that affects ≈5% to 8% of pregnancies in the United States and ≈8 million pregnancies worldwide.[2] Otherwise healthy women who develop preeclampsia during pregnancy are at a significantly (2–7×) greater risk for the development of CVD[3] and develop primary hypertension at a younger age and with greater frequency than women who have healthy pregnancies.[4–7] Furthermore, women who have had preeclampsia are significantly more likely to die of CVD[8–10] and represent an important at-risk cohort that requires early mechanism-specific intervention strategies to prevent or mitigate CVD morbidity and mortality.

Although the association between preeclampsia and chronic elevated CVD risk is apparent, the mechanism(s) responsible for this association are unknown. One emerging hypothesis for this association is irreversible endothelial damage sustained during the preeclamptic pregnancy. In support of this hypothesis, healthy women with a history of preeclamptic pregnancy demonstrate increased arterial stiffness[11–14] and attenuated brachial artery flow-mediated dilation[11,15] compared with women with a history of normal pregnancy. However, despite this compelling evidence for lasting endothelial damage in formerly preeclamptic women, few, if any, in vivo human studies investigated the mechanism(s) responsible for this persistent vessel dysfunction in postpartum women.

Vascular studies in animal models of preeclampsia demonstrate an increased vascular sensitivity to angiotensin II. This

Received March 13, 2017; first decision April 3, 2017; revision accepted May 1, 2017.

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increased sensitivity to angiotensin II is mediated, in part, through an increased concentration of agonistic autoantibodies (AT1-AA) to the angiotensin II type I receptor (AT_1R) and contributes to the sequelae of vascular symptoms experienced during preeclampsia.[16,17] Similarly, pregnant women with preeclampsia have an exaggerated presser response to angiotensin II[18–20] and exhibit elevated AT1-AA.[21,22] Despite the remission of symptoms postpartum, women who have had preeclampsia exhibit an increased sensitivity to systemic angiotensin II infusion.[23] However, to date, the role of increased angiotensin II sensitivity in persistent endothelial dysfunction has not been mechanistically examined in vivo in postpartum women who have had preeclampsia.

The purpose of this study was to systematically examine the mechanisms mediating persistent microvascular dysfunction in women who have had a preeclamptic pregnancy. Specifically we examined endothelium-dependent dilation, angiotensin II sensitivity, and the therapeutic effect of AT_1R blockade (losartan) on endothelium-dependent dilation in vivo in the cutaneous microvasculature—a validated in vivo bioassay of systemic microvascular function[24–29]—of women with a history of preeclampsia compared with control women who had a healthy pregnancy (HC). Complementary to our functional measures, we performed ex vivo analysis of receptor expression and endothelium-dependent signaling pathways on cutaneous biopsy samples obtained from HC and preeclampsia. We hypothesized that preeclampsia would have (1) reduced endothelium-dependent dilation, (2) reduced NO-mediated dilation, and (3) increased sensitivity to angiotensin II. We further hypothesized that localized losartan treatment would increase endothelium-dependent dilation in preeclampsia and that this increase would be mediated by an increase in NO-dependent dilation.

Methods

Study Population

Twenty-four healthy, normotensive, postpartum women who had delivered within the past 12 months participated in the study. Twelve of these women had a history of preeclampsia diagnosed by their obstetrician and confirmed according to the American College of Obstetricians and Gynecologists criteria for severe preeclampsia.[30] Experimental protocols were approved by the Institutional Review Board of the Pennsylvania State University and the Food and Drug Administration. Written and verbal consent were obtained voluntarily from all subjects before participation according to the Declaration of Helsinki and the US Code of Federal Regulations, Part 46. Subjects were screened for neurologic, cardiovascular, and metabolic diseases and underwent a complete medical screening, including physical examination, lipid profile, and blood chemistry (Quest Diagnostics, Pittsburgh, PA). All subjects were normally active, nonhypertensive, nondiabetic, healthy nonsmokers who were not taking prescription medications with primary or secondary vascular effects (eg, statins, antihypertensives, anticoagulants, etc). All women met the inclusion criteria of no history of gestational diabetes mellitus and no history of hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg[31]) before or after pregnancy. Subjects were matched for age, time postpartum (months), body mass index, and blood chemistry. Control women had higher blood urea nitrogen and blood urea nitrogen/creatinine ratio; however, all values were within clinically normal range. Subject characteristics are presented in Table.

