The Dahl salt-sensitive rat is a spontaneous model of superimposed preeclampsia

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Gillis EE, Williams JM, Garrett MR, Mooney JN, Sasser JM. The Dahl salt-sensitive rat is a spontaneous model of superimposed preeclampsia. Am J Physiol Regul Integr Comp Physiol 2015. First published April 22, 2015; doi:10.1152/ajpregu.00377.2014.—The mechanisms of the pathogenesis of preeclampsia, a leading cause of maternal morbidity and death worldwide, are poorly understood in part due to a lack of spontaneous animal models of the disease. We hypothesized that the Dahl salt-sensitive (S) rat, a genetic model of hypertension and kidney disease, is a spontaneous model of superimposed preeclampsia. The Dahl S was compared with the Sprague-Dawley (SD) rat, a strain with a well-characterized normal pregnancy, and the spontaneously hypertensive rat (SHR), a genetic model of hypertension that does not experience a preeclamptic phenotype despite preexisting hypertension. Mean arterial pressure (MAP, measured via telemetry) was elevated in the Dahl S and SHR before pregnancy, but hypertension was exacerbated during pregnancy only in Dahl S. In contrast, SD and SHR exhibited significant reductions in MAP consistent with normal pregnancy. Dahl S rats exhibited a severe increase in urinary protein excretion, glomerulomegaly, increased placental hypoxia, increased plasma soluble fms-like tyrosine kinase-1 (sFlt-1), and increased placental production of tumor necrosis factor-α (TNF-α). The Dahl S did not exhibit the expected decrease in uterine artery resistance during late pregnancy in contrast to the SD and SHR. Dahl S pups and litter sizes were smaller than in the SD. The Dahl S phenotype is consistent with many of the characteristics observed in human superimposed preeclampsia, and we propose that the Dahl S should be considered further as a spontaneous model to improve our understanding of the pathogenesis of superimposed preeclampsia and to identify and test new therapeutic targets for its treatment.

Preeclampsia is characterized by hypertension, proteinuria, and endothelial dysfunction after the twentieth week of pregnancy. Preeclampsia is currently among the leading causes of maternal morbidity and death worldwide, affecting approximately 5% of all pregnancies in the United States (35, 38). Preexisting chronic hypertension or chronic kidney disease dramatically increases the risk of developing superimposed preeclampsia during pregnancy, with an estimated 25% of chronically hypertensive women (39) and 30% of women with chronic kidney disease (23) developing superimposed preeclampsia. Furthermore, women with superimposed preeclampsia have an even greater risk of adverse maternal and fetal outcomes compared with women with de novo preeclampsia (8). Despite the severity and incidence of this disease, the mechanisms of the pathogenesis of preeclampsia remain unclear. There is currently no effective treatment available, and the only known “cure” for preeclampsia is the delivery of the placenta. There is also a potential for consequences of preeclampsia to linger as women who develop preeclampsia and their offspring are at an increased risk of cardiovascular disease later in life (13, 41). Therefore, it is critical that we develop a better understanding of the underlying causes of preeclampsia as this will lead to new therapeutic options to ameliorate the maternal symptoms while being safe for the growing fetus. Unfortunately, the lack of adequate animal models of preeclampsia has been an obstacle in the identification of the mechanisms and potential therapeutic targets of preeclampsia.

Preeclampsia can be described as a two-stage disease process: abnormal placentalation and maternal syndrome (17, 36, 41). The initiating event in the pathogenesis of preeclampsia is thought to be abnormal placentalation, theoretically caused by insufficient trophoblast invasion that results in a failure of the spiral arteries to invade the uterine wall to promote vascular remodeling. Spiral artery remodeling is essential during pregnancy to increase blood flow to the growing fetus. As a result of inadequate blood flow, the placenta becomes hypoxic, thereby triggering the release of various vasoactive substances, including soluble fms-like tyrosine kinase-1 (sFlt-1), tumor necrosis factor-α (TNF-α), and endothelin-1 (ET-1) that contribute to systemic endothelial dysfunction (16, 17). The most significant consequence of abnormal placentalation is that it contributes to the development of the maternal syndrome, which is characterized by hypertension and glomerular injury (proteinuria). The maternal syndrome becomes evident during the second half of pregnancy, and the resulting inadequate blood flow to the fetus can result in intrauterine growth restriction and the associated increased cardiovascular risk in the long term (18, 38).

