Cerebral Vascular Adaptation to Pregnancy and its Role in the Neurological Complications of Eclampsia

by

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Running Head: PPARγ and cerebral hemodynamics in pregnancy

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Abstract

The cerebral circulation has a central role in mediating the neurological complications of eclampsia, yet our understanding of how pregnancy and preeclampsia affect this circulation is severely limited. Here, we show that pregnancy causes outward remodeling of penetrating arterioles and increased capillary density in the brain due to activation of peroxisome proliferator-activated-γ (PPARγ), a transcription factor involved in cerebrovascular remodeling and highly activated in pregnancy. Pregnancy-induced PPARγ activation also significantly affected cerebral hemodynamics, decreasing vascular resistance and increasing cerebral blood flow by ~40% in response to acute hypertension that caused breakthrough of autoregulation. These structural and hemodynamic changes in the brain during pregnancy were associated with substantially increased BBB permeability, an effect that could promote passage of damaging proteins into the brain and cause the neurological complications of eclampsia, including seizure.

Key words: Pregnancy, cerebral blood flow, cerebral vascular resistance, eclampsia, acute hypertension, peroxisome proliferator-activated receptor γ, vascular remodeling
Introduction

Preeclampsia is a serious complication of pregnancy that becomes life-threatening when neurological symptoms, including severe and persistent headache, cortical blindness, and seizure, accompany hypertension and proteinuria in the last half of pregnancy (14,32,40). While it is seizure that distinguishes eclampsia, numerous neurological symptoms can precede the eclamptic convulsion in what is considered severe preeclampsia (32,40). The primary explanation for the neurological symptoms of severe preeclampsia and eclampsia is that they represent a form of posterior reversible encephalopathy syndrome in which an acute elevation in blood pressure, secondary to the preeclamptic state, causes breakthrough of autoregulation, blood-brain barrier (BBB) disruption and passage of damaging protein and serum constituents into the brain (14,23,40). In addition to BBB disruption, the eclamptic seizure is associated with intracerebral hemorrhage that can further promote neurological complications and threaten the life of the mother and fetus (40).

Although the cerebral circulation has a central role in the pathogenesis of the neurological complications of severe preeclampsia and eclampsia, our understanding of how pregnancy and preeclampsia affect this unique circulation is severely limited. Because women who develop preeclampsia and eclampsia are by definition asymptomatic and normotensive prior to and for the first half of pregnancy, we have hypothesized that the cerebrovascular adaptation to normal pregnancy predisposes the brain to neurological complications when blood pressure is acutely elevated, as in severe preeclampsia and eclampsia.
Pregnancy is a condition of profound cardiovascular adaptation with major alterations in both local and systemic hemodynamics (13,15,29,30). The pregnant state is characterized by decreased vascular resistance, hyperpermeability and increased cardiac output, that are necessary to accommodate the considerable expansion of plasma volume and perfuse vital organs such as the uterus and placenta (13,15,29,30). While such changes are considered adaptive for the cardiovascular system in general, similar hemodynamic changes in the brain during pregnancy (including diminished vascular resistance and hyperpermeability), increase the potential for edema formation, elevated intracranial pressure, and other neurological complications. This risk becomes even greater under conditions of severely elevated blood pressure, such as that seen during severe preeclampsia and eclampsia (14,23,40).

Here we investigated how normal pregnancy affects the cerebral circulation under normotensive and acutely hypertensive conditions to mimic hydrostatic pressure changes that occur during severe preeclampsia and eclampsia (14,23,40). We used in vivo and in vitro models to study the effect of pregnancy, with and without acute hypertension, on BBB permeability and hemodynamic alterations in the brain, including cerebral vascular resistance (CVR), cerebral blood flow (CBF), and capillary density. We found that pregnancy alone increases BBB permeability in response to acute hypertension through an effect that appears to be due to decreased CVR driven by outward remodeling of small penetrating arterioles and increased capillary density in the brain.

Peroxisome proliferator-activated receptor-γ (PPARγ) is a transcription factor that
has an important role in pregnancy, including placental development and changes in maternal metabolism (20,35,37). The presence of dominant negative mutations of PPARγ in humans produces early onset hypertension and preeclampsia (2). PPARγ also has prominent effects on the cardiovascular system (34), particularly in the cerebral circulation where it affects both structure and function (3,9,21). Thus, we also investigated the role of PPARγ as an underlying mechanism by which pregnancy causes outward remodeling, increased capillary density and decreased vascular resistance in the brain.
**Materials and Methods**

**Animal model of pregnancy.** All experiments were conducted using virgin nonpregnant (NP) female (280-320g) or timed-pregnant Sprague Dawley rats (Charles River). Rats were housed in the Animal Care Facility at the University of Vermont, an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility. Rats are a useful model of pregnancy because they have similar placentation (hemochorial) and cerebrovascular anatomy as humans and a short gestation period (22 days). Pregnant rats were used for experimentation as late-pregnant (LP; day 19 or 20) as this is when neurological complications of severe preeclampsia and eclampsia occur most often (14,40). All animal protocols and procedures were approved by the Institutional Animal Care and Review Board at the University of Vermont.

