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Alterations in Endothelin Type B Receptor Contribute to Microvascular Dysfunction in Women Who Have Had Preeclampsia

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Abstract

Microvascular dysfunction originating during a preeclamptic pregnancy persists postpartum and likely contributes to increased CVD risk in these women. One putative mechanism contributing to this dysfunction is increased vasoconstrictor sensitivity to endothelin-1 (ET-1), mediated by alterations in ET-1 receptor type-B (ET_BR). We evaluated ET-1 sensitivity, ET_AR and ET_BR contributions to ET-1-mediated constriction, and the mechanistic role of ET_BR in endothelium-dependent dilation *in vivo* in the microvasculature of postpartum women who had preeclampsia (PrEC, n=12) and control women who had a healthy pregnancy (HC, n=12). We hypothesized that 1) PrEC would have a greater vasoconstrictor response to ET-1, and 2) reduced ET_BR-mediated dilation. We further hypothesized that ET_BR-blockade would attenuate endothelium-dependent vasodilation in HC, but not PrEC. Microvascular reactivity was assessed by measurement of cutaneous vascular conductance responses to graded infusion of ET-1 (10⁻²⁰-10⁻⁸mol/L), ET-1+500nmol/L BQ-123 (ET_AR-blockade), and ET-1+300nmol/L BQ-788 (ET_BR-blockade), and during graded infusion of acetylcholine (ACh, 10⁻⁷-10⁻²mmol/L) and a standardized local heating protocol with and without ET_BR-inhibition. PrEC had an increased vasoconstriction response to ET-1 (P=0.02). PrEC demonstrated reduced dilation responses to selective ET_BR stimulation with ET-1 (P=0.01). ET_BR-inhibition augmented ET-1 mediated constriction in HC (P=0.01) but attenuated ET-1 mediated constriction in PrEC (P=0.003). ET_BR-inhibition attenuated endothelium-dependent vasodilation responses to 100mmol/L ACh (P=0.04) and local heat (P=0.003) in HC but increased vasodilation (ACh: P= 0.01; local heat: P=0.03) in PrEC. Women who have had preeclampsia demonstrate augmented vasoconstrictor sensitivity to ET-1, mediated by altered ET_BR signaling. Furthermore, altered ET_BR function contributes to diminished endothelium-dependent dilation in previously preeclamptic women.

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AUTHOR CONTRIBUTIONS

AES, conceived and designed research, data collection, analysis, interpretation and manuscript preparation; SJ, data collection, analysis, and interpretation; LS, data collection, analysis, interpretation, and manuscript preparation; LMA, funding and research design, data interpretation, and manuscript preparation. All authors approved the final version of the manuscript.

CONFLICTS OF INTEREST/DISCLOSURES

None.

Keywords

endothelin-1; microvascular; endothelial dysfunction; preeclampsia

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death for women in the United States(1), and CVD risk in women is an important public health concern. In addition to the classical risk factors for the development of CVD observed in both men and women, history of preeclampsia during pregnancy has recently emerged as a female sex-specific risk factor for the development of CVD(2, 3). Preeclampsia is a hypertensive disorder of pregnancy that affects ~5-8% of pregnancies in the United States and ~8 million pregnancies worldwide (4). Otherwise healthy women who develop preeclampsia during pregnancy are at a significantly (2-7 times) greater risk for the development of CVD (5). These women develop CVD at a younger age, and with greater frequency than women who have healthy pregnancies(6-9), and are significantly more likely to die of CVD as a result(10-12). As such, women who have had preeclampsia represent an important at-risk population and investigation of their unique vascular dysfunction may identify appropriate and novel intervention strategies to prevent and/or mitigate exaggerated CVD morbidity and mortality in these women.

While the association between preeclampsia and elevated lifetime CVD risk is clear, the mechanism(s) responsible for this association remain unclear. One evolving hypothesis for this association is irreversible microvascular damage sustained during the preeclamptic pregnancy, which persists postpartum despite the remission of clinical cardiovascular symptoms (e.g. hypertension, proteinuria, edema). In support of this hypothesis, otherwise healthy women with a history of preeclampsia demonstrate increased arterial stiffness (13-16), attenuated brachial artery flow-mediated dilation(13, 17), increased retrograde shear rate(18), increased coronary artery calcification(19), and augmented vasoconstrictor responses(20, 21) compared to women with a history of normal pregnancy. However, few *in vivo* human studies have examined the specific mechanistic underpinnings of this dysfunction.

We recently demonstrated that otherwise healthy women who have had preeclampsia exhibit attenuated microvascular endothelium-dependent vasodilation, mediated in part by a reduction in nitric oxide (NO)-dependent dilation(20). Animal models of preeclampsia suggest that the potent endogenous vasoconstrictor endothelin-1 (ET-1) is involved in mediating the microvessel dysfunction associated with preeclamptic pregnancy(22-24). Plasma ET-1 concentrations are elevated in preeclamptic women(25), and experimental preparations in rodents which mimic preeclampsia demonstrate increased vasoconstrictor sensitivity to ET-1 compared to normal pregnancy(26). This increased vasoconstriction is an endothelium-dependent effect mediated, in part, by a downregulation of the vasodilatory ET-1 receptor type-B (ET_BR)(26). In these animals, downregulation of ET_BR results in attenuated NO production and reduced endothelium-dependent vasodilation, and removes the auto-inhibitory role of NO on ET-1 production by the endothelium. However, to date, no *in vivo* human study has mechanistically examined the contribution of augmented ET-1

signaling to persistent microvascular dysfunction in postpartum women who have had preeclampsia.

The purpose of this study was to systematically examine the ET-1 signaling pathway in the microvasculature of postpartum women who have had a preeclamptic pregnancy (PrEC) compared to control women who had a healthy pregnancy (HC). Specifically, we examined ET-1-mediated vasoconstriction, the relative contributions of ET-1 receptor type-A (ET_AR) and ET_BR to ET-1-mediated vasoconstriction, and the mechanistic role of ET_BR in endothelium-dependent dilation *in vivo* in the cutaneous microvasculature, a validated *in vivo* bioassay of systemic microvascular function(27-32). Complementary to our functional measures, we performed *ex vivo* analysis of circulating ET-1 concentrations and endothelin-1 receptor expression on plasma samples and cutaneous biopsy samples, respectively, obtained from HC and PrEC women. We hypothesized that PrEC would have an augmented vasoconstriction response to ET-1 compared to HC, and that this augmented response would be due to an attenuated ET_BR-mediated vasodilation in PrEC. We further hypothesized that ET_BR-inhibition would attenuate endothelium-dependent vasodilation in HC, but would have no effect on vasodilation in PrEC.