Most current animal models of preeclampsia mimic the maternal syndrome associated with preeclampsia through experimental interventions during pregnancy. Models such as the reduced uterine perfusion pressure (RUPP) rat (3) and the sFlt-1 expressing adenovirus rat (25) are therefore not able to exhibit the abnormal placentalation that is thought to be the origin of this disease process. Thus the identification of a spontaneous animal model of preeclampsia that exhibits both abnormal placentalation and the maternal syndrome would be a critical advancement to understanding early mechanisms of preeclampsia. A spontaneous animal model of preeclampsia could aid in identification of new therapeutic targets and discovery of early biomarkers for preeclampsia, provide insight into genetic contributions present in preeclampsia, and contribute to systemic endothelial dysfunction (16, 17).

In this study, we tested the hypothesis that the Dahl salt-sensitive (Dahl S) rat spontaneously exhibits a phenotype of preeclampsia superimposed on chronic hypertension during pregnancy. We compared the changes in blood pressure, proteinuria, renal histology, and uterine artery resistance during...
pregnancy in the Dahl S as well as fetal growth to those in the Sprague-Dawley (SD) rat, a strain that has a well-characterized normal pregnancy and is used as the basis for the RUPP model, and the spontaneously hypertensive rat (SHR), a genetic model of hypertension, to distinguish chronic hypertension during pregnancy from the proposed preeclamptic phenotype in the Dahl S rat.

METHODS

Animals. Dahl salt-sensitive S (SS/jr) and spontaneously hypertensive rat (SHR SHR/NHsd or SHR) were obtained from the colonies maintained by Dr. Michael Garrett at the University of Mississippi Medical Center. SD rats were purchased from Harlan Laboratories (Indianapolis, IN). All rats were maintained on normal rodent chow (TD7034, 0.3% NaCl, Harlan Teklad, Madison, WI) and water ad libitum on a 12-h light-dark cycle. Timed breeding was performed for each strain, and presence of sperm in vaginal smears was indicative of gestational day (GD) 1. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved and monitored by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Mean arterial blood pressure measurements. At ~16 wk of age, a subset of rats was implanted with telemetry devices via the femoral artery (Data Sciences) for continual blood pressure monitoring as previously described (37). Rats were allowed to recover for 10 days before baseline measurements (before mating) were performed. Female rats were then mated with males of their same strain. Blood pressure measurements were obtained during early, mid, and late pregnancy (GD5-6, GD11-12, and GD19-20, respectively).

Urinary measurements. Rats were placed in metabolic cages for 24 h for urine collection at baseline (before mating) and during early, mid, and late pregnancy. Urine collections were also performed for age-matched virgin controls in all three rat strains. Urinary protein excretion was determined by Bradford Assay (Bio-Rad Laboratories), and proteinuria was defined as >20 mg protein/24 h. A subset of rats (n = 10) was further monitored through a second pregnancy to assess changes in proteinuria in a subsequent pregnancy.

Uterine artery resistance index. Power Doppler velocimetry measurements were performed on pregnant dams at an imaging station with a Vevo 770 unit (FUJIFILM VisualSonics) using a 30-Hz transducer and an insonating angle <30 degrees on GD14 and GD18. The peak systolic flow velocity (PSV) and end-diastolic flow velocity (EDV) was recorded using the uterine artery Doppler waveform, and the uterine artery resistive index (UARI) was calculated using the following formula: UARI = (PSV − EDV)/PSV. UARI was determined for the uterine artery bilaterally at three different locations along the uterine artery in each rat and the mean UARI was calculated (46).

Tissue collection. Rats were euthanized on GD20 while under isoflurane anesthesia (Piramal Healthcare). A blood sample was taken from the abdominal aorta, and the organs were then perfused blood free with saline. Placentas were collected and snap frozen in liquid nitrogen for later analysis, and a section of the left kidney was fixed in 10% buffered Formalin (Fisher Scientific). The number of viable and resorbed fetuses was recorded from each uterine horn, and placental weight, fetus weight, and fetus length were recorded for each viable fetus. The number of fetal resorptions was also noted when applicable.