**PPARγ inhibition/activation.** To investigate the role of PPARγ in changes in hemodynamics associated with pregnancy, two groups of animals were used and compared to NP and LP control animals. First, a group of NP animals were treated with the PPARγ activator rosiglitazone (20 mg/kg) daily in food for 3 weeks to mimic the increase in PPARγ activation that occurs during pregnancy. Second, a group of pregnant animals were treated with the PPARγ inhibitor GW9662 (10 mg/kg) daily in food for 10 days from day 9 or 10 of pregnancy. We chose to inhibit PPARγ after implantation and the establishment of pregnancy because PPARγ activation has a significant role in placental development (20,35) and earlier treatment might have terminated pregnancy.
Acute hypertension. A model of acute hypertension was used to mimic changes in hydrostatic pressure and autoregulatory breakthrough that have been shown to occur during severe preeclampsia and eclampsia. Acute hypertension was induced by infusion of phenylephrine (PE) under anesthesia, as previously described (16,17). Briefly, animals were anesthetized using isoflurane (3% in oxygen) for placement of the laser Doppler probe, arterial and venous catheters and a tracheostomy. Isoflurane anesthesia was discontinued and anesthesia maintained by intravenous infusion of pentobarbital (60 mg/kg/hr). Animals were mechanically ventilated with air and supplemental oxygen to maintain blood gases within the normal range (Table 1). The femoral artery catheter was used to obtain blood samples for blood gas measurements and to measure systemic blood pressure via a pressure transducer (Living Systems Instrumentation, Burlington, VT). Phenylephrine (0.01g/10mL Lactated Ringer’s solution, Sigma, St. Louis, MO) was infused i.v. and adjusted as needed to ensure a consistent rise in arterial pressure. This model of acute hypertension raises blood pressure through a pressor effect on the systemic circulation and increased peripheral vascular resistance, similar to that of preeclampsia (33). This model of acute hypertension was used in conjunction with the in situ carotid perfusion model to measure BBB permeability in response to autoregulatory breakthrough and in conjunction with microspheres to quantify absolute CBF, both described below.

In situ BBB permeability during acute hypertension. Permeability of the BBB was assessed using previously described methods (16), with modifications. Briefly, animals
were anesthetized and acute hypertension was induced as described above. Once autoregulatory breakthrough was observed on the laser Doppler (an obvious large, steep increase in CBF), fluorescent tracers were infused into the left ventricle of the heart through the carotid artery at a rate of 0.5 mL/min for 1 minute. Sodium fluorescein (MW=476; Stokes-Einstein radius ~ 0.45 nm) in lactated Ringer's and 70 kD Texas Red dextran (Stokes-Einstein radius ~7.0 nm) were used to distinguish size selectivity to small and large tracers, respectively. The infusion pump was then stopped and a reference sample taken from the carotid artery to determine the flux of tracer into the brain. The reference infusion pump was stopped, the animal's chest was opened, and the right atrium cut to allow for drainage of the tracer and lactated Ringer's while the circulation was flushed with 60 mL of lactated Ringer's at a rate of 2 mL/min. The animal was then decapitated and the brain removed and processed for measurement of fluorescence intensity in brain as follows. After sectioning the cortex into anterior and posterior regions and weighing, each brain section was homogenized in PBS and trichloroacetic acid, vortexed for 1 minute and centrifuged at 4 °C for 10 minutes at 4500 rpm. The supernatant was removed and the pellet centrifuged again. The fluorescence of the remaining pellet was determined at excitation and emission wavelengths of 460 and 515 nm for sodium fluorescein and 595 and 615 nm for Texas Red dextran. The reference sample was processed similarly and the flux calculated as: \( Q_T = \frac{C_T \times Q_R}{C_R} \); where \( Q_T \) and \( Q_R \) are the flux of the brain tissue and reference samples, respectively and \( C_T \) and \( C_R \) are the fluorescence counts of the brain tissue and references samples, respectively.
**CBF measurement with microspheres.** Absolute CBF was determined as previously described, with modifications (10). Briefly, once the appropriate blood pressure was attained (measured from the femoral artery catheter), either during the phenylephrine infusion or at baseline for control animals, a catheter connected to a pressure transducer was placed into the right common carotid artery and advanced into the left ventricle of the heart (appropriate placement was evident as the pressure recording profile dropped from arterial to ventricular). Microspheres labeled with a stable isotope (15 μm; Biopal Spheres, Worcester, MA) were injected within a period of 10-20 seconds. Microspheres were vortexed and sonicated for several minutes prior to injection. Arterial reference samples were taken from the femoral artery at a known flow rate (0.85 mL/min), starting 10-15 seconds before microsphere infusion and continued for 90 seconds. The animals were then euthanized by decapitation and the brains removed, sectioned, weighed and dried overnight at 90 °C. The dried brain samples were sent to Biopal for reading of isotopes and calculation of CBF and CVR, as previously described (10).

**In vitro BBB permeability.** Permeability of isolated cerebral arteries from NP and LP animals to Lucifer Yellow (LY) in response to intravascular pressure was performed as previously described (11). Isolated posterior cerebral arteries (PCA) were used for these studies because they have BBB properties (5) and can be studied pressurized in their physiological state. This method also allowed for permeability to be assessed in response to changes in hydrostatic pressure. Arteries were perfused with 0.5 mg/ml
Lucifer Yellow-CH (LY; Molecular Probes, Eugene, OR, USA) in HEPES buffer for three minutes and the HEPES buffer outside the vessel wall (superfusate) was sampled to measure baseline fluorescence intensity using an ultrasensitive fluorescent spectrophotometer (Photon Technology International, Birmingham, NJ, USA). To obtain pressure vs. permeability curves, the superfusate was sampled after step increases in pressure from 60 to 200 mmHg to determine the change in fluorescence intensity. The concentration of LY was quantified from a linear standard curve plotted from known amounts of LY in HEPES.

