Exposure to Experimental Preeclampsia in Mice Enhances the Vascular Response to Future Injury

Dafina Pruthi,* Eliyahu V. Khankin,* Robert M. Blanton, Mark Aronovitz, Suzanne D. Burke, Amy McCurley, S. Ananth Karumanchi, Iris Z. Jaffe

Abstract—Cardiovascular disease (CVD) remains the leading killer of women in developed nations. One sex-specific risk factor is preeclampsia, a syndrome of hypertension and proteinuria that complicates 5% of pregnancies. Although preeclampsia resolves after delivery, exposed women are at increased long-term risk of premature CVD and mortality. Pre-existing CVD risk factors are associated with increased risk of developing preeclampsia but whether preeclampsia merely uncovers risk or contributes directly to future CVD remains a critical unanswered question. A mouse preeclampsia model was used to test the hypothesis that preeclampsia causes an enhanced vascular response to future vessel injury. A preeclampsia-like state was induced in pregnant CD1 mice by overexpressing soluble fms-like tyrosine kinase-1, a circulating antiangiogenic protein that induces hypertension and glomerular disease resembling human preeclampsia. Two months postpartum, soluble fms-like tyrosine kinase-1 levels and blood pressure normalized and cardiac size and function by echocardiography and renal histology were indistinguishable in preeclampsia-exposed compared with control mice. Mice were then challenged with unilateral carotid injury. Preeclampsia-exposed mice had significantly enhanced vascular remodeling with increased vascular smooth muscle cell proliferation (180% increase; \( P<0.01 \)) and vessel fibrosis (216% increase; \( P<0.001 \)) compared with control pregnancy. In the contralateral uninjured vessel, there was no difference in remodeling after exposure to preeclampsia. These data support a new model in which vessels exposed to preeclampsia retain a persistently enhanced vascular response to injury despite resolution of preeclampsia after delivery. This new paradigm may contribute to the substantially increased risk of CVD in women exposed to preeclampsia.

Key Words: cardiovascular diseases ■ hypertension ■ preeclampsia ■ vascular remodeling ■ VEGF receptor flt-1 protein

Despite substantial advances in prevention and treatment strategies, cardiovascular disease (CVD) remains the leading killer of women in the developed world. In the United States, deaths from CVD in women exceed that of men and of the next 7 leading causes of death in women combined.1 One sex-specific CVD risk factor is a history of preeclampsia, a hypertensive complication during pregnancy.2 Preeclampsia is one of the most common and serious pregnancy complications affecting 5% of all pregnancies or >200,000 women annually in the United States. Preeclampsia is diagnosed by the development of hypertension, proteinuria, and other features of end organ damage during pregnancy such as hemolysis, elevated liver enzymes and low platelets syndrome, eclampsia (preeclampsia with associated seizures), and even maternal mortality.3 Delivery of the placenta cures the immediate preeclampsia episode, yet substantial epidemiological data demonstrate that affected women have an increased risk of chronic hypertension, premature CVD, and death from heart attack or stroke many years postpartum.4–8 Recurrent preeclampsia is associated with an even greater CVD risk and women who have had preeclampsia before 34 weeks of gestation or preeclampsia associated with fetal growth restriction, have a risk of cardiovascular death that is 4 to 8 times that of women who had a normal pregnancy.2 Overall, the risk of CVD after preeclampsia is increased by 2 to 4×, an increase comparable with the risk induced by smoking. This strong correlation has prompted the American Heart Association to include a history of preeclampsia as an independent risk factor in the 2011 guideline for the prevention of CVD in woman.1

Substantial progress has been recently made in understanding the pathogenesis of preeclampsia. Abnormal placentation results in placental expression of antiangiogenic factors,
including soluble fms-like tyrosine kinase-1 (sFlt-1), an endogenous inhibitor of vascular endothelial growth factors. Serum/plasma levels of sFlt-1 are increased in women with preeclampsia, rise weeks before the appearance of clinical manifestations of preeclampsia, and correlate with the severity of disease. High sFlt-1 levels produce endothelial dysfunction, which contributes to abnormal vascular tone and hypertension, increased glomerular vascular permeability leading to proteinuria, and consumptive coagulopathy. When injected into pregnant rodents, sFlt-1 reproduces systemic endothelial dysfunction resulting in a syndrome that phenocopies human preeclampsia, supporting the concept that sFlt-1 is a pathogenic mediator of preeclampsia.