In Vivo Microvascular Reactivity Studies

Each subject participated in 2 separate laboratory visits. Intradermal microdialysis fibers (CMA Microdialysis, Holliston, MA) were placed into the dermal layer of the ventral forearm for the local delivery of pharmacological agents.[32] Pharmacological agents were mixed just before use, dissolved in lactated Ringer solution, sterilized using syringe microfilters (Acrodisc; Pall, Ann Arbor, MI), wrapped in foil to prevent degradation because of light exposure, and perfused through the microdialysis fibers at a rate of 2 µL/min (Bee Hive controller and Baby Bee microinfusion pumps; Bioanalytical Systems). Each protocol commenced after an initial hyperemia-resolution period (≈60–90 minutes). Cutaneous red blood cell flux was continually measured directly over each microdialysis site with an integrated laser Doppler flowmetry probe placed in a local heating unit (Moor Instruments, Wilmington, DE), which was set to thermoneutral (33°C) unless otherwise noted. Automated brachial blood pressure (Cardiocap; GE Healthcare, Milwaukee, WI) was measured every 5 minutes throughout each protocol.

Table. Subject Characteristics

Characteristics	HC (n=12)	PrEC (n=12)
Age, y	30±1	28±2
Time postpartum, mo	6±2	8±2
MAP, mm Hg	85±1	89±3
SBP, mm Hg	113±2	118±3
DBP, mm Hg	72±1	74±3
Height, cm	167±2	166±2
Weight, kg	71±4	82±6
BMI, kg/m^{-2}	27±1	30±2
Total cholesterol, mg/dL^{-1}	156±11	178±13
HDL, mg/dL^{-1}	54±4	53±7
LDL, mg/dL^{-1}	89±8	97±11
BUN, mg/dL^{-1}	16±1.3	12±0.6*
Creatinine, mg/dL^{-1}	0.8±0.1	0.8±0.1
BUN/creatinine ratio	20±1	16±1*
Fasting glucose, mg/dL^{-1}	86±2	86±2
HbA1c, %	5.3±0.1	5.3±0.1

Values are means±SEM. BMI indicates body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; HbA1C, glycohemoglobin; HC, healthy pregnancy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAP, mean arterial pressure; PrEC, preeclampsia; and SBP, systolic blood pressure.

*$P<0.05$ PrEC vs HC.

Cutaneous Vasodilator Responsiveness to Exogenous Acetylcholine and Local Heat

Three intradermal microdialysis fibers were placed and randomly assigned for the delivery of lactated Ringer (control), 15 mmol/L L-NAME (N^G-nitro-L-arginine methyl ester; Calbiochem; EMD Millipore, Billerica, MA) for the inhibition of NO synthase (NOS), or 43 µmol/L losartan (United States Pharmacopeial, Rockville, MD) for inhibition of AT_1R.[33]

Acetylcholine Dose–Response Protocol

After baseline measurements, ascending concentrations of acetylcholine (10^{-7}–10^{2} mmol/L; United States Pharmacopeial), mixed with the site-specific inhibitor, were perfused sequentially for 5 minutes each to ensure a plateau. After acetylcholine doses, 28 mmol/L sodium nitroprusside (United States Pharmacopeial) was perfused and local temperature increased to 43°C to elicit maximal dilation (CVC_{max}).[34]

Local Heating Protocol

After baseline measurements, the temperature of the local heaters was raised to 42°C following a standardized local skin heating protocol.[35–37] Once skin blood flow reached a plateau (≈30–40 minutes),

15 mmol/L L-NAME was perfused at each site to quantify in-site NO-dependent dilation. After a post-L-NAME plateau was established, maximal dilation was achieved as described above.

Cutaneous Vasoconstrictor Responsiveness to Exogenous Angiotensin II and Norepinephrine

Two intradermal microdialysis fibers were placed and randomly assigned for the local delivery of angiotensin II or norepinephrine. After baseline measurement (lactated Ringer perfusion) sites received ascending concentrations of angiotensin II (10^{-20}–10^{-4} mol/L; Tocris, Ellisville, MO) or norepinephrine (10^{-12}–10^{-2} mol/L; Sigma, St. Louis, MO), doses were perfused sequentially for 5 minutes each to ensure a plateau.