**PCR analysis of cerebral arteries.** Real-time PCR was performed on isolated cerebral arteries from NP and LP rats using standard techniques for real time quantitative PCR (qPCR) and was performed by the DNA Facility at the University of Vermont. Briefly, cerebral arteries and penetrating arterioles were dissected separately from NP and LP brains and rapidly frozen in RNAlater. The vessels were then placed in an RNase free microcentrifuge tube, homogenized in the presence of RLT buffer, and total RNA isolated using an RNeasy Micro Kit animal tissue protocol (Qiagen, Valencia, CA). Samples were not DNAase treated. The concentration of total RNA was measured using a NanoDrop ND-1000 Spectrophotometer (Wilmington, DE ) and its integrity analyzed by an Agilent 2100 Bioanalyzer (Santa Clara, CA). cDNA was made from total RNA using SuperScript III Kit (Invitrogen, Carlsbad, CA). The cDNA reaction was according to standard protocol. All target genes (claudin-1, claudin-5, occludin, ZO-1, PPARγ and B2m for an endogenous control) were assessed using Assays on Demand
from Applied Biosystems (Foster City, CA) that were validated for efficiency and did not amplify genomic DNA. All samples were run in technical duplicates using a 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Only Ct duplicate values of <35 were used (if replicates were >1.0 apart, they were rerun). Ct values were averaged and used for comparison using the \(2^{-\Delta\Delta \text{Ct}}\) method (26).

**Isolated arteriole reactivity and structural measurements.** Penetrating brain arterioles were dissected and mounted in an arteriograph chamber, as previously described (17). Lumen diameter and wall thickness were measured by video microscopy under active conditions in HEPES buffer at pressures from 25 to 200 mmHg and again under passive conditions in the presence of papaverine (0.1 mM) and diltiazem (10\(^{-5}\) M). Circumferential wall tension (T) was calculated by the equation \(T = \text{pressure} \times \text{radius}\). Before \(T\) was calculated, pressure in mmHg was converted to dynes per square centimeter (1 mmHg = 1333.2 dynes/cm\(^2\)) and radius in microns was converted to cm. Circumferential wall stress was then calculated by the equation \(T/\text{wall thickness}\).

**Capillary density.** Capillary density of the anterior and posterior cerebral cortex was determined by immunohistological staining for von Willebrand factor (vWF) and morphometric analysis, as previously described (9). Briefly, brain sections were paraffin embedded and processed for immunohistochemistry. A polyclonal rabbit anti-human vWF primary antibody (dilution 1:400, Dako, Glostrup, Denmark) and an Ultravision LP
horseradish peroxidase kit (Thermoscientific, Fremont, CA) were used according to specifications. Sections of anterior and posterior cerebral cortex were imaged at 20X and the images imported into Metamorph for counting of capillaries per area. Counting was done blinded relative to group. Four images from each section and two sections from each animal were counted. Images were counted twice and the results averaged.

**Determination of PPARγ activity in brain tissue and vasculature.** PPARγ activity was determined in nuclear extracts using a commercially available immunoassay from brains in which the vasculature was separated from the brain tissue and then the vasculature was further separated into pial vessels vs. microvessels. First, cerebral arteries that were on the top of the brain (pial) were dissected and kept on ice in PBS until nuclear extraction. The brain was then homogenized in PBS for separation of brain tissue and microvessels. The homogenate was centrifuged at 3150 rpm at 4 °C for 10 minutes and the pellet resuspended in 10 mL of PBS, layered over 10 mL of 15% dextran and centrifuged again at 4400 rpm for 60 minutes. The top layer was discarded and the second layer containing the brain tissue was carefully collected using a serologic pipette. The third layer containing dextran and microvessels was poured over an 80 μm sieve and microvessels collected in cold PBS. Nuclear extracts of brain tissue, pial arteries, microvessels and spleen were obtained using a commercially available kit (Caymen Chemicals, Ann Arbor, MI) and stored at -80 °C until use in the PPARγ assay. PPARγ activity was determined in nuclear extracts using a PPARγ transcription factor assay kit (Caymen Chemicals, Ann Arbor, MI) following
manufacturer’s instructions.

Drugs and solutions. All isolated vessel experiments were performed using HEPES buffered saline solution, the composition of which was (mM): NaCl (142), KCl (4.7), MgSO\(_4\) (1.71), EDTA (0.50), CaCl\(_2\) (2.8), HEPES (1.0), KH\(_2\)PO\(_4\) (1.2), and glucose (5.0). HEPES, papaverine and diltizem were purchased from Sigma (St. Louis, MO).

Data analysis and statistics. Data are presented at mean ± standard error of the mean. One-way analysis of variance was used for most analysis with a post-hoc Tukey’s test for multiple comparisons where appropriate. To determine the effect of pressure or pregnancy on BBB permeability in vitro, two-way repeated measures analysis of variance was used. Statistical significance was considered at p<0.05.
Results

Blood-brain barrier permeability is increased during pregnancy

Brain edema occurs in patients with severe preeclampsia and eclampsia and is thought to be a primary contributor to the neurological complications of these conditions (14,23,25,40). Because increased permeability of the BBB is a necessary event in the development of vasogenic brain edema (36), we measured BBB permeability to different-sized solutes in nonpregnant (NP) and late-pregnant (LP) rats using a well-established in situ carotid perfusion model after acute hypertension. We found that pregnancy caused a significant increase in BBB permeability to both sodium fluorescein and 70 kD Texas Red dextran following acute hypertension (Figure 1a and 1b).