Western blot analysis. One placenta per pregnant rat was selected from 11 Dahl S, 9 SD, and 7 SHR rats. Total soluble protein was extracted in radioimmunoprecipitation assay (RIPA) lysis buffer containing PMSF in dimethyl sulfoxide, sodium orthovanadate, and a protease inhibitor mixture (Santa Cruz Biotechnology). Total protein concentration for each homogenized placenta was determined using DC protein assay (Bio-Rad Laboratories). Homogenized samples of placenta, standardized by their protein concentrations (130 μg), or 5 μl of plasma samples were separated by electrophoresis (100 V, 120 min 4–15% TGX Stain-Free Precast gel, Bio-Rad Laboratories) and transferred onto nitrocellulose membranes (Bio-Rad Trans-Blot Turbo). Membranes were then stained with Ponceau Red (Sigma) to determine transfer efficiency/uniformity and equal loading, blocked and washed then incubated overnight with the primary antibody [1:500 hypoxia-inducible factor-1 α (HIF-1α), Novus Biologicals or 1:500 Flt-1, Sigma] at 4°C on a shaker. Membranes were then incubated with the secondary antibody (1:2,000 for HIF-1α samples, 1:5,000 for Flt-1 samples, goat α-mouse, Bio-Rad Laboratories) for 1 h at room temperature and then developed with enhanced chemiluminescent reagents (Thermo Scientific). Bands were then quantified by densitometry using the VersaDoc Imaging System and Image Lab 3.0 Software (Bio-Rad Laboratories). Protein abundance was calculated as integrated optical density of the protein of interest (after subtraction of background), factored for Ponceau Red stain (a measure of total protein loading for each sample), and normalized to the SD control average (to quantitatively compare results between different membranes).

Renal histology. A section of the left kidney was fixed in 10% buffered Formalin and embedded in paraffin, cut into 4-μm sections, and stained with Masson’s trichrome. Glomerular area and diameter were measured in 20 randomly selected glomeruli from each slide (n = 5–8 dams, ×40 magnification, Nikon Eclipse 80i microscope with digital camera, Nikon Instruments) using Nis-Elements image analysis software (version 3.03; Nikon Instruments).

TNF-α ELISA. Plasma and placental TNF-α (placental samples homogenized in RIPA as described above) were measured via commercially available ELISA (R&D Systems) according to manufacturer’s instructions.

Statistical analysis. All data are presented as means ± SE. Statistical analyses were performed by one-way or two-way analysis of variance (among strains) followed by Tukey test or Holm Sidak multiple comparisons method, respectively, using Sigma Plot 12 (Systat Software). Repeated measures two-way ANOVA and the Holm Sidak multiple comparisons method were used for analysis of blood pressure and urinary protein excretion measurements over the course of pregnancy and among strains. Means were considered significantly different if P < 0.05.

RESULTS

Increased mean arterial pressure in the Dahl S during pregnancy. Mean arterial pressure (MAP) significantly decreased during mid (MP) and late pregnancy (LP) in the SD rats (n = 8), as expected during normal pregnancy. The SHR also exhibited normal pregnancy patterns with a significant decrease in MAP by late pregnancy (n = 3). However, the Dahl S rat did not exhibit a drop in blood pressure but rather a steady increase in blood pressure during the pregnancy (n = 7). The change in MAP during the pregnancy for all three strains is displayed in Fig. 1A. In Fig. 1B, the change in MAP from baseline to late pregnancy reveals a significant difference in the blood pressure response to pregnancy between the Dahl S (~9 mmHg rise) compared with the SD and SHR (~11 and ~20 mmHg drop, respectively), representing a 20- to 30-mmHg differential in blood pressure (BP) between groups by LP.

Exacerbation of proteinuria in the Dahl S. In addition to worsening hypertension, the Dahl S rat also exhibited a severe increase in urinary protein excretion during pregnancy (Fig. 2A). The SD had a small, but significant, increase in urinary protein excretion at midpregnancy, while urinary protein excretion was unchanged in the SHR (n = 4–18). Since urinary
protein excretion increases with age in the S strain, we also compared urinary protein excretion to age-matched virgin rats in each strain. Urinary protein excretion was modestly increased, but still below the pathological range, in the SD rats but greatly increased in the Dahl S rats compared with age-matched virgin rats (Fig. 2B). Proteinuria was further assessed in a subsequent pregnancy in a subset of Dahl S rats. Proteinuria was exacerbated to an even greater extent during second pregnancy (536 ± 97 mg/day), but significantly dropped 1 wk postpartum (210 ± 33 mg/day, n = 10, P < 0.05 vs. late second pregnancy), suggesting that this is a transient, pregnancy-specific renal injury in the Dahl S rat that worsens with repeated pregnancies.