In contrast, the pathogenesis for increased CVD risk late after preeclampsia is not well understood. Traditional CVD risk factors, including pre-existing hypertension, obesity, and diabetes mellitus, are also associated with an increased risk of developing preeclampsia during pregnancy. As a result of these shared risk factors, it has been proposed that the development of preeclampsia uncovers a pre-existing condition that would have resulted in CVD later in life. However, another possibility is that preeclampsia-induced vascular damage contributes directly to the future development of maternal CVD. Women with preeclampsia demonstrate an increased blood pressure response to angiotensin II that persists after delivery supporting the presence of subclinical vascular dysfunction that could persist after preeclampsia. The increased risk of CVD in women with recurrent preeclampsia and a decreased incidence of hypertension in the siblings of women with preeclampsia who might be expected to be at similar CVD risk to their preeclampsia-exposed siblings also support this hypothesis. Recent studies in a mouse model of preeclampsia induced by sFlt-1 reveal no detectable differences in blood pressure or in vascular contractile and relaxation function 6 months after delivery, although alterations in circulating proteins persist. Thus, whether the predisposition to CVD after preeclampsia is caused by exposure to preeclampsia or because of shared risk factors remains an important unanswered question.

Vascular disease and dysfunction develop in response to damage to the vascular endothelium that may be initiated by exposure to risk factors such as hypertension and diabetes mellitus, direct mechanical damage during vascular procedures, and other causes. The damaged endothelium promotes thrombosis and vascular inflammation and also acts in a paracrine fashion on underlying smooth muscle cells (SMCs) to activate SMC proliferation and migration that contribute to adverse vascular remodeling. Vascular remodeling is the process in which vascular hyperplasia and fibrosis compromise vessel function by reducing compliance and inhibiting normal blood flow. Excessive vascular remodeling is an important feature of atherosclerosis and also contributes to the failure of therapies for CVD including stent and vein graft failure and transplant vasculopathy. Mouse models of vascular remodeling, including the wire carotid injury model, have been used for 2 decades to explore mechanisms driving vascular remodeling. Here, we use the classical mouse carotid injury model after exposure to a mouse model of sFlt-1–induced preeclampsia to test the hypothesis that prior exposure to preeclampsia results in an enhanced vascular response to future vessel injury as this could contribute directly to the enhanced risk of CVD in women exposed to preeclampsia.

Methods

Mouse Model of Preeclampsia

All animals were handled in accordance with the National Institutes of Health standards and all procedures were approved by the Institutional Animal Care and Use Committees. Female CD1 mice were randomly assigned to receive either sFlt-1 adenovirus or cytomegalovirus promoter–null virus at gestational day 9 of pregnancy resulting in recurrent preeclampsia. The increased risk of CVD in women with shared risk factors persists between pregnancies.

Mouse Carotid Injury Model

Two months postpartum, left common carotid artery endothelial denudation vascular injury was produced with an angioplasty wire and a bromodeoxyuridine infusion pump was placed subcutaneously. The right carotid artery served as an uninjured control in each mouse. Two weeks after injury, both the carotid arteries were processed for histology and medial area, bromodeoxyuridine-positive cells, and fibrosis were each quantified as described. The overall experimental paradigm is depicted in Figure 1.

For detailed methods descriptions, see the online-only Data Supplement.

