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Administration of recombinant human placental growth factor (rhPIGF) decreases blood pressure in obese hypertensive pregnant rats

Ana C. Palei¹, Adelene Y. Tan², Woo S. Joo², Paul Kussie², Christopher D. Anderson¹, Barbara A. Wilson¹, Frank T. Spradley¹

¹Department of Surgery, Cardiovascular-Renal Research Center, University of Mississippi Medical Center, Jackson, MS;

²Aggamin Biologics, New York, NY

Abstract

Objectives: While epidemiological studies have shown that obesity is associated with increased incidence of hypertension during pregnancy, the mechanisms linking these two co-morbidities are not as well studied. Previous studies detected lower levels of the anti-hypertensive and pregnancy-related factor, placental growth factor (PIGF), in obese hypertensive pregnancies. Therefore, we examined if obese hypertensive pregnant rats have reduced PIGF and whether increasing its levels by administering recombinant human (rh)PIGF reduces their blood pressure.

Methods: We utilized a genetic model of obesity characterized to be heavier, hypertensive, and fertile, namely rats having heterozygous deficiency of the melanocortin-4 receptor (MC4R-def).

Results: MC4R-def obese rats had lower circulating levels of PIGF compared to wild-type lean controls at gestational day 19. Also, assessment of the PIGF receptor, Flt-1, in the vasculature showed that its levels were reduced in aorta and kidney glomeruli but increased in small mesenteric arteries. Chronic intraperitoneal administration of rhPIGF from gestational day 13 – 19 significantly increased circulating PIGF levels in both obese and lean rats, but reduced blood pressure only in the obese pregnant group. The rhPIGF treatment did not alter maternal body and fat masses or circulating levels of the adipokines, leptin and adiponectin. Additionally, this treatment did not impact average fetal weights but increased placental weights regardless of obese or lean pregnancy.

Conclusions: PIGF is reduced in MC4R-def obese hypertensive pregnant rats, which is similar to findings in obese hypertensive pregnant women, while increasing its levels with exogenous rhPIGF reduces their blood pressure.

Corresponding author: Frank T. Spradley, PhD, Department of Surgery, Cardiovascular-Renal Research Center, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, fspradley@gmail.com.

Conflicts of interest:

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We have nothing to disclose.

Keywords

Adipose Tissue; Angiogenic Factor; Obesity; Placenta; Women's Health

INTRODUCTION

Overweight and obesity are becoming more prevalent worldwide and both constitute a significant risk factor for the development of hypertension during pregnancy [1,2]. Hypertensive disorders of pregnancy are categorized based on the clinical manifestation: chronic hypertension, gestational hypertension, preeclampsia, and superimposed preeclampsia [3]. Regardless of the type of hypertensive disorder, they increase the odds for immediate harm to the mother and her baby [4–7]. Moreover, accumulating evidence indicates that hypertension during pregnancy predisposes for future chronic disease like hypertension in both generations [8,9]. According to the CDC, the rates of hypertensive disorder of pregnancy have been on the rise during the past several decades. The immediate and long-term deleterious outcomes for hypertensive pregnancies and their increasing incidence call for more basic research in this area. Focusing on obesity and blood pressure regulation in pregnancy is important, as obesity is associated with increased risk for all types of hypertensive disorders of pregnancy [10–14].

The mechanisms linking obesity and hypertension during pregnancy have not been heavily investigated beyond assessment of circulating biomarkers. These include measurements of angiogenic factors like placental growth factor (PlGF) and anti-angiogenic factors like soluble fms-like tyrosine kinase (sFlt-1). The latter, also known as soluble Vascular Endothelial Growth Factor (VEGF) Receptor-1 (sVEGFR-1), is the soluble receptor of PlGF that quenches its bioavailability [15]. Although increases in the sFlt-1:PlGF ratio predict the development of hypertension during pregnancy with high confidence [16–18], this finding has not been as consistent in the overweight and obese population. Some investigators found no difference in circulating levels of PlGF with a reduction of sFlt-1:PlGF ratio [19,20] whereas others found reductions in PlGF levels [21–24] or no change in the sFlt-1:PlGF ratio [25] in obese hypertensive pregnancies. Thus, it is unknown whether alterations in these circulating angiogenic factors play a role in regulating blood pressure in obese hypertensive pregnancies.