Ex Vivo Skin Biopsy Sample Analysis

Two 3 mm^3 ventral forearm skin samples were obtained under local anesthesia (2% lidocaine without epinephrine) as previously described.[35] Samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis. The expression levels of different proteins were determined by Western blotting. Samples were lysed in ice-cold 1× radioimmunoprecipitation assay buffer (Upstate) containing protease inhibitors (Roche), and total protein concentration was determined (Bio-Rad protein assay reagent). Equal amounts of lysate proteins (25 μg) from each sample were resolved by Sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose. Blots were blocked with 3% nonfat dry milk for 1 hour at room temperature. The membrane was incubated with primary antibody followed by horseradish peroxidase–conjugated antirabbit or antimouse antibody (1:1 000) for 1 hour at room temperature. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was used as loading control. Rabbit monoclonal antiphospho (Ser1177) endothelial NO synthase (eNOS; 1:1 000; CellSignaling), mouse anti-eNOS (1:1 000; BD Bioscience), Rabbit antiphospho (Ser239) VASP (vasodilator-stimulated phosphoprotein; 1:1 000; CellSignaling), rabbit monoclonal anti-VASP (1:1 000; Cell Signaling), rabbit anti-AT_1R (1:1 000; Abcam), and mouse anti-GAPDH (1:5 000; Novus Biologicals) were used. Blots were developed using enhanced chemiluminescence using a ChemiDoc Touch Imaging system (Bio-Rad) and quantified using ImageJ (National Institutes of Health).

Data and Statistical Analysis

All data collection and analysis procedures were standardized before testing. Blood flow data were digitized at 40 Hz, recorded, and stored for offline analysis using Windaq software and Dataq data acquisition system (Dataq Instruments, Akron, OH). Blood flow data were analyzed by 2 independent investigators blinded to microdialysis drug treatment. CVC (cutaneous vascular conductance) was calculated (CVC=laser Doppler flux/mean arterial pressure) and normalized to a percentage of site-specific maximum (%max) for dilation and site-specific baseline (%base) for vasoconstriction. Acetylcholine-mediated NO-dependent dilation was calculated as the difference between L-NAME treated and lactated Ringer sites at 10^2 mmol/L acetylcholine administration. In the local heating protocol, within-site NO-dependent dilation was calculated as the difference between the local heating plateau and the post-L-NAME plateau.

Sample size (n=12 in each group) was determined a priori by power analysis (power=0.08; α=0.05), and significance was set a priori at α=0.05. Briefly, using previously published data with similar primary outcomes in subjects with microvascular dysfunction,[33,35] we determined that 12 subjects per group would be sufficient to measure a meaningful physiological difference of at least 15% between microdialysis treatment sites (within subject) and between groups. Student unpaired *t* tests were used to compare subject characteristics and to examine differences in the densitometry analysis of Western blots. Dose–response CVC data were analyzed using sigmoidal dose–response curve with variable slope.[38] Absolute CVC data and %NO-dependent dilation were analyzed using a 2-way repeated measures ANOVA (group×pharmacological site; SAS 9.4, Cary, IN) with post hoc Bonferroni corrections applied for specific planned comparisons when appropriate. Values are presented as mean±SEM.

Results

Figure 1 presents cutaneous vasodilation (cutaneous conductance, %max) responses to acetylcholine dose–response and local heating protocols in control (A and D) and NOS-inhibited (L-NAME; B and E) sites and %NO-dependent dilation ($\%CVC_{max}$) with acetylcholine (C) and local heating (F) in postpartum women who have had a HC and women who have