Figure 1. Experimental paradigm. Pregnant female CD1 mice were randomized to experimental preeclampsia (PE) by injection of soluble fms-like tyrosine kinase-1 (sFlt-1) adenovirus (n=8) or control pregnancy by injection of cytomegalovirus promoter (CMV) virus (n=6) on gestational day (GD) 9. Plasma sFlt-1 levels were measured on GD16 and mice were allowed to deliver. Mice were aged for 2 months and plasma sFlt-1 and cardiovascular function by noninvasive tail cuff plethysmography and cardiac ultrasound were measured. The mice then underwent unilateral wire–induced carotid artery injury and implantation of a bromodeoxyuridine (BrdU) infusion pump. Fourteen days later, mice were euthanized and tissues processed for carotid and renal histology.
Results

Mouse Model That Reproduces the Preeclampsia Phenotype During Pregnancy With Resolution Postpartum

To examine the long-term vascular response to injury after prior exposure to preeclampsia, we used a well-validated mouse model of preeclampsia induced by injection of adenovirus expressing sFlt-1 into pregnant mice at gestational day 9 (Figure 1). Pregnant mice were randomized to receive sFlt-1 (experimental preeclampsia) or cytomegalovirus promoter adenovirus (control) under otherwise identical conditions. sFlt-1 levels were significantly elevated at gestational day 16 (7 days after virus injection) in the experimental preeclampsia group compared with controls (Figure 2A) and correlated with levels found in women with preeclampsia. A second group of mice (n=3 control, n=5 experimental preeclampsia) had telemetry devices inserted before pregnancy exclusively to confirm the preeclampsia phenotype. In this subset of mice, overexpression of sFlt1 was associated with the characteristics of preeclampsia including significantly elevated systolic blood pressure and characteristic renal pathology in late gestation in the preeclampsia group similar to what has been described by other groups (Figure 1 in the online-only Data Supplement). The post–preeclampsia carotid injury mice (n=6 control, n=8 experimental preeclampsia) were allowed to deliver pups and then aged for 2 months. Plasma sFlt-1 levels measured 2 months postpartum had returned to within the normal range in both groups with no significant difference between the experimental preeclampsia and the controls (Figure 2B). Before carotid injury, systolic and diastolic blood pressures were normal and were not significantly different in mice exposed to preeclampsia compared with controls (Figure 2C). Because preeclampsia has also been associated with cardiac dysfunction in this model and in women, cardiac structure and function were measured by noninvasive ultrasound 2 months postpartum. No significant difference was detectable in cardiac chamber dimensions (end-diastolic dimension and end-systolic dimension), cardiac thickness (anterior and posterior wall thickness), or fractional shortening (a measure of cardiac contractile function) between mice exposed to preeclampsia or control pregnancy (Figure 2D). Finally, renal histology at the termination of the study reveals resolution of the renal endotheliosis 2 months after exposure to experimental preeclampsia (Figure 2E). Thus, this mouse model phenocopies human preeclampsia including the transient hypertension and...
renal pathology during pregnancy, which resolve after delivery along with normalization of plasma sFlt-1 levels and of noninvasive measures of cardiovascular function.

**Vascular Remodeling in Response to Injury Is Enhanced After Distant Exposure to Preeclampsia**

To examine whether preeclampsia exposure has lasting effects on the vasculature that could contribute to future vascular responses to injury, mice were exposed to unilateral wire-induced endothelial injury 2 months after either experimental preeclampsia or control pregnancy. Two weeks after carotid injury, the injured and uninjured carotid arteries were isolated and vascular thickness quantified in histological sections stained to visualize elastin fibers. Vessel medial area of the uninjured right carotid arteries is not different in mice exposed to preeclampsia compared with controls (Figure 3, uninjured). However, when exposed to wire-induced endothelial injury, vessels undergo hypertrophic remodeling and this response is significantly enhanced in the arteries of mice exposed to prior preeclampsia (Figure 3, injured). These data support the concept that although vessel structure seems histologically normal after recovery from preeclampsia, when the vessel is exposed to an injury stimulus, the adverse remodeling response is accentuated after preeclampsia exposure.