We previously characterized an experimental animal model of obese hypertensive pregnancy; this is a genetic model of obesity with heterozygous deficiency of the melanocortin-4 receptor (MC4R) gene in the rat [26]. Research has shown that this receptor is important for control of food intake, and reduced MC4R signaling produces hyperphagia and increased body weight and fat mass [27]. Toward the end of pregnancy, MC4R-deficient (MC4R-def) rats have significantly increased body weight, total body and visceral fat masses, and elevated blood pressure by gestational day 19, similar to the findings in obese hypertensive pregnant women [26]. In the present study, we measured PlGF-2 levels, which is the predominant rodent isoform of PlGF, in this animal model. Our first aim was to test the hypothesis that obese hypertensive pregnant rats have lower circulating PlGF levels. Our

second aim was to determine whether administration of recombinant human (rh)PIGF reduces blood pressure in obese hypertensive pregnant rats.

METHODS

Transparency

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animal model and genotyping

All animal experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* with all animal-use protocols approved by The University of Mississippi Medical Center's Institutional Animal Care and Use Committee. Male and female MC4R^{+/K314X} (MC4R heterozygous-deficient) rats were obtained from Charles River Laboratories (Wilmington, MA, USA) and used as founders for our colony. Experimental MC4R-def and wild-type (WT) rats were produced from 3 different heterozygous breeding pairs. All rats were maintained on standard chow diet (Envigo 7013/NIH31; Indianapolis, IN, USA) and water *ad libitum*. At weaning (3 weeks old), offspring were placed on NIH31 diet and tail biopsies were collected for genotyping as previously described [14].

Timed pregnancies and chronic rhPIGF administration

At ~13 weeks of age, WT or MC4R-def females were mated with genotype-matched males for the generation of timed-pregnant experimental rats. The observation of sperm in vaginal smears was indicative of gestational day (GD) 0. On GD 13, each rat was anesthetized with 2–4% isoflurane and a 2ML1 ALZET mini-osmotic pump (DURECT Corporation, Cupertino, CA, USA) loaded with 180 µg/kg per day of rhPIGF in Phosphate-Buffered Saline (PBS), pH 7.4 was implanted in the intraperitoneal space or the untreated control group underwent sham surgery.

Production and purification of rhPIGF

The production of rhPIGF began with cDNA encoding human PIGF131 (GenBank Accession number [P49763-2](#)) that was cloned into an expression vector and electroporated into Chinese Hamster Ovary (CHO) cells. The rhPIGF-1 protein was purified to >90% purity via column chromatography and formulated in Dulbecco's PBS, pH 7.4. The infused dose was chosen according to our previous study [28].

Blood pressure measurements

On GD 18, untreated and rhPIGF-treated WT and MC4R-def rats were anesthetized with 2–4% isoflurane and provided 5 mg/kg body weight of Carprofen for analgesia followed by implantation of an indwelling catheter into the left carotid artery and exposure of it at the nape of the neck. Catheters consisted of V/1 tubing attached to V/3 tubing (Scientific Commodities, Lake Havasu City, AZ, USA). Approximately 2.5 cm of the V/3 end of the catheter was inserted into the carotid. Catheters were filled with sterile heparin-0.9% saline

solution (300 mg/mL; Pfizer, New York City, NY, USA) and stoppered with a stainless-steel plug (SP22/12; Instech Laboratories, Plymouth Meeting, PA, USA) to maintain patency. On GD 19, conscious mean arterial blood pressure was measured, as described previously [29]. For this purpose, rats were placed in restrainers (Kent Scientific Corp, Torrington, CT, USA) and catheters connected to pressure transducers (MLT0699; ADInstruments, Colorado Springs, CO, USA) coupled to a computerized data acquisition system (PowerLab, ADInstruments). Readings were calibrated on every rat then data acquired at 1 k/s. Once blood pressure readings stabilized (~1 h), ~10 min of mean arterial blood pressure (MAP), systolic blood pressure (SBP), and diastolic blood pressure (DBP) data were collected and averaged. The % decrease in MAP in response to rhPIGF within lean or obese groups was calculated by: % decrease = ((individual rhPIGF-treated MAP - average untreated pregnant MAP)/(average untreated pregnant MAP))*100.

Blood, vessel, and adipose tissue harvest and pregnancy biometrics

On GD 19, untreated and rhPIGF-treated WT and MC4R-def rats were anesthetized with 2–4% isoflurane. Maternal body mass was recorded. A midline incision was used to exteriorize the uterine horns with fetuses. Blood was collected from the abdominal aorta into Corvac Integrated Serum Separator Tubes (Covidien, Dublin, Ireland) and Vacutainer K₂EDTA tubes (BD, Franklin Lakes, NJ, USA), spun at 2500 rpm for 12 min at 4°C, and serum and plasma were stored at –20°C. Thoracic aortas and the small mesenteric artery arcade, which included primary, first-, second-, third-, and fourth-order arteries, were isolated in physiological saline solution (PSS). Kidney glomeruli were isolated in PSS from both kidneys by progressive sieving of cortical tissue through 150, 106, and 75 µm screens. Tissue samples were immediately snap-frozen in liquid nitrogen and stored at –80°C.