METHODS

Study Population

24 healthy normotensive postpartum women who had delivered within the past 16 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 (33). Of these 12 participants, 5 (42%) had early onset preeclampsia, diagnosed before 34 weeks of gestation, and 7 (58%) had late-onset preeclampsia, diagnosed ≥ 34 weeks of gestation. Experimental protocols were approved by the institutional review board of The Pennsylvania State University and the Federal Drug Administration (IND 124294). Written and verbal consent were obtained voluntarily from all subjects prior to participation according to the Declaration of Helsinki and the U.S. Code of Federal Regulations, Part 46. Subjects were screened for neurological, 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, non-hypertensive, non-diabetic, healthy non-smokers who were not taking prescription medications with primary or secondary vascular effects (e.g. statins, antihypertensives, anticoagulants, etc). All women met the inclusion criteria of no history of gestational diabetes, and no history of hypertension prior to pregnancy. Subjects were matched for age, time postpartum (months), body mass index, and blood chemistry. Subject characteristics are presented in Table 1.

In Vivo Microvascular Reactivity Studies

Intradermal microdialysis fibers (CMA, Holliston, MA) were placed into the dermal layer of the ventral forearm for the local delivery of pharmacological agents(34). Pharmacological agents were mixed just prior to use, dissolved in lactated Ringer's solution, sterilized using syringe microfilters (Acrodisc; Pall, Ann Arbor, MI), wrapped in foil to prevent degradation

due to light exposure, and perfused through the microdialysis fibers at a rate of 2 μ L/min (Bee Hive controller and Baby Bee microinfusion pumps; Bioanalytical Systems). Each protocol commenced after an initial hyperemia-resolution period (~60-90 mins). 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 SHO2) which was set to thermoneutral (33°C) unless otherwise noted. Automated brachial blood pressure (Cardiicap; GE Healthcare, Milwaukee, WI, USA) was measured every 5 minutes throughout each protocol.

Cutaneous vasoconstrictor responsiveness to exogenous ET-1 and norepinephrine (NE)

Four intradermal microdialysis fibers were placed and randomly assigned for the local delivery of lactated Ringer's solution (two sites), 500nmol/L BQ-123 (ET_AR-inhibition; Sigma, St. Louis, MO)(35), or 300nmol/L BQ-788 (ET_BR-inhibition; Sigma, St. Louis, MO)(35, 36). Following baseline measurements, one Ringer's site and the two inhibited sites received ascending concentrations of ET-1 (10⁻²⁰-10⁻⁸ mol/L; Tocris, Ellisville, MO) while the other Ringer's site received ascending concentrations of norepinephrine (NE; 10⁻¹²-10⁻² mol/L; Sigma, St. Louis, MO).

Cutaneous vasodilator responsiveness to sarofotoxin S6c (SRTX 6c)—One intradermal microdialysis fiber was placed in the forearm skin of subjects who were not breastfeeding (HC n=6; PrEC n=9) and, following baseline measurements, received ascending concentrations of the ET_BR-specific agonist sarofotoxin S6c (SRTX 6c, 10⁻¹²-10⁻⁵ mol/L; Tocris, Ellisville, MO). Following SRTX 6c doses, 28mmol/L sodium nitroprusside (USP, Rockville, MD) was perfused and local temperature increased to 43°C to elicit maximal dilation (CVC_{max})(37).

Cutaneous vasodilator responsiveness to exogenous acetylcholine (ACh) and local heat—Two intradermal microdialysis fibers were placed for the local delivery of lactated Ringer's (control), or 300nmol/L BQ-788 for local inhibition of ET_BR(36).

ACh dose-response protocol: Following baseline measurements, ascending concentrations of ACh (10⁻⁷-10² mmol/L; USP, Rockville, MD), mixed with the site-specific inhibitor, were perfused at each site. Following ACh doses, maximal dilation was achieved as described above.

Local heating protocol: Following baseline measurements, the temperature of the local heaters was raised to 42°C following a standardized local skin heating protocol(38-40). Once skin blood flow reached an established plateau (~30-40 min), 15mmol/L N^G-nitro-L-arginine methyl ester (L-NAME, Calbiochem, EMD Millipore, Billerica, MA) was perfused at each site to quantify in-site NO-dependent dilation. After a new post-L-NAME plateau was established, maximal dilation was achieved as described above.

Ex Vivo Skin Biopsy Sample Analysis

Two 3mm³ ventral forearm skin samples were obtained under local anesthesia (2% lidocaine without epinephrine) as previously described(38). Samples were immediately frozen in

liquid nitrogen and stored at -80°C until analysis. The expression levels of $\text{ET}_{\text{A}}\text{R}$ and $\text{ET}_{\text{B}}\text{R}$ were determined by Western blotting. Samples were lysed in ice-cold $1\times$ radioimmunoprecipitation assay buffer (Upstate) containing protease inhibitors (Roche), and total protein concentration was determined (Bio-Rad protein assay reagent). Equal amounts of lysate proteins ($25\text{ }\mu\text{g}$) 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 anti rabbit or anti mouse antibody (1:1 000) for 1 hour at room temperature. Actin was used as loading control. Mouse monoclonal anti- $\text{ET}_{\text{A}}\text{R}$ (1:1 000, Abcam), mouse monoclonal anti- $\text{ET}_{\text{B}}\text{R}$ (1:1 000; Abcam), and mouse monoclonal anti actin (1:5 000; Santa Cruz Biotech) were used. Blots were developed using enhanced chemiluminescence using a ChemiDoc Touch Imaging system (Bio-Rad) and quantified using ImageJ (National Institutes of Health).

Ex Vivo Plasma Sample Analysis

Samples were collected in EDTA treated collection tubes and plasma was immediately aliquoted and stored at -80°C until analysis. Plasma ET-1 concentrations were determined by commercial ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Each sample was run in duplicate and data are reported as $\text{pg}\cdot\text{ml}^{-1}$.

Data and Statistical Analysis

Laser Doppler flowmetry data were digitized at 40Hz, recorded, and stored for offline analysis using Windaq software and Dataq data acquisition system (Windaq; Dataq Instruments, Akron, OH). Cutaneous vascular conductance was calculated ($\text{CVC} = \text{laser Doppler flux}/\text{mean arterial pressure}$) and normalized to a percentage of site-specific baseline (% base) for constriction and site-specific maximum (% max) for vasodilation as per standard normalization procedures(20, 41-43). 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 ($n=12$ each group) was determined *a priori* by power analysis (power=0.08, $\alpha=0.05$) and significance was set *a priori* at $\alpha=0.05$. Student's unpaired t-tests were used to compare subject characteristics. Mann-Whitney tests were used to examine differences in the densitometry analysis of Western blots. 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. ACh dose-response CVC data were analyzed using sigmoidal dose-response curve with variable slope as previously described(44). Values are presented as mean $\pm$ SEM.

