RESEARCH ARTICLE | Hypertensive Disorders of Pregnancy: Effects on Mother and Baby

Adverse metabolic phenotype of female offspring exposed to preeclampsia in utero: a characterization of the BPH/5 mouse in postnatal life

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Sutton EF, Lob HE, Song J, Xia YW, Butler S, Liu C, Redman LM, Sones JL. Adverse metabolic phenotype of female offspring exposed to preeclampsia in utero: a characterization of the BPH/5 mouse in postnatal life. Am J Physiol Regul Integr Comp Physiol 312: R485–R491, 2017. First published January 25, 2017; doi:10.1152/ajpregu.00512.2016.—Preeclampsia (PE) is a devastating disorder of pregnancy that classically presents with maternal hypertension and proteinuria after 20 wk of gestation. In addition to being a leading cause of maternal and fetal morbidity/mortality, epidemiological and prospective studies have revealed long-term consequences for both the mother and baby of preeclamptic pregnancies, including chronic hypertension as well as other cardiovascular diseases and metabolic derangements. To better understand the effect of in utero exposure of PE on offspring, we utilized the BPH/5 mouse, a spontaneous model of the maternal and fetal PE syndrome. We hypothesized that young BPH/5 offspring would have altered metabolic and cardiovascular phenotypes. Indeed, BPH/5 growth-restricted offspring showed excess catch-up growth by early adulthood due to hyperphagia and increased white adipose tissue (WAT) accumulation, with inflammation markers isolated to the reproductive WAT depot only. Both excessive WAT accumulation and the inflammatory WAT phenotype were corrected by pair-feeding young BPH/5 female mice. We also found that young BPH/5 female mice showed evidence of leptin resistance. Indeed, chronic hyperleptinemia has been shown to characterize other rodent models of PE; however, the maternal metabolic profile before pregnancy has not been fully understood. Furthermore, we found that these mice show signs of cardiovascular anomalies (hypertension and cardiomegaly) and altered signaling within the reproductive axis in early life. Future studies will involve challenging the physiological metabolic state of BPH/5 mice through pair-feeding to reduce WAT before pregnancy and determining its causal role in adverse pregnancy outcomes.

Preeclampsia affects 2–8% of pregnancies in the US and is the leading cause for maternal and perinatal morbidity and mortality worldwide (12, 25). This pregnancy-specific disorder is characterized at or after 20 wk of gestation by the onset of hypertension and one other accompanying sign or symptom, including proteinuria, thrombocytopenia, impaired liver function, pulmonary edema, and headache or visual deficits (14). Despite its high incidence and severe consequences, a cure for preeclampsia still eludes us. As employed beginning 150 yr ago, preterm delivery of the fetus and placenta still remains the only curative treatment for preeclampsia. Indeed, preeclampsia is responsible for ~15–20% of preterm births per year (11). In addition to prematurity, preeclampsia is also a leading cause of fetal growth restriction, with up to 25% of growth-restricted and small-for-gestational age babies born to preeclamptic mothers (11).

Beyond the perinatal period lie significant long-term consequences of preeclampsia for both mother and baby. For the mother, risk for developing cardiovascular disease later in life increases two- to eightfold after a preeclamptic pregnancy (10). For the offspring, in utero development within a preeclamptic or hypertensive environment increases the risk for hypertension, cardiovascular diseases, and metabolic derangements in adolescence and adulthood (18, 19). This phenomenon exemplifies the Developmental Origins of Health and Disease (DOHaD) hypothesis (1), theorizing that in utero exposures during early development program an individual’s risk for health and disease later in life.

Given the profound short- and long-term consequences as well as the prevalence of preeclampsia, further research is crucial to prevent, treat, and advance our understanding of this pregnancy-specific disorder. However, studying preeclampsia in humans presents numerous challenges, such as limitations for research within a vulnerable population and an obvious inability for in vivo mechanistic studies. There are limited animal models to study preeclampsia, with most replicating single aspects of the disease state and failing to imitate the full maternal and fetal syndrome (24). The BPH/5 model, initially described in 2002 by Davisson et al. (5), was the first spontaneous mouse model of preeclampsia mimicking key maternal and fetal adverse outcomes. In midgestation (E9.5–E12.5), BPH/5 mice begin to exhibit uterine/placental abnormalities, including poor trophoblast invasion, decreased trophoblast remodeling of decidual vasculature, and increased vascular uterine artery resistance (6). By late gestation (E14.5–E20.5), BPH/5 mice display elevated mean arterial pressure (MAP) and proteinuria, endothelial dysfunction, and renal glomerulosclerosis as well as reduced fetal growth, decreased litter sizes, and...
small-birth-weight offspring (6). Although these mice have been described during pregnancy, no studies to date have characterized these offspring in early life or before pregnancy.