Retroperitoneal white adipose tissue (RWAT) was collected and wet weights recorded to assess visceral fat mass. As a measure of perivascular adipose tissue (PVAT), periaortic fat was collected from the thoracic aorta and normalized to the weight of each thoracic aorta as previously described [30]. Average fetal and placenta weights were calculated per rat then averaged for each experimental group.

Placental villous explant cultures

On GD 19, placental explant cultures were conducted. Random placentas from untreated WT and MC4R-def rats were used to excise villous bundles from the trophospongium and labyrinth areas in ice-cold PBS, pH 7.4. The individual bundles were placed on top of Corning Matrigel Basement Membrane Matrix (Corning, NY, USA) containing 20% of unsupplemented Gibco Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12; Life Technologies, Grand Island, NY, USA) in a 24-well culture plate. A drop of Matrigel was placed over the explant to ensure adherence. Then, 500 µL of DMEM/F-12 (supplemented with 100 µg/mL streptomycin, 100 U/mL penicillin, and 25 µg/mL ascorbic acid) was added to each well. Explant cultures were incubated at 37°C, 6% O₂, and 5% CO₂ for 48 h, after which the conditioned media were harvested. PIGF-2 and sFlt-1 protein levels were quantified by Enzyme-Linked ImmunoSorbent Assay (ELISA) using Quantikine kits from R&D Systems (Minneapolis, MN, USA; catalogue # MP200 and

MVR100, respectively) and normalized to the total protein concentration in each sample (bicinchoninic acid (BCA) method, Thermo Scientific, Rockford, IL, USA).

Quantification of leptin, adiponectin, PIGF, and Flt-1

Plasma levels of leptin and adiponectin were quantified using Quantikine ELISA kits (R&D Systems; catalogue # MOB00 and RRP300, respectively). Serum levels of PIGF-2 and Flt-1 were assessed with mouse Quantikine ELISA kits (R&D Systems; catalogue # MP200 and MVR100, respectively), while serum rhPIGF was assessed with human DuoSet ELISA kits (R&D Systems; catalogue # DY264).

To determine vascular levels of Flt-1, thoracic aortas, small mesenteric arteries, and kidney glomeruli from a subset of rats were homogenized in 2 mL Lysing Matrix D Tubes using a Fast- Prep-24 5G Instrument for 2X40 seconds at a speed of 6.0 m/sec (MP Biomedicals, Santa Ana, California, USA). Each sample was homogenized in 300–400 μ L of radio-immunoprecipitation assay lysis buffer with protease inhibitor cocktail as per manufacturer's instructions (sc-24948; Santa Cruz Biotechnology, Santa Cruz, California, USA).

Homogenates were quickly centrifuged at 13,200 rpm at room temperature, after which the supernatants were collected and stored at -80°C . Flt-1 levels in vessel homogenate supernatants were quantified using the aforementioned mouse Flt-1 Quantikine ELISA kit (R&D Systems). Vascular Flt-1 values were normalized to the total protein concentration in each sample (BCA method, Thermo Scientific).

The intra-assay and inter-assay variabilities, respectively, for the ELISA kits were 3–7.9% and 8.7–9.5% for PIGF-2; 3.2–7.2% and 6.3–8.4% for Flt-1; 3.3–4.3% and 4.7–7.6% for leptin; and 2.2–3.6% and 7–9.4% for adiponectin.

Statistical analysis

Data were graphed and analyzed using GraphPad Prism version 8 (La Jolla, CA, USA). Data are presented as mean $\pm$ standard error of the mean (SEM). Statistically significant differences were defined as $P < 0.05$. For comparisons of mean between untreated lean and obese pregnant groups, a two-tailed Student's t-test was used. Whereas, two-way analysis of variance (ANOVA) tests were conducted followed by Tukey's multiple comparisons tests to analyze the two factors of obesity and rhPIGF treatment. The results of the two-way ANOVAs are inset within graphs. Correlative statistics were calculated to assess the impact of circulating PIGF on blood pressure levels using rhPIGF + PIGF-2 as the independent variable and MAP as the dependent variable then analyzed via two-tailed Student's t-test.

RESULTS

Maternal circulating levels of PIGF-2 and sFlt-1

Serum levels of PIGF-2 were significantly lower ($P = 0.006$) by 22% in MC4R-def vs. WT pregnant rats on GD 19 (Figure 1A). The assay used to quantitate PIGF-2 measures free bioavailable levels of this factor. In contrast, serum levels of the PIGF-2 receptor and blocker, sFlt-1, were similar ($P = 0.5$) between MC4R-def and WT pregnant rats (Figure 1B). The assay used to quantitate this anti-angiogenic factor detects free sFlt-1. Also, there were

no significant differences ($P=0.7$) when comparing sFlt-1:PIGF-2 ratio in MC4R-def and WT pregnant rats (Figure 1C).