RESULTS

ET-1 mediated vasoconstriction is augmented in the microvasculature of women who have had preeclampsia

In order to determine if postpartum women who have had preeclampsia have increased microvascular vasoconstriction responses to ET-1, we first examined responses to exogenous ET-1 and the adrenergic-agonist NE in the cutaneous circulation of postpartum women who had preeclampsia and women who had a healthy pregnancy. PrEC had an increased vasoconstriction (cutaneous conductance, %base) response to ET-1 ($P=0.02$, Fig. 1A). There were no group differences in the vasoconstriction response to NE ($P>0.05$, Fig. 1B) suggesting that the augmented constriction response to ET-1 observed in PrEC was specific to ET-1-dependent mechanisms and not reflective of a generalized increase in constrictor tone in this cohort.

Alterations in ET_BR signaling and expression contribute to exaggerated ET-1-mediated constriction in the microvasculature of women who have had preeclampsia

Vascular responses to ET-1 are mediated by ET_A- and ET_B-receptor subtypes. In order to examine the functional contribution of each receptor subtype in the microvascular response to exogenous ET-1, we assessed vasoconstriction responses to ET-1 in control, ET_AR-inhibited (+BQ-123), and ET_BR-inhibited (+BQ-788) sites in HC and PrEC. PrEC had attenuated vasodilation responses when ET_AR was inhibited compared to HC at ET-1 doses 10^{-14} ($P<0.05$, Fig. 2). ET_BR-inhibition increased ET-1 mediated constriction in HC ($P=0.01$, Fig. 2) but attenuated ET-1-mediated constriction in PrEC ($P=0.003$, Fig. 2). These data suggest that ET_BR-mediated dilation is attenuated and/or functionally absent in postpartum women who have had preeclampsia and that ET_BR is contributing to the exaggerated vasoconstriction response to ET-1 in these women.

To probe potential alterations in ET_AR and ET_BR expression in women who have had preeclampsia, Western blotting and densitometric analysis for ET_AR and ET_BR were performed in cutaneous biopsy samples from HC and PrEC. There were no group differences in ET_AR expression ($P=0.13$, Fig. 3B). However, PrEC had greater ET_BR protein expression compared to HC ($p=0.02$, Fig. 3C). The smaller number of samples included in this analysis is limited by the number of cutaneous tissue samples collected, since some subjects declined to participate in that part of the experiment because the procedure is more invasive.

To further examine ET_BR-mediated microvascular responses in these cohorts, we locally perfused the ET_BR-specific agonist SRTX 6c in HC ($n=6$) and PrEC ($n=9$) women who were not breastfeeding at the time of the experimental visit. SRTX 6c induced cutaneous vasodilation in both groups (Fig. 4). There were no statistical differences between groups, but PrEC tended to have attenuated responses compared to HC ($P=0.1$ main effect of group). We acknowledge that these data are limited by the smaller sample size. We were limited in the number of participants we could test for figure 4, because we could not utilize SRTX 6c in women who were breastfeeding. However, these data agree with our finding that ET_BR-

mediated dilation is attenuated in PrEC (Fig. 2) and suggest that alterations in ET_BR-signaling contribute to microvascular dysfunction in women who have had preeclampsia.

ET_BR-inhibition improves microvascular endothelial function in women who have had preeclampsia

ET_BR contributes to endothelium-dependent dilation in healthy, premenopausal women. In order to examine the mechanistic role of ET_BR in endothelial function in women who have had preeclampsia we measured the cutaneous vascular responses to exogenous ACh administration (endothelium-dependent pharmacological stimulus) and local heat (endothelium-dependent physiological stimulus) in control and ET_BR-inhibited (+BQ-788) microdialysis sites. PrEC had an attenuated logEC₅₀ ($P<0.001$) and maximum ($P<0.001$) response to ACh compared to HC in the control site (Table 2). ET_BR-inhibition did not effect logEC₅₀ compared to control in either group (both $P>0.05$). ET_BR-inhibition attenuated maximum vasodilation compared to control in HC ($P=0.04$) but increased maximum vasodilation compared to control in PrEC ($P=0.01$; Table 2). Similarly, PrEC had attenuated vasodilation ($P<0.001$) and %NO-dependent dilation ($P=0.01$) responses to local heating at the control site compared to HC (Fig. 5). ET_BR-inhibition attenuated vasodilation and %NO-dependent dilation during local heating compared to the control site in HC (plateau: $P=0.003$, %NO: $P<0.001$; Fig. 5). Similar to our findings with ACh, ET_BR-inhibition increased the vasodilation response to local heating in PrEC ($P=0.02$, Fig. 5). However, ET_BR-inhibition had no effect on %NO-dependent dilation compared to control in PrEC ($P=0.6$, Fig. 5). Collectively, these data agree with previous findings that ET_BR contributes to endothelium-dependent dilation in healthy premenopausal women, but suggest that this vasodilatory role for ET_BR is absent in healthy premenopausal women who have had preeclampsia. Furthermore, inhibiting ET_BR augments endothelium-dependent dilation in PrEC, suggesting that alterations in ET_BR contribute to endothelial dysfunction postpartum in women who have had preeclampsia.

DISCUSSION

The primary findings of this study are that women who have had a preeclamptic pregnancy exhibit increased vasoconstrictor responses to ET-1, mediated by alterations in ET_BR signaling, compared to parity-matched women who had a healthy pregnancy. Furthermore, these alterations in ET_BR signaling contribute to endothelial dysfunction in women who have had preeclampsia. *Ex vivo* analysis showed that PrEC have no changes in ET_AR expression but do demonstrate elevated ET_BR expression compared to HC, corroborating our functional data and suggesting that increased sensitivity to ET-1 is mediated by alterations in ET_BR. We also found that PrEC had no changes in circulating plasma ET-1 concentrations, suggesting that the increased sensitivity to ET-1, rather than elevated circulating ET-1, likely contributes to the accelerated progression of CVD in otherwise healthy premenopausal women who have had a preeclamptic pregnancy. Furthermore, targeting of the ET-1 pathway may be a clinically relevant therapeutic strategy for the management of elevated CVD risk in this population.