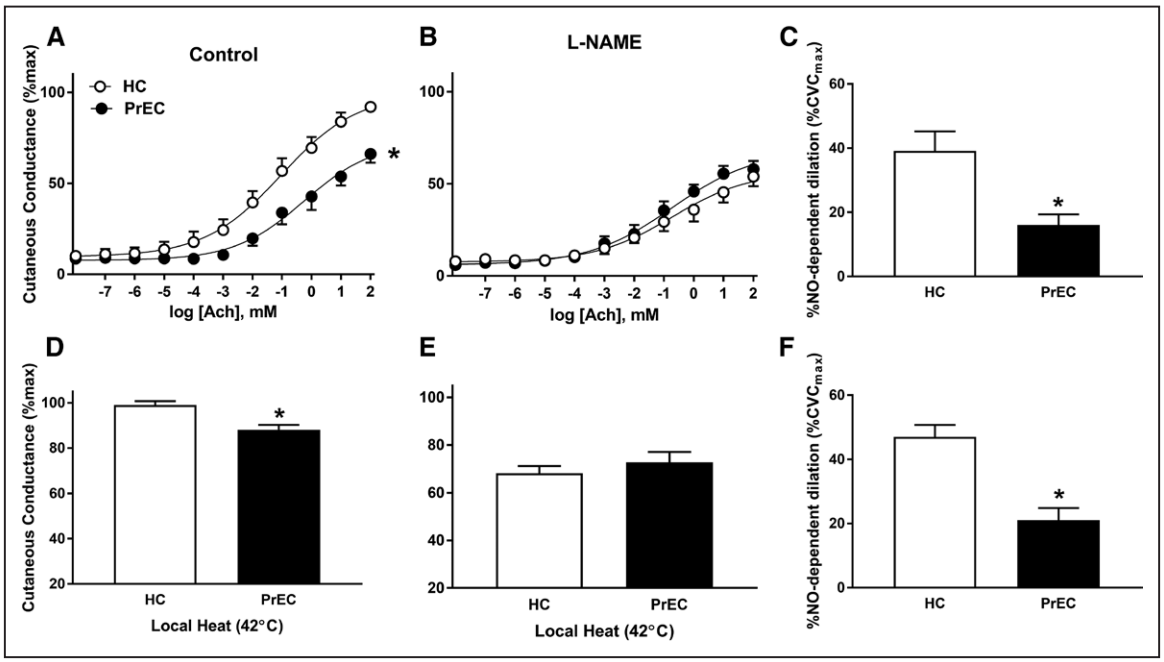

Figure 1. Mean±SEM vasodilation (cutaneous conductance; %max) responses to acetylcholine (ACh) and local heating in control (**A** and **D**) and NO synthase-inhibited (L-NAME [N^G-nitro-L-arginine methyl ester]; **B** and **E**) sites, and mean±SEM %NO-dependent dilation ($\%CVC_{max}$) with acetylcholine (**C**) and local heating (**F**) in postpartum women who have had a healthy pregnancy (HC) and women who have had preeclampsia (PrEC). CVC indicates cutaneous vascular conductance. **P*<0.05 PrEC vs HC.

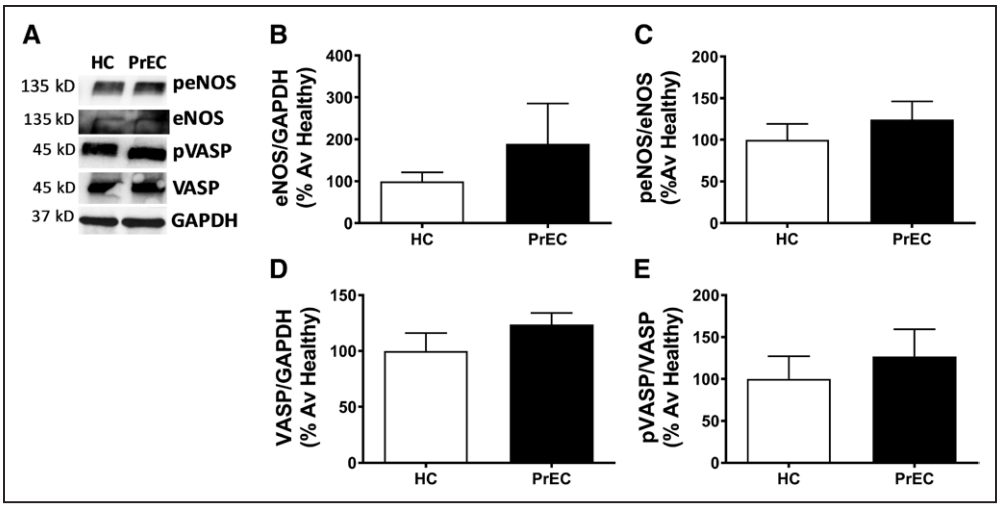

Figure 2. Mean±SEM protein expression of endothelial NO synthase (eNOS; **B**) phosphorylated eNOS (peNOS; **C**), vasodilator-stimulated phosphoprotein (VASP; **D**) phosphorylated VASP (pVASP; **E**), in cutaneous biopsy samples of postpartum women who have had a healthy pregnancy (HC; n=8) and women who have had preeclampsia (PrEC; n=8). Sample blot is shown in **A**. Densitometry analysis was performed using ImageJ software (National Institutes of Health). There were no statistical differences between HC and PrEC (all $P>0.05$). GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase.

had preeclampsia. Preeclampsia had an attenuated vasodilation response to acetylcholine (preeclampsia −0.3±0.5 versus HC −1.0±0.4 $\log_{EC50}$; $P<0.001$) and local heating (preeclampsia 89±2 versus HC 99±2 $\%CVC_{max}$; $P=0.002$) in the control sites. However, there was no difference between groups at the NOS-inhibited sites (all $P>0.05$). %NO-dependent dilation was attenuated in preeclampsia with both pharmacological (acetylcholine preeclampsia 16±3% versus HC 39±6%; $P=0.006$) and physiological (local heating preeclampsia 21±4% versus HC 47±3%; $P<0.001$) endothelium-dependent stimuli.