Secretion of sFlt-1 and PIGF-2 from placental villi

We examined sFlt-1 and PIGF-2 secretion from placental villi by performing explant culture studies. It was found that sFlt-1 (Figure 2A) and PIGF-2 (Figure 2B) levels in culture media from placental villous explants of MC4R-def pregnant rats were similar ($P=0.9$ and $P=0.2$, respectively) to WT pregnant rats.

Maternal vascular levels of PIGF-2 receptor, Flt-1

Vascular levels of the PIGF-2 receptor, Flt-1, were examined in isolated thoracic aortas, small mesenteric arteries, and kidney glomeruli. There were lower levels of Flt-1 in aortas (Figure 3A) ($P=0.02$) and glomeruli (Figure 3B) ($P=0.03$) from MC4R-def vs. WT pregnant rats on GD19. In contrast, there was higher Flt-1 in small mesenteric arteries (Figure 3C) ($P=0.03$) from MC4R-def vs. WT pregnant rats.

Effect of chronic rhPIGF infusion on maternal blood pressure

Because, by GD 19, the circulating levels of PIGF-2 were reduced in MC4R-def compared to WT pregnant rats, we hypothesized that chronic treatment of rhPIGF would attenuate the blood pressure in MC4R-def obese pregnant rats that we have previously published to be hypertensive [31]. The rhPIGF was infused at the rate of 180 $\mu\text{g/kg}$ per day from GD 13 to 19 in MC4R-def and WT rats, which led to detectable levels of human PIGF in the serum from both treated groups but was undetectable in the untreated groups (Figure 4A). It was then examined whether exogenous administration of rhPIGF affected levels of endogenous PIGF-2. In response to rhPIGF treatment, serum levels of endogenous PIGF-2 were observed to be significantly elevated in WT (rhPIGF-treated: 122 ± 18 vs. untreated: 78 ± 7 pg/mL; $P=0.008$) but not in MC4R-def pregnant rats (rhPIGF-treated: 82 ± 6 vs. untreated: 61 ± 6 pg/mL; $P=0.2$). Moreover, circulating PIGF-2 levels were still lower ($P=0.02$) in rhPIGF-treated MC4R-def (82 ± 6 pg/mL) vs. rhPIGF-treated WT (122 ± 18 pg/mL) pregnant rats. The administration of rhPIGF did not result in significant differences in serum levels of sFlt-1 between the treated groups (WT+rhPIGF: 621 ± 332 pg/mL vs. MC4R-def+rhPIGF: 229 ± 101 pg/mL, $P=0.7$) or sFlt-1:PIGF-2 ratio (WT+rhPIGF: 4.3 ± 1.8 vs. MC4R-def+rhPIGF: 2.8 ± 1.4 , $P=0.2$).

Regarding the blood pressure responses, foremost, post-hoc analysis revealed that MAP was significantly elevated ($P=0.0009$) in MC4R-def over WT pregnant rats at GD 19 (Figure 4B). Although the rhPIGF did not significantly alter ($P=0.7$) MAP in WT pregnant rats (treated: 101 ± 2 mmHg vs. untreated: 105 ± 2 mmHg), its administration decreased ($P<0.0001$) MAP in the MC4R-def pregnant group (treated: 102 ± 2 mmHg vs. untreated: 115 ± 2 mmHg). Figure 4C illustrates that the blood pressure-lowering effect of rhPIGF administration was significantly greater ($P=0.008$) in the MC4R-def compared to the WT rats.

To further understand the extent to which rhPIGF administration affected MAP in the MC4R obese versus WT lean pregnant rats, we performed correlative statistical analyses using

PlGF-2 + rhPlGF as the independent variable and MAP as the dependent variable. It can be appreciated from Figure 5 that increased levels of circulating rhPlGF was not significantly associated ($P=0.3$) with blood pressure in lean pregnant rats (Figure 5A), but was negatively associated ($P=0.0001$) with blood pressure in the obese pregnant rats (Figure 5B).

We also assessed SBP and DBP values. The rhPlGF treatment reduced SBP and DBP in MC4R-def (SBP in untreated: 125 ± 2 vs. treated: 113 ± 2 mmHg, $P=0.0005$; DBP in untreated: 104 ± 2 vs. treated: 93 ± 2 mmHg, $P=0.002$) but did not alter these levels in WT (SBP in untreated: 114 ± 2 vs. treated: 111 ± 4 mmHg, $P=0.5$; DBP in untreated: 93 ± 2 vs. treated: 90 ± 2 mmHg, $P=0.3$).