Pregnancy-induced pathological changes in the Dahl S. The Dahl S rat also showed significant changes in renal histology during pregnancy consistent with changes seen in human preeclamptic patients (42). The pregnant Dahl S rats showed a significant increase in glomerular area (Fig. 3A) and glomerular diameter (Fig. 3B) compared with their virgin controls (n = 6 rats, 20 glomeruli/rat). There was no significant difference in glomerular size between pregnant and virgin SD or SHR rats (n = 4 – 8). Representative images are shown in Fig. 3, C–H. There was also evidence of arteriolar thickening and hyalinosis in small renal vessels of the pregnant Dahl S rat (Fig. 3, I–K) that was not observed in the virgin Dahl S or pregnant or virgin SD or SHR.

Increased UARI in the Dahl S. The Dahl S rat also has a reduction in uteroplacental blood flow, as measured by UARI. UARI is high during midpregnancy (GD14) in all three strains, and there is no difference in UARI among the strains at this time point (Fig. 4A). During normal pregnancy, UARI drops during late pregnancy as a result of systemic vasodilation as seen on GD18 in both the SD and SHR rat in Fig. 3B (n = 6 and 7, respectively). However, UARI remains elevated during LP in the Dahl S rat (n = 12) and is significantly higher than both the SD and SHR rat. Figure 4B illustrates the drop in UARI between GD 14 and 18 in the SD and SHR, in contrast to the slight rise in UARI in the Dahl S. Furthermore, the waveforms for uterine artery flow are distinctively different in the Dahl S rat compared with the SD and SHR. The waveforms in the Dahl S rat resemble those seen in human preeclamptic patients (44) with a steep decline from the peak systolic flow and subsequent rebound in diastolic flow, resulting in a notch formation (denoted by arrow in Fig. 4C). In the SD and SHR, there is a smooth curve between peak systolic and end-diastolic flow as is observed in normal human pregnancies.

HIF-1α and TNF-α are increased in the Dahl S and SHR placenta. The Dahl S placenta exhibits hypoxia as measured by a significant increase in the abundance of HIF-1α in the placenta compared with the SD control [1.4 ± 0.1 vs. 1.0 ± 0.1 relative densitometric units (RDU)]. The SHR exhibits even
greater placental hypoxia compared with the SD control (2.3 ± 0.2 vs. 1.0 ± 0.1 RDU) despite having a normal phenotype during pregnancy (Fig. 5A).

The pregnant Dahl S rat exhibits a significant increase in TNF-α in plasma compared with the virgin control (2.1 ± 0.2 pg/ml vs. 1.4 ± 0.2 pg/ml), but there was no observed significant difference in pregnant SD or SHR compared with their virgin counterparts (Fig. 5B). TNF-α was also increased in the Dahl S and SHR placenta compared with the SD (152 ± 8 and 144 ± 11 pg/g, respectively, vs. 92 ± 5 pg/g, Fig. 5C).

Circulating sFlt-1 is significantly elevated in the pregnant Dahl S rat. Plasma concentrations of sFlt-1 were also significantly increased in the pregnant Dahl S rat during late pregnancy (1.83 ± 0.16 RDU) compared with the pregnant SD and SHR (1.00 ± 0.09 and 1.27 ± 0.14 RDU, respectively, Fig. 5D).

Intrauterine growth restriction in the Dahl S. The Dahl S rats had smaller litters than the SD or the SHR and also had significantly more fetal resorptions (Fig. 6, A and B, data from 5 to 9 litters). Dahl S and SHR pups were significantly smaller compared with the SD pups, as measured by both fetal weights and lengths (Fig. 6, C and D). Because the Dahl S dams (281 ± 13 g) are larger than the SD (229 ± 6 g) and SHR (226 ± 5 g) before pregnancy (P < 0.05), pup weight and pup length were also normalized to maternal size. When normalized, the Dahl S pups were smaller than both the SD and SHR. Placental weight significantly varied among the strains, with the greatest placental weight observed in the Dahl S rat (0.61 ± 0.02 g, P < 0.05 vs. SD: 0.55 ± 0.01 g and SHR: 0.48 ± 0.01 g). The SHR placental weight was the smallest of the three strains (P < 0.05 vs. SD and Dahl S). Therefore, the relative increase in placental weight and decrease in fetal weight resulted in a greater ratio of placental weight to pup weight in the Dahl S compared with both the SD and the SHR (Fig. 6E).