Animal models of preeclampsia have identified augmented vascular responses to ET-1 as a major mediator of vascular dysfunction during preeclamptic pregnancy(22, 26, 45-47). These studies have provided compelling evidence to suggest that increased ET-1 synthesis, coupled with dysregulation of the ET receptor subtypes, are centralized mechanisms contributing to multi-system vascular dysfunction during a preeclamptic pregnancy(25). *In vivo* human studies demonstrate that plasma ET-1 concentrations are elevated during pregnancy in preeclamptic women compared to healthy pregnant women(23, 25, 48), and increases in ET-1 may serve as a predictor of disease progression in preeclampsia(22, 23). However, despite the growing evidence for a critical role of the ET system in vascular dysfunction during a preeclamptic pregnancy, few, if any, human studies have examined the mechanistic role of ET-1 signaling in persistent vascular dysfunction postpartum in women who have had preeclampsia. In the current study, we utilized 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(27-32), and *in vivo* mechanistic studies can be performed in the skin that would not otherwise be possible on a systemic level in humans(49). Consistent with previous reports of augmented ET-1 mediated constriction during preeclampsia, the current study shows that microvascular vasoconstriction responses to exogenous ET-1 are augmented postpartum in otherwise healthy women who have had preeclampsia. To test whether this enhanced constriction is specific to ET-1, 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 ET-1 observed in formerly preeclamptic women is specific to ET-1 activated receptor(s) and signaling mechanisms, and not generalized to all receptor mediated vasoconstrictor stimuli. These findings suggest that microvascular alterations in ET-1 signaling that occur during a preeclamptic pregnancy persist postpartum and likely contribute to CVD progression despite the remission of clinical symptoms in these women.

ET-1-induced vascular responses are the sum result of vasoconstriction via both ET_AR and ET_BR located on the vascular smooth muscle, and vasodilation via ET_BR located on the vascular endothelium(50). In healthy premenopausal women, ET_BR is predominately found on the vascular endothelium, and mitigates overall ET-1 mediated vasoconstriction via buffering from an endothelium-dependent vasodilation response(35, 36, 51, 52). Our functional data in HC demonstrate this balance, as ET_AR-inhibition resulted in a mild ET_BR-mediated vasodilator response to exogenous ET-1, while ET_BR-inhibition increased the vasoconstrictor response to ET-1 by removing the vasodilatory balance presumably provided by the endothelial ET_BR (Figure 2A). Animal models of preeclampsia, specifically the reduced uterine perfusion pressure (RUPP) rat model, suggest that the increased vasoconstriction response to ET-1 is mediated by a pathological imbalance between ET_AR-mediated constriction and ET_BR-mediated dilation during pregnancy(26). In the RUPP rat, downregulation of the vasodilatory ET_BR results in augmented constrictor responses to exogenous ET-1 and attenuated NO production(26). This alteration in ET_BR leads to reduced endothelium-dependent vasodilation and removes the auto-inhibitory role of NO on ET-1 production by the endothelium(26). Similarly, microvascular endothelial cells of

preeclamptic women have attenuated ET_BR expression compared to healthy pregnant controls(53). Our functional data build on these findings and demonstrate that ET_BR-mediated dilation – i.e. increases in cutaneous conductance in response to 1) exogenous ET-1 administration during ET_AR-inhibition (Figure 2B), and 2) the ET_BR-specific agonist SRTX 6c (Figure 4) - is attenuated postpartum in women who have had preeclampsia.

Interestingly, we found that ET_BR-inhibition attenuated the exaggerated constriction response to exogenous ET-1 in PrEC (Figure 2B). These data suggest that ET_BR may be contributing to exaggerated ET-1-mediated vasoconstriction in women who have had preeclampsia. *Ex vivo* analysis of tissue samples obtained from these women indicate that women who have had preeclampsia have similar ET_AR but increased ET_BR protein expression compared to women who have had a normal pregnancy. Previous studies have demonstrated a reduced ET_BR protein expression in endothelial cells of RUPP rats(26) and preeclamptic women(53). However, increased ET_BR expression in vascular smooth muscle may explain the increased overall ET_BR protein expression in our homogenized tissue samples, as well as our functional finding that ET_BR-mediated constriction contributes to the overall exaggerated constriction response to ET-1. In agreement with our findings, Mazzuca et al found greater ET_BR expression in endothelium-denuded microvessels from RUPP rats, suggesting a role for vascular smooth muscle ET_BR in their model of preeclampsia(26). Taken together, these data suggest that alterations in ET_BR expression and/or location likely contribute to exaggerated vasoconstriction and microvascular dysfunction, despite the remission of clinical symptoms, in women who have had preeclampsia.

We recently demonstrated that women who have had preeclampsia have an attenuated microvascular endothelium-dependent dilation, mediated in part by reductions in NO-dependent dilation(20). Consistent with this previous finding, the current study found that PrEC had attenuated endothelium-dependent dilation and reduced NO-dependent dilation compared to HC. In order to examine the role of augmented ET_BR function in this attenuated microvascular vasodilator response, we locally administered the ET_BR-specific antagonist BQ-788 during assessments of pharmacologically (exogenous ACh) and physiologically (local heating of the skin) stimulated endothelium- and NO-dependent dilation. Consistent with previous reports of an endothelium-dependent vasodilator role of ET_BR in healthy premenopausal women(35, 36, 43), we found that ET_BR-inhibition attenuated endothelium-dependent dilation via reduced NO-dependent dilation in HC. However, we found that ET_BR-inhibition increased endothelium-dependent dilation, independent of NO-dependent mechanisms, in PrEC. These data further demonstrate that ET_BR-mediated dilation is functionally absent in formerly preeclamptic women, and suggest that alterations in ET_BR contribute to the tonic vasoconstrictor tone and attenuated microvascular endothelial function that persists postpartum in women who have had preeclampsia.

Along with alterations in ET-1 synthesis and signalling, the renin-angiotensin system plays a major role in the pathophysiology of preeclampsia. Women who have preeclampsia exhibit a greater pressor response to angiotensin II (ang II), increased dimerization of the angiotensin type 1 receptor (AT₁R), and elevated circulating agonistic antibody to AT₁R. Similarly, increased sensitivity to ang II likely contributes to microvasculalr dysfunction in these

women postpartum. Women who have had preeclampsia have an exaggerated pressor response to systemic ang II infusion (21), and we have recently demonstrated that women who have had preeclampsia have increased microvascular vasoconstrictor sensitivity to ang II (20). Furthermore, in that study we found that locally inhibiting AT₁R increased endothelial function in the microvasculature of these women. Increased production of ET-1 is a downstream consequence of endothelial AT₁R activation, and it is likely that the pro-oxidant and pro-inflammatory milieu of the preeclamptic pregnancy are a common upstream link between the alterations observed in ang II and ET-1 signalling mechanisms. Collectively, ang II and ET-1 are tightly coupled within the vasculature and the microvascular dysfunction that persists postpartum in women who have had preeclampsia is likely due to alterations in both systems which feed forward on each other. Future *in vivo* studies are warranted to explore these interactions, and to determine if interventions which mechanistically target one pathway may also have beneficial effects on the other.