Figure 2 presents representative Western blots (A) and densitometric analysis for eNOS (B), phosphorylated eNOS (C), VASP (D), and pVASP (E) in cutaneous biopsy samples from HC and preeclampsia. There were no group differences (all $P>0.05$).

Figure 3 presents cutaneous vasoconstriction (cutaneous conductance; %base) responses to angiotensin II (A) and norepinephrine (B) dose–response protocols in HC and preeclampsia. Preeclampsia had greater vasoconstriction responses to angiotensin II compared with HC (preeclampsia −10.2±1.3 versus HC −8.3±0.5; $P=0.006$). There were no group differences in the vasoconstriction responses to norepinephrine ($P>0.05$).

Figure 4 presents representative Western blots and densitometric analysis for AT_1R in cutaneous biopsy samples from HC and preeclampsia. Preeclampsia had greater AT_1R expression compared with HC ($P=0.04$).

Figure 5 presents cutaneous vasodilation (cutaneous conductance; %max) responses to acetylcholine (A and B), and plateau and %NO-dependent dilation response to local heating (C and D), in control and AT_1R-inhibited (+losartan) sites in HC and preeclampsia. AT_1R inhibition augmented vasodilation in response to acetylcholine in preeclampsia (+losartan −1.7±0.6 versus control −0.3±0.5; $P<0.001$) but had no effect in HC ($P>0.05$). AT_1R inhibition also augmented the vasodilation response (+losartan 95±1 versus control 89±2; $P=0.02$) and the %NO-dependent dilation response (+losartan 37±7% versus control 21±4%; $P=0.03$) to local heating in preeclampsia but had no effect in HC (all $P>0.05$).

Discussion

The primary findings of this study are that women who have had a preeclamptic pregnancy exhibit reduced endothelium-dependent vasodilation and exaggerated vasoconstrictor sensitivity to angiotensin II postpartum, compared with parity-matched women who had a HC. Furthermore, the reduced endothelium-dependent dilation is mediated, in part, by reductions in

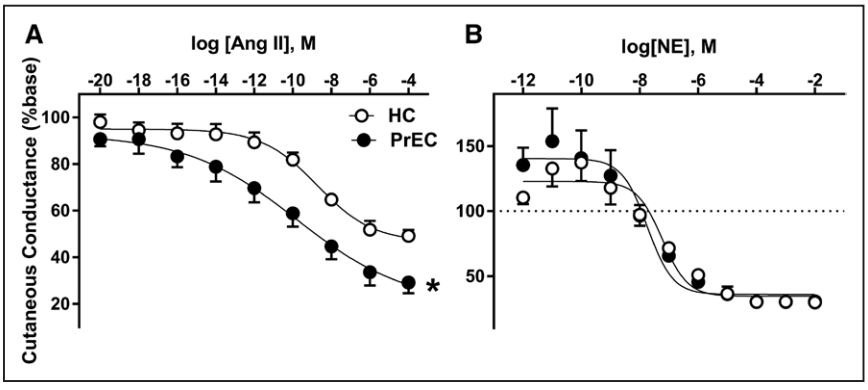

Figure 3. Mean±SEM vasoconstriction (cutaneous conductance; %base) responses to angiotensin II (ang II; **A**) and norepinephrine (NE; **B**) in postpartum women who have had a healthy pregnancy (HC) and women who have had preeclampsia (PrEC). *$P<0.05$ PrEC vs HC.

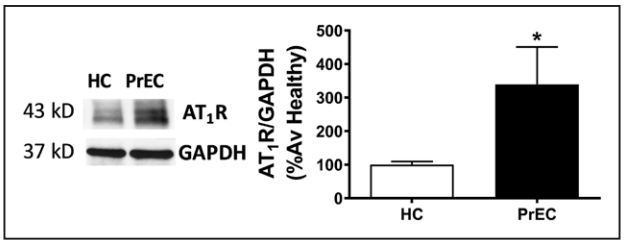

Figure 4. Representative blot (**left**) and mean±SEM protein expression of angiotensin II type 1 receptor (AT_1R) in cutaneous biopsy samples of postpartum women who have had a healthy pregnancy (HC; n=8) and women who have had preeclampsia (PrEC; n=8). **P*=0.04 PrEC vs HC. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase.