DISCUSSION

Preeclampsia is a serious complication of pregnancy, and there is a vital need for the discovery of new therapeutic options for women with preeclampsia that ameliorate the maternal symptoms and are also safe for the growing fetus (31). Unfortunately, the lack of adequate animal models of preeclampsia has been an obstacle in the identification of the mechanisms and potential therapeutic targets of preeclampsia. The present study reports novel findings that the Dahl S rat spontaneously displays a preeclamptic phenotype during pregnancy characterized by increased BP, exacerbation of proteinuria, glomerulomegaly, increased UARI and placental hypoxia, increased production of TNF-α, increased plasma sFlt-1, and evidence of intrauterine growth restriction and intrauterine fetal demise. Furthermore, the preeclamptic phenotype observed in the Dahl S rat is distinctive from the pregnancy patterns observed in the SHR despite both strains exhibiting pregestational hypertension, indicating that these distinct dif-

Fig. 3. Glomerulomegaly during late pregnancy in the S rat compared with age-matched virgin control. A: comparison of glomerular area at late pregnancy compared with age-matched virgin rats of each strain. B: comparison of glomerular diameter at late pregnancy compared with age-matched virgin rats of each strain. C–H: representative histological images of glomeruli in a virgin (C–E) and pregnant (F–H) SD (C, F) SHR (D–G), and Dahl S (E, H) rats. I–K: representative images of renal arterioles with increased wall-to-lumen ratios (I) and hyaline deposits (J) in the pregnant Dahl S rat compared with a virgin Dahl S rat (K).
ferences in the Dahl S are not solely due to the preexisting hypertension in this model.

Most of the previous animal models of preeclampsia, such as the RUPP rat model (3), chronic inhibition of nitric oxide synthase (51), and infusion of the sFlt-1 expressing adenovirus (25), rely on surgical or pharmacological interventions to induce a preeclamptic phenotype. While these models have been useful to investigate many possible mechanisms involved in the progression of the maternal syndrome observed in preeclampsia, they have not been able to recreate the onset of the pathogenesis of preeclampsia. In the Dahl S rat, we see a spontaneous development of a preeclamptic phenotype that is distinct from the normal progression of increased blood pressure and renal injury in this strain and provides many advantages to further research in this field by allowing the study of early time points in the development of preeclampsia that are not possible in other animal models. The preeclamptic phenotype in the Dahl S rat is similar to phenotype observed during pregnancy in the BPH/5 mouse, a genetic mouse model of hypertension (14). It has been proposed that abnormal placentation is the initiating event in the pathogenesis of this preeclamptic phenotype (16); however, definitive evidence to support this theory has been limited by a lack of a spontaneous model of preeclampsia. As both the Dahl S rat and the BPH/5 mouse mimic the maternal syndrome of superimposed preeclampsia, these rodent models may provide insights into the initiating events in the development of placental ischemia; however, it has been reported that trophoblast invasion during placentation in the rat better mimics human placentation than the mouse (2). Our current findings that the Dahl S rat develops the maternal syndrome of superimposed preeclampsia (increased blood pressure, proteinuria, renal histological changes, and uterine artery resistance) along with fetal growth restriction suggest that the Dahl S rat should be considered as a useful model to study early placentation and how the placenta changes during pregnancy. In addition, this model will aid in identifying changes in other vasoactive factors that contribute to the maternal syndrome of superimposed preeclampsia during the Dahl S pregnancy.