Permeability of the BBB was size-selective as the smaller molecule (sodium fluorescein) had considerably greater flux than the larger 70 kD dextran in both types of animals. However, permeability to both solutes was significantly greater in LP animals compared to NP. Similar to our previous study (16), permeability to the larger dextran tended to be greater in the posterior cortex, but this difference was not statistically significant (Figure 1b).

The increase in BBB permeability during pregnancy could be due to decreased CVR and subsequent increases in hydrostatic pressure in the microcirculation or to direct changes in barrier properties of cerebral endothelium. To examine the question of whether pregnancy decreases barrier properties independent of hydrostatic pressure, we used an in vitro method that allows measurement of BBB permeability in cerebral arteries from NP and LP rats in response to changes in hydrostatic pressure. Figure 1c
shows that BBB permeability was increased in response to pressure in arteries from LP and NP animal, suggesting pressure did increase permeability during pregnancy. In addition, there was an increase in permeability in arteries from LP versus NP rats. This difference was present over a range of pressures but was only statistically significant at the highest pressure studied. It therefore appears that pregnancy moderately enhances BBB permeability in response to hydrostatic pressure.

We next assessed whether pregnancy affected mRNA levels for key tight junction proteins. Reductions in expression of these proteins at the BBB increases permeability and may explain the increase in permeability seen \textit{in situ} during pregnancy (27). We used real-time PCR to assess expression of claudin-1, claudin-5, occludin and ZO-1 in cerebral vessels from LP rats compared to NP and found no change with pregnancy (Figure 1d).

\textbf{Cerebral blood flow and vascular resistance during pregnancy}

Acute hypertension, similar to what occurs during severe preeclampsia and eclampsia, causes decreased CVR and breakthrough of autoregulation that severely increases hydrostatic pressure at the BBB, causing an increase in permeability and edema formation (16-19,24). Because pregnancy is known to have significant effects on vascular resistance and blood flow to many organs (13,15,25,30), we hypothesized that pregnancy causes a greater decrease in CVR and that this difference contributes to increased BBB permeability in response to acute hypertension. To test this hypothesis, we measured CBF and determined CVR in NP and LP rats under control conditions and
in response to acute hypertension with breakthrough of autoregulation. Pregnancy did not affect CVR or CBF under normotensive conditions whereas acute hypertension significantly reduced CVR and caused considerable hyperperfusion in both NP and LP rats (Figure 2). Notably, there was a significantly greater decrease in CVR during pregnancy compared to NP animals and a much greater increase in CBF in response to acute hypertension. Changes in CBF during acute hypertension were similar between anterior and posterior cerebral cortex (Figure 2b).

**Pregnancy promotes outward remodeling of penetrating brain arterioles and decreases CVR through activation of PPARγ**

To gain insight into how pregnancy causes a decrease in CVR in response to acute hypertension, we measured the lumen diameter of different segments of the cerebral circulation. The cerebral circulation is unique compared to most vascular beds in that the large cerebral (pial) arteries contribute significantly to CVR (18,22). Because large cerebral arteries are key contributors to CVR, these vessels contribute to regulation of CBF and protect downstream vessels during changes in systemic blood pressure (18). Similar to previous studies, we found that the diameter of large cerebral arteries was not different during pregnancy (Figure 3a). Importantly, penetrating brain arterioles - segments of the vasculature that branch off pial vessels and penetrate into the brain parenchyma - had considerably larger lumen diameters both actively, when myogenic tone was present, and in the presence of papaverine and diltiazem to inactive smooth muscle contraction (Figure 3b and 3d). The larger lumen diameters of the arterioles
during pregnancy was not due to a decrease in myogenic tone. The percent tone at 50 mmHg (calculated as a percent decrease in diameter from fully relaxed) for NP and LP PA was: 44.6 ± 2.7 and 44.9 ± 2.7; p>0.05). Thus, the larger lumen was due to outward remodeling as vessel diameters were significantly larger under passive conditions.

Notably, outward remodeling of penetrating arterioles was hypotrophic in nature as wall thickness was significantly decreased (Figure 3c), but outer diameter was unchanged (data not shown).

PPARγ is highly activated in pregnancy due to increased production of endogenous ligands, and has an important role in both the establishment and maintenance of pregnancy (6,20,31,35,37). PPARγ has also been shown to inhibit inward remodeling of cerebral arterioles under nonpregnant conditions (3,21). Thus, we hypothesized that increased activation of PPARγ during pregnancy contributes to outward remodeling of penetrating arterioles in the brain. To test this hypothesis, we treated NP animals with the PPARγ agonist rosiglitazone for 3 weeks to mimic the increase in activation seen in pregnancy. In addition, we treated pregnant animals with the PPARγ inhibitor GW9662 during the last half of pregnancy. Rosiglitazone administration in NP animals caused outward hypotrophic remodeling similar to that seen during pregnancy. In contrast, inhibition of PPARγ during pregnancy prevented outward remodeling altogether (Figure 3a-c). These results suggest that outward remodeling of brain arterioles during pregnancy is mediated by PPARγ.