**Enhanced Vascular SMC Proliferation in Response to Wire Injury After Prior Preeclampsia**

One component of the vascular response to endothelial injury is activation of the normally quiescent vascular SMCs to proliferate until the endothelium heals. Bromodeoxyuridine infusion pumps were inserted at the time of injury and proliferation of carotid SMCs during the 2 weeks after injury was
quantified by counting bromodeoxyuridine-positive nuclei in the vessel media. As expected, in the absence of an injury stimulus, vascular SMC proliferation is minimal in control, uninjured vessels. Exposure to prior preeclampsia did not alter the low level of SMC proliferation in uninjured vessels (Figure 4, uninjured). However, after exposure to wire-induced endothelial injury, SMC proliferation is activated and this proliferative response is significantly enhanced (180% increase; \( P < 0.01 \)) in vessels exposed to prior preeclampsia (Figure 4, injured).

Increased Vascular Fibrotic Response to Injury After Distant Exposure to Preeclampsia

Another component of the vascular injury response is extracellular matrix deposition leading to vascular fibrosis. In the absence of a vascular injury stimulus, medial vessel fibrosis is minimal and not different in vessels exposed to prior preeclampsia compared with control pregnancy (Figure 5, uninjured). However, the fibrotic response to wire-induced endothelial injury is substantially and significantly increased (216% increase; \( P < 0.001 \)) in vessels exposed to prior preeclampsia (Figure 5, injured).

**Discussion**

A mouse model that faithfully reproduces the syndrome of preeclampsia was used to explore whether there is a direct causative effect of preeclampsia on future vascular remodeling in response to injury. Mice without underlying cardiovascular risk factors were randomly assigned to exposure to preeclampsia or normal pregnancy. Because preeclampsia is associated with hypertension and with increased risk of cardiac systolic dysfunction both in women and in this mouse model of preeclampsia,22,23 resolution of hypertension and normal cardiac function after delivery was confirmed by noninvasive methods similar to those used to follow women after preeclampsia. Two months postpartum, mice were challenged with a vascular injury stimulus and mice exposed to prior preeclampsia had a substantially increased vascular remodeling response. The increase in vessel remodeling was because of both an increase in SMC proliferation and enhanced vessel fibrosis in injured vessels exposed to prior preeclampsia. These data support a new paradigm in which vessels exposed to preeclampsia during...
pregnancy retain an enhanced vascular response to future injury (Figure 6). The lack of changes in vascular structure in uninjured vessels after exposure to preeclampsia supports the idea that an additional vascular injury stimulus is necessary and synergistic with previous preeclampsia exposure. Prepregnancy cardiovascular risk factors are associated with increased risk of preeclampsia although the mechanism for this predisposition is not clear. In this new paradigm, continued exposure to hypertension, obesity, or diabetes mellitus, or the development of these or other cardiovascular risk factors (smoking, dyslipidemia) later in life which damage the vasculature would then elicit an enhanced remodeling response in women with a history of preeclampsia. Damage to the endothelium, by denudation or by CVD risk factors, results in endothelial dysfunction with decreased nitric oxide production that activates the normally quiescent SMCs to proliferate and migrate into the intima (as seen in Figure 4 in the injured vessel exposed to prior preeclampsia). This pathology contributes to atherosclerotic plaque development, stent restenosis after vascular interventions, vein graft failure, and transplant vasculopathy. The associated vascular hypertrophy and fibrosis also contribute to increased vascular stiffness, which has been demonstrated in woman with a history of preeclampsia and is associated with increased risk of CVD. Therefore, this new mechanism may be contributing to the substantially increased risk of future CVD in women exposed to prior preeclampsia.