The Dahl S rat has been used extensively as a model of salt-sensitive hypertension and chronic kidney disease (33). As hypertension and preexisting renal disease are strong risk factors for the development of preeclampsia, we hypothesized that this model would develop superimposed preeclampsia during pregnancy. In addition, the Dahl S rat has impaired nitric oxide production (9, 10, 43), increased oxidative stress (27, 47, 48), and immune activation (24, 28), all of which are factors proposed to contribute to the pathogenesis of preeclampsia (29). Indeed, we observed that the Dahl S rat does exhibit worsening hypertension in late pregnancy, in contrast to both the normotensive SD and the hypertensive SHR. Healthy pregnancy is characterized by a decrease in systemic vascular resistance and blood pressure, likely due to increased nitric oxide-mediated vasodilation (40), and this typical response is observed in both the SD and SHR (4, 15, 19, 37). However, the Dahl S rat exhibits a slight but significant increase in MAP, resulting in a ~20 mmHg difference in pressure compared with the expected drop that should occur at late pregnancy. In the current study, the Dahl S rats were maintained on standard rodent chow (0.3% NaCl); however, a high-salt diet will exacerbate hypertension and renal injury in this strain. A recent report by Takushima et al. (45) demon-

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Fig. 4. Uterine artery resistance index (UARI) decreases at late pregnancy in the SD and SHR rats but not in the Dahl S rats. A: UARI at gestational day 14 and 18 (D 14 and D 18, respectively). B: percent change in UARI from D 14 to D 18. C: representative Doppler ultrasound waveforms for each strain. Arrow denotes “notch” in waveform of Dahl S. *P < 0.05 vs. D 14 in same strain, †P < 0.05 vs. SD; #P < 0.05 vs. SHR.
Stratified that high-salt feeding during pregnancy resulted in hypertension, fetal growth restriction, vascular thickening in the decidua, and impaired nitric oxide-cGMP signaling (45). Preliminary observations in our laboratory also indicate that high-salt intake during early pregnancy (GD 2–11) exacerbates the superimposed preeclampsia phenotype in the Dahl S rat as we see increased urinary protein excretion during late pregnancy (492 ± 38 mg/day, n = 3) and increased MAP (from ~147 mmHg at baseline to ~172 mmHg at midpregnancy, n = 2, unpublished data; Gillis EE, Williams JM, Garrett MR, Mooney JN, Sasser JM).

In addition to the increase in blood pressure during late pregnancy, the Dahl S rat exhibits a severe increase in urinary protein excretion between mid and late pregnancy; a change of over 100 mg/day in less than a week. This increase in urinary protein excretion is much greater than we have observed when age-matched virgin female rats are placed on a high-salt diet for 3 wk (8% NaCl, final urinary protein excretion of 101 ± 10 mg/day, unpublished data; Sasser JM, Garrett MR, and Mooney JN), and this rise is greater than would be predicted based on the change in blood pressure observed in the pregnant Dahl S. Furthermore, this increase in urinary protein excretion is greatly exacerbated in a subsequent pregnancy and reverses after delivery. These rapid changes in proteinuria (with a relatively modest increase in BP) suggest that increased glomerular permeability occurs during pregnancy in the Dahl S rat. The mechanism for this severely exacerbated proteinuria in the Dahl S rat warrants further research and could be related to circulating factors released from the placenta (such as TNF-α or the anti-angiogenic factor sFlt-1) that directly affect the glomerular endothelium or podocytes. This is evidenced by the dramatic changes in glomerular diameter/area observed in the pregnant Dahl S rat compared to the age-matched virgin rat. Interestingly, we also observed renal arteriolar thickening and hyaline deposition in some vessels of kidneys isolated from pregnant Dahl S rats that was not present in the virgin Dahl S rats or pregnant or virgin SD or SHR. These renovascular changes are similar to those previously characterized in women with preexisting hypertension who develop superimposed preeclampsia (12, 32). The changes in renal histology observed in this model are remarkable considering the very rapid time course of the change (less than 3 wk of pregnancy). The increase in proteinuria and renal injury in the Dahl S is in stark contrast to the SHR which, although hypertensive before pregnancy, exhibits no proteinuria and minimal renal injury before or during pregnancy. In fact, the SHR has no proteinuria or renal injury even after three successive pregnancies (5).