To further assess the effect of pregnancy and PPARγ activation on the structure of brain arterioles, we calculated circumferential wall stress at 75 mmHg, the
approximate pressure that these vessels would experience in vivo during acute hypertension (17,19). During pregnancy, brain arterioles experienced significantly greater wall stress than NP arterioles due to the larger lumen and thinner wall: 0.83 ± 0.08 vs. 1.43 ± 0.1 dynes/cm² (p<0.01). A similar effect on wall stress was found in NP animals treated with rosiglitazone as arterioles from these animals underwent similar outward hypotrophic remodeling (1.15 ± 0.06 dynes/cm²; p<0.01 vs. NP). Further, inhibition of PPARγ during pregnancy with GW9662 decreased wall stress. This difference was even greater than that seen in the NP state due to the smaller lumen and thicker vascular wall (0.67 ± 0.04 dynes/cm²; p<0.01 vs. LP).

To determine if remodeling of downstream arterioles during pregnancy and PPARγ activation was associated with changes in cerebral hemodynamics, we measured CBF under normal conditions and during acute hypertension in NP animals treated with rosiglitazone and pregnant animals treated with GW9662. These hemodynamic parameters were then compared to vehicle-treated NP and LP rats (Table 1). We found that there was no difference in CVR or CBF under normotensive conditions between any of the groups. As expected, CVR was significantly decreased with acute hypertension in all groups compared to normotensive animals and was accompanied by a significant increase in CBF. Compared to normotensive animals, all animals had significantly decreased CVR and substantially increased CBF during acute hypertension, suggesting breakthrough of autoregulation had occurred. PPARγ activation tended to decrease CVR and increase CBF similar to pregnancy, but this did not reach statistical significance (p=0.07).
Pregnancy increases capillary density in the posterior cerebral cortex

Changes in capillary density in the brain occur under certain conditions that can affect CVR (28), demonstrating the dynamic nature of the cerebral microcirculation. To determine if pregnancy and PPAR\(\gamma\) affect a key feature the microcirculation, we measured capillary density in brains from NP and LP animals with and without activation or inhibition of PPAR\(\gamma\), respectively. We assessed capillary number in the anterior vs. posterior cerebral cortex because of the propensity for edema to form in the posterior brain region during eclampsia (14,23,40). There was a significant increase in capillary density in the posterior cerebral cortex in all groups compared to anterior, except LP animals treated with GW9662 (Figure 4a and 4b). However, the percent change in capillary density in the posterior brain region was significantly greater in all groups compared to NP (Figure 4c).

PPAR\(\gamma\) expression and activation in cerebral cortex and brain vasculature during pregnancy

It was unclear how pregnancy and PPAR\(\gamma\) activation could selectively affect penetrating brain arterioles during pregnancy and increase brain capillary density, with no apparent effect on upstream cerebral arteries (a previous study from our laboratory showed that inhibition of PPAR\(\gamma\) during pregnancy had no effect on the structure of cerebral arteries, [7]). Because the difference in response to pregnancy and PPAR\(\gamma\) in these two vascular segments could be due to differences in expression of PPAR\(\gamma\), we measured mRNA levels of PPAR\(\gamma\) in arteries and microvessels pooled from LP pregnant animals. We...
found that expression of PPARγ was considerably less in microvessels compared to larger upstream arteries (Figure 5a). Thus, there does not appear to be a direct correlation between the level of PPARγ mRNA expression in whole vessels and vascular responsiveness to pharmacological activation of PPARγ. One major difference between cerebral parenchymal microvessels and arteries on the brain surface is the close association with non-vascular cells which express PPARγ (1,42). To determine if the effect of PPARγ during pregnancy might be affecting the microvessels through a paracrine effect on the vasculature, we used a commercially available immunoassay to measure PPARγ activation of nuclear extracts of isolated brain tissue (without vasculature), cerebral (pial) arteries, and microvessels from LP animals. We found that baseline PPARγ activity was higher in brain tissue compared to either vascular segment (Figure 5b). To confirm that pregnancy was associated with an increase in PPARγ activation, we measured PPARγ activity in nuclear extracts from spleen, an organ with high PPARγ expression (4), and found that the activity of PPARγ was significantly increased in LP animals vs. NP.
Discussion

Increased BBB permeability and subsequent formation of vasogenic brain edema is considered a major contributor to the neurological complications of severe preeclampsia and eclampsia (14,23,36,40). Here, we show that normal pregnancy is associated with an increase in BBB permeability when blood pressure is acutely elevated to mimic the change in hydrostatic pressure that occurs during these conditions (14,23,40). The increase in BBB permeability during pregnancy does not appear to be related to changes in expression of tight junction proteins but to an effect on barrier properties and a pregnancy-induced decrease in CVR that increases hydrostatic pressure on the BBB. The cerebral circulation actively increases vascular resistance in the brain in response to increases in cerebral perfusion pressure, providing a protective mechanism that limits transmission of hydrostatic pressure to the microcirculation (18,19,22). Thus, diminished CVR during pregnancy would be expected to increase CBF and BBB permeability and promote vasogenic brain edema during acute hypertension. These results are in agreement with our previous study that showed significant brain edema formation in LP but not NP animals after acute hypertension (17).

Changes in cerebral hemodynamics during pregnancy in humans have been highly debated and are still largely unknown, mostly because of the difficulties in obtaining accurate quantification of CBF in pregnant women. Our results suggest that pregnancy is associated with decreased resistance in small, but not large vessels in the brain during acute hypertension, a hemodynamic change that has been predicted to occur (but not measured previously) in human studies (41). The prominent contribution
of large cerebral arteries to vascular resistance is unique to the brain. One advantage of this arrangement is that small arterioles can adjust their diameter or undergo remodeling without an appreciable change in total CVR under normotensive conditions (18,19,22). The greater decrease in CVR and hyperperfusion during pregnancy was unmasked only during acute hypertension that caused breakthrough of autoregulation when large vessel resistance was also decreased (19).