Maternal body and fat masses

The untreated MC4R-def pregnant rats were significantly heavier than their WT counterparts by ~10% ($P=0.002$) and rhPlGF did not impact this measurement ($P=0.88$) (Figure 6A) taken at GD19. Body length measured from nose to anus was not different within untreated and treated groups for WT (21.1 ± 0.1 and 20.6 ± 0.2 cm, $P=0.1$) and MC4R-def (21.3 ± 0.1 and 21.7 ± 0.2 cm, $P=0.4$) pregnant rats, but overall was greater ($P<0.05$) in the obese groups. Furthermore, maternal fat mass data for RWAT (Figure 6B) and PVAT (Figure 6C) were higher by 84% ($P<0.0001$) and 34% ($P=0.001$), respectively, in the MC4R-def vs. WT pregnant rats and these values were not significantly altered ($P=0.70$ and $P=0.74$, respectively) by administration of rhPlGF.

Circulating adiponectin and leptin

Plasma adiponectin levels were similar ($P=0.06$) within WT and MC4R-def pregnant rats but rhPlGF administration led to an overall increase ($P=0.009$) in these levels regardless of rat strain by GD 19 (Figure 7A). Plasma levels of leptin were significantly higher ($P=0.003$) in the MC4R-def vs. WT pregnant rats but these levels were not altered ($P=0.1$) by the rhPlGF treatment (Figure 7B).

Food intake

Cumulative food intake was assessed between GD 13 – 19 in animals that were housed individually in their home cages. Although the two-way ANOVA indicated that cumulative food intake from GD 13 – 19 was not different ($P=0.3$) between untreated rat strains (WT: 84 ± 2 g vs. MC4R-def: 94 ± 5 g), it was statistically different by a t-test ($P=0.046$). Food intake was reduced ($P<0.0001$) in both rhPlGF-treated WT (62 ± 4 g) and rhPlGF-treated MC4R-def (59 ± 3 g) pregnant rats. When grams of food intake were normalized to grams of body weight, the values were: WT (0.29 ± 0.01), WT+rhPlGF (0.20 ± 0.01), MC4R-def (0.27 ± 0.01), and MC4R-def+rhPlGF (0.17 ± 0.01); overall, the MC4R-def groups had increased ($P=0.008$) whereas the rhPlGF-treated groups had reduced food intake ($P<0.0001$), with no interaction ($P=0.6$) between these two factors.

Fetal and placental biometrics

On GD 19, average fetal weights (Figure 8A) were similar ($P=1.0$) between MC4R-def and WT pregnant rats and were not altered ($P=0.7$) by rhPlGF administration. The number of live fetuses was similar ($P=0.8$) between all groups (WT-untreated: 13 ± 1 , WT+rhPlGF:

12±1, MC4R-def-untreated: 11±1, and MC4R-def+rhPIGF: 11±1). The number of absorbed fetuses average 1 in each of the experimental groups. Overall, average placental weights (Figure 8B) were greater (P=0.02) following rhPIGF administration but were not different (P=0.3) between MC4R-def and WT pregnant rats.

DISCUSSION

In this study, we show that obese hypertensive (MC4R-def) pregnant rats had significantly lower circulating levels of bioavailable PIGF-2 towards the end of gestation. MC4R-def pregnant rats also presented with greater body weight, increased visceral and vessel fat masses, and elevated blood pressure, thereby demonstrating their obese hypertensive phenotype. Administration of rhPIGF attenuated the blood pressure levels in these obese hypertensive pregnant rats without affecting their body weight or fat mass. In contrast, administration of rhPIGF did not alter the blood pressure levels in their lean WT counterparts. These findings suggest that PIGF is involved in the pathophysiology of hypertension in obese pregnancies.

The goal of this study was to develop a better understanding of the underlying pathophysiology of elevated blood pressure during obese hypertensive pregnancies. Associational studies have found that obese pregnant women have reduced levels of circulating PIGF [21–24], but whether approaches to increase this factor lead to attenuation of blood pressure levels in obese hypertensive pregnancies had not been examined yet. We utilize a relevant obese hypertensive pregnant rat model that has approximately double the visceral adipose tissue compared to their normal pregnant controls. This is considered obese and is similar to the greater amount of visceral fat found in humans with high versus low body mass index (BMI) [32]. The cohort of obese pregnant rats used in this study had reduced circulating PIGF-2 levels at GD 19, which is a novel finding and mimics what is seen in obese pregnant women [23].