NO-mediated mechanisms and is rescued by AT_1R blockade. Ex vivo analysis showed that preeclampsia has preserved eNOS and VASP expression and phosphorylation, but has greater expression of AT_1R compared with HC, suggesting that the increased sensitivity to angiotensin II is likely mediated by increased AT_1R expression. Together, these findings suggest that despite the remission of clinical symptoms postpartum, women who have had a preeclamptic pregnancy exhibit persistent microvascular dysfunction, which may contribute to increased lifetime CVD risk in these patients. Furthermore, AT_1R inhibition may be a clinically relevant therapeutic strategy for the management of elevated CVD risk in this population.

The few in vivo human studies of vessel dysfunction after preeclamptic pregnancy have been qualitative, linking conduit vessel dysfunction with circulating vasoactive factors. These studies demonstrate attenuated endothelium-dependent vasodilation after preeclampsia, evidenced by attenuated flow-mediated dilation[15] and increased retrograde shear rate.[39] In the current study, we used the human cutaneous circulation as an in vivo bioassay to examine mechanisms of microvascular dysfunction. There is a significant relation between microvascular dysfunction assessed in the cutaneous microcirculation and that measured invasively in deeper microvascular beds,[24–29] and in vivo mechanistic studies can be performed in the skin that would not otherwise be possible on a systemic level.[40] Consistent with previous reports of dysfunction in large arteries, the current study shows that endothelium-dependent dilation responses to both pharmacological and physiological stimuli are attenuated in the microvasculature of otherwise healthy postpartum women who have had preeclampsia. Furthermore, the present data suggest that this reduced endothelial function is due, in part, to reductions in functional NO-mediated dilation. These findings suggest that women who have had preeclampsia may have a reduced NO bioavailability or secondary mechanisms are inhibiting NO-mediated relaxation of the vascular smooth muscle.

Interestingly, ex vivo analysis of cutaneous tissue samples indicate that women who have had preeclampsia have similar eNOS protein expression and phosphorylation to that of women who have had a HC. We also did not detect group differences in VASP expression or phosphorylation. It is important to note that the skin biopsy tissue represents a mixture of tissue types (cutaneous vessels, keratinocytes, nerve endings, etc), and we are limited by our inability to specifically assess protein expression in the vascular endothelium or vascular smooth muscle alone. However, these ex vivo data complement our functional findings and suggest that women who have had preeclampsia do not have attenuated NO production and that the NO is reaching the vascular smooth muscle. As such, it is likely that mechanisms secondary to NO production and diffusion to the vascular smooth muscle, such as increased vasoconstrictor tone, are affecting both endothelium- and NO-dependent dilation in these women.

Vasoconstrictor sensitivity to circulating angiotensin II is attenuated in normal pregnancy and this reduced sensitivity