PPARγ activity is an important determinant of cerebrovascular structure (3,9,21). For example, genetic interference with PPARγ produces inward remodeling of cerebral arterioles (3). Consistent with this concept, our results suggest that outward remodeling of cerebral microvessels during pregnancy occurs through a mechanism involving PPARγ. Both pregnancy and PPARγ activation caused outward remodeling of penetrating arterioles that was at the expense of the vascular wall, i.e., hypotrophic remodeling, thus substantially increasing circumferential wall stress. This elevation in wall stress in cerebral arterioles from LP animals may predispose these vessels to increased permeability or rupture, especially under conditions of acute hypertension such as that seen in severe preeclampsia and eclampsia. Increases in capillary density during pregnancy, which were also observed in our studies, may also contribute to reductions in CVR with acute hypertension. Such changes may provide a mechanistic basis for the appearance of edema and hemorrhage during eclampsia ([40], Figure 6).

Pregnancy is a state of tremendous hormonal changes that drive hemodynamic adaptations systemically, including decreased total peripheral vascular resistance (13), increased renal blood flow and glomerular filtration rate (29), and increased uterine
blood flow in the uteroplacental vascular bed (30). In addition to changes in multiple hormones, pregnancy is associated with increased activation of PPARγ by endogenous ligands (6,31,37). While the effect of PPARγ activation has been studied extensively in the placenta during pregnancy (20,35), the present study is the first to suggest that PPARγ activation during pregnancy promotes outward remodeling of microvessels within the brain that is associated with changes in cerebral hemodynamics. However, unlike other organs in which there are substantial increases in blood flow over the course of gestation, brain blood flow during normal pregnancy remains relatively constant (28). Maintenance of normal blood flow is not surprising for a vital organ that relies almost exclusively on oxidative metabolism. Thus, it appears that the adaptation of the brain or cerebral vasculature to pregnancy includes the ability to maintain stable levels of CBF and oxygen delivery despite marked changes in hormone concentrations and systemic hemodynamics.

PPARγ has been shown to have an important role in vascular structure and function and promotes outward remodeling in the cerebral microcirculation during pregnancy. The cell type(s) in which PPARγ exerts these effects is not clear. Our results raise the question of whether there are paracrine effects of PPARγ from non-vascular cells, rather than a direct effect of PPARγ on the vasculature, that promotes outward remodeling of cerebral microvessels during pregnancy. This possibility is based on several findings, including that cerebral microvessels, but not arteries, were highly responsive to pregnancy and activation of PPARγ yet they expressed considerably lower levels PPARγ than upstream arteries - vessels that did not respond to pregnancy
(shown in this study) or PPARγ activation (7). In addition, PPARγ activity was increased in brain tissue compared to either vascular segment, suggesting that PPARγ responsive genes in neurons and astrocytes within the brain parenchyma that are closely associated with microvessels may be affecting vascular structure.

Our results also demonstrate that both pregnancy and PPARγ activation with rosiglitazone increased capillary density in the posterior cerebral cortex, an effect that may contribute to the propensity for edema formation to occur in this region of the brain during severe preeclampsia and eclampsia (14,23,40). The finding that PPARγ inhibition with GW9662 did not prevent the increase in capillary density during the last half of pregnancy may be because changes in capillary density occurred earlier in gestation, prior to GW9662 treatment. We assessed changes in vessel structure and capillary density during late-gestation because this is when severe preeclampsia and eclampsia occur most often (40). However, we do not know at what stage of gestation changes in capillary density occurred.

We characterized cerebral hemodynamic changes during normal pregnancy which may have important implications for understanding the development of neurological complications associated with severe preeclampsia and eclampsia. Preeclampsia is defined as hypertension after the 20th week of gestation with significant proteinuria (32,40). Thus, elevated arterial pressure associated with preeclampsia may be present for a few weeks, but is not truly chronic like many forms of hypertension. While chronic hypertension has profound effects on the structure of the cerebral circulation, including causing inward remodeling and capillary rarefaction that would
increase CVR, both pregnancy and PPARγ activation prevent and reverse these structural changes (9,12). Thus, structural changes in the cerebral circulation during preeclampsia may not be fundamentally different from those seen during pregnancy. However, the endogenous ligands for PPARγ have been shown to be decreased in serum from preeclamptic women (38), an effect that may prevent decreases in small vessel resistance and as a result, increase CVR instead of producing a reduction as shown here. It is worth noting that how the preeclamptic state truly affects the cerebral circulation is not known and may be very difficult to accurately assess using animal models because to our knowledge, preeclampsia occurs spontaneously only in bipedial species and thus no true animal model has been developed. In addition, the role of PPARγ in pregnancy and preeclampsia may be further complicated by the recent finding that PPARγ is phosphorylated in disease states such as obesity, preventing transcription of specific PPARγ targets (8). Whether PPARγ is phosphorylated in other disease states such as preeclampsia remains to be determined.