Pharmacological inhibition of ET-1 receptors is used in a number of cardiovascular diseases, most notable pulmonary arterial hypertension. While a number of animal models of preeclampsia have demonstrated that ET receptor antagonism attenuates the development of the disorder (46, 54-56), ET receptor antagonism has been demonstrated to be teratogenic and is currently contraindicated during pregnancy. Our data suggest that women who have had preeclampsia may benefit from systemic treatment with ET-1 antagonists. However, despite the high therapeutic potential of these pharmacotherapies demonstrated in the clinical literature, these antagonists are not commonly prescribed for the treatment of systemic hypertension (57), a phenomenon mainly attributed to their side effects, particularly fluid retention. Compounding these limitations is the fact that sex-specific differences in the ET system (not discussed here but elegantly reviewed in (58)) obfuscate the efficacy of these pharmacotherapies in women. Given the emerging role of the endothelin pathway(s) in women-specific CVD, particularly preeclampsia and post-menopausal hypertension, further clinical studies focusing on sex-targeted or sex-tailored interventions for the treatment or prevention of CVD are necessary if systemic ET receptor antagonists are to be more commonly prescribed in clinical practice.

For the purpose of this study, we specifically recruited women who had delivered within the past 16 months in order to examine persistent vascular dysfunction within this shortened timeframe following a preeclamptic pregnancy. Importantly, our groups were matched for time postpartum; however, the variability of time postpartum (2 -16 months, Table 1) may have modified our results. Our data clearly indicated that women who have had preeclampsia exhibit microvascular dysfunction, even following the remission of clinical symptoms. However, our smaller sample size precluded sub-analysis to explore the effect of time-since-delivery on our microvascular outcomes, and it remains unclear if microvascular 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(16, 59-61). As such, the vascular injury that occurs during a preeclamptic pregnancy likely persists throughout the patient's life. Although our mechanistic findings focused on a shortened window postpartum, it is likely that increased sensitivity to ET-1 and endothelial

dysfunction persist in the years following the incident pregnancy and, in the absence of intervention, contribute to the development of overt CVD in these women.

In order to isolate the preeclamptic pregnancy as the primary factor contributing to vascular dysfunction, we limited our sample to women who were otherwise free of clinical cardiovascular and metabolic disease prior to pregnancy. However, our investigation is limited by our inability to characterize vessel function in these women pre-pregnancy and we cannot rule out the possibility that these subjects had subclinical alterations in microvascular function prior to pregnancy. There is some debate as to whether the vascular dysfunction observed in women who have had preeclampsia is a cause or a consequence of the disorder. Hypertensive disorders of pregnancy, particularly preeclampsia, and CVD share several similar risk factors (e.g. obesity, metabolic disease, renal disease) and these commonalities may suggest that the cardiovascular strain of the pregnancy serves as a “stress test” that reveals an underlying weakness that may have gone unnoticed until later in life. Alternatively, hypertension in pregnancy could induce long-term metabolic and vascular abnormalities that might increase the overall risk of CVD later in life. While our discussion and hypotheses fall on the side of this second approach, we cannot discount the possibility that subclinical dysfunction preceded the development of preeclampsia in our cohort. This is an important area of inquiry that deserves future study.

Clinical Perspectives

CVD is the leading cause of death among women worldwide and women who develop preeclampsia during pregnancy are at a significantly greater risk for the development of CVD(6, 12, 19). These women exhibit CVD at a younger age and with greater frequency than women who have healthy pregnancies(2, 6-8), and are significantly more likely to die as a result(10-12). The clinical symptoms of preeclampsia (high blood pressure, proteinuria, edema, etc.) typically resolve within 12 weeks following delivery. However, our data demonstrate that despite this absence of clinical CVD symptoms, otherwise healthy women who have had preeclampsia demonstrate pronounced microvascular dysfunction postpartum, indicating the need for early intervention. To our knowledge, we are the first to systematically examine the ET-1 signaling pathway *in vivo* in postpartum women who have had preeclampsia. Our data show that 1) otherwise healthy women who have had preeclampsia have a greater vasoconstrictor sensitivity to ET-1 mediated by alterations in ET_BR, 2) functional ET_BR-mediated dilation is absent following preeclamptic pregnancy, and 3) local ET_BR-inhibition improves microvascular endothelium-dependent vasodilation postpartum in these women. Targeting of the ET-1 pathway, may be a mechanistically specific approach to simultaneously mitigate exaggerated microvascular vasoconstriction and improve endothelial function in order to slow or prevent the development of clinical CVD in this at-risk population.

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References

1. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. *Circulation*. 2011; 123(11):1243–62. Epub 2011/02/18. [PubMed: 21325087]
2. Drost JT, Arpaci G, Ottervanger JP, de Boer MJ, van Eyck J, van der Schouw YT, et al. Cardiovascular risk factors in women 10 years post early preeclampsia: the Preeclampsia Risk Evaluation in FEMales study (PREVFEM). *European journal of preventive cardiology*. 2012; 19(5): 1138–44. Epub 2011/08/24. [PubMed: 21859777]
3. Yinon Y, Kingdom JC, Odutayo A, Moineddin R, Drewlo S, Lai V, et al. Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: insights into future vascular risk. *Circulation*. 2010; 122(18):1846–53. Epub 2010/10/20. [PubMed: 20956209]
4. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi JM. Pre-eclampsia: pathophysiology, diagnosis, and management. *Vascular health and risk management*. 2011; 7:467–74. Epub 2011/08/09. [PubMed: 21822394]
5. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ*. 2007; 335(7627):974. Epub 2007/11/03. [PubMed: 17975258]
6. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort. *Hypertension*. 2010; 56(1):166–71. Epub 2010/06/03. [PubMed: 20516394]
7. Ahmed R, Dunford J, Mehran R, Robson S, Kunadian V. Pre-eclampsia and future cardiovascular risk among women: a review. *Journal of the American College of Cardiology*. 2014; 63(18):1815–22. Epub 2014/03/13. [PubMed: 24613324]
8. Haukkamaa L, Salminen M, Laivuori H, Leinonen H, Hiilesmaa V, Kaaja R. Risk for subsequent coronary artery disease after preeclampsia. *The American journal of cardiology*. 2004; 93(6):805–8. Epub 2004/03/17. [PubMed: 15019902]
9. Ghossein-Doha C, van Neer J, Wissink B, Breetveld NM, de Windt LJ, van Dijk AP, et al. Pre-eclampsia: an important risk factor for asymptomatic heart failure. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2017; 49(1):143–9. Epub 2016/11/03.
10. Jonsdottir LS, Arngrimsson R, Geirsson RT, Sigvaldason H, Sigfusson N. Death rates from ischemic heart disease in women with a history of hypertension in pregnancy. *Acta obstetrica et gynecologica Scandinavica*. 1995; 74(10):772–6. Epub 1995/11/01. [PubMed: 8533558]
11. Arnadottir GA, Geirsson RT, Arngrimsson R, Jonsdottir LS, Olafsson O. Cardiovascular death in women who had hypertension in pregnancy: a case-control study. *BJOG : an international journal of obstetrics and gynaecology*. 2005; 112(3):286–92. Epub 2005/02/17. [PubMed: 15713141]
12. Alvarez-Alvarez B, Martell-Claros N, Abad-Cardiel M, Garcia-Donaire JA. [Hypertensive disorders during pregnancy: Cardiovascular long-term outcomes]. *Hipertension y riesgo vascular*. 2016 Epub 2016/07/11. Trastornos hipertensivos en el embarazo: repercusion a largo plazo en la salud cardiovascular de la mujer.
13. Chambers JC, Fusi L, Malik IS, Haskard DO, De Swiet M, Kooner JS. Association of maternal endothelial dysfunction with preeclampsia. *JAMA : the journal of the American Medical Association*. 2001; 285(12):1607–12. Epub 2001/04/13. [PubMed: 11268269]
14. Hausvater A, Giannone T, Sandoval YH, Doonan RJ, Antonopoulos CN, Matsoukis IL, et al. The association between preeclampsia and arterial stiffness. *Journal of hypertension*. 2012; 30(1):17–33. Epub 2011/12/03. [PubMed: 22134391]
15. Evans CS, Gooch L, Flotta D, Lykins D, Powers RW, Landsittel D, et al. Cardiovascular system during the postpartum state in women with a history of preeclampsia. *Hypertension*. 2011; 58(1): 57–62. Epub 2011/05/25. [PubMed: 21606389]