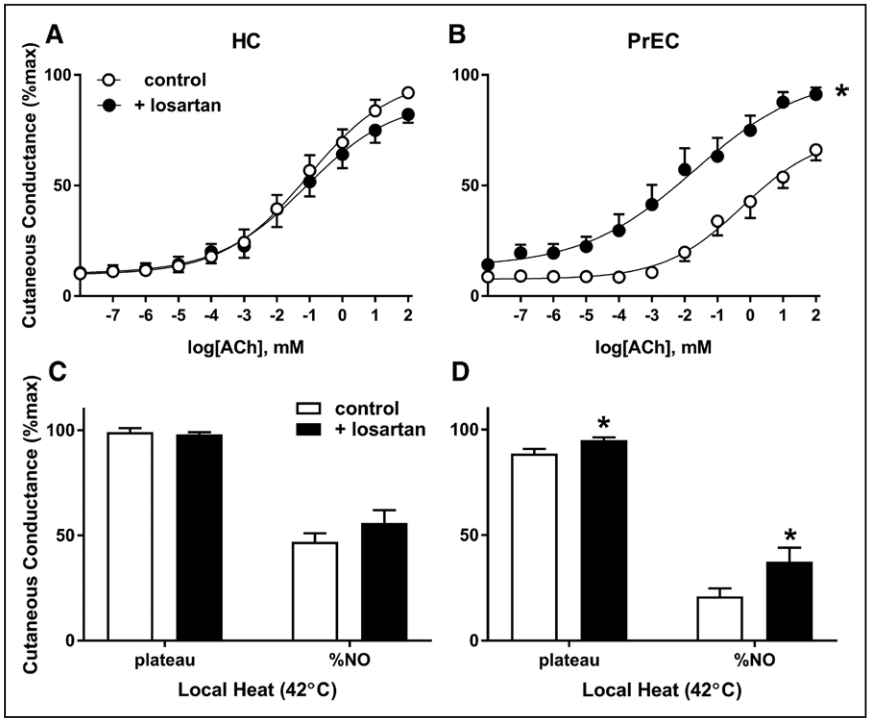

Figure 5. Mean±SEM vasodilation (cutaneous conductance; %max) responses to acetylcholine (ACh; **A** and **B**) and plateau and %NO-dependent dilation response to local heating (**C** and **D**) in control and angiotensin II type I receptor-inhibited (+losartan) sites in postpartum women who have had a healthy pregnancy (HC) and women who have had preeclampsia (PrEC). Control sites from Figure 1 are included for reference. **P*<0.05+losartan vs control within group.

contributes to the normal reduction in systemic vascular resistance observed in HC.[19] In contrast, women who develop preeclampsia have an exaggerated pressor response to angiotensin II during pregnancy—an effect that is likely mediated by increased circulating inflammatory cytokines and concomitant increases in circulating AT1-AA.[16,17,41] Similarly, previously preeclamptic women demonstrate an augmented presser response to systemic angiotensin II infusion postpartum, suggesting that this augmented sensitivity that arises during pregnancy persists postpartum.[23] Our data build on these findings and clearly demonstrate that women who have had preeclampsia exhibit a greater microvascular vasoconstrictor response to angiotensin II than control women matched for age and time postpartum. To test whether this enhanced constriction is specific to angiotensin II, we also assessed vasoconstrictor sensitivity to the adrenergic agonist norepinephrine. The lack of difference in norepinephrine-mediated vasoconstriction between groups suggests that the increased sensitivity to angiotensin II observed in formerly preeclamptic women is specific to angiotensin II-activated receptor and signaling mechanisms, and not generalized to receptor-mediated vasoconstrictor stimuli. Furthermore, ex vivo analysis showed that women who have had preeclampsia have increased AT_1R protein expression. It is likely that this increased AT_1R expression contributes, at least in part, to the increased sensitivity to angiotensin II observed in these women.

To examine the role of increased angiotensin II sensitivity in the attenuated microvascular vasodilator response in women who have had preeclampsia, we locally administered the AT_1R inhibitor losartan during assessments of endothelium- and NO-dependent dilation. We found that localized losartan treatment augmented endothelium-dependent and NO-dependent dilation in formerly preeclamptic women but had no effect in women with a history of HC. These data further demonstrate that augmented angiotensin II sensitivity contributes to persistent microvascular dysfunction in women who have had preeclampsia.

Although circulating angiotensin II is not elevated during or postpregnancy in women with preeclampsia,[42] AT1-AA concentrations increase in women during a preeclamptic pregnancy[21,22,43] and remain elevated postpartum.[44] Although we did not assess circulating angiotensin II or AT1-AA in our subjects, it is likely that chronically elevated AT1-AA contributes to persistent endothelial dysfunction in women who have had preeclampsia, independent of endogenous angiotensin II concentrations. In animal models of preeclampsia, AT1-AA induces a dose-dependent vasoconstriction response, which is abolished by AT_1R blockade.[45] As such, a possible alternative explanation for our finding that localized losartan treatment improved endothelial function is that AT_1R inhibition ameliorated chronic constrictor tone attributable to AT1-AA signaling. It is likely that a combination of both chronically elevated AT1-AA and increased AT_1R sensitivity contribute to persistent endothelial dysfunction in women who have had preeclampsia, and the mechanisms mediating this balance require further investigation.