In summary, normal pregnancy was associated with increased BBB permeability during acute hypertension that was not associated with changes in expression of tight junction proteins. Pregnancy caused selective outward remodeling of brain arterioles and increased capillary density due to PPARγ activation. Structural remodeling of brain arterioles during pregnancy and PPARγ activation was also associated with a significant decrease in total CVR and hyperperfusion during acute hypertension compared to NP animals, suggesting hemodynamic changes during pregnancy may contribute to increased BBB permeability and promote the neurological complications of eclampsia.
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Titles and Legends to Figures

Figure 1: Effect of pregnancy on the blood-brain barrier. In situ brain perfusion was used to compare BBB permeability in nonpregnant (NP) and late-pregnant (LP) rats after acute hypertension induced by phenylephrine. Pregnancy was associated with a significant increase in BBB permeability to both sodium fluorescein (a) and 70 kD Texas Red dextran (b). Isolated cerebral arteries were used to measure BBB permeability to Lucifer Yellow (LY) in response to the same changes in hydrostatic pressure (c) and tight junction protein expression (d). Both arteries from NP and LP animals modestly increased permeability in response to pressure, however, the increase was greater in LP vs. NP. There was no change in mRNA expression of tight junctional proteins; *p<0.05 vs. NP HTN, †† p<0.01 vs. 80 mmHg.

Figure 2: Effect of pregnancy on cerebral vascular resistance and cerebral blood flow. Microspheres were used to measure CBF in nonpregnant (NP) and late-pregnant (LP) animals under basal normotensive conditions and in response to acute hypertension induced by phenylephrine. Pregnancy did not alter CVR (a) or CBF (b) under normotensive conditions, but significantly decreased CVR and increased CBF in response to acute hypertension. **p<0.01 vs. control; †† p<0.01 vs. NP.

Figure 3: Effect of pregnancy and PPARγ activation on remodeling of brain penetrating arterioles. Penetrating brain arterioles isolated from nonpregnant control (NP), late-pregnant control (LP), NP treated with the PPARγ agonist rosiglitazone for 3
weeks to mimic pregnancy (NP+Rosi) or LP and treated with the PPARγ inhibitor GW9662 (LP+GW9662) for the last half of pregnancy were used to measure lumen diameter and wall thickness under pressurized conditions. (a) Passive pressure vs. diameter curves of cerebral (pial) arteries from NP and LP rats. Pregnancy did not affect the luminal size of cerebral arteries. (b) Penetrating brain arterioles from LP and NP rosiglitazone-treated animals had significantly larger lumen diameters compared to NP control and LP GW9662-treated animal; **p<0.01 vs. NP; †† p<0.01 vs. LP-GW. (c) Wall thickness was significantly decreased in penetrating arterioles during pregnancy and PPARγ activation; **p<0.01 vs. NP; †† p<0.01 vs. LP-GW. Thus, pregnancy and PPARγ activation cause outward hypotrophic remodeling of brain penetrating arterioles. (d) Active pressure vs. diameter curves of penetrating arterioles shows that all vessels had myogenic reactivity within the autoregulatory pressure range from 25 to 100 mmHg then underwent forced dilatation. Arterioles from LP and rosiglitazone-treated NP animals had larger lumens than NP and GW9662-treated LP animals; **p<0.01 vs. NP; †† p<0.01 vs. LP-GW.

Figure 4: Effect of pregnancy and PPARγ activation on brain capillary density. (a) Representative photomicrographs (X20) from von Willebrand-stained cerebral cortex from nonpregnant (NP), late-pregnant (LP), rosiglitazone-treated nonpregnant (NP+Rosi) and GW9662-treated late-pregnant (LP+GW) rats. (b) Brain capillary density was significantly increased in the posterior vs. anterior cerebral cortex in all groups except LP+GW9662; *p<0.05 vs. anterior cortex; **p<0.01 vs. anterior cortex. (c) All
groups had a significant increase in capillary density in the posterior cerebral cortex compared to NP; *p<0.05 vs. NP; **p<0.01 vs. NP.

Figure 5: Effect of pregnancy on PPARγ expression and activity in brain tissue vs. cerebral vasculature. (a) Relative expression of PPARγ in cerebral pial arteries compared to penetrating arterioles (PA) pooled from late-pregnant (LP) rats. Microvessels had considerably less expression of PPARγ compared to upstream arteries. (b) Selective PPARγ activation in nuclear extracts from LP rat brain microvessels, arteries, and brain tissue (without vasculature) compared to spleen. PPARγ activation was significantly elevated in brain tissue compared to vasculature and spleen; ** p<0.01 vs. both vascular segments and spleen. (c) PPARγ activation was significantly increased in spleen from LP vs. nonpregnant (NP) rats. * p<0.05 vs. NP

Figure 6: Summary diagram of cerebral vascular adaptation to pregnancy and the effect of acute hypertension. (a) Cerebral arteries and arterioles that lie on top of the brain give rise to smaller arterioles that penetrate into the brain tissue, passing first through the Virchow-Robin Space. After passing through this space, arterioles are closely associated with multiple cell types, including pericytes, astrocytes and neurons. Penetrating arterioles are long and largely unbranched segments of the vasculature that connect upstream vessels to the capillary microcirculation and as such contribute significantly to cerebrovascular resistance in the brain. (b) During pregnancy, penetrating arterioles undergo outward hypotrophic remodeling under the influence of
PPARγ activation that is increased during pregnancy. In addition to outward remodeling of arterioles in the brain, capillary density increases. These alterations in structure appear to occur only in the vasculature associated with brain parenchyma, a segment of the vasculature in which expression of PPARγ is relatively low. Thus, we speculate that PPARγ-dependent mechanisms in brain parenchyma have a paracrine influence on the associated vasculature. We further speculate that upstream cerebral arteries, that appear unaffected by pregnancy and PPARγ activation, maintain vascular resistance that is protective in downstream microvessels in relation to hydrostatic pressure. (c) During acute hypertension, similar to what occurs during severe preeclampsia and eclampsia, forced dilatation of large cerebral arteries occurs, decreasing vascular resistance and allowing greater transmission of hydrostatic pressure to downstream arterioles and capillaries. Because the arterioles have undergone outward hypotrophic remodeling, wall stress is significantly elevated, an effect that could promote increases in permeability as well as rupture and hemorrhage (denoted in the figure by the black arrows). Increases in hydrostatic pressure also affect the capillary bed to increase transcapillary filtration and promote edema formation that is greater during pregnancy due to decreased vascular resistance and increased vascular volume and capillary density.
Figure 1