The Dahl S rat also exhibits changes in uterine artery blood flow waveforms that are characteristic of those observed in women with preeclampsia (30, 44). In a healthy pregnancy,
uterine artery resistance is decreased, as observed here in both the SD and SHR pregnancy. However, the Dahl S does not demonstrate a drop in UARI between GD14 and 18 and exhibits a distinctive “notch” in the Doppler waveform, similar to what is observed in the RUPP rat model of preeclampsia (46) and human preeclamptic pregnancies (30, 44). Some studies suggest that increased uterine artery resistance index with notching during the second trimester is a predictor for the later development of preeclampsia (26). This finding, along with the observed increase in HIF-1α abundance in the placenta, suggests that blood flow to the uteroplacental unit is compromised during the Dahl S pregnancy. Therefore, we speculate that increased hypoxia within the placenta initiates the maternal syndrome (exacerbated hypertension and proteinuria) observed in this strain. The increased abundance of placental HIF1-α in the Dahl S rat supports this theory that a decrease in uterine blood flow creates a hypoxic environment, driving the maternal syndrome in the Dahl S rat, but does not explain our findings in the SHR, which exhibits normal cardiovascular adaptations to pregnancy despite an increase in placental hypoxia. Further research is necessary to determine what other factors may compensate for the increases in placental HIF-1α and TNF-α in the SHR. The reduction in blood flow to the fetus could also contribute to the observed intrauterine growth restriction observed in the Dahl S. Despite having smaller litters and larger placentas than the other strains, the Dahl S pups were smaller than the SD and, when normalized to maternal weight to account for differences in the size of the strains, the SHR. The Dahl S rat exhibits an increased placenta weight-to-fetal weight ratio compared with the SD and SHR. Increased placental weight-to-birth weight ratios in human patients have previously been described as a marker of placental inefficiency and inadequate nutrient supply to the fetus (22). Furthermore, epidemiological studies have shown that preeclamptic women tend to have larger placental weight-to-birth weight ratios compared with normotensive women (34).

One factor that has been implicated in the progression of the maternal syndrome of preeclampsia is the inflammatory cytokine TNF-α. Women with preeclampsia have higher levels of
TNF-α compared with women who have a normal pregnancy or those who develop gestational hypertension (11), and hypoxia increased the production of TNF-α by villous explants from the human placenta in ex vivo experiments (6). In addition, in the RUPP model of preeclampsia, serum TNF-α levels are increased compared with normal pregnancy (21), and blockade of the actions of TNF-α with etanercept reduced blood pressure in this model (20). In the current study, we observed an increase in placental concentrations of TNF-α in both the Dahl S and the SHR compared with the SD; however, plasma levels of TNF-α were only increased during pregnancy in the Dahl S. This increase in circulating TNF-α in the Dahl S may contribute to the observed maternal symptoms of preclampsia. Interestingly, placental TNF-α levels were comparable between the Dahl S and the SHR, although plasma TNF-α did not increase in the SHR, and the SHR does not exhibit a preeclamptic phenotype. Similar to a previous study in women with preeclampsia (7), our findings suggest that there are other sources of TNF-α in addition to the placenta that contribute to increased circulating TNF-α in preeclampsia. Further research is needed to determine the upstream and downstream factors that link placental ischemia, TNF-α production, and systemic endothelial dysfunction.

The antiangiogenic factor sFlt-1 has also been extensively studied in preeclamptic pregnancies (1, 42, 49). In addition to human studies showing elevated sFlt-1 during and even preceding the clinical symptoms of preeclampsia, studies in mice have shown that administration of sFlt-1 is capable of recapitulating the maternal syndrome and fetal outcomes of this disease process (42). Though there is a general consensus in the field that sFlt-1 is increased in preeclampsia, the timing of this increase is not yet well understood (50). In the current study, we found that sFlt-1 is increased during late pregnancy in the Dahl S rat, but future studies will determine the timeline of sFlt-1 changes during early to midpregnancy in this model to gain further understanding of the pathogenesis of this disease process and the mechanisms upstream of sFlt-1 that stimulate its production.

**Perspectives and Significance**

As a spontaneous model of superimposed preeclampsia, the Dahl S rat provides a powerful model to gain a greater understanding of the pathogenesis of this disease. The identification of a spontaneous rat model of preeclampsia allows for the analysis of time-dependent changes throughout pregnancy in the placenta, vasculature, and kidney, as well as the discovery of new biomarkers that are present early during pregnancy. This model of preeclampsia superimposed on hypertension and chronic kidney disease will aid in identification of new therapeutics targets and biomarkers for preeclampsia in populations with preexisting cardio-renal diseases as well as provide insights into the genetic contributions present in preeclampsia and long term cardiovascular outcomes for both mothers who have experienced preeclampsia and their offspring.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