16. Orabona R, Sciatti E, Vizzardi E, Bonadei I, Valcamonico A, Metra M, et al. Endothelial dysfunction and vascular stiffness in women with previous pregnancy complicated by early or late pre-eclampsia. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2017; 49(1):116–23. Epub 2016/02/27.
17. Hamad RR, Eriksson MJ, Silveira A, Hamsten A, Bremme K. Decreased flow-mediated dilation is present 1 year after a pre-eclamptic pregnancy. *Journal of hypertension*. 2007; 25(11):2301–7. Epub 2007/10/09. [PubMed: 17921826]
18. Scholten RR, Spaanderman ME, Green DJ, Hopman MT, Thijssen DH. Retrograde shear rate in formerly preeclamptic and healthy women before and after exercise training: relationship with endothelial function. *American journal of physiology Heart and circulatory physiology*. 2014; 307(3):H418–25. Epub 2014/06/08. [PubMed: 24906915]
19. White WM, Mielke MM, Araoz PA, Lahr BD, Bailey KR, Jayachandran M, et al. A history of preeclampsia is associated with a risk for coronary artery calcification 3 decades later. *American journal of obstetrics and gynecology*. 2016; 214(4):519 e1–8. Epub 2016/02/14. [PubMed: 26874301]
20. Stanheiwicz AE, Jandu S, Santhanam L, Alexander LM. Increased Angiotensin II Sensitivity Contributes to Microvascular Dysfunction in Women Who Have Had Preeclampsia. *Hypertension*. 2017; 70(2):382–9. Epub 2017/06/28. [PubMed: 28652473]
21. Saxena AR, Karumanchi SA, Brown NJ, Royle CM, McElrath TF, Seely EW. Increased sensitivity to angiotensin II is present postpartum in women with a history of hypertensive pregnancy. *Hypertension*. 2010; 55(5):1239–45. Epub 2010/03/24. [PubMed: 20308605]
22. Verdonk K, Saleh L, Lankhorst S, Smilde JE, van Ingen MM, Garrelds IM, et al. Association studies suggest a key role for endothelin-1 in the pathogenesis of preeclampsia and the accompanying renin-angiotensin-aldosterone system suppression. *Hypertension*. 2015; 65(6):1316–23. Epub 2015/04/15. [PubMed: 25870197]
23. Aggarwal PK, Chandel N, Jain V, Jha V. The relationship between circulating endothelin-1, soluble fms-like tyrosine kinase-1 and soluble endoglin in preeclampsia. *J Hum Hypertens*. 2012; 26(4):236–41. Epub 2011/04/01. [PubMed: 21451568]
24. Brewer J, Liu R, Lu Y, Scott J, Wallace K, Wallukat G, et al. Endothelin-1, oxidative stress, and endogenous angiotensin II: mechanisms of angiotensin II type I receptor autoantibody-enhanced renal and blood pressure response during pregnancy. *Hypertension*. 2013; 62(5):886–92. Epub 2013/09/18. [PubMed: 24041954]
25. George EM, Palei AC, Granger JP. Endothelin as a final common pathway in the pathophysiology of preeclampsia: therapeutic implications. *Current opinion in nephrology and hypertension*. 2012; 21(2):157–62. Epub 2012/01/20. [PubMed: 22257796]
26. Mazza MQ, Li W, Reslan OM, Yu P, Mata KM, Khalil RA. Downregulation of microvascular endothelial type B endothelin receptor is a central vascular mechanism in hypertensive pregnancy. *Hypertension*. 2014; 64(3):632–43. Epub 2014/06/11. [PubMed: 24914193]
27. Walther G, Obert P, Dutheil F, Chapier R, Lesourd B, Naughton G, et al. Metabolic syndrome individuals with and without type 2 diabetes mellitus present generalized vascular dysfunction: cross-sectional study. *Arteriosclerosis, thrombosis, and vascular biology*. 2015; 35(4):1022–9. Epub 2015/02/07.
28. Holowatz LA, Thompson-Torgerson CS, Kenney WL. The human cutaneous circulation as a model of generalized microvascular function. *Journal of applied physiology*. 2008; 105(1):370–2. Epub 2007/10/13. [PubMed: 17932300]
29. Kenney WL, Cannon JG, Alexander LM. Cutaneous microvascular dysfunction correlates with serum LDL and sLOX-1 receptor concentrations. *Microvasc Res*. 2013; 85:112–7. Epub 2012/11/10. [PubMed: 23137925]
30. Khan F, Patterson D, Belch JJ, Hirata K, Lang CC. Relationship between peripheral and coronary function using laser Doppler imaging and transthoracic echocardiography. *Clin Sci (Lond)*. 2008; 115(9):295–300. Epub 2008/03/15. [PubMed: 18338981]
31. Coulon P, Constans J, Gosse P. Impairment of skin blood flow during post-occlusive reactive hyperhemia assessed by laser Doppler flowmetry correlates with renal resistive index. *J Hum Hypertens*. 2012; 26(1):56–63. Epub 2011/01/21. [PubMed: 21248780]