The aim of our study was to investigate the offspring of BPH/5 pregnancies to better understand the consequences of preeclampsia exposure in utero. We found that homozygous BPH/5 female offspring from preeclamptic BPH/5 pregnancies exhibit excessive catch-up growth in early life, which results in increased body weights, elevated adiposity, adipose tissue inflammation, hyperleptinemia, and leptin resistance as early as 8 wk of age. At early adulthood, female BPH/5 offspring exhibit adverse cardiovascular phenotypes (elevated arterial blood pressure and cardiomegaly) and perturbed reproductive axes.

MATERIALS AND METHODS

Animals

All animal experiments were approved by the Cornell University Institutional Animal Care and Use Committee and conducted in accordance with the guidelines of the National Institutes of Health. For all studies we used young, virgin 6- to 12-wk-old C57BL/6 and BPH/5 mice from our house colony (5). Animals were housed in a climate-controlled barrier facility with a 12-h light-dark cycle and fed a standard chow diet. At the age of 8 wk, some animals were housed in Comprehensive Laboratory Animal Monitoring System (CLAMS) cages for 72 h. After 48 h of acclimatization were allowed, energy expenditure and spontaneous locomotor activity were measured in these cages.

Leptin ELISA

Blood was collected via cardiac puncture, allowed to clot at room temperature for 90 min, centrifuged at 3,500 rpm for 20 min, and then stored at −80°C until it was assayed. A commercially available leptin ELISA was performed according to manufacturer’s instructions (Cayman Chemicals, Ann Arbor, MI). The sensitivity of the assay was 50 pg/ml.

Leptin Administration

Mice were administered leptin (intraperitoneal injection, 30 μg twice/day) over a 3-day period, as described previously (30).

Measurements of Food Intake and Pair-Feeding Protocol

Normal Chow food intake of 6-wk-old nonpregnant C57BL/6 and BPH/5 mice was measured concurrently for 14 days. Some BPH/5 mice received food intake matched to C57BL/6 counterparts (~25% less calories than their ad libitum-fed BPH/5 littermates during that time).

Quantitative Real-Time Polymerase Chain Reaction

All tissues were removed immediately after decapitation and flash-frozen. RNA was extracted using TRIzol reagent according to the manufacturer’s protocol (Invitrogen, Carlsbad, CA). RNA quality and quantity were assessed by spectrophotometry (NanoDrop), and 1,000 ng was used for reverse transcription using the qScript cDNA kit (Quanta BioSciences, Beverly, MA). Quantitative RT-PCR was performed in triplicate with an ABI 7500 Fast Thermocycler (Applied Bioscience) using SYBR Green (Quanta BioSciences). Data were analyzed using the ΔΔCT method, and results were normalized to 18s (15). Gene targets and primer sequences are listed in Table 1.

Table 1. Target genes and primer sequences for real-time PCR

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Primer Sequence</th>
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<tbody>
<tr>
<td>18s</td>
<td>5'-GTA ACC COT TGA ACC CCA TT-3'</td>
</tr>
<tr>
<td>TNFa</td>
<td>5'-GCA TGC TAG TAG CGG TAG GD-3'</td>
</tr>
<tr>
<td>IL-6</td>
<td>5'-GGC CCT ACT TGG CCA CCA GTA AGG ACT-3'</td>
</tr>
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Determination of Estrous Cycle

Vaginal cytology was performed on adult female nonpregnant C57BL/6 and BPH/5 mice daily, as described (28a). After a complete estrous cycle was documented (proestrus, estrus, and diestrus), tissue was collected for uterine wet weight to confirm vaginal cytology staging (28a). Serum was collected at proestrus and on the first day of diestrus and used for measurement of circulating 17β-estradiol with a commercially available rodent ELISA kit according to manufacturer’s instructions (CalBiotech, El Cajon, CA) and as published previously (7a).