We also examined circulating levels of the soluble receptor for PIGF, sFlt-1. Although we detected reduced serum PIGF levels in our obese hypertensive rats, there was no significant alteration in serum sFlt-1 levels or the sFlt-1/PIGF ratio on GD 19. These results are in line with clinical studies since circulating sFlt-1 levels have been shown to be similar or even lower in overweight/obese versus normal weight pregnant women [33]. As circulating sFlt-1 and PIGF-2 during pregnancy is largely derived from the placenta [34,35], we assessed the secretion of these factors in placental villous explants from our obese and lean pregnant rats. It was found that secretion of sFlt-1 and PIGF-2 were similar between these groups. We reported previously that Flt-1 levels in placental homogenates were similar between obese and lean pregnant rats [31]. Future studies should delve deeper to compare other potential sources of PIGF-2 in obese and lean pregnant rats [36].

Most studies have focused on measuring circulating angiogenic and antiangiogenic factors, like PIGF-2 and sFlt-1 respectively, in hypertensive pregnancies [34]. Whereas, fewer studies have probed levels of the PIGF-2 receptor, Flt-1, in cardiovascular tissues. We examined levels of Flt-1 within the maternal vasculature at GD 19 in MC4R-def and WT pregnant rats. For this purpose, Flt-1 levels were assessed in both large and small arteries by

respectively utilizing the thoracic aorta and small mesenteric arterial bed of the gut. Flt-1 levels in kidney glomeruli were also examined as the kidney is involved in long-term control of blood pressure [37]. In the aorta and glomeruli, Flt-1 levels were lower in obese pregnant rats. Reduced Flt-1, reduced circulating PlGF-2, or the combination of these findings, would result in endothelial dysfunction in these vessels, and future studies should examine this. In contrast, Flt-1 levels were higher in small mesenteric arteries from obese vs. lean pregnant rats; this could be a mechanism to promote blood flow to this circulation to assist in digestion, because MC4R-def rats are hyperphagic. In summary, our findings of reduced circulating PlGF-2 and no difference in circulating sFlt-1, which is similar to obese pregnant humans, further highlight the translational importance of our experimental animal model. Additionally, our experiments have provided novel information of changes in the amounts of Flt-1 within the vasculature in obese hypertensive pregnant rats.

To determine whether lower levels of PlGF and altered amounts of its receptor within the vasculature have a biological effect on blood pressure regulation in obese hypertensive pregnant rats, we next tested the hypothesis that administration of rhPlGF to increase its circulating levels reduces their blood pressure. To increase circulating PlGF levels, we administered rhPlGF from GD 13 to 19. Rats have a gestational period of ~22 days, thus, our treatment regimen was during the last trimester of rat pregnancy. In humans, monitoring blood pressure in the third trimester is important to prevent immediate and deleterious impacts on mother and fetus [38]. Because we previously observed that rhPlGF administration at a dose of 180 µg/kg per day abolished placental ischemia-induced hypertension [28], we utilized the same dose in the present study. The rhPlGF treatment significantly reduced blood pressure in the obese pregnant rats while there was no impact on blood pressure in the lean pregnant rats. We further reported correlative studies that greater levels of rhPlGF and PlGF-2 were associated with blood pressure decreases in the obese pregnant but not in lean pregnant rats. Ours is the first study to support that methods to increase circulating PlGF levels in obese hypertensive pregnancies results in reduced blood pressure.

Our findings that obese hypertensive pregnant rats have lower circulating PlGF; blood pressure responses to rhPlGF; but that sFlt-1 was similar between obese hypertensive vs. lean normotensive pregnancies suggest that the pathogenesis of hypertension in obese pregnancies might be different from other forms of hypertensive disorders of pregnancy, like preeclampsia. In preeclampsia, placental abnormalities and ischemia/reperfusion injury likely lead to elevated sFlt-1 in the circulation that sequesters bioavailable PlGF and VEGF to increase maternal blood pressure [39]. In the instance where patients are both obese and preeclamptic, it could be that Flt-1 signaling is diminished by both of these defects thereby explaining the increased severity of hypertension and/or exacerbating the symptoms observed in a cohort of these patients [40]. Our studies suggest that measuring both PlGF levels in the circulation and Flt-1 levels in blood vessels may be more reflective of altered maternal angiogenic signaling in obese hypertensive pregnancies. Furthermore, increasing circulating PlGF may compensate for the lower number of Flt-1 receptors in systemic vessels in organs like the kidney that are important for the long-term control of blood pressure. This might then restore Flt-1 signaling, which may explain the beneficial effect of rhPlGF treatment on blood pressure in obese hypertensive pregnant rats.