Although noninvasive and histological measures of cardiac and vascular structure and function are indistinguishable after preeclampsia compared with normal pregnancy in this animal model, these data suggest that vascular physiological changes persist that exacerbate the vascular response to injury. Whether preeclampsia exposure enhances other vascular responses to endothelial injury such as vascular inflammation and hypercoagulability remains to be determined using additional models of CVD. Moreover, the detailed molecular mechanism by which preeclampsia exposure leads to such sustained changes in the vasculature remains to be elucidated. Vascular remodeling involves multiple cell types with the intact healthy endothelium acting in a protective fashion to prevent excessive SMC proliferation. Thus, exposure to preeclampsia could enhance vascular remodeling in this model by slowing the rate of endothelial healing and by directly affecting SMC proliferative capacity. There are several pathways that could be implicated in the mechanism that warrant additional study. Vascular contractile responses to renin–angiotensin–aldosterone system activation are enhanced during and just after a pregnancy complicated by preeclampsia both in animal models and in humans. The mechanism for enhanced angiotensin II sensitivity and whether it persists long after delivery remain to be examined. Although 1 study in the same mouse preeclampsia model revealed no difference in vascular contractile responsiveness to adrenergic agonists, thromboxane, or serotonin 6 months postpartum, responsiveness to renin–angiotensin–aldosterone system agonists was not explored. In the same model, persistent alterations in the plasma proteome were identified 6 months after experimental preeclampsia despite normalization of serum sFlt-1 levels. Such persistent alterations in plasma proteins could directly contribute to future vascular injury responses and also may be studied as novel biomarkers of cardiovascular risk in this population. Increased circulating autoantibodies to the angiotensin receptor that occurs in preeclampsia could contribute to long-term cardiovascular risk. Other possibilities include epigenetic changes in the vasculature that occur as a consequence of vascular injury that may lead to long-term CVD. These results support the need for future basic research exploring the underlying mechanism for preeclampsia-induced cardiovascular damage.

Based on the substantial clinical data supporting prior preeclampsia as an important sex-specific cardiovascular risk factor for heart attack, stroke, and cardiovascular death in women, the American Heart Association guidelines recommend screening woman based on pregnancy history. However, at this time, no specific therapies are available to prevent these outcomes in woman identified to have increased risk by such screening. Future clinical studies are needed to address whether inclusion of a history of preeclampsia into risk prediction algorithms might be beneficial in determining treatment goals for traditional risk factors including cholesterol lowering and blood pressure targets in women. Based on this study, it might be postulated that woman exposed to prior preeclampsia would be at higher risk of instent restenosis after percutaneous vascular procedures or of vein graft failure after bypass surgery. This remains to be explored in epidemiological studies but if confirmed, it might be beneficial to choose coated stents or arterial conduits in this patient population. In addition, the current study supports the need for further exploration of the molecular mechanisms by which preeclampsia directly contributes to the pathophysiology of CVD. Elucidation of the mechanisms could identify novel sex-specific treatment targets to prevent the progression of CVD in women identified early as having increased risk based on their pregnancy history. Such novel therapies could substantially reduce CVD morbidity and mortality in this population of woman at high risk.

Perspectives

Exposure to preeclampsia during pregnancy is associated with increased future risk of CVD in women. This has been attributed to pre-existing CVD risk factors in patients with preeclampsia. This study demonstrates that in a mouse model without pre-existing risk factors, preeclampsia exposure during pregnancy potentiates the adverse vascular remodeling response to injury later in life. The vascular injury response in mice is enhanced after preeclampsia despite complete normalization of noninvasive cardiovascular parameters after delivery, as in women with preeclampsia. Moreover, examination of uninjured vessels reveals that in the absence of a vascular injury stimulus, there is no difference in vascular remodeling after preeclampsia. This supports a new paradigm in which preeclampsia causes changes in vascular physiology that enhance the response to future vascular damage that may be mediated by pre-existing and new risk factors to which women are exposed after preeclampsia. Because woman
can be identified early as being at high risk because of their exposure during pregnancy, this new understanding sets the stage for basic and clinical studies to determine treatments that can prevent the rapid progression of CVD in these high-risk women either by aggressively treating cardiovascular risk factors or by modulating the underlying physiology.

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Disclosures
Dr Karumanchi is a coinventor on patents related to the use of angiogenic markers in preeclampsia, is a consultant to Siemens, and has financial interest in Aggamin Therapeutics. The other authors report no conflicts.

References


**Novelty and Significance**

**What Is New?**
- This study demonstrates for the first time that exposure to preeclampsia (PE) during pregnancy enhances the adverse vascular remodeling response to future injury in a mouse model.
- PE exposure enhances the vascular injury response independent of preexisting cardiac risk factors in this model.