A  
*In situ* BBB permeability - NaFl

![Graph showing sodium fluorescein flux comparison between NP HTN (n=6) and LP HTN (n=6) in anterior and posterior cortex.](image)

B  
*In situ* BBB permeability - 70kD Texas Red

![Graph showing 70kD Texas Red dextran flux comparison between NP HTN (n=6) and LP HTN (n=6) in anterior and posterior cortex.](image)

C  
*In vitro* BBB permeability

![Graph showing concentration change of LY (ng/mL) in NP (n=7) and LP (n=7) at various intravascular pressures.](image)

D  
Tight junction protein expression

![Bar graph showing change in expression (RQ value) for Claudin-1, Claudin-5, Occludin, and ZO-1 in NP (n=5) and LP (n=5).](image)
Figure 2

A  CVR during pregnancy

B  CBF during pregnancy
Figure 3

A. Passive diameters (arteries)

B. Passive diameters (arterioles)

C. Passive wall thickness (arterioles)

D. Active diameters (arterioles)
Figure 4

A

NP  LP  NP + Rosi  LP + GW

B

Capillary Density (counts/mm²)

Anterior Cortex  Posterior Cortex

C

Percent Change from NP Anterior Cortex

NP  LP  NP + Rosi  LP + GW

(n=8)  (n=8)  (n=8)  (n=7)
Figure 5

A  PPARγ mRNA expression in brain vasculature

B  PPARγ activity in brain, vasculature and spleen

C  PPARγ activity in spleen during pregnancy
Figure 6

A  Nonpregnant

B  Late-pregnant

C  Late-pregnant during acute hypertension
Table 1. Physiological parameters for cerebral blood flow studies with microspheres

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>BP start (mmHg)</th>
<th>BP max (mmHg)</th>
<th>Arterial $P_{CO2}$ (mmHg)</th>
<th>Arterial $P_{O2}$ (mmHg)</th>
<th>Arterial pH</th>
<th>Total CBF (ml/100g•min)</th>
<th>CVR (mmHg/(ml/100g•min))</th>
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<tbody>
<tr>
<td>NP CTL (n=8)</td>
<td>292±6</td>
<td>116±5</td>
<td>129±5</td>
<td>38.2±2.2</td>
<td>141±10</td>
<td>7.38±0.01</td>
<td>92.4±9.3</td>
<td>1.34±0.15</td>
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<tr>
<td>LP CTL (n=8)</td>
<td>356±12a</td>
<td>111±4</td>
<td>119±5</td>
<td>41.2±2.5</td>
<td>159±7</td>
<td>7.35±0.02</td>
<td>88.0±6.6</td>
<td>1.26±0.07</td>
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<tr>
<td>NP Rosi CTL (n=6)</td>
<td>327±7a,b</td>
<td>124±6</td>
<td>128±6</td>
<td>34.2±1.4</td>
<td>162±6</td>
<td>7.38±0.01</td>
<td>94.7±20.5</td>
<td>1.49±0.25</td>
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<tr>
<td>LP+GW CTL (n=6)</td>
<td>375±11a,c</td>
<td>100±4c</td>
<td>104±4</td>
<td>36.7±2.4</td>
<td>88±6</td>
<td>7.39±0.02</td>
<td>106.7±15.3</td>
<td>1.08±0.24</td>
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<tr>
<td>NP HTN (n=8)</td>
<td>276±6</td>
<td>122±4</td>
<td>203±3a</td>
<td>36.6±1.1</td>
<td>141±9</td>
<td>7.39±0.02</td>
<td>256.6±30.0a</td>
<td>0.72±0.07a</td>
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<tr>
<td>LP HTN (n=8)</td>
<td>353±15e</td>
<td>100±5e</td>
<td>193±3.0b</td>
<td>40.7±2.0</td>
<td>141±6</td>
<td>7.35±0.03</td>
<td>398.9±45.7b</td>
<td>0.45±0.05b,e</td>
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<tr>
<td>NP Rosi HTN (n=8)</td>
<td>337±9e</td>
<td>125±2j</td>
<td>192±4c</td>
<td>35.2±0.9</td>
<td>135±8</td>
<td>7.38±0.01</td>
<td>394.8±45.9c</td>
<td>0.53±0.06c</td>
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<tr>
<td>LP+GW HTN (n=8)</td>
<td>378±11e,d</td>
<td>100±8e,g</td>
<td>179±5d,e,f,g</td>
<td>34.7±2.2</td>
<td>100±10</td>
<td>7.41±0.02</td>
<td>396.1±55.5d</td>
<td>0.51±0.10d</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

$^a$p<0.05 vs. NP CTL; $^b$p<0.05 vs. LP CTL; $^c$p<0.05 vs. NP Rosi CTL; $^d$p<0.05 vs. LP GW CTL; $^e$p<0.05 vs. NP HTN; $^f$p<0.05 vs. LP HTN; $^g$p<0.05 vs. NP Rosi HTN; $^h$p<0.05 vs. GW HTN