In addition to maternal outcomes, fetal and placental weights following rhPIGF administration were evaluated. The untreated lean normotensive and obese hypertensive dams had similar values for average fetal weights. Thus, we did not necessarily predict that there would be need for rhPIGF to improve fetal weight values. As for placental weights, these values were similar between untreated lean and obese dams. However, the overall effect of rhPIGF treatment was to increase placental weights regardless of the dam being lean or obese hypertensive. This study did not go beyond assessment of placenta and fetal weights; therefore, it is unknown whether rhPIGF-induced reductions in blood pressure and/or increased placental weights are beneficial to the long-term cardio-metabolic health of the offspring. Other investigators using offspring from MC4R-def pregnant rats suggest that only the MC4R homozygous-def females [41], but not males [42], show disease phenotypes, including elevated blood pressure. Future studies should examine whether rhPIGF treatment of obese hypertensive pregnancies has any benefit in protecting their offspring against chronic diseases later in life.

There are limitations to this study. Foremost, detection of PIGF and sFlt-1 levels is constrained by the assays available. The immunosorbent assays utilized here detect free PIGF (i.e. not in a complex with sFlt-1) and free sFlt-1. Due to limitations in currently available assays, we could not measure the total levels (free plus in complex) for these factors in pregnant MC4R-def versus WT rats. Therefore, at this point, we do not know exactly how much circulating endogenous PIGF is in a complex with sFlt-1.

Another limitation is that we did not measure PIGF-2 levels prior to pregnancy or as pregnancy progressed in our MC4R-def obese versus WT lean groups. It was previously shown that PIGF-2-induced vasorelaxation and vascular levels of Flt-1 are lower in non-pregnant rats but are increased in the pregnant state [43]. Others have examined mechanisms whereby PIGF induces vasorelaxation, which is mediated by Flt-1 in an endothelium-dependent manner to activate nitric oxide synthase (NOS) and endothelial hyperpolarization factor (EDHF) in pregnant rats [43–45]. Our present study found that circulating PIGF-2 levels were reduced on GD 19, and PIGF infusion normalized the blood pressure of obese hypertensive pregnant rats; therefore, we predict that they have reduced PIGF-2-mediated control of vascular function. Future investigations should examine the underlying mechanisms whereby PIGF mediates the control vascular tone in obese hypertensive versus lean normotensive pregnant rats.

In summary, using an experimental animal model of obese hypertensive pregnancy, we found that dams have reduced serum levels of PIGF and altered levels of its receptor, Flt-1 in the vasculature. Although reduced circulating PIGF has been detected in obese human pregnancies, this is one of the first experimental animal studies to implicate that lower PIGF levels are pathologic under obese situations. Indeed, we report that administration of rhPIGF decreases blood pressure in obese hypertensive pregnant rats. Our studies indicate that PIGF production, its regulation of maternal vascular function and blood pressure during pregnancy, and the impact by obesity on the processes, are worth investigating.

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ABBREVIATIONS LIST

ANOVA =	analysis of variance
DBP =	diastolic blood pressure
GD =	gestational day
ELISA =	enzyme-linked immunosorbent assay
MAP =	mean arterial blood pressure
MC4R-def =	melanocortin-4 receptor-deficient
PVAT =	perivascular adipose tissue
rhPIGF =	recombinant human placental growth factor
RWAT =	retroperitoneal adipose tissue
SBP =	systolic blood pressure
sFlt-1 =	soluble fms-like tyrosine kinase
sVEGFR-1 =	vascular endothelial growth factor receptor-1
VEGF =	vascular endothelial growth factor
WT =	wild type

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FIGURE 1.

Maternal serum levels of PlGF-2 (A), sFlt-1 (B), and sFlt-1:PlGF-2 ratio (C) in obese hypertensive MC4R-def (n=16) and lean WT (n=14) pregnant rats at GD 19. GD, gestational day; MC4R-def, melanocortin-4 receptor-heterozygous deficient; PlGF-2, placental growth factor-2; SEM, standard error of the mean; sFlt-1, soluble fms-like tyrosine kinase; WT, wild-type. Bars in scattered dot plots are mean $\pm$ SEM. A: *P=0.006 for MC4R-def vs. WT, B: P=0.5 for MC4R-def vs. WT, and C: P=0.7 for MC4R-def vs. WT.

FIGURE 2.

Levels of sFlt-1 (A) and PlGF-2 (B) in pg/mg of total protein, in media from placental villous explants isolated from MC4R-def (n=16) and lean WT (n=14) pregnant rats at GD 19. GD, gestational day; MC4R-def, melanocortin-4 receptor-heterozygous deficient; PlGF-2, placental growth factor-2; SEM, standard error of the mean; sFlt-1, soluble fms-like tyrosine kinase; WT, wild-type. Bars in scattered dot plots are mean $\pm$ SEM. A: P=0.9 for MC4R-def vs. WT and B: P=0.2 for MC4R-def vs. WT.