Radiotelemetric Measurement of Blood Pressure and Heart Rate

Nonpregnant BPH/5 and C57BL/6 female mice underwent carotid implantation of telemetry (Data Sciences International) according to published methods (3, 5). Briefly, female mice were anesthetized for placement of a telemeter in the left carotid artery and transmitter body in the subcutaneous space. Mice were allowed to recover for 10 days, followed by 5 days of heart rate (beats/min) and MAP recording.

Statistical Analysis

Data are presented as means ± SE. To determine statistical significance, we employed two-way ANOVA with Sidak’s multiple-comparisons test or Student’s t-test when appropriate. P values <0.05 were considered significant.

RESULTS

Metabolic Phenotype of Young BPH/5 Female Offspring

Body weight and adiposity. Offspring of preeclamptic BPH/5 dams have been described previously as growth restricted in utero (E12.5, E14.5, and E18.5) and at birth compared with C57BL/6 offspring (5, 6, 23). In the present study, we observed accelerated catch up growth of female BPH/5 pups. Compared with wild-type female counterparts, body weight of BPH/5 females was significantly less on postnatal (PN) day 1, normalized at 3 wk of age, and higher by 8 wk of age (Fig. 1A). Concomitantly, BPH/5 female offspring had comparable adipose tissue mass at 3 wk of age and increased visceral (reproductive and perirenal) and subcutaneous white adipose tissue (WAT) mass by 8 wk of age (Fig. 1, B–D). Brown adipose tissue (BAT) mass was comparable at 3 and 8 wk of age in C57BL/6 and BPH/5 females (87 ± 2.4 vs. 97 ± 17.3 and 80.4 ± 11.7 vs. 89.6 ± 7.8 mg, respectively; n = 3–15).

Energy intake and expenditure. To investigate whether the observed increased adiposity is driven by an increase in energy intake and/or a decrease in energy expenditure, food
intake and energy expenditure were assessed over 14 days and 72 h, respectively. Eight-week-old BPH/5 female offspring consume ~25% more food when fed normal chow ad libitum compared with age-matched C57BL/6 female mice without differences in oxygen consumption or heat production as determined by CLAMS cages (Fig. 1, E–G). The respiratory exchange ratio was significantly lower in BPH/5 female mice, indicating that these animals rely on fat as an energy substrate more than C57BL/6 female controls (Fig. 1H). Indeed, pair-fed BPH/5 offspring (matched to C57BL/6 ad libitum food intake) exhibited significantly decreased body weights and adipose tissue similar to C57BL/6 controls (Fig. 1, A–D).

**Leptin resistance.** Consistent with elevated adiposity, 8-wk-old BPH/5 female offspring have elevated serum leptin levels compared with their C57BL/6 counterparts (Fig. 2A). After 3 days of leptin administration, 8-wk-old BPH/5 female offspring exhibited severely blunted reductions in food intake and body weight
loss compared with age-matched C57BL/6 female mice (Fig. 2, B and C). This suggests that BPH/5 female mice are resistant to the antihyperphagic effects of leptin, which is indicative of leptin resistance.

Adipose inflammation. Increased adiposity is linked to increased adipose tissue inflammation (27). Reproductive WAT of 8-wk-old BPH/5 female offspring displayed a seven- and fourfold increase in TNF-α and IL-6 mRNA, respectively, compared with their wild-type counterparts (Fig. 3, A and B). Reversing this adiposity phenotype with 2 wk of pair-feeding BPH/5 offspring resulted in a significant blunting of these inflammatory markers (Fig. 3, A and B).