32. Debbabi H, Bonnin P, Ducluzeau PH, Leftheriotis G, Levy BI. Noninvasive assessment of endothelial function in the skin microcirculation. *American journal of hypertension*. 2010; 23(5): 541–6. Epub 2010/02/20. [PubMed: 20168305]
33. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*. 2002; 77(1):67–75. Epub 2002/07/04.
34. Bruning RS, Santhanam L, Stanhewicz AE, Smith CJ, Berkowitz DE, Kenney WL, et al. Endothelial nitric oxide synthase mediates cutaneous vasodilation during local heating and is attenuated in middle-aged human skin. *Journal of applied physiology*. 2012; 112(12):2019–26. Epub 2012/04/14. [PubMed: 22500004]
35. Wenner MM, Taylor HS, Stachenfeld NS. Androgens influence microvascular dilation in PCOS through ET-A and ET-B receptors. *American journal of physiology Endocrinology and metabolism*. 2013; 305(7):E818–25. Epub 2013/08/08. [PubMed: 23921139]
36. Wenner MM, Taylor HS, Stachenfeld NS. Endothelin B receptor contribution to peripheral microvascular function in women with polycystic ovary syndrome. *The Journal of physiology*. 2011; 589(Pt 19):4671–9. Epub 2011/08/10. [PubMed: 21825025]
37. Johnson JM, O’Leary DS, Taylor WF, Kosiba W. Effect of local warming on forearm reactive hyperaemia. *Clin Physiol*. 1986; 6(4):337–46. Epub 1986/08/01. [PubMed: 3527533]
38. Smith CJ, Santhanam L, Bruning RS, Stanhewicz A, Berkowitz DE, Holowatz LA. Upregulation of inducible nitric oxide synthase contributes to attenuated cutaneous vasodilation in essential hypertensive humans. *Hypertension*. 2011; 58(5):935–42. Epub 2011/09/21. [PubMed: 21931069]
39. Minson CT, Berry LT, Joyner MJ. Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *J Appl Physiol (1985)*. 2001; 91(4):1619–26. Epub 2001/09/25. [PubMed: 11568143]
40. Minson CT. Thermal provocation to evaluate microvascular reactivity in human skin. *Journal of applied physiology*. 2010; 109(4):1239–46. Epub 2010/05/29. [PubMed: 20507974]
41. Greaney JL, Stanhewicz AE, Kenney WL, Alexander LM. Lack of limb or sex differences in the cutaneous vascular responses to exogenous norepinephrine. *J Appl Physiol (1985)*. 2014; 116(10):3575–82. Epub 2014/10/25.
42. DuPont JJ, Greaney JL, Wenner MM, Lennon-Edwards SL, Sanders PW, Farquhar WB, et al. High dietary sodium intake impairs endothelium-dependent dilation in healthy salt-resistant humans. *Journal of hypertension*. 2013; 31(3):530–6. Epub 2012/12/25. [PubMed: 23263240]
43. Wenner MM, Sebzda KN, Kuczmarski AV, Pohlig RT, Edwards DG. ETB receptor contribution to vascular dysfunction in postmenopausal women. *American journal of physiology Regulatory, integrative and comparative physiology*. 2017; 313(1):R51–R7. Epub 2017/04/26.
44. Wenner MM, Wilson TE, Davis SL, Stachenfeld NS. Pharmacological curve fitting to analyze cutaneous adrenergic responses. *J Appl Physiol (1985)*. 2011; 111(6):1703–9. Epub 2011/08/27. [PubMed: 21868682]
45. Roberts L, LaMarca BB, Fournier L, Bain J, Cockrell K, Granger JP. Enhanced endothelin synthesis by endothelial cells exposed to sera from pregnant rats with decreased uterine perfusion. *Hypertension*. 2006; 47(3):615–8. Epub 2006/01/05. [PubMed: 16391174]
46. Alexander BT, Rinewalt AN, Cockrell KL, Massey MB, Bennett WA, Granger JP. Endothelin type a receptor blockade attenuates the hypertension in response to chronic reductions in uterine perfusion pressure. *Hypertension*. 2001; 37(2 Pt 2):485–9. Epub 2001/03/07. [PubMed: 11230323]
47. LaMarca B, Speed J, Fournier L, Babcock SA, Berry H, Cockrell K, et al. Hypertension in response to chronic reductions in uterine perfusion in pregnant rats: effect of tumor necrosis factor- α blockade. *Hypertension*. 2008; 52(6):1161–7. Epub 2008/11/05. [PubMed: 18981324]
48. Bernardi FC, Vuolo F, Petronilho F, Michels M, Ritter C, Dal-Pizzol F. Plasma nitric oxide, endothelin-1, arginase and superoxide dismutase in the plasma and placenta from preeclamptic patients. *Anais da Academia Brasileira de Ciencias*. 2015; 87(2):713–9. Epub 2015/05/21. [PubMed: 25993363]
49. Fuchs D, Dupon PP, Schaap LA, Draijer R. The association between diabetes and dermal microvascular dysfunction non-invasively assessed by laser Doppler with local thermal hyperemia:

- a systematic review with meta-analysis. *Cardiovascular diabetology*. 2017; 16(1):11. Epub 2017/01/21. [PubMed: 28103890]
50. Ergul A, Shoemaker K, Puett D, Tackett RL. Gender differences in the expression of endothelin receptors in human saphenous veins in vitro. *J Pharmacol Exp Ther*. 1998; 285(2):511–7. Epub 1998/05/15. [PubMed: 9580591]
 51. Kitada K, Ohkita M, Matsumura Y. Pathological Importance of the Endothelin-1/ET(B) Receptor System on Vascular Diseases. *Cardiology research and practice*. 2012; 2012:731970. Epub 2012/08/18. [PubMed: 22900227]
 52. Kellogg DL Jr, Liu Y, Pergola PE. Selected contribution: Gender differences in the endothelin-B receptor contribution to basal cutaneous vascular tone in humans. *J Appl Physiol* (1985). 2001; 91(5):2407–11. discussion 389-90. Epub 2001/10/20. [PubMed: 11641388]
 53. Lee DK, Nevo O. Microvascular endothelial cells from preeclamptic women exhibit altered expression of angiogenic and vasopressor factors. *American journal of physiology Heart and circulatory physiology*. 2016; 310(11):H1834–41. Epub 2016/05/21. [PubMed: 27199113]
 54. Tam KB, George E, Cockrell K, Arany M, Speed J, Martin JN Jr, et al. Endothelin type A receptor antagonist attenuates placental ischemia-induced hypertension and uterine vascular resistance. *American journal of obstetrics and gynecology*. 2011; 204(4):330 e1–4. Epub 2011/04/05. [PubMed: 21458623]
 55. LaMarca B, Parrish M, Ray LF, Murphy SR, Roberts L, Glover P, et al. Hypertension in response to autoantibodies to the angiotensin II type I receptor (AT1-AA) in pregnant rats: role of endothelin-1. *Hypertension*. 2009; 54(4):905–9. Epub 2009/08/26. [PubMed: 19704104]
 56. Murphy SR, LaMarca BB, Cockrell K, Granger JP. Role of endothelin in mediating soluble fms-like tyrosine kinase 1-induced hypertension in pregnant rats. *Hypertension*. 2010; 55(2):394–8. Epub 2009/12/23. [PubMed: 20026766]
 57. Laffin LJ, Bakris GL. Endothelin antagonism and hypertension: an evolving target. *Seminars in nephrology*. 2015; 35(2):168–75. Epub 2015/05/13. [PubMed: 25966348]
 58. Gohar EY, Giachini FR, Pollock DM, Tostes RC. Role of the endothelin system in sexual dimorphism in cardiovascular and renal diseases. *Life sciences*. 2016; 159:20–9. Epub 2016/03/05. [PubMed: 26939577]
 59. Orabona R, Sciatti E, Vizzardi E, Bonadei I, Prefumo F, Valcamonico A, et al. Maternal endothelial function and vascular stiffness after HELLP syndrome: a case-control study. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2016 Epub 2016/12/23.
 60. Weissgerber TL, Milic NM, Milin-Lazovic JS, Garovic VD. Impaired Flow-Mediated Dilation Before, During, and After Preeclampsia: A Systematic Review and Meta-Analysis. *Hypertension*. 2016; 67(2):415–23. Epub 2015/12/30. [PubMed: 26711737]
 61. Henriques AC, Carvalho FH, Feitosa HN, Macena RH, Mota RM, Alencar JC. Endothelial dysfunction after pregnancy-induced hypertension. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*. 2014; 124(3):230–4. Epub 2013/12/12.

Nonstandard Abbreviations

Ach	acetylcholine
CVC	cutaneous vascular conductance
ET-1	endothelin-1
ET_AR	endothelin type-A receptor
ET_BR	endothelin type-B receptor
NO	nitric oxide

eNOS endothelial nitric oxide synthase
SRTX 6c sarofotoxin S6c

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PERSPECTIVES

- Women who have had a preeclamptic pregnancy have augmented microvascular function postpartum, which may be due, in part, to increased vasoconstrictor sensitivity to endothelin-1 (ET-1), mediated by alterations in ET-1 receptor type-B (ET_BR).
- The results of this study suggest that women who have had preeclampsia have augmented vasoconstrictor sensitivity to ET-1, mediated by altered ET_BR signaling, and that altered ET_BR function also contributes to endothelial dysfunction in these women.
- Targeting of the ET-1 pathway may be a clinically relevant approach to simultaneously mitigate exaggerated vasoconstriction and improve endothelial function in order to slow or prevent the development of CVD in this at-risk population.

Figure 1. Mean $\pm$ SEM vasoconstriction (cutaneous conductance, %base) responses to endothelin-1 (ET-1, A) and norepinephrine (NE, B) in postpartum women who have had a healthy pregnancy (HC) and women who have had preeclampsia (PrEC). * $P = 0.02$ main effect of PrEC vs HC.

Figure 2. Mean $\pm$ SEM vasoconstriction (cutaneous conductance, %base) responses to endothelin-1 (ET-1) in control, ET_AR-inhibited (+ BQ-123), and ET_BR-inhibited (+ BQ-788) 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. * $P < 0.05$ PrEC vs. HC. ‡ $P < 0.05$ main effect of + BQ-788 vs. control within group.

Figure 3.

Representative blot (A) and mean ± SEM protein expression of endothelin type-A receptor (ET_AR, B) and endothelin type-B receptor (ET_BR, C) in cutaneous biopsy samples of postpartum women who have had a healthy pregnancy (HC, n=7) and women who have had preeclampsia (PrEC, n=7). *P=0.02 PrEC vs. HC.

Figure 4.

Mean $\pm$ SEM vasodilation (cutaneous conductance, %max) responses to the ET_BR-agonist sarafotoxin 6c (SRTX 6c) in a subset of postpartum women who have had a healthy pregnancy (HC, n=6) and women who have had preeclampsia (PrEC, n=9). P=0.1 main effect for PrEC vs. HC.

Figure 5. Mean $\pm$ SEM vasodilation plateau (cutaneous conductance, %max) and %NO-dependent dilation responses to local heating in control and ET_BR-inhibited (+ BQ-788) sites in postpartum women who have had a healthy pregnancy (HC) and women who have had preeclampsia (PrEC). * $P < 0.05$ PrEC vs. HC control site. ‡ $P < 0.05$ + BQ-788 vs. control within group.

Table 1

Subject characteristics

	HC (n=12)	PrEC (n=12)
Age (years)	33 ± 1 (25-39)	29 ± 2 (20-39)
Time postpartum (months)	6 ± 2 (2-15)	8 ± 2 (2-16)
MAP (mmHg)	85 ± 1 (77-91)	89 ± 3 (75-103)
SBP (mmHg)	113 ± 2 (110-122)	119 ± 3 (100-138)
DBP (mmHg)	71 ± 1 (60-80)	74 ± 3 (62-90)
BMI (kg·m ⁻²)	27 ± 1 (20-33)	30 ± 3 (20-36)
Total cholesterol (mg·dl ⁻¹)	157 ± 8 (106-195)	178 ± 13 (115-189)
HDL (mg·dl ⁻¹)	54 ± 4 (35-72)	48 ± 3 (34-66)
LDL (mg·dl ⁻¹)	89 ± 5 (58-115)	106 ± 9 (59-144)
HbA1c (%)	5.3 ± 0.1 (5.1-5.7)	5.3 ± 0.1 (5.0-5.5)
Plasma ET-1 (pg·ml ⁻¹)	1.8 ± 0.3	1.2 ± 0.4

Values are means ± SEM (range). MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; ET-1, endothelin-1.

Table 2

Parameters of acetylcholine dose-response curves in control and ET_BR-inhibited (+BQ-788) sites.

	HC		PrEC	
	control	+BQ-788	control	+BQ-788
minimum	9.9±3.7	8.2±1.9	9.0±2.0	12.4±3.4
logEC50	-1.5±0.4	-1.4±0.2	-0.7±0.3 [*]	-0.5±0.6
maximum	92.8±8.5	79.5±3.9 [‡]	68.7±6.2 [*]	92.4±15.7 [‡]

Values are means ± SEM. HC, women with a history of normal pregnancy; PrEC, women who have had preeclampsia.

^{*} P<0.05 PrEC vs. HC within the control site.

[‡] P<0.05 +BQ-788 vs. control within group.