FIGURE 3.

Flt-1 levels in pg/mg of total protein in thoracic aorta (A), small mesenteric arteries (B), and kidney glomeruli (C) isolated from obese hypertensive MC4R-def (n=10–16) and lean WT (n=7–14) pregnant rats at GD 19. Flt-1, fms-like tyrosine kinase; GD, gestational day; MC4R-def, melanocortin-4 receptor-heterozygous deficient; SEM, standard error of the mean; WT, wild-type. Bars in scattered dot plots are mean $\pm$ SEM. A: *P=0.02 for MC4R-def vs. WT, B: *P=0.03 for MC4R-def vs. WT, and C: *P=0.03 for MC4R-def vs. WT.

FIGURE 4.

Impact of chronic rhPIGF infusion on maternal blood pressure in obese hypertensive MC4R-def (n=16 for untreated and n=12 for treated) and lean WT (n=14 for untreated and n=8 for treated) pregnant rats at GD 19. Serum levels of rhPIGF (A) and mean arterial blood pressure (B). Bars in scattered dot plots are mean $\pm$ SEM; insets are the results from the two-way ANOVA tests. Results from Tukey's multiple comparisons test for the two-way ANOVA in Figure B: *P=0.0009 for untreated MC4R-def vs. untreated WT; &P<0.0001 for rhPIGF-treated MC4R-def vs. untreated MC4R-def. C: % mean arterial blood pressure effect

of rhPlGF. The bar histogram represents mean $\pm$ SEM; #P=0.008 for % blood pressure effect of rhPlGF-2 in MC4R-def vs. untreated WT. GD, gestational day; MAP, mean arterial pressure; MC4R-def, melanocortin-4 receptor-heterozygous deficient; rhPlGF, recombinant human placental growth factor; SEM, standard error of the mean; WT, wild-type.

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FIGURE 5.

Correlative statistical analyses revealed that increased levels of circulating rhPIGF + PIGF-2 were not significantly associated ($P=0.3$) with blood pressure in lean WT pregnant rats (A), but was associated ($P<0.0001$) with greater decreases in blood pressure in the obese MC4R-def pregnant rats (B) at GD 19. Unfilled circles = untreated WT ($n=14$), unfilled triangles = rhPIGF-treated WT ($n=8$), filled circles = untreated MC4R-def rats ($n=16$), and filled triangles = rhPIGF-treated MC4R-def rats ($n=12$). GD, gestational day; MAP, mean arterial

pressure; MC4R-def, melanocortin-4 receptor-heterozygous deficient; rhPIGF, recombinant human placental growth factor; WT, wild-type.

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FIGURE 6.

Impact of chronic rhPIGF infusion on maternal body and fat masses in obese hypertensive MC4R-def (n=11–16 for untreated and n=8–12 for treated) and lean WT (n=14 for untreated and n=5–8 for treated) pregnant rats at GD19. Absolute body mass (A), visceral RWAT mass (B), and PVAT mass from the thoracic aorta (C). GD, gestational day; MC4R-def, melanocortin-4 receptor-heterozygous deficient; RWAT, retroperitoneal white adipose tissue; PVAT, perivascular adipose tissue; rhPIGF, recombinant human placental growth factor;

SEM, standard error of the mean; WT, wild-type. Bars in scattered dot plots are mean $\pm$ SEM. Inset is the results from the two-way ANOVA tests.

FIGURE 7.

Impact of chronic rhPIGF infusion on maternal plasma adiponectin (A) and leptin (B) in obese hypertensive MC4R-def (n=16 for untreated and n=12 for treated) and lean WT (n=14 for untreated and n=8 for treated) pregnant rats at GD 19. GD, gestational day; MC4R-def, melanocortin-4 receptor-heterozygous deficient; rhPIGF, recombinant human placental growth factor; SEM, standard error of the mean; WT, wild-type. Bars in scattered dot plots are mean $\pm$ SEM. Inset is the results from the two-way ANOVA tests.

FIGURE 8.

Impact of chronic rhPIGF infusion on fetal and placental biometrics in obese hypertensive MC4R-def (n=16 for untreated and n=12 for treated) and lean WT (n=14 for untreated and n=8 for treated) pregnant rats at GD 19. Average fetal weight (A) and average placenta weight (B). GD, gestational day; MC4R-def, melanocortin-4 receptor-heterozygous deficient; rhPIGF, recombinant human placental growth factor; SEM, standard error of the

mean; WT, wild-type. Bars in scattered dot plots are mean $\pm$ SEM. Insets are the results from the two-way ANOVA tests.

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