For the purpose of this study, we specifically recruited women who had delivered within the past year to examine persistent vascular dysfunction within the first 12 months after preeclamptic pregnancy. Within that timeframe, the variability of time postpartum may have modified our results. Importantly, our groups were matched for time postpartum. Our data clearly indicated that women who have had preeclampsia exhibit microvascular dysfunction postpartum, even after the remission of clinical symptoms. However, our smaller sample size precluded subanalysis to explore the effect of time postpartum on indices of endothelial dysfunction. These studies speak solely to the first year postdelivery, and it remains unclear whether endothelial function may recover in these women over time. Vascular studies of conduit vessel endothelial function and arterial stiffness suggest that vascular impairments are detectable in women who have had preeclampsia ≤4 years, and possibly even 20 years after delivery.[14,46–48] As such, the vascular injury that occurs during a preeclamptic pregnancy likely persists throughout the patient's life. Although our mechanistic findings focused on the first year postpartum, it is likely that increased sensitivity to angiotensin II and endothelial dysfunction persist in the years following, and in the absence of intervention, contribute to the development of overt CVD in these women.

Aside from its primary antihypertensive effects, systemic AT_1R inhibition prevents the production of inflammatory mediators[49,50] and reduces biomarkers of inflammation in populations with known CVD.[51–53] Similarly, chronic AT_1R inhibition improves endothelium-dependent dilation in patients with vascular dysfunction[54,55] independent of reductions in blood pressure.[56] Within this context, our findings suggest that women who have had preeclampsia may benefit from chronic AT_1R inhibition to prevent the progression of atherosclerosis and mitigate elevated CVD risk.

To isolate history of preeclampsia as the primary factor contributing to vascular dysfunction, we limited our sample to women who were otherwise free of clinical, cardiovascular, and metabolic disease before, and post, pregnancy. However, our investigation is limited by our inability to characterize vessel function in these women prepregnancy and we cannot rule out the possibility that these subjects had subclinical alterations in microvascular function before pregnancy. Although not statistically different, our preeclamptic cohort did tend to have slightly elevated total cholesterol, low-density lipoprotein, and body mass index compared with our control group, and these differences may reflect an increased risk of developing preeclampsia and may contribute to increased risk of CVD postpartum. Classical CVD risk factors, such as hypertension,[57] obesity,[58] and hyperlipidemia,[59] have all been implicated in the development of preeclampsia and elevated CVD risk postpartum. Recent evidence suggests that women who have had preeclampsia demonstrate increased cardiovascular reactivity to sympathetic stressors, which may also contribute to increased risk of cardiovascular morbidity and mortality.[60] Overall, our data indicate that angiotensin II sensitivity is increased and contributes to impaired microvascular function in otherwise healthy women who have had preeclampsia; however, additional contributing mechanisms should not be discounted.

Perspectives

The clinical symptoms of preeclampsia (high blood pressure, proteinuria, edema, etc) typically resolve within 12 weeks postpartum. However, our data demonstrate that despite this absence

of clinical CVD symptoms, otherwise healthy women who have had preeclampsia demonstrate attenuated endothelium-dependent dilation and augmented angiotensin II sensitivity postpartum, indicating the need for early intervention. Given that women who have had preeclampsia have a greater sensitivity to angiotensin II and that local AT_1R inhibition rescues microvascular endothelium-dependent vasodilation in these women, systemic AT_1R inhibition may be a mechanistically specific intervention strategy to improve endothelial function and slow or prevent the development of clinical CVD in this at-risk population.

Acknowledgments

We would like to acknowledge the participants for contributing their time and for their dedication to the completion of the project. We would also like to acknowledge M.S. Jane Pierzga and R.N. Susan Slimak for their assistance throughout the project.

Sources of Funding

This project was supported by National Institutes of Health HL129677-02 (A.E. Stanhewicz) and NIH HL093238-06 (L.M. Alexander).

Disclosures

None.

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Novelty and Significance

What Is New?

- Women who have had a preeclamptic pregnancy have reduced vasodilator function in their microvessels even after their blood pressure has returned to normal.
- These women also have increased vasoconstriction of the microvessels in response to angiotensin II.
- When we inhibit the actions of angiotensin II, we improve vasodilator function in these patients.

What Is Relevant?

- Women who have had preeclampsia are more likely to develop and die of cardiovascular diseases.
- Inhibiting the actions of angiotensin II may be an effective strategy to prevent the development of cardiovascular disease in these women.

Summary

Our study suggests that vessel function remains altered postpartum in women who have had preeclampsia and provides evidence that inhibiting angiotensin II receptors may improve vessel function in these patients.

Hypertension
Journal of the American Heart Association

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Hypertension. 2017;70:382-389; originally published online June 26, 2017;
doi: 10.1161/HYPERTENSIONAHA.117.09386
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563