Reproductive Phenotype of Young BPH/5 Female Offspring

Because reproductive WAT showed a profound increase in inflammatory markers and it has been shown previously that BPH/5 mice have excessive uterine inflammation early in pregnancy (23), the reproductive axis was investigated in BPH/5 female offspring. BPH/5 and C57BL/6 female mice were housed individually, and daily vaginal cytologies were performed to determine the stage of estrous cycle, as described previously by Caligioni (4). BPH/5 showed unpredictable and irregular estrous cycles, whereas C57BL/6 followed a regular 4- to 5-day cycle length (representative examples: Fig. 4, A and B). Circulating ovarian hormones and uterine wet weights were obtained on the first day of cytologic diestrus (presence of leukocytes) and proestrus (lack of leukocytes among vaginal epithelial cells) from BPH/5 and C57BL/6 cycling females. BPH/5 females had significantly lower levels of circulating 17β-estradiol compared with C57BL/6 female mice in proestrus and diestrus (Fig. 4C) and comparable circulating progesterone levels (data not shown). Uterine wet weights were increased significantly in C57BL/6 females in diestrus versus proestrus as anticipated (Fig. 4D). Whereas BPH/5 uteri showed a similar increase from diestrus to proestrus, their diestrus uterine wet weights were significantly higher compared with C57BL/6 (Fig. 4D). This suggests an alternative source of uterine edema, i.e., inflammation, rather than ovarian hormones in the cycling BPH/5 female.

Cardiovascular Phenotype of Young BPH/5 Female Offspring

Exposure to preeclampsia and gestational hypertension in utero has been shown to program adverse cardiovascular phenotypes in offspring, including hypertension (18, 19). As we have reported previously, adult BPH/5 female offspring have elevated central blood pressure measured by radiotelemetry at 8 wk of age compared with age-matched C57BL/6 female mice (Fig. 5, A and B) (5). This is important because 26% of women entering pregnancy with preexisting chronic hypertension will develop superimposed preeclampsia in their pregnancies (2). Additionally, herein we report decreased heart rate and elevated heart weight-to-body weight ratios in 8-wk-old female BPH/5 offspring versus wild-type controls, which is indicative of cardiomegaly (Fig. 5C).

DISCUSSION

Preeclampsia is a pregnancy-specific disorder that can impart severe, life-threatening short- and long-term effects on both the mother and her baby. The BPH/5 mouse is an inbred strain that spontaneously demonstrates both the maternal and fetal syndromes of preeclampsia, presenting with a significant rise in late-gestational MAP and proteinuria in pregnancy and resolution postpartum. This strain has been extensively characterized during pregnancy (5, 6) and is an exceptional model for mechanistic studies of preeclampsia. Furthermore, because prepregnancy risk factors for preeclampsia, including hyper-
tension, obesity, and elevated cholesterol have been identified (16), characterization of the BPH/5 model before pregnancy presents a unique and important opportunity for investigation. In addition, an early-in-life characterization of the BPH/5 offspring allows for inferences of the effects of in utero preeclampsia exposure (i.e., DOHaD hypothesis). Our study extends our understanding of in utero preeclampsia exposure on BPH/5 female offspring and describes the young and adult virgin BPH/5 female mouse. This is an important consideration, as a strong genetic component is associated with preeclampsia in humans. A higher frequency of preeclampsia is observed in daughters of preeclamptic mothers versus daughters-in-law (22). While appearing healthy, BPH/5 females presented with a severely altered metabolic phenotype early in life (excessive catch-up growth, increased adiposity, adipose tissue inflammation, and leptin resistance by 8 wk of age) along with adverse cardiovascular outcomes, including cardiomegaly and elevated blood pressure, as well as abnormal reproductive signaling.

We have identified the BPH/5 offspring as a model for early-life catch-up growth. As is common in offspring of preeclamptic pregnancies (17), BPH/5 offspring are growth restricted in utero and are born small. However, these mice experience a burst of growth in early life associated with increased food intake, body weight, and adipose tissue mass by 8 wk of age; no alterations in energy expenditure were observed. This catch-up growth also coincides with reproductive WAT inflammation, hyperleptinemia, and leptin resistance. High-fat diet and overeating are the most common triggers for obesity; these lead to rapid increases in circulating leptin, which in turn can cause tissue leptin resistance (20). Considered together, these data describe an adverse metabolic phenotype strongly driven by excess adipose tissue mass.