**What Is Relevant?**
- Women exposed to PE during pregnancy have an increased risk of heart attack, stroke, and cardiovascular death that is comparable with a history of smoking.
- Although this has been attributed to shared risk factors for PE and cardiovascular disease, this study suggests that PE may also cause persistent vascular dysfunction that directly contributes to future risk of cardiovascular disease and may lead to future novel sex-specific therapeutic opportunities.

**Summary**

This study used a mouse model of PE to explore potential mechanisms for the substantial increased risk of cardiovascular disease in women with a history of PE. PE induced in mice by soluble fms-like tyrosine kinase-1 injection reproduces the human syndrome during pregnancy with normalization of cardiovascular structure and noninvasive measures of function 2 months postpartum. Mice exposed to PE demonstrate enhanced vascular responsiveness to future injury. This new paradigm may contribute to the substantially increased risk of cardiovascular disease in women exposed to PE.
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SUPPLEMENTAL MATERIAL

Exposure to Experimental Preeclampsia in Mice Enhances the Vascular Response to Future Injury

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SUPPLEMENTAL MATERIAL

Supplemental Methods:

**Mouse Model of Preeclampsia:** All animals were handled in accordance with NIH standards, and all the procedures were approved by the Beth Israel Deaconess Medical Center or the Tufts Medical Center Institutional Animal Care and Use Committee. Adenovirus encoding extracellular domain of murine Flt-1 (first generation, E1 and E3 deleted) (1–3) and CMV-null empty vector (Vector Labs, CA) were injected into pregnant mice to create a PE-like animal model as described previously(1-4). Such overexpression of sFlt-1 in mice and rats during pregnancy leads to hypertension, proteinuria and glomerular endothelial damage resembling human PE(1-4). Female CD1 mice (Charles River Laboratories International, Wilmington, MA) were randomized to receive either sFlt-1 Adenovirus or CMV-null virus at gestational day (GD)9 of pregnancy. PE animals received 10 microliters of sFlt-1 adenovirus at initial viral particle (VP) titer of 5.0X10^{12} VP/ml diluted to final volume of 100 microliters in PBS via tail vein injection. Control animals at the same pregnancy stage received comparable VP concentrations and volumes of CMV-null adenovirus. On GD16 and two months following delivery, 100 microliter blood samples were collected in EDTA tubes from the facial vein of the mice under isoflurane anesthesia and plasma separated by centrifugation and used to measure plasma sFlt-1 levels using mouse VEGF-R1/Flt-1 Quantikine ELISA Kit (R&D Systems, Inc. Minneapolis, MN) according to manufacturer’s instructions.

**Blood Pressure Measurement:**

Telemetry during PE: TA11PA-C10 radiotelemetry devices (DataSciences International™, St. Paul, MN) were surgically implanted in 25 gram female mice as described(5). Briefly, under isoflurane anesthesia (2.5% induction and 1.5% maintenance via nose cone) a midline incision overlying the throat is made. The left common carotid artery is isolated, occluded and cannulated with the pressure-sensing tip of the catheter. The catheter tip is placed within the arch of the aorta, secured with suture at the level of the carotid and the body of the transmitter is placed in a subcutaneous pocket created on the flank. Five mg/kg meloxicam is provided for analgesia and recovery of 14 days is permitted prior to entry into experiments. Recovered mice were recorded as non-pregnant, and were mated overnight with CD1 studs. Copulation plug was designated as GD0. Telemetry data were collected continuously throughout pregnancy. Data were acquired every four minutes for 10-seconds using DataQuest ART Software (DSI), and then exported for analysis. The average pre-randomization systolic blood pressure on GD0-6 and the average late pregnancy systolic blood pressure on GD15-19 are reported.