Next, we examined the cardiovascular health of young BPH/5 female offspring. Exposure to preeclampsia or gestational hypertension has been shown to correlate with elevated blood pressure in adolescence and adulthood (18, 19), and
small size at birth has been shown to correlate with hypertension, cardiovascular disease, and death by cardiovascular disease later in life in humans (7, 9, 21, 26). At 8 wk of age, BPH/5 female offspring exhibit cardiomegaly, elevated mean arterial blood pressure, and lower heart rate compared with age-matched C57BL/6 mice. These data support the hypothesis that in utero exposure to the preeclampsia syndrome imparts cardiovascular effects that present early in life.

Finally, we investigated the reproductive axes of BPH/5 female offspring, as altered cardiovascular and metabolic phenotypes are interwoven with ovarian hormone signaling and cyclicity in females (28). BPH/5 female mice showed decreased serum 17β-estradiol throughout the estrous cycle in combination with inappropriately excessive uterine wet weight during diestrus. Ongoing studies involve characterizing uterine inflammation in virgin BPH/5 females and determining the impact on pregnancy outcomes in BPH/5 mice. Interestingly, although BPH/5 female offspring have increased reproductive, perirenal, and subcutaneous WAT, only the reproductive adipose tissue exhibits increased inflammatory markers. Moreover, correction of this adiposity phenotype by pair-feeding reduces evidence of inflammation and excessive fat mass. We hypothesize that this increased adipose tissue mass, in particular the reproductive WAT, which is singularly inflamed, may be the primary driver for the abnormal reproductive and potentially whole body phenotype of the female BPH/5 mice described herein.

The BPH/5 hypertensive profile during late gestation has been well characterized using radiotelemetry throughout pregnancy and tested with a number of interventions (5, 8, 23, 29). Although documenting the increase in maternal MAP in every pregnant BPH/5 female would be advantageous, this type of characterization is not feasible in every BPH/5 pregnant mouse and represents a limitation of this study. Studies involving longitudinal maternal blood pressure monitoring with paired feeding during pregnancy are being undertaken to further understand the role of obesity and adiposity in the pathogenesis of preeclampsia. An additional limitation of this study is clarification of a programming effect versus a litter effect. Because of small litter sizes in BPH/5 mice, all females within a litter were utilized for analysis to increase sample size and power of findings. Ideally, one female per litter would be used for studies; however, these kind of approaches are not practical in mouse models that exhibit significant fetal demise, such as BPH/5 (5, 23).

Future directions for this research should focus on expounding the genetic contribution to this spontaneous, inbred model by high-throughput molecular analyses of BPH/5 female mice and their WAT. Understanding the BPH/5 genotype will enable future studies to decipher between genotypic effects and the effects of in utero exposure to preeclampsia during fetal development. Furthermore, maternal versus paternal contributions could be investigated by characterizing pregnancy outcomes after heterozygous breeding, i.e., BPH/5 females with C57BL/6 males and C57BL/6 females and BPH/5 males. Recent evidence supports the role for sex-specific differences in metabolic alterations and offspring in which female infants were fed high-fat diets (13). Our unpublished observations do not support evidence for obesity in male offspring, but this warrants additional investigations. In summary, our studies here show that BPH/5 female offspring exhibit an adiposity-driven adverse metabolic phenotype by 8 wk of age, with early-life cardiovascular and reproductive alterations. Further studies are required to understand the impact of these alterations on the incidences of preeclampsia in women and offspring health.

Perspectives and Significance

Metabolic phenotypes, including obesity, affect a large portion of reproductive-age women. The contribution of parental genotype versus maternal phenotype on pregnancy outcomes is an area of much interest. The use of obese animal models that mimic the human condition of overeating by spontaneous hyperphagia are useful in elucidating the central mechanisms that drive obesity, increased adiposity, and adverse pregnancy outcomes such as preeclampsia, preterm birth, and neonatal morbidity/mortality. The application of different dietary interventions on obese animal models in the context of pregnancy provides a unique opportunity to test whether 1) preconception nutritional counseling and/or 2) minimization of gestational weight gain will ameliorate adverse pregnancy outcomes. These types of longitudinal studies in pregnant women are logistically difficult, and thus animal models such as BPH/5 will aid in our global understanding of obesity and adiposity in the pathogenesis of preeclampsia.

ACKNOWLEDGMENTS

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REFERENCES