Tail cuff plethysmography during carotid injury: Since telemetry devices are inserted via the carotid artery, this method of blood pressure measurement cannot be used in conjunction with the carotid wire injury model. Blood pressure was therefore measured in the mice prior to carotid injury by tail cuff plethysmography using the Kent coda system by a well validated protocol that correlates closely with telemetry results, as described(6). Briefly, mice were trained twice daily with 20 tail cuff inflations for at least 3 days. The morning after training, blood pressure was measured with 20 cuff inflations,
the values averaged, rare outliers (±2 SD from mean) eliminated, and systolic and diastolic blood pressure recorded as the mean of the remaining measurements (provided there were at least 5 remaining values). Blood pressure data analysis was performed by a treatment-blinded investigator.

**Mouse Cardiac Ultrasound:** Echocardiography was performed under isoflurane anesthesia (2.5% induction and 1.5% maintenance). Left ventricular end-diastolic and end-systolic diameters (EDD and ESD, respectively) and anterior and posterior wall thickness were measured in the short axis view by averaging values measured from five cardiac cycles. Fractional shortening (FS) was calculated using the following equation: \( \text{FS} = \left[ \frac{\text{EDD} - \text{ESD}}{\text{EDD}} \right] \times 100 \). Echocardiography and image analyses were performed by treatment blinded investigators.

**Renal Histology:** Kidneys harvested from mice were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with H&E, and PAS stain and examined by light microscopy for glomerular endotheliosis as described previously(7).

**Mouse Wire Carotid Injury Model and Carotid Histology:** The wire injury protocol was performed 2 months post-partum using methods previously described in detail(6;8-12). The rationale for performing the carotid injury 2 months post-partum is based on the mouse life expectancy of approximately 18-24 months compared to a human woman’s life expectancy of about 80 years. From this we extrapolated that 2-3 months in the mouse is roughly equivalent to a decade in women, the time at which epidemiologic studies begin to reveal increased CVD risk in woman after exposure PE (13). Briefly, left common carotid artery endothelial denudation vascular injury was produced with an angioplasty wire and a Bromodeoxyuridine (BrdU, Sigma-Aldrich, 25 mg/kg/d) infusion pump (Alzet) was placed subcutaneously. The right carotid artery served as an uninjured control in each mouse. Two weeks after injury, both the injured and uninjured control carotid arteries were perfusion-fixed, processed for histology, and medial area, BrdU positive cells, and fibrosis were each quantified as described(6;8-12). All measurements were made by treatment-blinded investigators on intact sections in which complete endothelial denudation could be first confirmed by BrdU staining of all luminal endothelial cells thereby verifying that all endothelial cells in the injured area were removed at the time of surgery and have been replaced by proliferating cells, a process that is complete in less than 2 weeks(14). N=6 control animals (CMV virus during pregnancy) and N=8 prior pre-eclampsia animals (sFlt-1 virus during pregnancy).

**Statistics:** Values are reported as mean ± SEM. Within-group differences were assessed with 2-way ANOVA with Student-Newman-Keuls post-hoc test. Telemetry data were analyzed using 1-way ANOVA (within and between groups) with Bonferroni’s post-test. Plasma sFlt-1 values were analyzed using paired t-test. P < 0.05 was considered significant.
Supplemental References


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Figure S1: Mouse model of experimental preeclampsia that phenocopies the human disorder. (A) Preeclampsia Confirmation Study Paradigm: At the same time as preeclampsia was induced for the carotid injury study, a PE confirmation study was performed with additional female CD1 mice with telemetric blood pressure monitors implanted 14 days before pregnancy. These mice were injection with virus expressing soluble Fms-like tyrosine kinase-1 (sFlt-1, N=5 Experimental Preeclampsia, black bars) or control virus (CMV, N=3 Control Pregnancy, grey bars) at gestational day (GD) 9. (B) Blood pressure was measured by telemetry and the average systolic blood pressure before randomization and on GD0-6 was recorded. Blood pressure rose beginning at day 15 and the average systolic blood pressure was significantly elevated on GD15-19 in the experimental preeclampsia group. (C) Renal histology was performed at the time of sacrifice on GD19. Characteristic renal pathology with endotheliosisis is observed in the experimental preeclampsia mice on GD19 of pregnancy.